Biochimica et Biophysica Acta 816 (1985) 411–414
Elsevier

BBA 70228

BBA Report

Intestinal sugar transport during ageing

Clara Lindi [a], Paola Marciani [a], Alide Faelli [b] and Giovanni Esposito [a,*]

[a] *Istituto di Fisiologia Generale e Chimica Biologica, Facoltà di Farmacia, via Saldini 50, 20133 Milano, and* [b] *Dipartimento di Fisiologia e Biochimica Generali, Università di Milano (Italy)*

(Received January 28th, 1985)

Key words: Ageing; Glucose transport; Na^+ transport; Brush-border membrane; Valinomycin; (Rat intestine)

Ageing effects on sugar intestinal transport were studied by using the everted sac and the brush-border membrane vesicle techniques. Four age groups of rats were used: very young, young, adult and old animals. Net transintestinal transport of D-glucose and intracellular sugar accumulation were greater in young than in very young, adult and old rats. Net Na^+ transport was high in very young and young animals and then it declined with age. In brush-border membrane vesicle experiments D-glucose overshoot was smaller in the groups of animals where net sugar transport was less. In old rats, however, the overshoot did not occur. Short-circuiting of vesicles with valinomycin showed that the driving forces for sugar accumulation, i.e. the chemical potential gradient of Na^+ and the electrical potential gradient, played different roles during ageing. In very young animals the chemical potential gradient seems to be responsible for D-glucose overshoot; in young rats both gradients are important while in adult animals the electrical potential gradient represents the main driving force.

Vectorial transintestinal sugar transport consists of an entry step across the luminal membrane, a cellular sugar accumulation and an exit across the basolateral membrane of the enterocyte.

Most studies were performed 'in vitro' on intact intestine by using, for instance, the everted sac or the brush-border membrane vesicles.

Sugar entry into the cell is a Na^+-dependent process which utilizes, as a driving force, the electrochemical potential gradient of Na^+ [1,2] while the exit consists of a Na^+-independent and carrier-mediated [3] sugar movement across the contraluminal membrane.

The aim of the present study is to see whether these processes are present also in different age groups of rats and whether the driving forces play the same role during ageing.

The experiments were performed in both the everted sac of rat jejunum which represents an integrated system and in the brush-border membrane vesicles of the same animal to study the sugar entry process in detail. These experiments were carried out in four age groups of rats, namely: very young (35–45 days old), young (2 month old), adult (7 month old) and old (20 month old) animals.

The everted sacs of rats (Wistar strain, Charles River Italiana) were prepared as described previously [4], i.e. cannulated and incubated in 50 ml of a Krebs-Ringer bicarbonate solution containing 5.56 mM D-glucose and gassed with 95% O_2 and 5% CO_2. The serosal medium was always recirculated. Each rat and each group of animals had their extracellular space determined in order to measure the intracellular water needed to calculate the mean intracellular sugar concentration. The extracellular space determination was performed as described previously [5,6].

The brush-border membrane vesicles were pre-

* To whom correspondence should be addressed.

pared with the Ca^{2+}-precipitation method [7]. Briefly, the mucosal scrapings, homogenized in a hypotonic solution (50 mM sorbitol/2 mM Tris-HCl (pH 7.1)) and incubated with 10 mM $CaCl_2$ for 15 min at 0°C, were centrifuged at $3000 \times g$ for 15 min. The supernatant was centrifuged at $27000 \times g$ for 30 min and the pellet recovered was resuspended in a solution containing 250 mM sorbitol, 10 mM Hepes-Tris, (pH 7.4) and centrifuged at $43000 \times g$ for 20 min. The final pellet, resuspended again in the above solution and passed three times through a 25-gauge needle, was regarded as brush-border membrane fraction. Homogenate and brush-border membranes were analyzed for proteins [8] as well as for γ-glutamyltransferase [9] and alkaline phosphatase [10] as membrane marker enzymes.

The uptake of 0.1 mM ^{14}C-labelled D-glucose (D-[U-^{14}C]glucose, 329 mCi/mol, New England Nuclear Corp. plus unlabelled D-glucose to 0.1 mM) was determined in triplicate at room temperature (20–22°C) with a rapid filtration technique (presoaked cellulose nitrate filters, 0.45 μm, M.F.S., Dublin, CA, U.S.A.). Further details are reported in the legend of Fig. 2.

When uptake was determined under short-circuited conditions, valinomycin (25 μg/ml) and KCl were added to the preincubation medium [11,12]. See also the legend of Fig. 2.

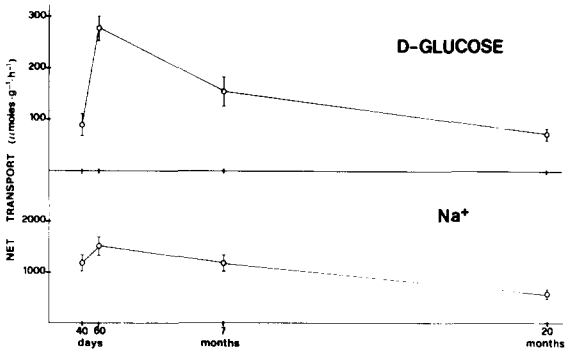

Fig. 1. Net transintestinal transport of D-glucose (above) and Na^+ (below) in the everted jejunum of rats of different age groups, incubated and perfused at 28°C for 30 min in a Krebs-Ringer bicarbonate solution containing 5.56 mM D-glucose. The different ages of rats are indicated on the abscissa. The net transport of D-glucose and Na^+ is expressed in μmol/g dry wt. of scraped mucosa per h (ordinate). Each point is a mean of six animals while the vertical bars represent the S.E.

The mechanisms of changes in the intestinal transport of solutes during ageing are, at present, not thoroughly understood. However, some studies concerning this topic have been recently performed, both 'in vivo' and 'in vitro', on the transport of electrolytes and non-electrolytes [13–16].

Our results, reported in Fig. 1, show that the intestinal transport activity undergoes substantial changes with age. Net transintestinal D-glucose transport is greater in young rats and then declines with age. However, a low transport activity is also present in very young animals.

The ratio between the cell vs. the mucosal fluid sugar concentration, indicates that this parameter is greater in young rats (3.1 ± 0.7, $n = 6$) than in very young and adult animals (1.4 ± 0.4, $n = 6$ and 1.7 ± 0.3, $n = 6$, respectively), while in old rats the ratio is 1.0 ± 0.4. Net Na^+ transport is prominent in young animals and then it declines. However, changes in Na^+ transport with age are less prominent than those of D-glucose transport, although the pattern of changes is the same in both instances.

The experiments with brush-border membrane vesicles (Fig. 2) indicate that D-glucose uptake is greater in young rats than in very young and adult animals. In these three age groups there is an overshoot due to the presence of a Na^+-gradient (Na^+ outside, no Na^+ inside the vesicle). Only in old rats the uptake shows no overshoot, but there is a continuous increase of sugar entry which levels off with time. The absence of overshoot might suggest a lack of significant stimulation by the electrochemical potential gradient.

By comparing everted sac experiments with brush-border membrane vesicle experiments it seems that the overshoot is smaller in the groups of animals where net D-glucose transport is smaller as well.

For very young rats the low sugar uptake might reflect the presence of a Na^+-dependent mechanism of sugar entry which is not fully developed yet.

Valinomycin experiments were performed to study the influence of the membrane electrical potential difference between the outside and the inside of the vesicle (internal side negative) on this Na^+-dependent sugar entry. By short-circuiting the vesicles with valinomycin, the following results

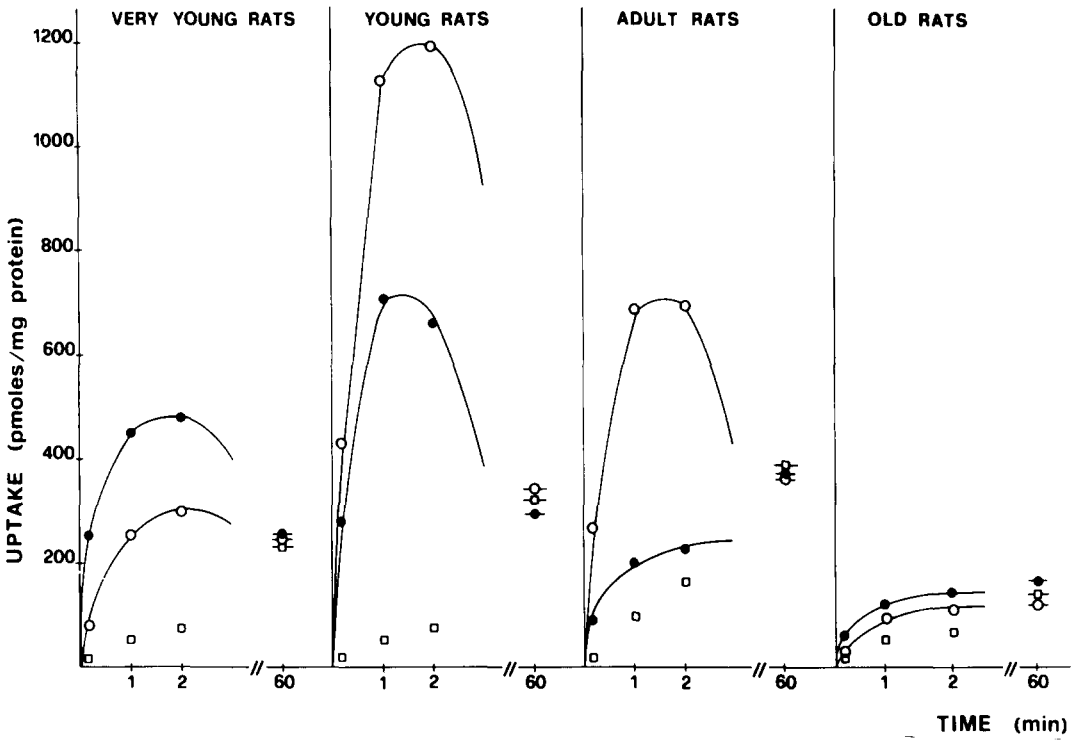

Fig. 2. The time-course of 0.1 mM D-[^{14}C]glucose uptake by brush-border membrane vesicles of rat jejunum enterocyte. The open squares (□) represent the uptake in the presence of a K^+-gradient. All other symbols indicate uptakes in the presence of a Na^+-gradient. The solid circles (●) represent the valinomycin uptakes (vesicles were pre-equilibrated in a solution containing 100 mM KCl, 250 mM sorbitol, 10 mM Hepes-Tris (pH 7.4) and 25 μg/ml valinomycin. The transport buffer solution with the radiolabelled sugar contained 100 mM KCl, 125 mM NaCl, 10 mM Hepes-Tris (pH 7.4)). The open circles (○) indicate control sugar uptake (the transport buffer solution with radiolabelled sugar contained 125 mM NaCl, 200 mM sorbitol and 10 mM Hepes-Tris (pH 7.4)). The time-course uptake (10 s, 1, 2 and 60 min) is reported on the abscissa; the uptake of D-glucose (ordinate) is expressed in pmol/mg protein. Each point represents a single measured uptake (in triplicate) selected from representative single experiments for each group of rats.

were obtained: for very young animals the overshoot is not reduced (It is even greater, even if not significantly, but the reason of this increase is not clear at present. One explanation might be that, at this age, the membrane is more permeable to Na^+ than to Cl^- thus causing an intravesicular positive potential which restrains the entry of a probable positively-charged ternary complex whose net charge depends on the Na^+ : sugar stoichiometry. Therefore, short-circuiting would abolish such hindrance and enhance the overshoot). The apparent non-influence of the electrical potential might suggest that the mean driving force for sugar entry is the chemical potential gradient of Na^+. In young rats the overshoot is reduced but not abolished, which seems to indicate that both the chemical and electrical potential gradients play a role in sugar accumulation. In adult animals the overshoot disappears, thus indicating that the electrical potential gradient is the main driving force. Old animals are affected neither by the electrical nor by the chemical potential gradient. In other words sugar uptake into these vesicles seems to be Na^+-independent.

In conclusion, it seems that ageing affects net sugar and Na^+ transport in the jejunum of rat. Brush-border membrane vesicle experiments indicate that D-glucose uptake parallels net transintestinal transport in the different age groups of animals. They also indicate that the driving forces for sugar accumulation, i.e. the chemical potential gradient of Na^+ and the electrical potential gradient, play different roles depending on the age of the animal.

This work was supported by a research grant of the Consiglio Nazionale delle Ricerche (C.N.R.), CT. 84.02303.56, Rome (Italy).

References

1 Crane, R.K. (1977) Rev. Physiol. Biochem. Pharmacol. 78, 99–159
2 Kimmich, G.A. (1981) Fed. Proc. 40, 2474–2479
3 Bihler, I. and Cybulsky, R. (1973) Biochim. Biophys. Acta 298, 429–437
4 Faelli, A., Esposito, G., Tosco, M. and Capraro, V. (1981) J. Physiol. (Paris) 77, 845–849
5 Esposito, G. and Csáky, T.Z. (1974) Am. J. Physiol. 226, 50–55
6 Esposito, G., Faelli, A., Tosco, M., Burlini, N. and Capraro, V. (1979) Pflügers Arch. 382, 67–71
7 Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98–112
8 Bradford, M.N. (1976) Anal. Biochem. 72, 248–252
9 Persijn, J.P. and Van der Slik, S. (1976) J. Clin. Chem. Clin. Biochem. 14, 421–427
10 Walter, K. and Schütt, C. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Vol. 2, pp. 856–864, Verlag Chemie, Weinheim
11 Levine, R., Hirayama, B. and Wright, E.M. (1984) Biochim. Biophys. Acta 769, 508–510
12 Wright, S.H., Hirayama, B., Kaunitz, J.D., Kippen, I. and Wright, E.M. (1983) J. Biol. Chem. 258, 5456–5462
13 Armbrecht, H.J., Lenser, T.V., Bruns, M.E.H. and Davies, B.B. (1979) Am. J. Physiol. 236, E769–E774
14 Jakab, L. and Penzes, L. (1981) Experientia 37, 740–742
15 Thomson, A.B.R. (1979) Am. J. Physiol. 236, E685–E691
16 Vinardell, M.P. and Bolufer, J. (1984) Exp. Gerontol. 19, 115–120