Biochimica et Biophysica Acta, 684 (1982) 146–148
Elsevier Biomedical Press

BBA 70003

BBA Report

TRANSPORT OF GLUCOSAMINE (ALDOHEXOSES) BY PREIMPLANTATION MOUSE BLASTOCYSTS

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(Received July 14th, 1981)

Key words: Glucosamine transport; Phloretin; (Mouse blastocyst)

A carrier-mediated system for uptake of glucosamine (and other aldohexoses) was identified in preimplantation mouse blastocysts. With early and late blastocysts, the values for K_m (525±82 and 651±137 μM, respectively) and V (0.29±0.02 and 0.29±0.04 pmol/h per ng protein, respectively) for glucosamine are statistically indistinguishable. The same carrier system, therefore, is operative at the two developmental stages. Glucosamine uptake was temperature sensitive and inhibited by structural analogues and phloretin.

During development of preimplantation mouse blastocysts a quantitative change in utilization of glucosamine for macromolecular synthesis occurs. Under identical pulse labeling conditions, [^{14}C]glucosamine incorporation is greater in late vs. early blastocysts [1]. Because a number of biochemical processes can impact on these values, the outlined studies were performed to determine whether translocation of exogenous glucosamine by mouse blastocysts is a carrier mediated process and whether kinetic parameters for glucosamine (aldohexoses) translocation change as a consequence of blastocyst development and transformation.

Embryos were flushed from the uteri of superovulated [2] Swiss-Webster pregnant mice to obtain early and late blastocysts, 88–92 h p.c. (postcoitum) or 98–101 h, respectively. The animals were 8–12 weeks old and all were progeny of a closed colony. Modified Brinster's medium [3] was used to flush uteri, wash the embryos and culture them in vitro.

For isotopic labeling, the embryos were incubated in modified Brinster's medium supplemented with the appropriate radioactive substance obtained from either New England Nuclear Co. (Boston, MA) or Amersham/Searle (Arlington Heights, IL). The amount of radioactive material used and duration of pulse labeling for the different experiments is indicated in the text. Procedures for measurements of uptake and incorporation of the labeled compounds are detailed elsewhere [3]. Thin-layer chromatograms for glucose and maltose were developed according to Hunt [4].

At 37°C, the time profiles of [^{14}C]glucosamine uptake by preimplantation blastocysts are linear for at least 10 min, in many cases significantly longer, over a wide range of glucosamine concentrations, 47.8 μM to 1.67 mM. From the relationship between concentrations and initial rates of uptake, expressed as pmol/h per embryo, Michaelis-Menten plots of glucosamine uptake by early and late blastocysts were constructed, Fig. 1. At both stages of development saturation by the metabolite is observed. Moreover, uptake is significantly diminished at 4°C, Fig. 1. Both proper-

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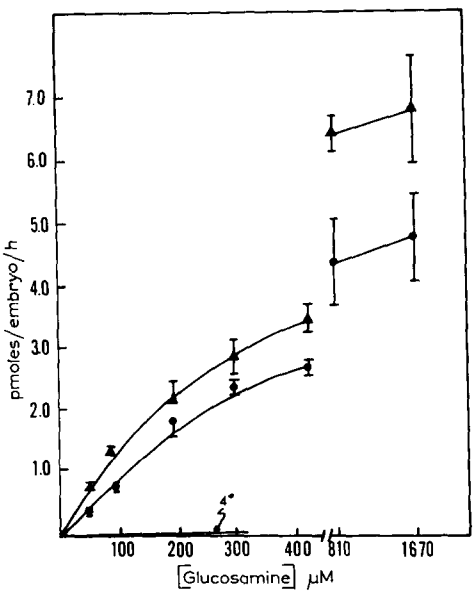

Fig. 1. Michaelis-Menten plots of glucosamine uptake by early (●) and late (▲) blastocysts. Results are expressed as pmol glucosamine per h per embryo, mean ± S.E.

ties, saturation and temperature dependency, are consistent with carrier mediated transport systems.

To obtain values for K_m, the concentration of glucosamine at half maximum velocity, and V, maximum velocity, the data in Fig. 1 were analyzed by means of a computer program described by Cleland [5]. The K_m values for early and late blastocysts are 525 ± 82 (S.E.) and 651 ± 137 μM, respectively; these values are statistically indistinguishable [5]. Thus, a change in affinity of the metabolite for a carrier does not appear to occur as a result of blastocyst development.

On the other hand, V values expressed a pmol/h per embryo (6.6 ± 0.05 and 10.0 ± 0.1 for early and late blastocysts, respectively) are statistically distinguishable ($P < 0.05$) [5]. If, however, the increase in total protein during development is taken into account and V is expressed as pmol/ng protein per embryo per h, the V values for early and late blastocysts are virtually identical, 0.29 ± 0.02 and 0.29 ± 0.04, respectively. On the basis of these results, it was concluded that glucosamine uptake occurs via the same system, or certainly an experimentally indistinguishable one, in early and late blastocysts. The increase in the number of active transport molecules paralleling the mass increase may have resulted from (i) increased synthesis of transport molecules, (ii) processing or activation of preformed carrier molecules, and/or (iii) membrane changes (affecting orientation in position) which may modify transport activity [6]. Finally, it should be noted that both K_m and V values refer to overall glucosamine uptake since translocation into the blastocoel may have occurred.

Since the kinetic parameters, K_m and V, for glucosamine were experimentally indistinguishable at the two extreme stages of blastocyst development (early and late), the experiments with competitors and inhibitors, which provide additional evidence for mediated transport, were performed only with early blastocysts. The embryos were pulse labeled with 14.8 μM D-[U-^{14}C]glucosamine in the presence of a single competing monosaccharide (4.63 mM). As a result of these experiments, the following order in inhibitory activity could be identified: glucose = mannose (97% inhibition, I) > mannosamine (88% I) > galactose (73% I) > 3-*O*-methylglucose (60% I) ≫ galactosamine (31%I). Among these compounds are 3-*O*-methylglucose, a non-metabolite, and galactosamine, which is neither phosphorylated nor incorporated into acid precipitable material by the embryos to a detectable extent. The observed effect of these two sugars on glucosamine uptake is, therefore, most likely due to competition for a carrier. To obviate competitive intracellular processing which could also have affected the rate of uptake [7], early blastocysts were incubated for 1 h in medium containing 4.63 mM 3-*O*-methylglucose, galactosamine or mannosamine prior to labeling for 1 h with 44.6 μM [^{14}C]glucosamine. No perturbation of glucosamine uptake was observed.

To address more fully the idea that glucose and glucosamine bind to the same transport molecule(s), early blastocysts were pulse-labeled with either [^{14}C]glucose or [^{14}C]glucosamine (47.6 μM, spec. act. 200 μCi/μmol) in the presence of different concentrations (10^{-8} to 10^{-2} M) of phloretin or maltose, established inhibitors of glucose transport in other systems [8,9]. The inhibition profiles of the two radioactive monosaccharides were similar. The greatest change in percent inhibition of uptake of either [^{14}C]gluco-

samine or [^{14}C]glucose per concentration unit of inhibitor occurred in the region of 10^{-6} to 10^{-4} M phloretin or maltose.

To determine whether maltose, like phloretin, represented the only substance in the medium competing for the carrier during the incubation period, chromatographic analysis for glucose was performed on samples of medium which had contained embryos plus $1.6 \cdot 10^{-3}$ M D-[U-^{14}C]maltose (spec. act. 7.8 μCi/μmol) and the corresponding controls. [^{14}C]Glucose, not present in the original maltose preparation, was found in medium which had contained blastocysts. Radioactive material was also taken up and incorporated (17 and 4 cpm per embryo, respectively) by early blastocysts which had been cultured for 15–18 h prior to labeling with [^{14}C]maltose, as indicated above. Therefore, either an enzyme on the trophoblast surface had hydrolyzed maltose to glucose which was subsequently utilized and/or remained in the medium or the disaccharide was internalized, hydrolyzed and some [^{14}C]glucose subsequently released into the medium.

When embryos were incubated in 10^{-4} M D-[U-^{14}C]maltose, the amount of radioactivity found as [^{14}C]glucose in the medium was 13% of the original input after 1 h and 27% after 2 h. After 1 h, the final molarity of glucose was 26 μM (2 mol glucose/mol maltose $\times 10^{-4}$ mol maltose/l $\times$ 0.13). Examination of these results in the light of experiments described previously indicate that it is unlikely that the glucose produced from maltose hydrolysis can account for the net inhibition of [^{14}C]glucose or [^{14}C]glucosamine uptake (70 and 65%, respectively) observed with maltose. Thus, maltose itself must compete for the glucose/glucosamine transport molecule(s). Moreover, the different inhibition studies described above appear to mutually support the conclusion that a number of aldohexoses share the same transport system in blastocysts.

The following characteristics of glucosamine/aldohexose uptake by preimplantation mouse blastocysts are consistent with a carrier mediated system: saturability, diminished activity at a lower temperature, competition by structural analogues, and inhibition by phloretin and maltose. The K_m value is of the order of magnitude reported for the low affinity glucose system found in fibroblasts [10]. In the latter case, only the low affinity system, but not the corresponding high affinity one, is inhibited by 3-*O*-methylglucose, which also inhibits the glucose-sharing system described here. Under the conditions of the outlined experiments, a high affinity system is not detectable.

As shown above, when one considers the change in mass during blastocyst development, V remains unchanged although late blastocysts have undergone developmental changes and transformation [11–14]. Consequently, the increased incorporation of [^{14}C]glucosamine found in late vs. early blastocysts which were pulse-labeled under identical conditions [1] must more closely reflect changes in gene expression, rates of post-translational processing and/or changes in turnover rates of the glycosylated products. The present studies, therefore, are part of a core of basic information required to more effectively assess and interpret labeling experiments with blastocysts that pertain to (i) qualitative and quantitative aspects of their development, (ii) carbohydrate metabolism and its utilization for macromolecular synthesis, as well as (iii) the effects of implantation delay on metabolite transport [15,16] and utilization.

These studies were supported, in part, by NIH grant RR-05384.

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