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An unusual active hexose transport system in human and mouse preimplantation embryos

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ABSTRACT **In a metabolic study of human and mouse preimplantation embryos (preembryos), we measured glucose uptake and phosphorylation with nonradioactive 2-deoxyglucose (DG) as tracer. Initial experiments indicated an active hexose transport capacity, a property thought to be restricted in mammals to intestinal villi and kidney tubules [Baly, D. L. & Horuk, R. (1988) *Biochim. Biophys. Acta* 947, 571–590]. Significant findings are as follows: (*i*) During a 60-min incubation with a low level of DG, mouse blastocyst DG rose to levels up to 30 times that of the medium. {The intestinal active system does not transport DG [Crane, R. K. (1960) *Physiol. Rev.* 40, 789–825].} (*ii*) Active preembryo transport was not blocked (as it would have been in the intestine) by phlorizin [Alvarado, F. & Crane, R. K. (1962) *Biochem. Biophys. Acta* 56, 170–172 and Sacktor, B. (1989) *Kidney Int.* 36, 342–350] or by replacement of Na^+ with choline$^+$ or K^+ [Crane (1960) and Sacktor (1989)]. (*iii*) Transport of DG was blocked by cytochalasin B (which is not true for the intestinal transporter). We conclude that a distinct active hexose transporter and at least one facilitated transporter are present in preembryos, perhaps appearing in tandem on different membranes during formation of the increasingly complex preembryo structure.**

During experiments to measure glucose consumption in human and mouse preimplantation embryos (preembryos), it was observed that 2-deoxyglucose (DG), which we were using as a tracer, was concentrated up to 30-fold by preembryos. This was intriguing because active glucose transport in mammals is generally thought to be restricted to kidney tubules and intestinal villi (1). The study was made with nonradioactive DG, which can be accurately distinguished from its predominant metabolite 2-deoxyglucose-6-phosphate (DG6P) with quantitative enzyme methods (2, 3).

MATERIALS AND METHODS

The *human* preembryos were discarded from an *in vitro* fertilization program because they were judged unsuitable for return to the donor. The *mouse* preembryos were obtained from superovulated animals at the two-cell stage followed by incubation *in vitro* until they reached the blastocyst stage or were obtained directly *in vivo* at the morula or blastocyst stage.

The ova were freeze-dried by a procedure adapted from Barbehenn *et al.* (4). Each ovum was transferred with 0.5–1 μl of culture medium onto a glass slide with a braking pipette. The medium was quickly spread out with the pipette tip over a circular area about 8 mm in diameter and was frozen immediately by dipping the slide into Freon-12 (CCl_2F_2) brought to its freezing point (−170°C) with liquid N_2. The specimens were freeze-dried on the slide at −35°C. The fluid depth on the slide is somewhat critical: if it is much less than 0.03 mm, the ovum may be dislodged and lost after drying; if it is much greater than 0.05 mm, it may be difficult to find the ovum after freeze-drying.

RESULTS

The first full experiment was made with five human preembryos (Fig. 1). These were starved for 20 min (incubated in a medium with no energy source) and then incubated for 60 min in the same medium containing 0.5 mM DG. This generated intracellular DG levels above that of the medium in four of the five cases, with parallel DG6P levels ranging from 4 to 17 times the corresponding DG concentrations (Fig. 1). Because of the scarcity and variability of available human preembryos, most of the further experiments were made with mouse preembryos.

The intestinal–renal active transport system requires Na^+ (5, 6). Fig. 2 explores this requirement for mouse preembryos with multiple samples from four different groups that ranged 7-fold in control DG uptake. The preembryos had been starved for 60 min in balanced salt solution with 0.1% bovine serum albumin and then incubated another 60 min in the same medium containing 0.2 mM DG (control) or in medium also containing 0.2 mM DG but with Na^+ replaced by choline$^+$ or K^+. Only in one case was DG significantly reduced by Na^+ replacement (choline substitution in one of the morula sets), and even in this case DG accumulated to more than twice that of the medium. In three instances Na^+ removal caused a significant *increase* ($P < 0.05$) in transport (Fig. 2): replacement of Na^+ with choline for one morula set and replacement with either choline or K^+ for the blastocyst set. [The large increase in *DG* uptake with maturation is similar to the increases in *glucose* uptake observed by others (7, 8)]. Fig. 3 supplies information about effects on DG active transport of several well-known hexose transport inhibitors. *Cytochalasin B*, a potent and specific inhibitor of the GLUT family of facilitated transporters (1), inhibited group A and group B blastocysts 87% and 96%, respectively. *Phloretin*, another less potent inhibitor of GLUT transporters (1), inhibited the two groups 58% and 80%, respectively. In contrast to these results, *phlorizin*, a specific inhibitor of the renal–intestinal *active* glucose transporter (6, 9), did not inhibit DG transport significantly in either group. DG accumulation was 97% of the control in group A. The levels in group B averaged 57% of the control, but the result is less conclusive because of a large standard error. Group A blastocysts were also incubated in a medium with the Na^+ replaced by choline (Fig. 3*A*). DG accumulation was still 82% of control, or 11 times that of the medium, in confirmation of the lack of a Na^+

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Abbreviations: DG, 2-deoxyglucose; DG6P, 2-deoxyglucose-6-phosphate.

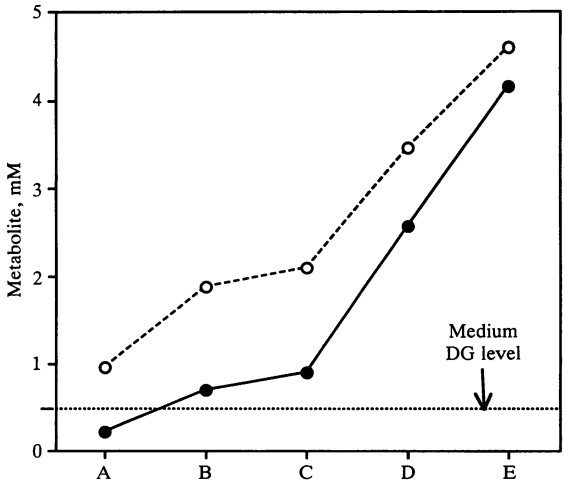

FIG. 1. Accumulation of DG (—) and DG6P (- - -) in five three-cell to eight-cell human preembryos (A–E) from the same donor. They were starved for 20 min (incubated in balanced salt solution with 0.1% bovine serum albumin without carbon source) and then incubated for 60 min in the same medium plus 0.5 mM DG. After a brief rinse in DG-free medium, they were frozen in Freon-12 at −150°C and dried overnight under vacuum at −35°C. Each preembryo was added to 0.4 μl of 0.02 M HCl under mineral oil in an oil well and heated 20 min at 80°C. From this droplet, 0.1 μl aliquots were taken for separate enzymatic measurements of DG and DG6P. The amount of metabolite in the separate aliquots ranged from 16 fmol for the lowest DG levels to 1.4 pmol for the highest DG6P levels. The DG6P values plotted are the measured DGPP concentrations divided by 4. Each assay yielded a stoichiometric amount of NADPH, which had to be amplified as much as 50,000-fold by enzymatic cycling to achieve the necessary sensitivity.

requirement for active transport. Three group B blastocysts were incubated with 1 mM galactose in addition to 0.2 mM DG. DG accumulation was 114% of control (Fig. 3*B*), a difference of no statistical significance, and an indication that the presence of 1 mM galactose in the medium that was used

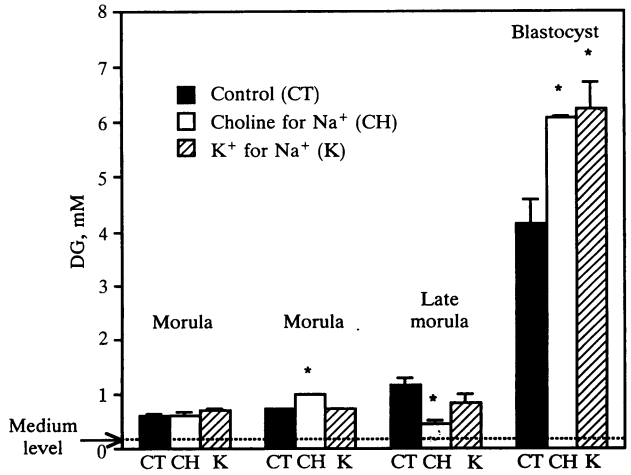

FIG. 2. Effect of Na^+ replacement on DG accumulation in mouse preembryos. Morulas from three mice and blastocysts from another were starved as in Fig. 1, but for 60 min, and then incubated in balanced salt solution with 0.1% bovine serum albumin that was supplemented with 0.2 mM DG alone (controls) or with 0.2 mM DG and the Na^+ replaced by equimolar choline$^+$ or K^+. Each bar represents the mean ± SE of four or five replicates except in the case of the second morula set for which there were only duplicates. Asterisks indicate significant differences from controls ($P < 0.05$). DG6P levels, also measured in each preembryo, were on average 3.5 times higher than those of DG with a close correlation between the two compounds. The assays were conducted as described for Fig. 1.

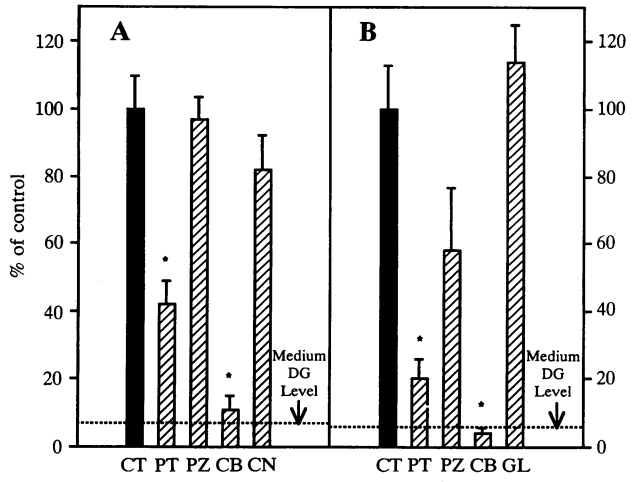

FIG. 3. Two groups of mouse blastocysts were starved for 60 min as for Fig. 2 and then incubated 60 min in the same medium supplemented with 0.2 mM DG. (*A*) For group A the medium also contained 1 mM galactose and either 0.5 mM phloretin (PT), 0.5 mM phlorizin (PZ), or 50 μM cytochalasin B (CB) or Na^+ was replaced by equimolar choline (CN). (*B*) Group B preembryos had the same basic treatment as those of group A except that the 1 mM galactose was omitted for all the preembryos except those of bar GL. PT, PZ, and CB concentrations were the same as for *A*. The solid bars (CT) are controls for each group. Asterisks indicate significant differences from controls ($P < 0.05$). Each bar represents the average ± SE of four blastocysts for group A and three blastocysts for group B except that there were only three control blastocysts for group A. The DG level in the medium (0.2 mM) is indicated by a dotted line in each panel, which is plotted as a percentage of the average DG concentration in the control blastocysts. DG6P is closely correlated to DG, but the ratio of DG6P to DG in group B varied from 7 for the controls to 2 for those with cytochalasin B.

for all the group A cells was of little consequence. Most of the preembryo experiments were conducted with a low level of DG in the medium (0.2 mM). A direct experiment with DG levels ranging from 0.1 to 1 mM indicated a very low K_m (0.05 mM). There was actually some *inhibition* with DG above 0.5 mM.

DISCUSSION

Our conclusions are that mammalian preembryos have an active hexose transporter that differs in several respects from that of the kidney and intestine and that they also have GLUT-type facilitated diffusion transporters that operate in tandem with the active transporter.

From the earliest developmental stages, preembryo cytoplasm is separated from the environment by more than one membrane; at later stages, as glucose uptake increases (7, 8, 10, 11), there may be more than two membranes intervening (12, 13). Our hypothesis is that a GLUT transporter is in the outermost membrane, explaining the almost total block of transport by cytochalasin B, whereas the active transporter is on an inner membrane where it would be shielded from phlorizin so that whether or not it is sensitive to this inhibitor it could not respond. The same might explain the failure of Na^+ replacement to block DG uptake if the active transporter does in fact require this cation. This could also accommodate the results of Wiley *et al.* (14, 15), who demonstrated by fluorescence staining that mouse preimplantation embryos express epitopes on blastomere surfaces that cross-react with antibodies to a 75-kDa subunit of the porcine renal brush border Na^+/glucose cotransporter system.

In regard to the active transporter itself, although to our knowledge no one has identified a mammalian transporter with the properties we describe, there is a protozoan parasite,

Leishmania donovani, that actively transports DG with very high affinity (K_m = 0.024 mM) and has a similar affinity for glucose (16, 17). It is driven by a proton symporter and does not require Na^+. Unlike the mammalian kidney–intestinal active transporter, it is at least partially inhibited by cytochalasin B. The authors point out that during some phases of their life cycle these protozoa are exposed to low concentrations of glucose on which their survival depends. A high-affinity glucose transporter is therefore essential. The situation is somewhat analogous for the mammalian preembryo, which cannot count on a constant high glucose concentration, particularly during the implantation process. We hesitate, however, to suggest that human preembryos may be parasites.

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