COUNTER-TRANSPORT BETWEEN SUGARS AND AMINO ACIDS IN RABBIT ILEUM*

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1. Introduction

A considerable controversy has arisen in recent years on the mechanism of interaction between sugars and amino acids for transport by the intestinal mucosa. Four main hypotheses have been forwarded to explain how sugars and amino acids mutually inhibit the transport of each other. The first, presented by Smyth and his collaborators [1] and supported by others [2], postulates a competition between amino acids and sugars for a common energy source for transport. A second hypothesis [3] implicates the action of toxic metabolites. The arguments against these two hypotheses are weighty and have been discussed in detail elsewhere [4]; they need not be repeated here. A third proposal is that the primary interaction occurs at the inner face of the membrane, apparent inhibition of uptake occurring by acceleration of efflux [5, 6]. The fourth suggestion is that an allosteric inhibition between the two groups of compounds occurs at the outer face of the membrane, this being caused by the contiguity of two binding sites in the membrane surface [7]. The apparent irreconcilability of the last two possibilities led Alvarado [8] to propose that dissimilar mechanisms may operate in different species, since strong evidence existed in favour of the fourth hypothesis in the case of the hamster intestine, whereas results from other laboratories appeared to place doubt on its applicability to the rabbit [5] and dogfish [6]. The unsatisfactory nature of this conclusion, however, provoked us to study the interactions between sugars and amino acids in one of the controversial species, the rabbit, by means of a method which theoretically should distinguish between events occurring at the level of the mucosal rather than other membranes of the intestine. Using the everted sac technique [9] and determining changes in the substrate concentration of the mucosal fluid, it is possible to monitor events occurring solely at the level of the brush-border membrane, where the transport carriers are believed to be located. The results indicate that sugars are able to elicit counter-transport of amino acids in sacs of rabbit ileum, and it is therefore proposed that in this species, as in the case of the hamster [7], interaction between the two series of compounds takes place at the carrier level.

2. Methods

Everted sacs [9] of rabbit ileum, of approximately

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1 g wet weight, were incubated in 10 ml of a labelled solution of L-phenylalanine in phosphate buffer [10]. The sacs were filled with air and anchored to glass rods to keep them fully submerged. 100% Oxygen was bubbled continuously through the solution at a rate of 2–3 ml per min. Aliquots were removed from the mucosal medium at minute 10 and every 5 min thereafter, and counted in a liquid scintillation spectrometer. 1 ml of a 0.3 M aqueous solution of the *elicitor* [11], the substance whose ability to provoke counter-transport was to be tested, was added immediately after removal of the sample at minute 30. To one sac was added D-mannitol as a control; to a second, L-methionine which would be expected to provoke strong counter-transport, since it is known to share the same transport system as the substrate; and to a third was added the test substance, D-galactose. A fourth sac was removed at minute 30 for analysis. At the end of the incubation (50 min), the sacs were blotted and weighed, and tissue samples were dissolved in 30% KOH and counted as described previously [12].

The results are presented as the percentage change in medium substrate concentration during the course of the incubation. The calculations include a correction for the dilution caused by the introduction of the elicitors at minute 30. Statistical evaluation was performed by means of the paired t test between values of medium concentration at different times. The final tissue/medium (T/M) concentration ratio for each sac was also determined. The experiment was repeated with four animals and the T/M values were subjected to an analysis of variance as an orthogonal two-way classification from which a value of D, the least significant difference between any two means at a given probability level, can be extracted [13].

Parallel experiments were performed to determine the effect of sugars on the initial uptake of L-phenylalanine by slices of rabbit intestine. Tissue samples were incubated for 1 min in 0.9 mM labelled phenylalanine in the presence of 20 mM sugar. After the incubation, the tissues were blotted, weighed, extracted in KOH [12], and counted in the liquid scintillation spectrometer. The results were evaluated statistically by the same technique as used for the sac tissues [13]. The extracellular space was determined using rings of intestine incubated with 3 mM labelled mannitol under similar conditions. The mannitol space was found to be about 9% of the medium substrate concentration; corrections for this space were not applied to the experimental results since such a correction was found not to affect the data significantly.

3. Results

The results of the sac experiment are shown in the figure. Equilibrium is not quite complete during the initial 30-min incubation period, as witnessed by the fact that the substrate concentration in the mucosal fluid continues to fall when mannitol is added. Furthermore, analysis of the tissues shows that those removed from the incubation at minute 50 (after addition of mannitol) have a significantly greater tissue/medium concentration ratio than those removal at minute 30.

When methionine is added to the bathing medium, an immediate steep rise in the medium substrate concentration occurs, and the concentration in the tissue falls; this can be interpreted as evidence for homologous counter-transport. When galactose is added to the bathing fluid, there is also an immediate increase in the external substrate concentration, as testified by the statistically significant rise in the curve during the first five minutes after addition of the elicitor; the increase continues at a decelerating rate for the rest of the incubation period. Moreover, analysis of the tissues at the conclusion of the incubation demonstrates that the sacs that had been treated with galactose had lost a significant quantity of phenylalanine when compared with the control sacs removed before addition of the elicitor. Therefore, these results provide strong evidence, not simply for interaction between sugars and amino acids at the membrane level, but also for heterologous counter-transport elicited by a member of one transport family on another.

The results presented in the table provide further evidence for an external locus of action of the heterologous inhibitors. Both glucose and galactose are shown to inhibit the initial uptake of phenylalanine by rabbit ileal strips during a one-minute incubation, the effect of glucose being significant at the 1% level and that of galactose at the 0.1% level. Since the inhibition by glucose and galactose is readily demonstrable during such short incubation periods, it can hardly be interpreted as taking place from the internal face of the cell membrane.

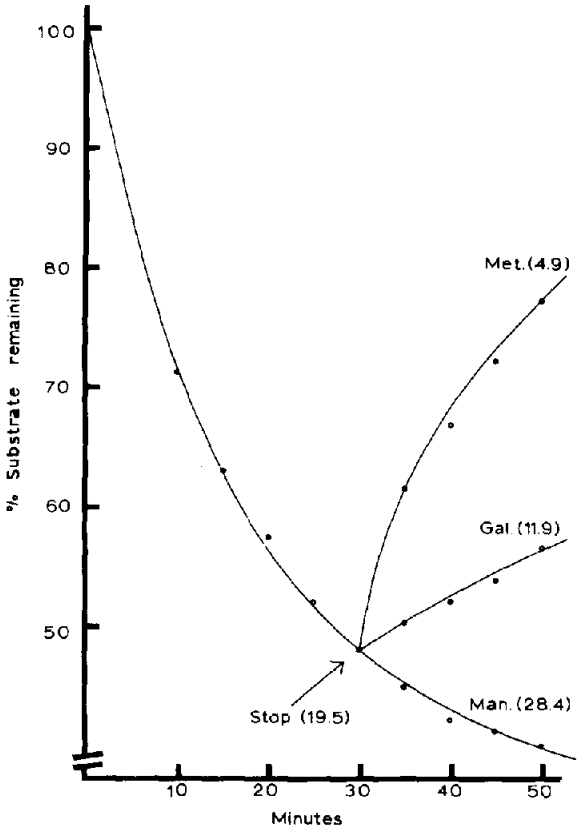

Fig. Sacs of rabbit ileum (mean wet wt. 926 mg) were incubated in 10 ml of a solution of 1 mM U-^{14}C-L-phenylalanine in oxygenated Krebs-Henseleit phosphate buffer [10]. At minute 30, one sac ("stop") was removed as a control, and to each of the remaining sacs was added 1 ml of a 0.3 M aqueous solution of the elicitors, D-mannitol, L-methionine and D-galactose respectively. At minute 50, these sacs were also removed for analysis. The curves show the mean changes in substrate concentration in the mucosal fluid during the incubation and are means of four identical experiments with different animals. Figures in parentheses indicate final tissue/medium concentration ratios. The rise in substrate concentration in the first five minutes after addition of the elicitor is significant at the 5% level, and between minutes 30 and 50 at the 1% level, according to the paired t test. Analysis of variance [13] of the tissue/medium concentration ratios shows the difference between "stop" and galactose to be significant at the 5% level, between "stop" and mannitol at the 2% level, and between "stop" and methionine at the 0.1% level.

Table
Inhibition of the initial uptake of L-phenylalanine by sugars.

Inhibitor		Phenylalanine uptake
D-Mannitol		0.941
D-Glucose		0.789
D-Galactose		0.718
	$D_{0.01}$	0.140
	$D_{0.001}$	0.194

Rabbit ileal slices were incubated for 1 min in 7.5 ml of 0.9 mM L-phenylalanine in phosphate buffer [10] in the presence of 20 mM sugar (Mannitol was used in the controls). The results which represent the means of six experiments with different animals are expressed in μmoles of amino acid absorbed per ml of tissue water. The values of D were obtained from an analysis of variance as an orthogonal two-way classification [13].

4. Discussion

The experiment presented in the figure demonstrates that addition of galactose to the mucosal fluid bathing an everted sac of rabbit ileum elicits an efflux of phenylalanine from the tissue, as witnessed by monitoring both the alterations in the concentration of the bathing fluid and the changes in substrate content of the tissue. The use of everted sacs restricts this interaction to the mucosal face of the cell, whereas previous demonstrations of such counter-transport in the intestine [7, 14] have been open to the criticism that both faces of the tissue plus the "cut edges" were in contact with the incubation medium.

Counter-transport is a specific property of membrane transport mechanisms [15], hence its provocation by galactose represents strong support for the hypothesis that sugars and amino acids interact at the carrier level in the mucosal (brush-border) membrane of the epithelial cell [7]. The similarity of the behaviour of hamster [7, 8] and rabbit intestine (present results) strongly suggests that all mammalian species behave in essentially the same manner.

There is a blatant contradiction between the present findings and those of Chez et al. [5]. These workers failed to demonstrate an inhibition of unidirectional influx of an amino acid (L-alanine) into mucosal cells of rabbit ileum from which result they deduced without direct evidence that the interaction of sugars and amino acids takes place at the *inner* face of the membrane, galactose stimulating amino acid efflux. We are quite unable to explain the lack of agreement between the two laboratories, using the same animal, but it may be pointed out that the work of Chez et al. [5] was carried out with a somewhat different technique and was limited to inhibition studies: no attempt was made to study counter-transport, nor to measure efflux directly (as has in fact been done in other heterologous-interaction studies [16]). In order to ascertain whether the discrepancies could possibly be due to the use of different substrates, we performed a series of counter-transport experiments analogous to those shown in the figure, but using alanine as substrate. The results were qualitatively similar but quantitatively inferior, in that no significant heterologous counter-transport effect could be demonstrated, although there was an obvious trend. It is possible, therefore, that alanine is not the most adequate substrate for the demonstration of these interactions.

Finally, it should be mentioned that other workers have attemped to demonstrate counter-transport of amino acids by sugars in rat intestine, but with negative [17] or poor [2] results. Munck's experiments [17] are however debatable, particularly since the incubation periods were inordinately long (rat intestine is especially fragile during prolonged incubations in the absence of added glucose [12]), and since the failed to prove the viability of his preparation by determining the substrate content of the tissue at the end of the incubation. Since amino acid flux from serosa to mucosa is slow, the presence of serosal/mucosal concentration ratios greater than one at the end of the incubations does not necessarily mean that the tissue is still viable. It is quite possible that, on changing the conditions, counter-transport between sugars and amino acids might become apparent in the rat intestine as well: evidence to this effect is being gathered in our laboratories [18].

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