REVISTA ESPAÑOLA DE FISIOLOGIA, 48 (2), 127-132, 1992

Role of −SH Groups in Rat Sugar Intestinal Transport *in vivo**

N. Diez, A. Barber** and F. Ponz

Departamento de Fisiología y Nutrición
Universidad de Navarra
31080 Pamplona (Spain)

(Received on March 8, 1992)

N. DIEZ, A. BARBER and F. PONZ. *Role of −SH Groups in Rat Sugar Intestinal Transport* in vivo. *Rev. esp. Fisiol.*, 48 (2), 127-132, 1992.

The effect of p-chloromercuribenzoic acid (pCMB), either alone or in the presence of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), on the 1 mM galactose absorption by *in vivo* perfused rat intestine has been studied. At 0.25 mM concentration, pCMB inhibits galactose absorption in about 32 % but it does not modify the absorption of this sugar when the transport is blocked by 0.5 mM phlorizin, or that of the non-transportable monosaccharide derivative 2-deoxy-D-glucose. This shows that only the active transport component of galactose absorption is inhibited. A 2 min preexposure period is required for the inhibition to appear. The inhibition was not reversed by washing with saline solution even when it contained 0.5 mM dithioerythritol, 10 mM cysteine or 5 and 10 mM EDTA. The simultaneous exposure to 0.25 pCMB and 0.25 mM DTNB inhibits the total galactose entry in about 50 %, an effect higher than the one exerted by each reagent separately and close to the one obtained with 0.5 mM phlorizin. Our results, *in vivo*, confirm the importance of the thiol groups in the cotransport of Na^+ and sugar. As DTNB is an SH-reagent of lesser liposolubility than pCMB, the existence of two populations of sulfhydryl groups related to sugar transport which differ in their location within the brushborder membrane and in accessibility from the intestinal lumen, is suggested.

Key words: pCMB, DTNB, Na^+-sugar cotransport, Intestinal absorption.

Sugar active transport by the intestine implies the cotransport of Na^+ and sugar across the enterocyte luminal membrane. The membrane protein responsible for this cotransport has been widely studied. In the case of rabbit intestine it seems to be constituted by an about 75 KDa polypeptide, which associates itself to form *in situ* an about 290 KDa homotetramer (14, 23) and whose aminoacid sequence has been established (6). Some sulfhydryl

* Supported by a grant from the Spanish DGICYT (Dirección General de Investigación Científica y Tecnológica) PB 86-0407.

** To whom all correspondence should be addressed. (Fax (948) 175500)

reagents disturb the cotransport in intestinal brush border membrane vesicles (2, 8, 9, 22) which suggests that the native state of these groups in the sodium-dependent cotransporter is essential for the translocation function. As the accessibility and reactivity of the thiol groups in the vesicle preparations may differ from those with intact epithelium, it seemed interesting to find out what would happen in closer physiological conditions.

Various heavy metals, which react with -SH groups, have been seen to inhibit sugar transport *in vivo* (12, 16, 17, 19) and *in vitro* (10, 11). The 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)*, an SH-reagent which inhibits sugar transport in brush border membrane vesicles (2, 9) has also been observed to inhibit galactose transport *in vivo* (5).

In the present paper the effect of the sulfhydryl reagent p-chloromercuribenzoic acid (pCMB) alone or jointly with DTNB, on sugar absorption by *in vivo* perfused rat intestine is studied.

Materials and Methods

Intestinal absorption by the jejunum of white Wistar rats (180-225 g), fasted for 24 h, has been measured according to the *in vivo* luminal perfusion technique of PONZ *et al.* (15). A proximal jejunum segment of about 20 cm was perfused *in situ* without recirculation (single pass), at 5.6 ml/min rate (peristaltic pump), with NaCl 0.9 % solution buffered with Tris-ClH (7.4 pH) to which sugar or reagents under study were added.

In each animal, the sugar absorption in control and in different experimental conditions was measured. Three successive absorption periods of two minute duration were used for each condition. Between the successive periods, washings of the jejunal segment with saline solution for 2 min were performed, and the residual content was emptied by pumping air for 2 min. In most of the experiments with pCMB, the reactive was previously perfused and exposed to the mucosa for 5 min. After emptying by pumping air, the sugar absorption was measured also in the presence of pCMB.

The absorbed sugar, determined by measuring radioactivity is expressed in $\mu mol \cdot cm^{-1} \cdot min^{-1}$. The sugars D-[1-^{14}C]-galactose (59.6 mCi/mmol) and 2-[1-^{14}C]-deoxy-D-glucose (58.0 mCi/mmol) were purchased from Radiochemical Dupont (Dreieich, Germany). Other products were D-galactose, L-cysteine (Merck), dithioerythritol (Aldrich Chemie), 2-deoxy-D-glucose and pCMB (Sigma).

Oxygen consumption measurements in everted intestinal rings (3) have been made according to Warburg's direct method (24).

Statistical analysis was carried out using the Student's t-test for paired values in *in vivo* experiments. In oxygen consumption experiments Student's t-test for unpaired values was applied. A probability < 0.05 was accepted as significant.

Results

Sugar transport inhibition by pCMB. — Results show that 0.25 mM pCMB inhibits absorption of 1 mM D-galactose in about 31.8 % (fig. 1), without significantly modifying the diffusional passive component obtained by blocking the active transport with 0.5 mM phlorizin. The passive absorption of the non-transportable sugar 2-deoxy-D-glucose is not altered either by the reagent. Then, the inhibition of galactose absorption by pCMB exclusively affects the active transport component, which would be reduced in about 54 %.

* *Abbreviations:* 2-D-glc, 2-deoxy-D-glucose; DTE, dithioerythritol; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); pCMB, p-chloromercuribenzoic acid.

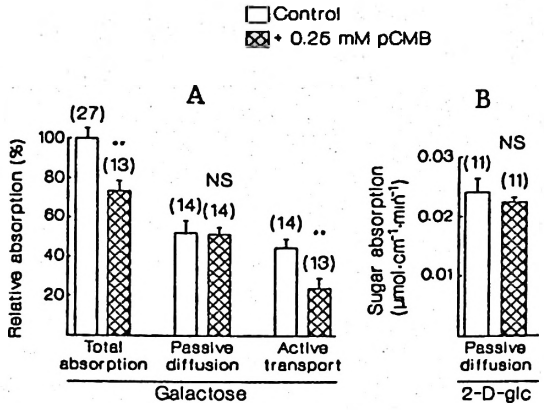

Fig. 1. *Effect of pCMB on the absorption of 1 mM D-galactose (A) and 1 mM 2-deoxy-D-glucose (2-D-glc) (B)*

Active transport values are obtained by difference between total and passive (+ 0.5 mM phlorizin) absorption. Before the absorption periods in the presence of the reagent, a previous 5 min perfusion with 0.25 mM pCMB was carried out. 100 % relative absorption of galactose was 0.0368 µmol · cm^{-1} · min^{-1}. Vertical bars denote standard error of the mean (SEM) of the number of separate determinations given on the top of the histograms. * $0.01 < p \leq 0.05$, ** $p \leq 0.01$. NS: non significant differences.

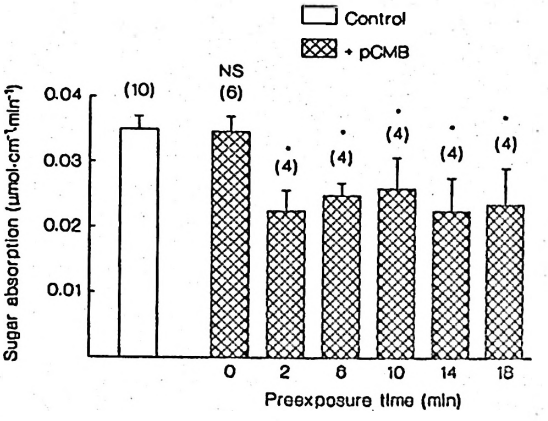

Fig. 2. *Influence of the preexposure time on the inhibition of 1 mM galactose absorption by 0.25 mM pCMB.*

After a pCMB perfusion during the indicated times, the sugar absorption (2 min) was measured, also in the presence of the reagent. Statistical significance and symbols as in figure 1.

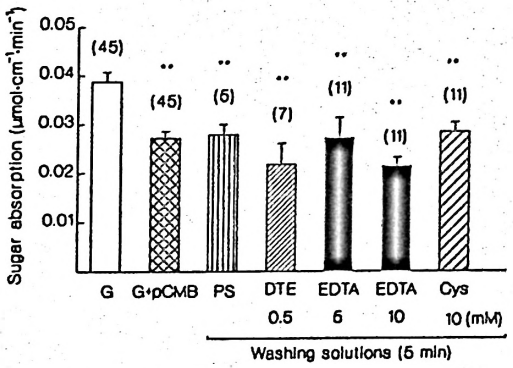

Fig. 3. *No reversion of the pCMB inhibition on galactose absorption by washing even in the presence of DTE, EDTA or Cys.*

After measuring galactose (G) absorption without and with 0.25 mM pCMB, a 5 min washing period with physiological solution (PS) or PS plus DTE, EDTA, or Cys was performed. Finally, the sugar absorption in the absence of pCMB was in each case obtained. Symbols as in figure 1.

The absorption inhibitions produced by 0.1 mM (30 %), 0.25 mM (32 %) or 0.5 mM (34 %) pCMB were not significantly different.

For the inhibition to appear, a previous period of mucosa exposure to pCMB is required. As fig. 2 shows, 2 min of preexposure to the reagent in the absence of sugar are sufficient, and the inhibition does not significantly increase with greater preexposure periods of up to 20 min. Most experiments, therefore, were carried out after a 5 min preexposure.

Reversibility of the effect of pCMB. — In order to verify the possible reversibility of the effect of pCMB, the intestinal segment in which galactose absorption had been determined both in absence and presence of the reagent, was washed with saline solution alone or containing 0.5 mM dithioerythritol (DTE), 5 and 10 mM EDTA or 10 mM cysteine. Subsequently galactose absorption was measured in the absence of pCMB. Results (fig. 3) show

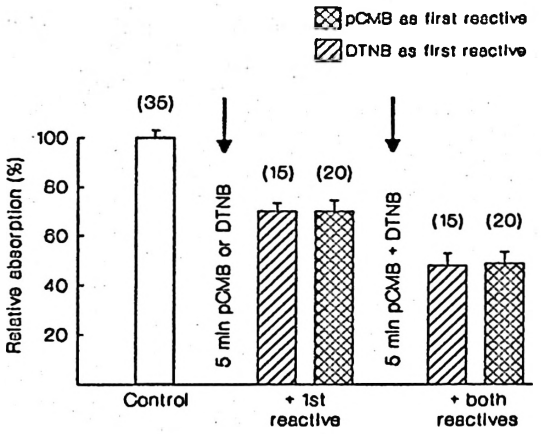

Fig. 4. *Additive inhibitory effect of pCMB and DTNB on galactose intestinal absorption* in vivo. After measuring galactose absorption in control conditions and in the presence of pCMB or DTNB (1st reactive, 5 min preexposition) the absorption in the presence of both reagents was determined (5 min preexposure with pCMB + DTNB). Symbols as stated in figure 1.

that in no case the inhibitory effect from pCMB was reversed.

Oxygen consumption by the tissue. — In order to determine if the action of the reagent might include alterations on the cellular oxidative metabolism, the oxygen consumption of everted jejunum rings has been evaluated. Oxygen consumption in the presence of 10 mM glucose is not significantly modified when the incubation medium contains 0.25 mM pCMB (data not shown).

Combined action of pCMB and DTNB. — Equivalent experiments *in vivo* had shown that DTNB inhibited D-galactose transport by reaction to thiol groups (5). As it is a sulfhydryl reagent of great specificity and low liposolubility (4, 9, 26) it might affect -SH groups other than those which react with pCMB. For this reason experiments to investigate the effect of the joint action of both reagents on sugar absorption have been carried out.

After measuring the total entry of 1 mM galactose in control conditions and in the presence of one of the reagents (pCMB or DTNB), the mucosa was also preexposed to the other reagent (DTNB or pCMB) during 5 min, and finally sugar absorption was determined in the simultaneous presence of pCMB and DTNB.

Results (fig. 4) show that the inhibition observed in the presence of both compounds, independently of the inhibitor used in the first place, is greater than the exerted by either of them when they were perfused separately. Thus it increases from about 30 % when only one reagent is used to about 50 % in the presence of both, an inhibition which is equivalent to that exerted by 0.5 mM phlorizin.

Discussion

Results show that, as it happens with heavy metals (10, 13, 17-20) and DTNB (5), pCMB lowers sugar intestinal absorption *in vivo*, by inhibiting the active transport component without affecting the passive entry. This action requires a preexposure time of the mucosa to the reagent and it does not vary with longer preexposures of up to 20 min, or with concentration changes between 0.1 mM and 0.5 mM, which suggests that all the -SH groups affecting the transport accessible to the reagent, react with 0.1 mM pCMB in 2 min or longer preexposures.

In this work short preexposure periods (5 min) and low reagent concentrations (0.25 mM) have been preferred to minimize possible secondary metabolic alterations and reactions with other than thiol groups (21, 26). As pCMB penetrates into the cells slowly (25) its action on sugar active transport might be thought to be due to inhibition of the oxidative metabolism, but in short periods oxygen consumption by the intestinal rings is not appreciably modified by the reagent.

Galactose transport inhibition by pCMB does not disappear after washing with saline solution, or when the latter contains EDTA, cysteine of DTE. This differs from DTNB inhibition (5) which reversed itself after washing with 0.5 mM DTE. This might be explained by the fact that the DTNB-sensitive -SH groups are more accessible to DTE on being located at the extracellular side of the membrane. In intestinal brush border membrane vesicles (9), pCMB effects are partially reversed by 5 mM DTE. This high concentration has not been reached in our experiments because 1, 5 or 10 mM concentrations of DTE were observed to provoke by themselves epithelial desquamation and galactose absorption inhibition.

The inhibition produced by pCMB (32 %) is of similar magnitude to that reported for DTNB (35 %). Nevertheless when both reagents act simultaneously, the inhibition of galactose intestinal absorption increases up to 50 %, a partially additive effect which suggests that each one of them acts on -SH group populations involved in the cotransporter, not completely coincident. Similar results have often been observed both for one protein (20, 26) and according to the protein arrangement in the membrane (1, 7-9) and are explained by differences in the chemical reactivity of the distinct agents or by the diverse accessibility of the reagents to the thiol groups according to their location in the membrane «geography» (20).

The 50 % inhibition by the combined presence of the two reagents is of a similar order to that obtained with 0.5 mM phlorizin which corresponds to the practically total blocking of the galactose active transport component. This seems to indicate that in those circumstances all the thiol groups essential for the process of the Na^+ and sugar cotransport are altered.

Our results *in vivo*, with intact epithelium, confirm the importance of the thiol groups in the sugar and Na^+ cotransport through the luminal membrane of the rat enterocytes. There seems to exist two populations of sulfhydryl groups essential for transport, those that react with DTNB, which could be located in hydrophilic regions of the membrane accessible from the intestinal lumen and those that react with pCMB, probably located in more internal hydrofobic regions, which could be reached by this reactive because of its greater liposolubility.

Resumen

Se estudia el efecto del ácido paracloromercuribenzoico (pCMB) sólo y en presencia del 5,5'-ditiobis(2-nitrobenzoico) (DTNB) sobre la absorción de galactosa 1 mM en intestino de rata, con una técnica de perfusión *in vivo*. El pCMB 0,25 mM inhibe sobre un 32 % la absorción total de galactosa, pero no modifica la absorción de este azúcar cuando se bloquea el transporte con florricina 0,5 mM, ni la del azúcar no transportable 2-deoxiglucosa. Ello indica que la inhibición de la absorción de galactosa se ejerce sobre el componente de transporte mediado. Se requiere un período de preexposición de 2 min para que se manifieste el efecto inhibidor, que no revierte por lavado con solución salina, ni siquiera cuando contiene ditioeritritol 0,5 mM, cisteína 10 mM o EDTA 5 o 10 mM. En presencia simultánea de pCMB y DTNB 0,25 mM, la absorción de galactosa se inhibe sobre un 50 %, efecto superior al obtenido por cada uno de los reactivos separadamente y similar al correspondiente al de la florricina 0,5 mM. Estos resultados *in vivo* confirman la importancia de los grupos tiol en el cotransporte intestinal de Na^+ y azúcar. Dada la menor liposolubilidad del DTNB respecto del pCMB, se sugiere la existencia de dos poblaciones de grupos sulfhidrilo relacionados con el transporte de azúcares que difieren en su localización en la membrana luminal y accesibilidad desde la luz intestinal.

Palabras clave: pCMB, DTNB, Cotransporte Na^+-azúcar, Absorción intestinal.

References

1. Adibi, S. A. and Kim, Y. S.: In «Physiology of the Gastrointestinal Tract» (Johnson, L. R., ed). Raven Press, New York, 1981, pp. 1073-1095.
2. Biber, J., Weber, J. and Semenza, G.: *Biochim. Biophys. Acta*, 728, 429-437, 1983.
3. Crane, R. K. and Mandelstam, P.: *Biochim. Biophys. Acta*, 45, 460-476, 1960.
4. Deuticke, B.: In «Reviews of Physiology Biochemistry and Pharmacology». Springer-Verlag, Berlin, 1977. Vol. 78, pp. 2-97.
5. Diez N., Barber, A. and Ponz, F.: *Rev. esp. Fisiol.*, 47, 69-74, 1991.
6. Hediger, M. A., Coady, M. J., Ikeda, T. S. and Wright, E. M.: *Nature*, 330, 379-381, 1987.
7. Heizer, W. D., Kerley, R. L. and Isselbacher, K. J.: *Biochim. Biophys. Acta*, 264, 450-461, 1972.
8. Klip, A., Grinstein, S., Biber, J. and Semenza, G.: *Biochim. Biophys. Acta*, 598, 100-114, 1980.
9. Klip, A., Grinstein, S. and Semenza, G.: *Biochim. Biophys. Acta*, 558, 233-245, 1979.
10. Lugea, A., Barber, A. and Ponz, F.: *Rev. esp. Fisiol.*, 44, 121-126, 1988.
11. Lugea, A., Barber, A. and Ponz, F.: Z. *Gastroenterol.*, 27, 296, 1989.
12. Ortiz, M. Absorción intestinal de azúcares *in vivo*. Tesis Doctoral. Facultad de Ciencias Biológicas. Universidad de Navarra. Pamplona, 1978.
13. Passow, H., Rothstein, A. and Clarkson, T. W.: *Pharmacol. Rev.*, 13, 185-224, 1961.
14. Peerce, B. E. and Clarke, R. D.: *J. Biol. Chem.*, 265, 1731-1736, 1990.
15. Ponz, F., Ilundain, A. and Lluch, M.: *Rev. esp. Fisiol.*, 35, 97-104, 1979.
16. Ponz, F., Lluch, M. and Alemany, I.: *Rev. esp. Fisiol.*, 13, 265-273, 1957.
17. Rodríguez-Yoldi, M. J., Lluch, M. and Ponz, F.: *Rev. esp. Fisiol.*, 43, 239-244, 1987.
18. Rodríguez-Yoldi, M. J. and Ponz, F.: *Rev. esp. Fisiol.*, 43, 39-44, 1987.
19. Rodríguez-Yoldi, M. J. and Ponz, F.: *Rev. esp. Fisiol.*, 43, 45-50, 1987.
20. Rothstein, A.: In «Current Topics in Membranes and Transport» (Bronner, F. and Kleinzeller, A., eds.). Academic Press, New York, 1970. Vol. I, pp. 135-176.
21. Russo, A.: In «Methods of Biochemical Analysis» (Glick, D., ed.). J. Willey and Sons, New York, 1988. Vol. 33, pp. 165-241.
22. Schaeffer, J. F., Preston, R. L. and Curran, P. J.: *J. Gen. Physiol.*, 62, 131-146, 1973.
23. Stevens, B. R., Fernández, A., Hirayama, R., Wright, E. M. and Kempher, E. S.: *Proc. Natl. Acad. Sci. USA*, 87, 1456-1460, 1990.
24. Umbreit, W. W., Burris, R. H. and Stauffer, J. F.: Manometric Techniques and Tissue Metabolism. Burgess Publ., Minneapolis, 1951.
25. Vansteveninck, J., Weed, R. I. and Rothstein, A.: *J. Gen. Physiol.*, 48, 617-632, 1965.
26. Webb, J. L.: In «Enzyme and Metabolic Inhibitors». Academic Press, New York, 1966. Vol. 2, pp. 635-683.