REVISTA ESPAÑOLA DE FISIOLOGIA, 43, (1), 45-50, 1987

Inhibition of Sugar Transport across Rat Jejunum, *in vivo*, by Cupric Ions

M.ª J. Rodríguez-Yoldi* and F. Ponz**

Departamento de Fisiología Animal
Universidad de Navarra
31080 Pamplona (Spain)

(Received on Septembre 12, 1986)

M. J. RODRIGUEZ-YOLDI and F. PONZ. *Inhibition of Sugar Transport across Jejunum, in vivo by Cupric Ions.* Rev. esp. Fisiol., 43 (1), 45-50, 1987.

Cupric ions inhibit galactose absorption by *in vivo* perfused rat jejunum. It takes some delay for the inhibitory action to display its maximal levels, and previous exposure of the mucosa to Cu markedly increases inhibition. Copper effects were only scarcely reversed by saline solution washing, more effectively by EDTA and more so by dithioerythritol, in no case reaching control values. Absorption of L-sorbose, or that of galactose in the presence of 0.5 mM phlorizin, are not modified by 0.5 mM cupric ions. Cu action may be understood as a selective impairment of the phlorizin-sensitive sugar transport system by binding of the metal to prevailing thiol chemical groups of proteins at the brush border, located at different depth within the thickness of the membrane.

Key words: Sugar transport, Galactose absorption, Copper.

It was shown some time ago (12) inhibition of intestinal absorption of 0.3 m glucose in rat, *in vivo*, by Cu^{2+}. This metal also inhibited galactose absorption but not that of arabinose, the inhibition being partially reverted by EDTA (10). Alanine absorption across rabbit ileon *in vitro* was also depressed by cupper (8).

When in luminal solutions, Cu is absorbed across intestine (17, 18) in a yet unclarified way (13, 9), perhaps involving a saturable component dependent on metabolic energy (2). Moreover, Cu can bind to proteins (7) through amino, carboxyl and thiol groups (15), so that this binding may be implicated in the intestinal absorption of the metal (4-6, 16). It was suggested that the inhibition of absorption of several substrates by copper could be related to its binding to thiol groups of proteins (1, 10, 12).

In the present paper, Cu action on the intestinal absorption of sugars and its partial reversibility by EDTA or DTE treatment, is studied in rat *in vivo*. It is shown that Cu inhibition exclusively affects the phlorizin-sensitive carrier transport and that the thiol groups are involved in that effect.

* Present address: Departamento de Fisiología, Facultad de Veterinaria. 50080 Zaragoza (Spain).

** To whom correspondence should be addressed.

Materials and Methods

Intestinal absorption of sugars has been studied *in vivo*, in anaesthetized Wistar rats, by the PONZ *et al.* perfusion technique (13) with jejunal segments about 20 cm long. Procedure specifications, sources of compounds, and sugar determinations were as recently described (14). Cu^{2+} was added to the perfusion solution as $CuCl_2$ (Merck).

Results

A series of three successive 1 min periods of galactose absorption in control conditions was first made on each animal, followed by series of three periods in the presence of 0.5 mM Cu, three more periods without Cu in the solution, three more with 5 mM Cu, and a final series of three periods in the absence of Cu. Between an absorption period and the next one, the lumen of the cannulated jejunum was thoroughly washed with 150 mM Na Cl saline solution. As fig. 1 shows, 0.5 mM Cu did not modify D-galactose absorption in this type of experiment, whilst a 5 mM metal concentration produced about 15% inhibition ($p < 0.03$).

The Cu action strongly increased when a sugar free solution containing the metal was allowed to perfuse the lumen for 5 min before measuring galactose absorption in the presence of Cu. After this exposure of the mucosa to the Cu solution, about 33% inhibition was found with 0.5 mM Cu, and a 43% one with 5 mM Cu (fig. 1). Washing the lumen with saline solution slightly restored galactose absorption.

In some experiments, after the measurement of the control absorption of galactose and the exposure of the mucosa to Cu solution for 5 min, sugar absorption in the presence of Cu was followed through 9 min of continuous perfusion, taking perfusate fractions every minute. The jejunum lumen was then perfused with 10 mM EDTA containing saline solution for 10 min and absorption in the

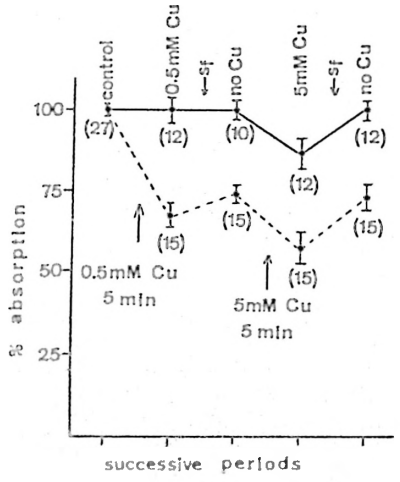

Fig. 1. *Effects of Cu on the intestinal absorption of 1 mM D-galactose.*
Full line, without previous exposure to Cu solution. Broken line, after 5 min exposure of the mucosa to the metal. sf, washing with 150 mM NaCl solution. Mean values ± SE. Data number in parenthesis.

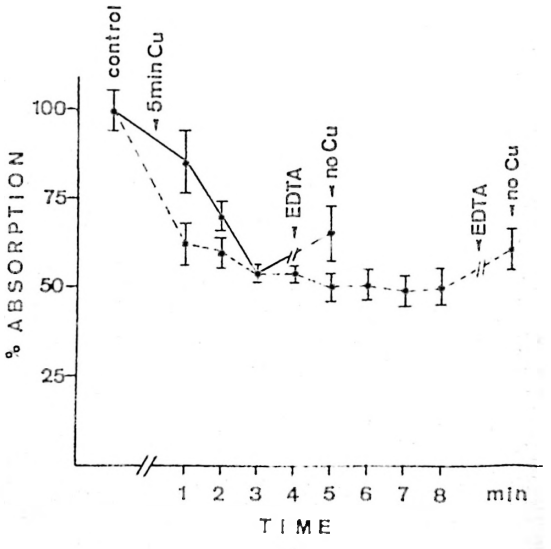

Fig. 2. *Time course of the inhibition of 1mM D-galactose absorption by Cu and partial reversion by 10 mM EDTA.*
Mean values ± SE. (—) 0.5 mM. (- - -) 5 mM Cu.

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

D-GALACTOSE (mM)	CONTROL	EXPOSURETO 0.5 mM Cu	+ 0.5 mM Cu	10 mM DTE	NO Cu
0.5	11.5 ± 0.9 (11)	+	6.0 ± 1 (11) % 47.8	+	8.51 ± 1.4 (12) % 26
1	17.3 ± 0.6 (12)	+	9.5 ± 0.8 (11) % 45	+	13.5 ± 1.7 (11) % 22
5	80.5 ± 7 (10)	+	52.3 ± 6 (10) % 35	+	69.0 ± 8 (12) % 14.3

absence of Cu was again determined. It could be shown (fig. 2) that Cu at 0.5 mM or 5 mM concentrations began to display its inhibitory action during the first minute of absorption, but this inhibition increased until it reached a steady level after 6-7 min. Washing the lumen with EDTA was followed by clear enhancement of galactose absorption without climbing to the control values.

Table I reports the effects of 0.5 mM Cu on galactose absorption after exposure of the mucosa to the same Cu concentration for 5 min at three different concentrations of the sugar, and their reversion by dithioerythritol (DTE) treatment. DTE, like EDTA, markedly improved the galactose absorption previously inhibited by Cu.

Other experiments were carried out to test if Cu affected galactose absorption when the phlorizin-sensitive transport component was excluded. After a series of control periods, galactose absorption in the presence of 0.5 mM phlorizin was measured, then the mucosa was exposed to Cu solution for 5 min and lastly sugar absorption in the presence of 0.5 mM phlorizin and 0.5 mM or 5 mM Cu was

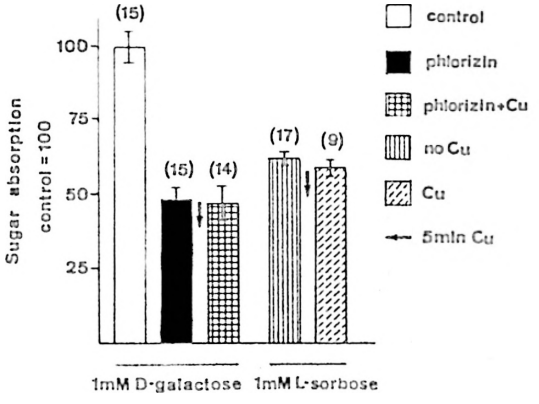

Fig. 3. *Ineffectiveness of 0.5 mM Cu on apparently diffusional absorption of sugars by rat jejunum.*
Mean values ± SE, referred in percent of control galactose absorption. Data number in parenthesis. Lack of Cu action on 1 mM D-galactose absorption in the presence of 0.5 mM phlorizin, and on 1 mM L-sorbose absorption.

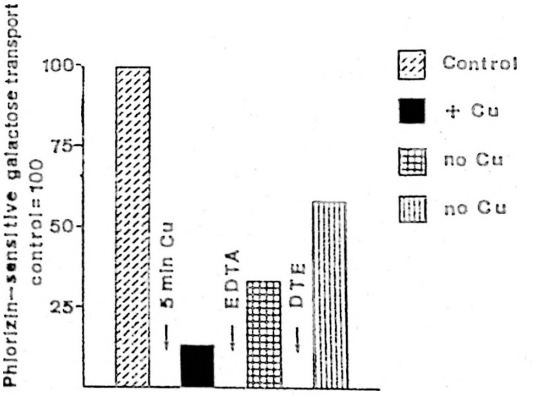

Fig. 4. *Effects of 0.5 mM Cu on the plorizin-sensitive transport component of 1 mM D-galactose absorption by rat jejunum and their partial reversibility by 10 mM EDTA or DTE.*
Mean values referred in percent of control transport.

determined. Fig. 3 shows that Cu did not modify the apparently diffusional component of absorption which continues after complete cessation of the phlorizin-sensitive transport.

If the Cu effect on galactose absorption were ascribable to a transport system inhibition, the metal would have to be ineffective on the absorption of L-sorbose, a non-transportable sugar, which was fairly the case as the same fig. 3 shows.

In fig. 4 the values for maximal sugar transport inhibitions by Cu are reported, under the assumption of an exclusive effect of the metal on the transport component. The transport has been estimated as the difference between galactose absorption in the absence and in the presence of 0.5 mM phlorizin. Inhibitions of sugar transport of about 85% were obtained by 0.5 mM Cu, which dropped to 67% or 42% after luminal washing with 10 mM EDTA or DTE, respectively.

Discussion

Cu inhibits galactose absorption by rat jejunum in agreement with old observations also *in vivo*, (10, 12) under very different experimental conditions (0.3 M sugar, 30 min absorption periods, disregard of Na concentration), and with recent preliminary assays (11). Inhibition of alanine transport by Cu across rabbit ileum *in vitro* had also been reported (8).

The present experiments reveal a great similarity between the sugar absorption inhibition by Cu and the one recently shown by Cd (14). Some interesting differences, however, are to be emphasized. With Cu the inhibitory action seems to be displayed more slowly than with Cd. Actually, in the first 1 min absorption period, 0.5 mM Cu concentrations were ineffective and those of 5 mM produced only a 15% diminution. Moreover, after exposure of the mucosa for 5 min to the Cu solution, a clear inhibition by 0.5 mM Cu was found, which kept increasing through a few more minutes of perfusion.

Essentially, this delay in the apparition of the Cu effect with respect to that of Cd may be explained by a slower fixation of Cu to the chemical groups of proteins at the epithelium surface or to a less accessible location of the involved groups to the metal.

Washing the mucosa with NaCl isotonic solution slightly improved galactose absorption after the Cu action. This improvement was clear when the saline solution contained 10 mM EDTA, a non-penetrating sequestering agent, and it was even greater when 10 mM DTE, a thiol group protector was present.

In the case of Cd (14), the inhibition was not modified by saline solution washing but completely disappeared by EDTA or DTE. These additional differences suggest that absorption inhibition by Cu involves its binding not only to chemical groups at the luminal surface, which can be easily reverted by EDTA, as it seems to be the case with Cd, but also to other more deeply located groups, not accessible to EDTA but partially set free by DTE treatment. Most of the binding groups are probably thiol groups.

Cupric ions inhibit galactose absorption by selective impairment of the phlorizin-sensitive transport system, without affecting the residual apparently diffusional passive component. This conclusion was favored by the lack of the Cu effect when galactose absorption was measured in the presence of 0.5 mM phlorizin, and when the absorbed sugar did not use that transport system, as in the case of L-sorbose. The inhibitions of galactose absorption may, therefore, refer to inhibitions of just the transport component, which reach 85% with 0.5 mM Cu.

Thus the impairment of sugar transport by Cu may be explained by binding of

the metal to prevailing thiol chemical groups of proteins, located at a different depth in the brush border membrane of enterocytes, pertaining or being functionally related to the phlorizin-sensitive transport system.

Resumen

El ion cúprico inhibe la absorción de la galactosa por yeyuno de rata perfundido *in vivo*. La acción inhibidora precisa de cierto tiempo para alcanzar su nivel máximo y la exposición previa de la mucosa al metal aumenta marcadamente la inhibición. Estos efectos del cobre se reversibilizan sólo en muy escasa proporción por lavado con solución salina, más ampliamente con EDTA y mejor aún con ditioeritritol, sin que se llegue a alcanzar en ningún caso los valores normales. La absorción de L-sorbosa y la de galactosa en presencia de florricina 0,5 mM no se modifican por Cu 0,5 mM. La acción del cobre puede comprenderse como perturbación selectiva del sistema de transporte de azúcares sensible a la florricina, por unión del metal a grupos químicos preferentemente tiólicos de proteínas del borde en cepillo, localizados a diferente profundidad en el espesor de la membrana.

Palabras clave: Transporte de azúcares, Absorción de galactosa, Cobre.

References

1. Bemner, I.: In «Biological Roles of Copper». Ciba Foundation Symposium. 79, (new series). Excerpta Medica, Amsterdam, 1980, pp. 23-48.
2. Crampton, R. F., Matthews, D. M. and Poisner, R.: *J. Physiol.*, 178, 111-126, 1965.
3. Evans, G. W.: *Physiol. Rev.*, 53, 535-570, 1973.
4. Evans, G. W. and Hahn, J.: *Adv. Exp. Med. Biol.*, 48, 285-297, 1973.
5. Evans, G. W. and Leblanc, F. N..: *Nutr. Rep. Intern.*, 14, 281-288, 1976.
6. Evans, G. W., Majors, P. F. and Cornatzer, W. E.: *Biochim. Biophys. Res. Commun.*, 40, 1142-1148, 1970.
7. Feeney, R. E. and Allison, R. G.: In «Evolutionary Biochemistry of Proteins». J. Wiley, London, 1969.
8. Frizzell, R. A. and Schultz, S. G.: *J. Gen. Physiol.*, 56, 462-490, 1970.
9. Gitlin, D., Hughes, W. L. and Janeway, C. A.: *Nature*, 188, 150-151, 1960.
10. Lluch, M. and Ponz, F.: *Rev. esp. Fisiol.*, 16, suppl. I., 309-316, 1960.
11. Ortiz, M.: Tesis doctoral, Facultad de Ciencias, Universidad de Navarra. Pamplona, 1978.
12. Ponz, F.: *Rev. esp. Fisiol.*, 9, 205-211, 1953.
13. Ponz, F., Ilundain, A. and Lluch, M.: *Rev. esp. Fisiol.*, 35, 97-104, 1979.
14. Rodríguez-Yoldi, M.ª J. and Ponz, F.: *Rev. esp. Fisiol.*, 43, 39-44, 1987.
15. Schubert, J.: In «Copper and peroxides in radiobiology and medicine». Charles C. Thomas Publisher, 1964.
16. Starcher, B. C.: *J. Nutr.*, 97, 321-326, 1969.
17. Van Berger Hennegouwen, G. P., Tangedahl, T. N., Hofmann, A. F., Northfield, T. C., La Russo, N. F. and McCall, J. T.: *Gastroenterology*, 72, 1228-1231, 1977.
18. Van Campen, D. R.: *J. Nutr.*, 88, 125-130, 1966.