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SUGAR TRANSPORT AT THE BASAL AND LATERAL ASPECT OF THE SMALL INTESTINAL CELL

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SUMMARY

A method has been developed for the study of sugar transport across the basal and lateral aspect of epithelial cells from mouse small intestine. Suspensions of isolated epithelial cells were prepared from everted intestine which had been treated with $HgCl_2$, an irreversible inhibitor of active sugar transport. Since sugar transport at the luminal aspect was blocked, transport at the other faces could be studied in isolation. This transport process is independent of Na^+ and insensitive to phlorizin. Its specificity differs from that of the Na^+-dependent active process.

INTRODUCTION

The absorptive epithelium of the small intestine is a tissue specialized for the transcellular transfer of a number of important nutrients. In the case of glucose and related monosaccharides, this process involves an active inward transport step at the brush border, followed by the outward transfer of the sugar across the basal and lateral faces of the epithelial cell, towards the underlying tissue and the blood circulation. The active transport step at the luminal aspect has been well characterized as a Na^+-dependent, phlorizin-sensitive process with a characteristic chemical specificity (for review, see Crane[1]). The pathway(s) whereby sugar exits at the basal and lateral aspect of the cell has not been studied in detail, for technical reasons. It is generally assumed, on the basis of indirect evidence, that this transport pathway is energy independent but rapid enough to mediate the outward transfer of sugar in the physiological process of absorption[1]. As sugars supplied from the blood or from the serosal side *in vitro* are able to support metabolic activity and Na^+ and water transfer[2,3], this transport pathway may also mediate the influx of sugars from the blood into the epithelial cell.

In earlier studies using suspensions of isolated epithelial cells (*e.g.* of Kimmich[4]) active transport at the brush border largely overshadowed the possible contribution of other transport pathways. We have, therefore, attempted to irreversibly block active sugar transport at the brush border before preparing isolated cells, thus making transport at other aspects of the cell available for direct examination.

Abbreviation: DNFB, 2,4-dinitrofluorobenzene.

METHODS

Preparation of isolated cells

It was found in preliminary experiments that mouse intestine was superior to that of hamster or rat in terms of cell yield and viability of isolated cells.

The mice were killed by cervical dislocation, the small intestine was removed, washed with 0.9% NaCl, everted onto a glass rod and immersed in oxygenated Krebs–Ringer phosphate buffer[5] at 33 °C, containing 0.15% hyaluronidase (430 I.U./mg, Miles Research Labs), 10 mM D-glucose and 0.25% serum albumin ("fatty acid poor", Miles Research Labs). The composition of the medium is as described by Perris[6]. Where indicated, the everted intact intestine was first exposed to various inhibitors as described in Results. It was then transferred to fresh medium and shaken by electrical vibration of the mounting rods at 60 Hz, as described by Harrison and Webster[7]. Best results were obtained by 20 min of vibration. The cells were collected by two centrifugations in the cold, the first followed by a wash with Krebs–Ringer phosphate buffer containing 1% albumin and the second by resuspension of the cells in the medium used for subsequent incubation.

Incubation of isolated cells

The cell suspension was added to incubation media containing ^{14}C-labelled sugars and [^{3}H]inulin serving as indicator of apparent extracellular space. For experiments in the presence of Na^+, Krebs–Ringer phosphate buffer (pH 7.4) was used. For experiments in the absence of Na^+, an isotonic mannitol–Tris–bicarbonate (25 mM) buffer (pH 7.4) was used. The media were equilibrated with 100% O_2. The suspensions were incubated at 37 °C for 10 min with rapid shaking. Incubation was terminated by placing the tubes in ice and centrifugation in the cold. The supernatant was decanted and the tube blotted, the cell pellet was removed from the tube, blotted, weighed and extracted by boiling water.

All containers coming in contact with the cells were made of polyethylene or polypropylene.

The radioactivity of tissue extracts and similarly treated incubation media was determined by double-label liquid scintillation spectrometry[8]. Results are expressed as penetration, *i.e.* the concentration in the intracellular water as a fraction of the concentration in the medium. The amount of intracellular water was determined by correcting for the inulin space determined in each experiment and for dry weight determined in a separate group of samples. Na^+ and K^+ were determined by emission flame photometry with Li^+ as internal standard. Results are expressed as concentrations (in mM) in the intracellular water.

At various stages of the procedure the cells were monitored by direct microscopic observation and their viability was estimated by the ability to exclude trypan blue[9].

Experiments with everted sacs were performed as described by Wilson and Wiseman[10]. The tissue was extracted by boiling and radioactivity of tissue extracts and media was measured as described above. Statistical evaluation was by Students *t* test.

RESULTS

Preparation of isolated epithelial cells

The method as described above yielded 0.3–0.4 ml packed cells per mouse intestine. Microscopic observation indicated that over 75% of the cells released were epithelial cells. The remainder were mainly lymphocytes and crypt cells. Over 85% of the cells survived the 70 min required to complete the whole procedure. Optimum cell yield was obtained by vibration for 20 min. After that the increment in cell yield diminished rapidly and the proportion of epithelial cells was reduced by the release of crypt cells.

The functional integrity of the isolated cells and, by inference, their good metabolic state is shown by the ability of the isolated cells to accumulate D-galactose against its gradient (shown in Table II). Thus, the active sugar transport mechanism at the luminal aspect of the cell was intact and functional.

Blockade of sugar transport at the luminal aspect

To study transport at the basal aspect in isolation, an inhibitor is required which irreversibly blocks sugar transport at the luminal aspect without at the same time causing other damage to the cell. Phlorizin was not a correct choice since, prior to the start of the experiments, its effect on sugar transport at the basal aspect was unknown. Also, its effect is partially reversible and it would therefore be ineffective as a pretreatment. Alterations in the ionic composition of the incubation medium or agents which alter the ionic content of the cell were also initially excluded since the role of intracellular and extracellular ions in sugar transport at the basal aspect was one of things to be studied.

To choose the correct inhibitor, experiments were performed with several SH-inhibitors in sacs of intact everted intestine. In this preparation only the luminal aspect of the cells was exposed to the inhibitor, and the tissue concentrations of sugar reflect only transport at the luminal aspect.

TABLE I

TRANSPORT OF 0.5 mM D-GALACTOSE IN EVERTED SACS

Everted intestinal sacs were pretreated with inhibitors as shown and then incubated for 15 min in fresh Krebs–phosphate medium containing 0.5 mM D-galactose. The figures are means of 6 experiments (with inhibitors) and of 19 experiments (control).

Inhibitor	*Pretreatment (min)*	*Transport of D-galactose (% of control)*	*Concentrations in intracellular water (mM)*	
			Na^+	K^+
None	—	100*	48.8	151.9
DNFB, 2 mM	2	27.1	125.0	62.7
N-Ethylmaleimide, 1 mM	10	10.0	198.5	71.8
$HgCl_2$, 2 mM	2	13.5	42.6	141.5

* The mean penetration of 19 controls was 3.038 ± 0.140.

The effect of *p*-chloromercuribenzene sulfonate was found to be reversible (not shown). As shown in Table I, pretreatment with 2,4-dinitrofluorobenzene (DNFB) and *N*-ethylmaleimide reduced transport effectively but inhibited the Na^+ pump as well.

A short pretreatment with $HgCl_2$ strongly inhibited sugar transport without affecting the internal levels of Na^+ and K^+. Longer treatments or higher concentrations, however, did adversely affect ion levels in the tissue. Pretreatment of the everted intestine before vibration with 3 mM $HgCl_2$ was therefore chosen to block transport at the luminal aspect of the isolated cells.

Effects of $HgCl_2$ and Na^+ on sugar transport in isolated cells

Table II compares sugar penetration in sacs and isolated cells. In sacs, the active transport observed with Na^+ was abolished in the absence of this ion. Pretreatment with $HgCl_2$ also strongly inhibited transport but not quite to the same extend as absence of Na^+. This suggests that there may remain some residual activity of Na^+-dependent sugar transport at the luminal aspect after $HgCl_2$ treatment.

TABLE II

THE EFFECTS OF $HgCl_2$ AND Na^+-FREE MEDIUM ON D-GALACTOSE TRANSPORT IN EVERTED SACS AND ISOLATED CELLS

Everted sacs and isolated cells were treated with 3 mM $HgCl_2$ for 2 min and then incubated for 10 min with 0.5 mM D-galactose. The figures are means ± S.E. (number of experiments).

Treatment		*Sugar penetration*
Sacs	Control	3.670 ± 0.302 (9)
	$HgCl_2$	0.345 ± 0.038 (8)
	Na^+-free medium	0.093 ± 0.016 (7)
Cells	Control	3.559 ± 0.404 (12)
	$HgCl_2$	0.606 ± 0.029 (13)
	$HgCl_2$ and Na^+-free medium	0.614 ± 0.100 (14)

Isolated cells not treated with $HgCl_2$ also showed active transport of D-galactose. As in sacs, in the isolated cells pretreated with $HgCl_2$, active transport was abolished but the penetration was still nearly double the corresponding value in sacs. As transport at the luminal aspect is largely abolished by $HgCl_2$ treatment, it appears that the relatively rapid entry of sugar into Hg^{2+}-treated isolated cells must occur *via* membrane sites not previously exposed to the inhibitor and presumably located at the basal and lateral aspect of the cells. Omission of Na^+ caused no further decrease in transport.

Fig. 1 shows the effect of Na^+ on the transport of several additional sugars by Hg^{2+}-treated cells. The penetration of none of these sugars was significantly affected by the absence of Na^+. It is known[1] that the luminal transport of 3-*O*-methyl-D-glucose, α-methyl-D-glucoside and D-xylose is active and Na^+ dependent. L-Rhamnose, L-arabinose and L-fucose are not actively transported, and their entry at the luminal aspect is as slow[1] as that of D-galactose in the absence of Na^+ (shown

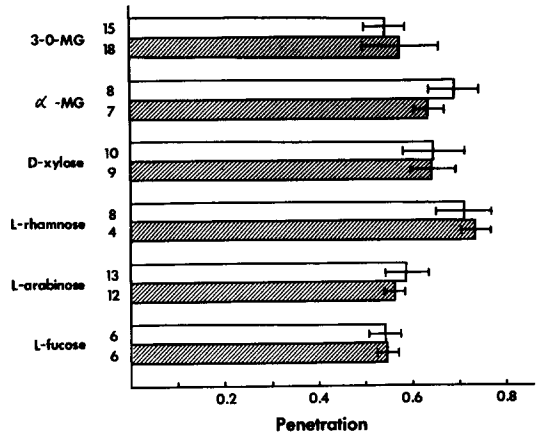

Fig. 1. The effect of Na^+ on sugar transport in isolated cells treated with 3 mM $HgCl_2$. Concentration of all sugars, 0.5 mM. Abbreviations: 3-*O*-MG, 3-*O*-methyl-D-glucose; α-MG, α-methyl-D-glucoside. Incubation for 10 min in Krebs–phosphate or in Na^+-free medium (shaded bars). The figures at left indicate the number of expts.

in Table II). Their transport at the basal aspect was about the same as of the first 3 sugars, which is 3–4 times faster than at the luminal aspect.

The effect of phlorizin

Fig. 2 shows that there was no effect of phlorizin on sugar transport in Hg-treated cells. At the luminal aspect the Na^+-dependent entry of these three sugars is virtually abolished by 0.5 mM phlorizin[1].

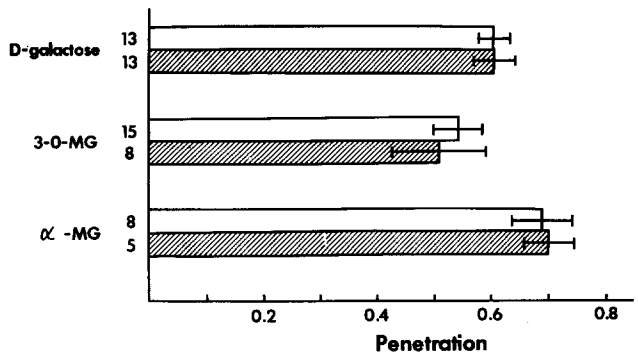

Fig. 2. The effect of phlorizin on sugar transport in isolated cells treated with 3 mM $HgCl_2$. Concentration of all sugars, 0.5 mM. Phlorizin, 0.5 mM (shaded bars). Incubation for 10 min in Krebs–phosphate medium. Other details and abbreviations as in Fig. 1.

The specificity of transport at the basal aspect

Because phlorizin had no effect on sugar transport in Hg^{2+}-treated cells, it was used instead of $HgCl_2$ treatment to inhibit the Na^+-dependent sugar transport mechanism at the luminal aspect of the cell. To investigate the specificity of transport at the basal aspect, various sugars were tested for their potential inhibitory

effect on the transport of 3-*O*-methylglucose in the presence of phlorizin. Fig. 3 shows that α-methyl-D-glucoside which is a strong competitor of D-glucose and 3-*O*-methyl-D-glucose transport at the luminal aspect of the cell, did not compete at the basal aspect. In contrast, 2-deoxy-D-glucose, D-fructose and D-mannose, which are ineffective as competitors of D-glucose and 3-*O*-methyl-D-glucose transport at the luminal aspect[1], were quite effective as competitors at the basal aspect. D-Galactose and D-glucose which are actively transported at the luminal aspect were also inhibitory.

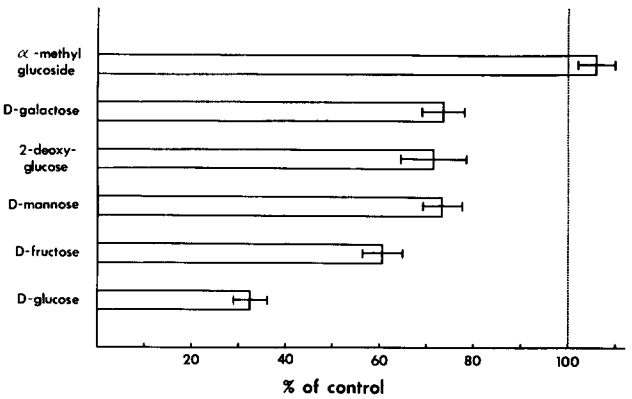

Fig. 3. Inhibition of 3-*O*-methyl-D-glucose transport by other sugars in isolated cells in the presence of phlorizin. Cells were incubated for 10 min with 0.5 mM 3-methyl-D-[^{14}C]glucose and 0.5 mM phlorizin. 5 mM other sugars (unlabelled) were added as indicated. The figures are percent of control ±S.E. (mean of 4–7 experiments). Results for each sugar were calculated with reference to controls from the same pool of cells.

DISCUSSION

The purpose of these experiments was to develop and test a procedure for the study of sugar transport at the basal and lateral aspect of isolated intestinal epithelial cells in isolation from the active, Na^+-dependent transport system located at the luminal aspect. The data show that the isolated cells were of satisfactory viability and unless treated with $HgCl_2$, capable of active transport. This irreversible inhibitor of sugar transport, was used to inhibit sugar transport in erythrocytes (Reinwein *et al.*[11]; LeFevre and McGinniss[12]). Under the conditions chosen for $HgCl_2$ treatment sugar transport across the luminal aspect of intact everted sacs was virtually abolished while the maintenance of normal levels of internal Na^+ and K^+ indicates that the cells did not suffer other functional damage. The treatment adopted seems therefore reasonably effective and selective for the desired purpose.

The procedure for preparation of isolated epithelial cells is based on mechanical vibration (Harrison and Webster[7]) and the use of a medium containing hyaluronidase, D-glucose and serum albumin (Perris[6]). This method is less liable to cause cell damage than incubation with trypsin[13], protracted exposure of the intestine

to lysozyme[14] or a Ca^{2+} chelator[15]. The provision of glucose and serum albumin[6] and the use of plastic vessels[4] all combine to enhance the survival of cells.

Comparison of results in sacs and cells (Table II) shows, firstly, that entry into Hg^{2+}-treated isolated cells is probably not mediated by residual Na^+-dependent transport at the luminal aspect. While it is true that Hg^{2+} treatment of sacs did not depress sugar entry in sacs to quite the same low level as seen in Na^+-free medium, it is also obvious that omission of Na^+ does not depress sugar transport in Hg^{2+}-treated isolated cells. Fig. 1 shows that several sugars apart from galactose also exhibit appreciable Na^+-independent entry. Some of these sugars, 3-*O*-methyl-D-glucose, α-methyl-D-glucoside and D-xylose are actively transported across the luminal aspect. The three others, L-rhamnose, L-arabinose and L-fucose are not but appear to enter the Hg^{2+}-treated isolated cells more rapidly than they cross the luminal membrane of intact intestinal tissue. These data suggest that the specificity of intestinal sugar transport in Hg^{2+}-treated isolated cells may differ from that at the luminal aspect.

Fig. 2 indicated that sugar transport in Hg^{2+}-treated cells is not affected by phlorizin which competitively inhibits active transport of these same sugars at the luminal aspect. High sensitivity to inhibition by phlorizin is characteristic for active sugar transport systems; facilitated diffusion of sugar in muscle, erythrocytes, *etc.* is far less sensitive, and sugar transport in the liver is even less affected[16].

More direct studies on the specificity of this transport system are shown in Fig. 3. The results indicate that there is some overlap in specificities between this system and the active one at the luminal aspect: both accept D-glucose and D-galactose. However, there are also important differences: 2-deoxy-D-glucose, D-fructose and D-mannose which are not actively transported by the Na^+-dependent system for glucose at the luminal aspect, are effective inhibitors at the basal and lateral aspect and, conversely, α-methyl-D-glucoside which is actively transported at the luminal aspect is not. Thus, the specificity pattern of Na^+-independent sugar transport in isolated epithelial cells, presumably at their basal and lateral aspect, resembles that mediated by facilitated diffusion systems in other types of cells.

The above studies are concerned with influx of sugars across the basal and lateral aspect of the cell, and it might be argued that the physiologically important flux is in the opposite direction. However, it should be kept in mind that glucose required for metabolism is supplied to these cells from the internal side except during the relatively short periods of absorption from the intestine. Furthermore, if transport occurs by facilitated diffusion, no asymmetry is to be expected. In preliminary experiments neither accumulation against a concentration difference, nor inhibition of transport by uncoupling of oxidative phosphorylation was observed, suggesting that this system may indeed be of the facilitated diffusion type. It is clear, however, that more stringent tests are needed to settle this question conclusively.

Another question still unanswered is whether the system studied here is located only at the basal and lateral border or also at the luminal aspect. Earlier experience[11,12] indicates that $HgCl_2$ inhibits facilitated diffusion of sugars. Further experiments are being done to confirm this point and to identify possible Na^+-independent transport systems at the luminal face. The specificity pattern, kinetics and sensitivity to hormonal or metabolic regulation are also under study.

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