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Independent modulation by food supply of two distinct sodium-activated D-glucose transport systems in the guinea pig jejunal brush-border membrane

(intestinal adaptation/semistarvation/fasting/methyl α-D-glucopyranoside/membrane potential)

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ABSTRACT **D-glucose transport across the intestinal brush-border membrane involves two transport systems designated here as systems 1 and 2. Kinetic properties for both D-glucose and methyl α-D-glucopyranoside transport were measured at 35°C by using brush-border membrane vesicles prepared from either control, fasted (48 hr), or semistarved (10 days) animals. The results show the following: (*i*) The sugar influx rate by simple diffusion was identical under either altered condition. (*ii*) Semistarvation stimulated D-glucose uptake by system 2 (both its V_{max} and K_m increased), whereas system 1 was untouched. (*iii*) Fasting increased the capacity of system 1 without affecting either K_m of system 1 or V_{max} and K_m of system 2. The effect of fasting on V_{max} of system 1 cannot be attributed to indirect effects from changes in ionic permeability because the kinetic difference between control and fasted animals persisted when the membrane potential was short-circuited with equilibrated K^+ and valinomycin. This work provides further evidence for the existence of two distinct sodium-activated D-glucose transport systems in the intestinal brush-border membrane, which adapt independently to either semistarvation or fasting.**

D-glucose transport in the small intestine responds to a variety of pathophysiological conditions that include qualitative and quantitative modifications of the diet, small-bowel resection, and diabetes. Often, however, published results are contradictory. For instance, under apparently similar conditions total sugar absorption has been described as either increasing, decreasing, or exhibiting no change (for reviews, see refs. 1–3). No satisfactory explanation of these discrepancies thus far exists, but many variables that have escaped appropriate control may be involved. Furthermore, the procedures of analysis and data expression differ widely and complicate the situation (3–7).

An additional source of confusion is the heterogeneity in intestinal transport. Although the suggestion that two D-glucose transport systems occur in the apical border of the intestine has existed since the work of Honegger and Semenza (for review, see ref. 8), D-glucose absorption is usually treated as involving a single homogeneous transport system, the D-glucose/Na^+ cotransporter identified in the sixties (for reviews, see refs. 8–11). Consequently, work on intestinal adaptation has been concerned with overall transport function of the intestine, providing no answers to whether individual transport systems were being selectively affected. In this paper we describe experiments permitting such a diagnosis.

We demonstrated recently that D-glucose transport across the intestinal brush-border membrane involves at least two distinct, sodium-activated transport agencies (12–14). Although we identified the first, system 1 (S-1), as being identical with the classical D-glucose/Na^+ cotransporter (12), the exact nature of system 2 (S-2) remains to be established. [In this paper we use our own classification (12–14); although two distinct D-glucose/Na^+ cotransport systems have been described in the kidney (for review, see ref. 8), present evidence indicates no ready equivalence between intestinal and renal systems.]

Preliminary work in our laboratory indicated that S-2, the low-affinity system thus far ignored by essentially all workers, is indeed the one most sensitive to modulation by the physiological state. Thus, whereas conditions leading to hyperphagia, such as lactation and cold-temperature adaptation, cause the overall transport function of S-2 to increase, these conditions leave S-1 unaffected (15). Indeed, around 1986 the suggestion seemed possible that S-2 was adaptive, whereas S-1 could be constitutive. We investigated this question by using three distinct sets of nutritional conditions that, even though involving very simple experimental setups, have pervasive, still unexplained, consequences in intestinal physiology; the conditions were as follows: (*i*) semistarvation, (*ii*) fasting, and (*iii*) fasting followed by refeeding. To avoid interpretational problems due, for instance, to indirect metabolic effects or to changes in intestinal tissue structure, we used isolated brush-border membrane vesicles as our experimental tool.

A preliminary account of this work has been given (16).

MATERIALS AND METHODS

Animals. Either adult female guinea pigs (white Dunkin–Hartley strain, Lessieux, Bray et Lu, France) or tricolor males bred in our laboratory were used. All animals were preadapted to controlled conditions of individual housing, constant temperature (24°C) and hygrometry (50%), alternating 12-hr light/darkness periods, and free access to water and vitamin C-containing food (guinea pig chow 114, Usine d'Alimentation Rationnelle, Epinay-sur-Orge, France).

At day 30 four animal subgroups were constituted: (*i*) controls, which continued to be fed ad libitum until day 40; (*ii*) semistarved animals, which from days 30 to 40 were given 25% (9 g/day) of the average daily food intake of the control group; (*iii*) fasted animals, which after a 38-day period of ad libitum feeding continued to receive water but no food from

Abbreviations: S-1 and S-2, D-glucose transport systems 1 and 2; αMeGlc, methyl α-D-glucopyranoside; V_{max1} and V_{max2}, V_{max} of system 1 and system 2, respectively; K_{m1} and K_{m2}, K_m of system 1 and system 2, respectively; K_{ds}, kinetic diffusion constant.

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days 38 to 40; and (*iv*) fasted and refed animals, which after a 48-hr fasting identical to group *iii*, were again given food ad libitum for an extra 24 hr.

At either day 40 (groups *i* to *iii*) or day 41 (group *iv*) the animals (*n* equaled four per group) were killed by a blow to the neck. The jejunum was removed, washed with saline, everted, and stored at −20°C. Less than 10 days afterward, brush-border membrane vesicles were prepared from the pooled tissues of four animals by using the Mg^{2+}/EGTA precipitation method of Hauser *et al.* (17) with slight modifications (12, 14). The final vesicle preparation, resuspended in mixture of 500 mM sorbitol, 4 mM lithium azide, and a metal ion-free 10 mM Hepes/7 mM 1-butylamine/7 mM maleic acid buffer, pH 7.4 (18), was stored in liquid nitrogen until the day of transport measurement.

Substrate uptake was measured by using a rapid filtration technique (19) in a medium containing the following: (*i*) 4 mM lithium azide and the Hepes/1-butylamine/maleic acid buffer, (*ii*) variable concentrations of the $^{14}C(U)$-labeled substrate, (*iii*) NaCl to give an initial metal-ion gradient (out/in) equal to 100/0 mM; and (*iv*) enough sorbitol to maintain an inward-directed net osmolarity gradient (out/in) of 600/500 mosM/liter. In certain experiments, under otherwise identical conditions, the membrane potential was short-circuited by preequilibrating the vesicles with 100 mM KCl and valinomycin (14).

Initial uptake rate measurements were done at 35°C with a short-time incubation apparatus (Innovativ Labor AG, Adliswil, Switzerland), as described (12). Total uptakes are expressed as absolute velocities (18). Uncorrected initial velocities as a function of the substrate concentration were fitted by nonlinear regression analysis to an equation containing two saturable, Michaelian transport terms plus a diffusional component (12, 20):

$$v = \frac{V_{max1}\cdot[S]}{K_{m1} + [S]} + \frac{V_{max2}\cdot[S]}{K_{m2} + [S]} + K_{ds}[S], \qquad [1]$$

where the suffixes 1 and 2 identify each of two distinguishable transport systems and K_{ds} is a kinetic diffusion constant (12). The nonlinear regression analyses included an *F* test (20). All calculations were done by using an Apple MacIntosh microcomputer.

RESULTS

Animal Growth Curves. In a typical experiment, the initial weight of the animals was 458 ± 25 g. Semistarvation induced a rapid weight loss, which after 48 hr tended to stabilize. At the end of 10 days, the animals had lost 16% of their initial weight—29% as compared to the controls. A 48-hr fasting induced a relative loss of ≈20%, but 58% of this loss was recovered after 24 hr of ad libitum refeeding.

Transport Results. Although the same protocol was repeated several times with essentially identical results, the data presented first (Figs. 1 and 2; initial entries in Table 1) were obtained with brush-border membrane vesicles derived from a single, female Dunkin–Hartley animal lot. Concordant results were obtained with male tricolor animals (final entries in Table 1), indicating that the effects evinced by our experiments are sex-independent.

The two Na^+-activated systems involved in D-glucose transport across the intestinal brush-border membrane were distinguished from their kinetic behavior (12, 14). Because the information obtained by using either D-glucose or methyl α-D-glucopyranoside (αMeGlc) as the substrate is complementary but different, the results with each of these two substrates will be separately described.

D-Glucose Transport Experiments. Semistarvation induced a significant increase in total D-glucose uptake (Fig. 1*A*),

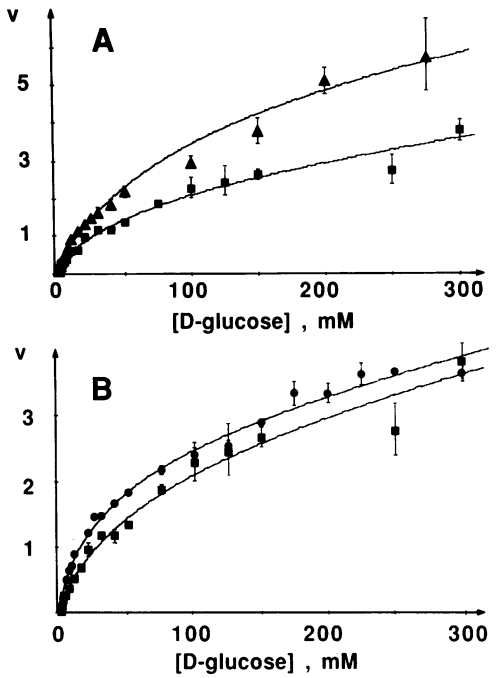

FIG. 1. Kinetics of D-glucose transport by isolated brush-border membrane vesicles from either control, semistarved, or fasted animals. D-glucose saturation curves in the presence of Na^+ were done under standard conditions, with substrate concentrations ranging from 0.1 to 300 mM. Initial velocities, *v*, were measured for 3 sec (12) and are expressed as the uncorrected total uptake rates in nmol·(mg of protein)$^{-1}$·sec^{-1} ± SEM (n = 3 for each point; for most points, SEM is smaller than the symbol). (*A*) Vesicles from either control (■) or semistarved animals (▲) are directly compared. (*B*) Control (■) and fasted (●) animals; note the difference in scale. The continuous lines show the theoretical total uptake curves calculated by applying Eq. 1 and the kinetic parameters estimated from these data on female guinea pigs listed in Table 1.

confirming work done by others with intact-tissue preparations (4–7, 21). Extending the older work, however, nonlinear regression analysis of our data reveals the transport increase induced by semistarvation to be highly specific: semistarvation affects only S-2. In fact (see Table 1), while V_{max1} and K_{m1} remained unchanged, V_{max2} and K_{m2} increased by 2.4 and 1.5 times, respectively.

Studies with fasted animals yielded the opposite result: S-2 remained constant but S-1 increased (Fig. 1*B*). Moreover, only the capacity parameter was here affected: V_{max1} increased by ≈1.6 times, whereas K_{m1} remained totally unaffected. A 48-hr fast followed by a 24-hr period of ad libitum feeding yielded results indistinguishable from those obtained with fasted animals (line F+R, Table 1).

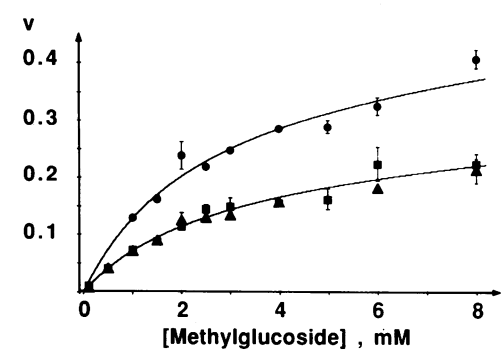

FIG. 2. Kinetics of αMeGlc transport. Conditions and symbols as in Fig. 1 except that the substrate was αMeGlc, and transport was measured for 10 sec in the concentration range from 0.1 to 8 mM (14).

Table 1. Kinetic parameters for D-glucose and αMeGlc transport: Effects of semistarvation, fasting, and fasting followed by refeeding

Condition	Substrate	V_{max1}	K_{m1}	V_{max2}	K_{m2}	K_{ds}	F[df]
			Female Dunkin–Hartley guinea pigs				
C	DGlc	0.23 ± 0.03	0.68 ± 0.14	2.32 ± 0.25	69 ± 15	5	1.57 (15–38)
C	αMeGlc	0.24 ± 0.03	2.44 ± 0.33	0	—	5	1.10 (9–21)
S	DGlc	0.27 ± 0.04	0.68 ± 0.17	5.52 ± 0.67	104 ± 23	5	1.00 (17–40)
S	αMeGlc	0.24 ± 0.02	2.63 ± 0.28	0	—	5	1.77 (9–22)
C+S	αMeGlc	0.24 ± 0.02	2.52 ± 0.30	0	—	5.1 ± 0.6	0.57 (8–54)
F+R	DGlc	0.41 ± 0.03	0.69 ± 0.06	2.30 ± 0.09	47 ± 5	5	1.79 (21–117)
F	αMeGlc	0.43 ± 0.02	2.48 ± 0.20	0	—	5	2.46 (9–22)
R*	αMeGlc	0.43 ± 0.02	2.60 ± 0.15	0	—	5	0.58 (6–29)
			Male tricolor guinea pigs				
C*	αMeGlc	0.47 ± 0.02	2.66 ± 0.20	0	—	5	0.52 (8–61)
F*	αMeGlc	0.67 ± 0.06	2.64 ± 0.40	0	—	5	1.63 (8–81)

Summary of the kinetic parameters estimated (±SD) by nonlinear regression analysis from sugar saturation curves. DGlc, D-glucose. F[df], F test and degrees of freedom (20). C, Control animals; S, semistarved animals; F, fasted animals; R, fasted and refed animals. C+S and F+R identify fits obtained with pooled data when the relevant groups were statistically indistinguishable. Asterisks identify results obtained with and without short-circuiting conditions, which were statistically indistinguishable and hence have also been pooled. Units: V_{max}, nmol·(mg of protein)$^{-1}$·sec^{-1}; K_m, mM; K_{ds}, nl·(mg of protein)$^{-1}$·sec^{-1}. In the V_{max2} column, 0 means that the data do not fit Eq. 1 unless this parameter equals 0—i.e., only S-1 exists, and consequently K_{m2} has no meaning (—). Because K_{ds} values were essentially identical in all fits performed [range, 4.5–5.5 nl·(mg of protein)$^{-1}$·sec^{-1}], this constant was fixed to the values shown in parentheses before doing the various final fits. An example of free fitting of K_{ds} is given in line C+S. For further details, see the figure legends and the text.

αMeGlc Transport Experiments. Because αMeGlc is not transported through S-2 (14), use of this substrate allows direct testing of the conclusions drawn in the preceding paragraph from the sugar D-glucose, which is transported by both S-1 and S-2. (*i*) The αMeGlc saturation curves exhibited by either the semistarved or the control animals (see Fig. 2) are indistinguishable—thereby confirming that semistarvation causes no change in S-1. (*ii*) In sharp contrast, fasting induced an overall increase in αMeGlc uptake, confirming that fasting affects S-1 specifically. The data from female guinea pigs in Table 1 ratify these conclusions.

Effect of Short-Circuiting the Electrical Membrane Potential on the Kinetics of αMeGlc Transport: Control Versus Fasted Animals. Because S-1 corresponds to the rheogenic D-glucose/Na^+ cotransport system of the brush-border membrane (12–14), two entirely different mechanisms can, in theory, explain the increase in V_{max1} exhibited by fasted animals. (*i*) Fasting may cause the effective number of S-1 transporters per membrane surface unit to increase. (*ii*) Fasting may act indirectly by modifying the membrane permeability to ions such that the driving force available for D-aldohexose transport increases (22–24). For instance, fasting could increase the permeability ratio P_{Cl^-}/P_{Na^+} so that, in the NaCl gradient used in our experiments (out/in = 100/0 mM), the generation of an inside-negative membrane potential would cause V_{max1} to increase while leaving K_{m1} unaffected (see ref. 14). This hypothesis is simply tested by short-circuiting the membrane potential, under which conditions the difference between control and fasted animals should disappear if unspecific effects on sugar-driving forces were the cause.

αMeGlc saturation curves were therefore done both without and with short-circuiting conditions. Complete analysis of the question with the same female guinea pig lot used for the experiments in Table 1 was impossible due to exhaustion of the vesicle material. Nevertheless, a preliminary experiment was run by using vesicles from the fasted and refed animal lot; we had seen that these vesicles behave identically to those derived from fasted animals. The results (Table 1, line R*) indicate that short-circuiting the membrane potential does not prevent the V_{max1} increase elicited by fasting.

The experiment was then repeated with vesicles derived from either control or fasted animals from a group of male tricolor guinea pigs. The results (see Table 1) confirmed the following: (*i*) Fasting specifically causes V_{max1} to increase, and (*ii*) the difference between control and fasted animals is unaffected by short-circuiting membrane potential.

DISCUSSION

The small intestine is known to be a maleable organ capable of adapting its absorptive capacity to a large variety of conditions. But the nature and site of these adaptations are the subjects of debate (1–3).

By using isolated brush-border membrane vesicles and two different sets of nutritional conditions, semistarvation and fasting, the present work aimed at answering two fundamental questions: (*i*) Is the brush-border membrane a key site in intestinal adaptation? (*ii*) Do these adaptations selectively involve one or the other of the two distinct D-glucose transport systems existing in this membrane?

During semistarvation, macroscopic changes in intestinal structure have been described (1, 2, 25). The intestinal wall, for instance, has been found to thin, which may create interpretation problems when absorption is measured in terms of transmural fluxes and similar indirect parameters. Use of isolated brush-border membrane vesicles would eliminate such problems, provided that this membrane is the site of the adaptation and, more important, that membranes derived from either control or experimental tissues are indeed comparable. If, for example, under a given condition the number of closed, effectively transporting vesicles per unit of membrane protein (vesicle yield, see ref. 18) dropped significantly, this fact would show operationally as a fall in the apparent transport V_{max} of a solute under study, even if the transport capacity of the relevant transport system were totally unaffected.

To rule out these potential complications, we ran a series of tests to verify that, in terms of protein and enzyme marker content, vesicles from either controls or experimental animals do not differ grossly. In agreement with other workers (6, 7), our results justify the conclusion that brush-border membrane vesicles derived from either animal group are all functionally similar (although the proportion of mucosal tissue per gram of intestine may decrease in both semistarvation and fasting). The relevant data, however, are not presented here, having been superseded by other observations better proving the point. In fact, the results of our transport studies intrinsically contain the necessary internal controls.

Stability of the Diffusion Parameter. To establish whether the effects seen were due, in whole or in part, to changes in the unspecific permeability of the membrane (see refs. 7 and 26), diffusion was monitored either by using L-glucose as a marker or by estimating by nonlinear regression analysis the

limiting slope of substrate saturation curves (12). The results (illustrated in part in Table 1) warrant the conclusion that the nutritional conditions studied here specifically affect D-glucose transport, not its passive diffusion.

Internal Controls. The results in Table 1 indicate that the best internal control for our experiments comes from the fact that the two key conditions tested, semistarvation and fasting, selectively affect one or the other of the two D-glucose transport systems; each condition never affects both systems at the same time.

Examining first the semistarvation results—because S-1 is totally unaffected, the activity of this system serves here as an internal control. Consequently, the observed increase in the overall activity of S-2 can be explained unequivocally in terms of a selective effect of semistarvation on this particular system. The validity of using S-1 as an internal control is further shown by the fact that αMeGlc uptake is totally unaffected under semistarvation. Furthermore, the V_{max1} data obtained by using either D-glucose or αMeGlc as the substrate are numerically identical (Table 1), a result to be expected if both sugars share this transport system in common (14).

Making the reverse argument, the activity of S-2 did not vary when we compared fasted animals with controls. Therefore, we conclude that the increase in activity of S-1 reflects a specific fasting effect on this system. The V_{max1} values seen with either fasted or fasted and refed animals are homogeneous and also independent of the substrate (see Table 1).

Possible Physiological and Molecular Significance of the Transport Effects Seen. Available information does not tell whether the selective increases in V_{max} caused by either semistarvation or fasting are due to greater mobility of the corresponding transporter or rather to increases in the number of transporters per membrane surface unit. For fasting, the experiments under short-circuiting conditions permit the conclusion that the observed increase in V_{max1} cannot be explained in terms of nonspecific changes in the efficacy of the Na^+ electrochemical gradient acting as a driving force. By default, the hypothesis that effective S-1 cotransporter density increases in the membrane gains preference in explaining the fasting effect.

The effect of semistarvation on S-2 is mixed because both V_{max2} and K_{m2} increase. Its high K_{m2} has been argued (13) to render S-2 most useful for intestinal absorption because at glucose concentrations at which S-1 would be saturated (e.g., 5 mM or higher), S-2 might not be saturated and therefore could still adapt its transport rate proportionally to the luminal sugar load. Teleologically, the observed mixed activation of S-2 would strongly enhance the overall absorption capacity of the semistarved animal. The double effect of semistarvation on each V_{max2} and K_{m2} is reminiscent of that seen in certain situations that induce hyperphagia—e.g., cold adaptation and lactation where again V_{max2} and K_{m2} both increase (15).

One point worth further consideration is the contrast existing between the just-mentioned effects—all on S-2—and that of fasting, the only condition found thus far where S-1 is selectively affected. Luminal nutrients are thought a key signal in enterocyte adaptation (1, 2, 27), which would explain the two opposite responses evidenced by our studies. Although during fasting the apical border of the enterocytes is not exposed to food, a radically different situation—presence of food in the lumen—prevails under each semistarvation, lactation, and cold adaptation.

On the other hand, these last three conditions involve long-term adaptations, which probably differ markedly in terms of the animal's general and hormonal state. What do these disparate situations have in common? This question cannot yet be answered with assurance, but a working hypothesis is apparent. In all three cases, the animals tend to lose weight, indicating that, either for absolute (semistarvation) or relative (lactation or cold adaptation) lack of food, the animals are in caloric deficit. Such a situation would signal an increase in the absorptive capacity of the small intestine—hence, an increase in S-2 (13). But, as is often the case in intestinal adaptation (1–3), the signals that elicit these effects remain to be identified.

Conclusion. The data presented in this paper confirm the existence in the brush-border membrane of guinea pig jejunum of two distinct sodium-activated D-glucose transport systems. These systems respond quite independently to the two nutritional conditions tested, semistarvation and fasting. Whether the difference between the two systems is genetic or phenotypic is an open question awaiting further studies.

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