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Interactions Between Monosaccharides and Leucine in Basolateral Membrane of Isolated Chick Intestinal Epithelial Cells *

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The characteristics of the interactions between 3-O-methyl-glucose (3-OMG) and monosaccharides or leucine transport were examined on chick isolated intestinal epithelial cells. In a Na^+-medium, the unidirectional influx of 1.5 mM 3-OMG was found to be already inhibited by 37.5 mM glucose and 37.5 mM leucine after 20 s incubation. In Na^+-free mannitol substituted medium, either glucose, galactose or leucine (37.5 mM) inhibited the unidirectional influx of 3-OMG. Theophylline, a drug that decreases the basolateral permeability to sugars, decreased the unidirectional influx of 1.25 mM leucine in Na^+-free medium but increased the steady-state uptake of the aminoacid in Na^+-medium.

The efflux of 3-OMG from preloaded cells into a Na^+-free medium was stimulated by extracellular galactose and leucine (37.5 mM). This was inhibited by theophylline. Our results indicate that sugars and leucine interactions at the Na^+-independent transport system could be produced by mutual competition for binding the same system.

Evidence has been accumulated that intestinal epithelial cells have two distinct transport systems for sugars, one at the brush-border is characterized as a N^+-dependent and phlorizin sensitive process (17, 20) in constrast to the other, located at the basolateral membrane, which exhibits Na^+-independence and phloretin sensitivity (4, 12, 22). In the first system, heterologous interactions between monosaccharides and amino acids, both *in vivo* (3, 5) and *in vitro* (1, 6, 16), have been shown. However, the possibility of heterologous interactions in the basolateral plasma membrane has not been studied.

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The aim of the present work is to study homologous and heterologous interactions in the brush-border and basolateral membranes of isolated chick intestinal epithelial cells, in order to compare the pattern of both transport systems.

Materials and Methods

Intestinal epithelial cells were isolated from 5-to 7 week-old male Broiler-Hubbard chicks using the hyaluronidase-mechanical agitation procedure developed by KIMMICH (9, 10). The final cell suspension had 10-20 mg cellular protein/ml. The incubation medium contained (mM): NaCl, 80; Tris-Cl (pH 7.4), 20; $MgCl_2$, 1; $CaCl_2$, 1; K_2HPO_4, 3; mannitol, 100; and 1 mg/ml bovine serum albumin. The medium without sodium was prepared by substituting it for mannitol in order to maintain osmolarity. When elicitors of substrate were added at 37.5 mM concentrations, osmolarity was maintained by exclusion of an appropriate amount of mannitol.

Measurement of substrate uptake. Substrate accumulation was initiated by adding 1 ml of the cell suspension to 3 ml incubation medium, containing either 3-O-(^{14}C)methyl-D-glucose or L-(^{14}C)leucine (Amersham). For measuring unidirectional influxes or steady-state uptake, 200 μl samples of the cell suspension were taken at appropriate intervals and diluted into 2 ml of ice-cold medium. The chilled, diluted samples were centrifuged for 1 min in a refrigerated centrifuge, in order to sediment the cells. The pellets were washed twice and dispersed in 0.2 ml 3% perchloric acid on a vortex mixer, centrifuged for 10 min and intracellular substrate accumulation was determined by adding 0.1 ml of supernatant to 10 ml of scintillation mixture. The radioactivity was quantitated in a Nuclear Chicago Mark II liquid scintillation counter.

Determination of substrate efflux. In some experiments, cells were preloaded with (^{14}C) 3-OMG incubating them at 37° C in a shaker bath for 10 min in Na^+-medium. The cells were then washed three times with Na^+-free medium to discard the extracellular sodium and radioactivity, and resuspended in a medium without sodium and with phlorizin 50 μM, in order to eliminate the Na^+-dependent transport system. 3-OMG efflux was monitored by taking 1 ml cell suspension and adding to 6 ml Na^+-free medium with the appropriate unlabelled elicitors. At indicated times, 3-OMG content was determined as described before.

In all experiments cellular protein was measured by the LOWRY method (15).

Results and Discussion

Uptake of 1.5 mM 3-OMG in isolated chick intestinal epithelial cells prepared and incubated both in the presence or in the absence of sodium, is shown in fig. 1. Phlorizin 200 μM completely inhibits Na^+-dependent transport system since mean steady-state (30 min) 3-OMG accumulation was 3.86 ηmoles/mg cellular protein, very similar to the value obtained in Na^+-free medium (4.15 ηmoles/mg cellular protein). Accumulation ratio

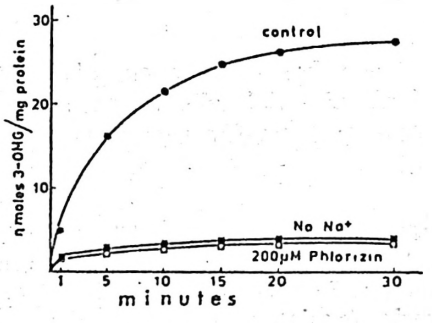

Fig. 1. *Uptake of 3-OMG (1.5 mM) into Isolated Intestinal epithelial cells.*
Effect of Na^+ absence or phlorizin (200 μM).

(amount of 3-OMG accumulated at steady-state in control relative to that accumulated with phlorizin) was 7, similar to that found by RANDLES and KIMMICH (19).

Unidirectional influx of 1.5 mM 3-OMG in Na^+-medium was linear in the first minute and the observed mean rate was 4.85 ηmoles/mg cellular protein, about 3.5 times faster than the influx in the Na^+-free medium (fig. 2). Addition of glucose or leucine (37.5 mM) produced an inhibition in the unidirectional influx of 75 % and 20 % respectively (fig. 2 *A*). To explain heterologous interactions in the brush-border membrane, two hypotheses have received detailed attention. In one, there is an allosteric interaction at the outer face of the matrix membrane (1, 2). In the other, the interaction results from dissipation of the electrochemical sodium gradient provoked by the co-transport of sodium with each substrate (16, 18, 21). Our findings on the unidirectional influx of 3-OMG in the presence of sodium and leucine indicate that heterologous interaction in the Na^+-dependent system was significant at short-time incubations. Therefore, it is not clear whether the second hypotesis can explain the results, since it would seem unlikely that significant changes in the electrochemical sodium gradient occur at that time.

Unidirectional influx of 1.5 mM 3-OMG in Na^+-free medium was also linear during the one minute interval of study. In the presence of 37.5 mM glucose, leucine or galactose, the influx was inhibited by 40 %, 22 % and 18 % respectively (figure 2 *B*). These findings show that in the basolateral membrane, where the Na^+-independent transport system for sugars is located (11, 22), interactions between 3-OMG and glucose, leucine or galactose occur. Since neutral amino acid transport has a similar cellular location to Na^+-independent sugar transport in intestine (8) and kidney (13), it was interesting to test the effect of theophylline, a drug that

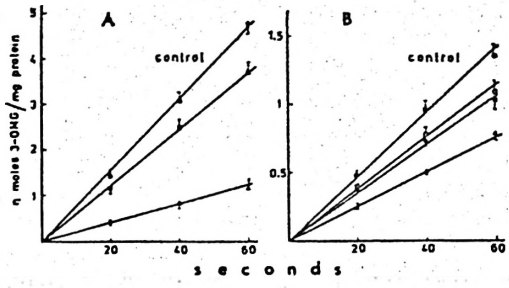

Fig. 2. *Effects of 37.5 mM glucose (▲), galactose (□) or leucine (■) on unidirectional influx of 3-OMG (1.5 mM) into isolated intestinal epithelial cells.*

A) cells were incubated in 80 mM Na^+-medium. *B*) incubation was in Na^+-free mannitol medium. At zero time, cells were added to media containing appropriate elicitors, and corrections were made in mannitol concentration to maintain the osmolarity. Values accompanying SE are means for five experiments.

decreased the basolateral permeability to sugars in rabbit ileum (7) and isolated chick intestinal epithelial cells (19), on Na^+-independent leucine transport system. Fig. 3, shows the effect of theophylline (7.5 mM) both in the presence or absence of extracellular sodium. Note that under these circumstances leucine uptake was

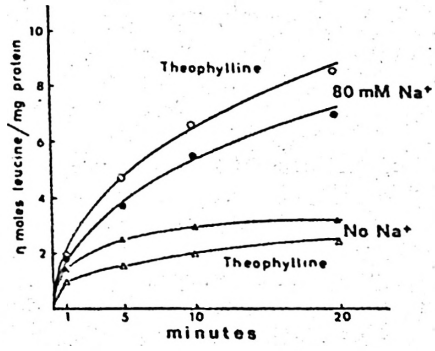

Fig. 3. *Effects of theophylline (7.5 mM) on Na^+-dependent and Na^+-independent uptake of L-leucine (1.25 mM), by isolated intestinal epithelial cells.*

affected by theophylline in the same way as 3-OMG (19), i.e. increased in Na^+-medium and decreased in Na^+-free medium. Since theophylline did not change mean cellular volume (J. Bolufer, unpublished observations), it is clear that this drug interferes with the function of the Na^+-independent leucine transport system and, consequently, unidirectional influx was not affected in Na^+-medium and was inhibited in Na^+-free medium. These results clearly indicate that leucine fluxes through basolateral membrane were inhibited by theophylline and suggest that sugars and neutral amino acids share carried-mediated Na^+-independent transport system.

In order to ascertain that heterologous interaction was due to competition for binding the Na^+-independent carrier, the effect of preloading the isolated cells with (^{14}C) 3-OMG in Na^+-medium, on the subsequent efflux into 50 μM phlorizin Na^+-free medium with appropriate substrates was studied. The rate of 3-OMG efflux from cells preloaded into this medium was compared to the similar cells to which medium theophylline (7.5 mM), galactose or leucine (37.5 mM) were added (fig. 4). Theophylline, as was to be expected, inhibit basolateral permeability and decreased 3-OMG efflux. Galactose and leucine increased 3-OMG efflux, both to the same extent. This accelerated efflux can be explained by counter-transport of the labelled intracellular 3-OMG with the unlabelled extracellular elicitors. Counter-transport of labelled and unlabelled D-glucose or L-valine by basolateral membrane vesicles from rat small intestine has been reported (8).

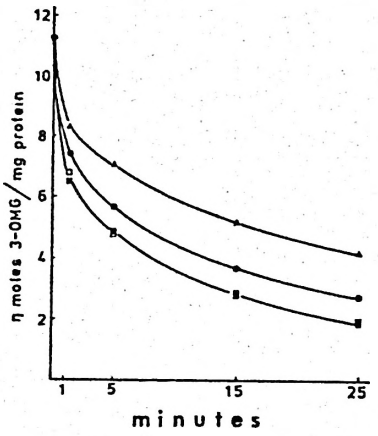

Fig. 4. *Efflux of 3-OMG from isolated intestinal cells.*
Cells were preloaded by incubation for 10 min in 5 mM (^{14}C) 3-OMG Na^+-medium. After, the cells were washed three times with Na^+-free, mannitol substituted medium plus 50 μM phlorizin. Cells were resuspended in the same medium in order to determine the efflux of 3-OMG into: (1) medium alone (●), (2) medium + 37.5 mM galactose (□), (3) medium + 37.5 mM leucine (■) or, (4) medium + 7.5 mM theophylline (▲). Uptake of 3-OMG at zero time was 11.25 ± 0.55 ηmoles/mg cellular protein. Results are the mean of five experiments.

Sufficient data in the literature show that the Na^+-independent sugar system catalyzes a facilitated diffusion of its substrates (11, 14, 22) and the results reported in this paper support the theory that this system in the basolateral membrane could be utilized by sugars and neutral amino acids.

Resumen

Se estudian las interacciones entre el transporte de 3-OMG y monosacáridos o leucina en enterocitos aislados de pollo. La entrada de 3-OMG 1,5 mM en presencia de Na^+, está ya inhibida por glucosa o leucina (37,5 mM) después de 20 s de incubación. En ausencia de Na^+, tanto la glucosa como la galactosa y leucina (37,5 mM) inhiben la entrada de 3-OMG.

La teofilina inhibe la entrada de leucina 1,25 mM en ausencia de Na^+ y aumenta el nivel intracelular del amino ácido en el estado estacionario en presencia de Na^+.

La salida de 3-OMG desde células precargadas, en ausencia de Na^+, es estimulada por la presencia de galactosa y leucina (37,5 mM) extracelular, mientras que la teofilina inhibe este flujo de salida. Nuestros resultados indican que las interacciones entre 3-OMG, azúcares y leucina, en el sistema de transporte independiente

de Na^+, podrían ser producidas por competencia en la utilización del mismo sistema.

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