Glutamine transport by mouse inner cell masses

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Mouse blastocysts take up glutamine by specific transport systems. Glutamine is an important precursor for macromolecular synthesis and a potential alternative fuel to glucose. This study compared glutamine uptake in blastocysts and isolated inner cell masses and characterized the major participating systems in the latter. Inner cell masses take up glutamine by facilitated transport systems. The identity of these was investigated using substrate competition and kinetic studies. Na^+ -dependent uptake of $13 \mu\text{mol glutamine l}^{-1}$ was inhibited by 60% by $1 \text{ mmol tryptophan l}^{-1}$, 25% by $1 \text{ mmol 2-amino-2-norbornanecarboxylic acid l}^{-1}$ and 50% by $1 \text{ mmol lysine l}^{-1}$. Furthermore, $1 \text{ mmol 2-methyl(amino)isobutyric acid (MeAIB) l}^{-1}$ inhibited uptake by 29%. Kinetic analysis of MeAIB-resistant uptake revealed a predominant Na^+ -dependent facilitated uptake system with K_m and $V_{\max}$ values of $434 \pm 72 \mu\text{mol l}^{-1}$ and $237 \pm 38 \text{ fmol per inner cell mass per } 10 \text{ min}$, respectively. The inhibition of Na^+ -dependent uptake by tryptophan, lysine and the analogue 2-amino-2-norbornanecarboxylic acid suggests that most uptake of glutamine by inner cell masses occurs via the same system that predominates in whole blastocysts, $\text{B}^{0,+}$. The period of assay was so brief that significant participation of the inner cell mass in whole blastocyst uptake was precluded showing that system $\text{B}^{0,+}$ is expressed by both the trophectoderm and inner cell mass components of the blastocyst. However, MeAIB inhibited uptake by inner cell masses but not by blastocysts. This MeAIB-sensitive uptake had a K_m value of $4.3 \pm 1.7 \text{ mmol l}^{-1}$ and a $V_{\max}$ value of $451 \pm 119 \text{ fmol per inner cell mass per } 10 \text{ min}$. These characteristics suggest the first embryonic appearance of system A, which is a common Na^+ -dependent transporter in many somatic cells. Significant inhibition of Na^+ -independent glutamine uptake by tryptophan suggests participation of the ubiquitous system L, while inhibition by lysine suggests that $\text{b}^{0,+}$ may also be expressed in inner cell masses. Thus glutamine uptake by inner cell masses occurs via several transport systems expressed in whole blastocysts, including Na^+ -dependent system $\text{B}^{0,+}$, and probably Na^+ -independent systems L and $\text{b}^{0,+}$, but is supplemented by the first expression of Na^+ -dependent system A in these cells from which the fetus later develops.

Introduction

Glutamine is a precursor for protein and pyrimidine biosynthesis in addition to being implicated in nitrogen metabolism. In many cells, conversion of glutamine to α -ketoglutarate can provide a significant source of energy and lead to conversion of the carbon backbone into other amino acids. Glutamine is necessary for the culture of many cells *in vitro* and is required at concentrations 5–10 times higher than other amino acids (Eagle, 1955).

During preimplantation development, glutamine may provide an alternative to glucose as an energy source for bovine blastocysts (Rieger and Guay, 1988) and it has been suggested that it is an energy source in preimplantation mouse embryos at all stages (Chatot *et al.*, 1990). In mice, glutamine is abundant in oviduct fluid (Gardner and Leese, 1990).

Mouse embryos express facilitated transport systems capable of carrying this amino acid across the plasma membrane from the two-cell stage, and uptake increases significantly as differentiation of the blastocyst occurs (Gardner *et al.*, 1989; Chatot *et al.*, 1990; Lewis and Kaye, 1992). However, it is not known whether, in the blastocyst, this glutamine can be provided to cells of the inner cell mass, since there is no knowledge of glutamine uptake activity in this tissue.

The aim of this study was to classify glutamine transport systems in inner cell mass cells, using studies of Na^+ requirement, substrate inhibition profiles and kinetics of uptake by immunosurgically isolated inner cell masses.

Materials and Methods

Embryos

Quackenbush mice were randomly bred in our own animal house with lights on between 06:00 and 18:00 h. At 10 weeks

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of age, mice were superovulated by an injection with 10 iu pregnant mares' serum gonadotrophin i.p. (Folligon: Intervet, Lane Cove, NSW) at 10:00 h; followed 48 h later by 10 iu hCG (Chorulon: Intervet). At this time, the injected females were placed with single males of the same strain and the presence of a vaginal plug 23 h later indicated mating. Blastocysts (96 h after hCG treatment) were collected from the mated mice and washed in M2 medium (Fulton and Whittingham, 1978; modified as described by Hobbs and Kaye, 1985). Kinetic studies were performed on inner cell masses isolated from blastocysts that had developed *in vivo* and blastocysts that had developed from two-cell embryos (48 h after hCG treatment) during 48–52 h culture *in vitro* in 20 μ l droplets of BMOC2 medium (Brinster, 1965), modified as described by Pemble and Kaye (1986), under paraffin oil in an atmosphere of H₂O-saturated 5% CO₂, 5% O₂ and 90% N₂ at 37°C. Previous experiments confirmed that under these conditions at least 50% of two-cell embryos (48 h after hCG treatment) developed to blastocysts by 96 h after hCG treatment.

Isolation of inner cell masses

The zonae pellucidae were removed by brief exposure to Tyrode's solution (pH 2.5; modified as described by Hogan *et al.*, 1986) before immediate transfer through three 2 ml washes of M2. The zonae-free blastocysts were then placed in rabbit anti-mouse spleen cell antiserum diluted 1:10 (v:v) in M2 for 13 min. The antiserum was produced in a New Zealand White rabbit, bled 10 days after three injections i.v., 6 days apart, of Quackenbush mouse spleen cells in 0.5 ml 0.9% (w/v) sterile saline (Solter and Knowles, 1975; Harvey and Kaye, 1990). The blastocysts were then washed with fresh M2 and placed in guinea-pig serum diluted 1:10 in M2 for 13 min (ICN Biomedicals, Costa Mesa), as a source of complement. Inner cell masses were freed of lysed trophectoderm cells by passage through a fine hand drawn pipette. About 75% of blastocysts yielded suitable inner cell masses.

Na⁺-free M2 medium

M2 medium, free of Na⁺ (M2–Na⁺), was prepared by equimolar replacement of NaCl with choline chloride and NaHCO₃ with KHCO₃. The hepes used to buffer this medium was adjusted to pH 7.4 with KOH, and sodium lactate was omitted. Previous experiments had shown that omission of lactate did not affect blastocyst development over 4 h *in vitro*, or amino acid transport rates in blastocysts (Hobbs and Kaye, 1986).

The medium was added to the freeze-dried radiolabelled substrate, mixed and then transferred to the culture dish and preincubated under paraffin oil at 37°C, for at least 1 h.

Glutamine uptake

Glutamine uptake was determined by transferring blastocysts or inner cell masses to 20 μ l drops of M2 containing 9–13 μ mol [³H]glutamine l^{−1} (33–55 Ci mmol^{−1}, Amersham International, Bucks) under liquid paraffin in a Petri dish that had been equilibrated at 37°C.

The blastocysts or inner cell masses were collected, washed thoroughly by transfer through four changes of 2 ml ice cold M2 and placed in separate vials containing 0.2 ml H₂O to which was added 1 ml of Optiphase 'Hisafe 3' scintillation cocktail (Optiphase 'Hisafe' 3: LKB; FSA Laboratory Supplies, Loughborough) before the radioactivity was determined in a Packard 1900 CA liquid scintillation counter at 40% efficiency, with an absolute background of 4–7 c.p.m.

Efflux of [³H]glutamine

Inner cell masses were preincubated for 10 min at 37°C in M2 containing 100 μ mol or 1 mmol [³H]glutamine l^{−1} (0.5 Ci l^{−1}), collected and rapidly washed in M2, before transfer to M2 lacking glutamine at 37°C. At various times inner cell masses were collected, washed in ice-cold M2 and radioactivity was determined as above.

Competition by other amino acids

Blastocysts or inner cell masses were incubated for 10 min in M2 containing 9–13 μ mol [³H]glutamine l^{−1} and one of the following at 1 mmol l^{−1}: bicyclic analogue, 2-amino-2-norbornanecarboxylic acid (BCH); lysine (Lys); tryptophan (Trp); or 2-methyl(amino)isobutyric (MeAIB), and [³H]glutamine uptake was determined as above.

Standardization

The specific radioactivity of each drop was determined from samples of the medium collected at the end of the uptake period and the known non-radiolabelled glutamine concentration, allowing for [³H]glutamine contribution. A sample of the final wash was used to determine the experimental background radioactivity, which was found to be the same as the absolute background.

Statistical analyses

In cases where the data were not normally distributed, they were normalized by logarithmic transformation. The statistical significance of differences between means was assessed by multiple-range test (Fisher's protected LSD) after analysis of variance of normalized data. Kinetic parameters for saturable glutamine uptake were determined by fitting data to the equation

$$v = K_0[\text{glyn}] + \{V_{\max}[\text{gln}]\} \{K_m + [\text{gln}]\}^{-1}$$

using non-linear regression, where v is the initial rate or uptake in fmol per inner cell mass per 10 min, K_0 is the rate constant for the non-saturable transport, and K_m (μ mol l^{−1}) and $V_{\max}$ (fmol per inner cell mass per 10 min) values are the kinetic parameters describing the saturable transport component (Hobbs and Kaye, 1990).

Results

Initial experiments confirmed that over 95% of 13 μ mol [³H]glutamine l^{−1} taken up by inner cell masses during 10 min

Fig. 1. Percentage of [^3H]glutamine remaining at 37°C from mouse inner cell masses preloaded with $100\ \mu\text{mol}$ (■), or $1\ \text{mmol}$ (□) [^3H]glutamine l^{-1} for 10 min at 37°C . Values are the percentage of initial radioactivity remaining based on means $\pm$ SEM of three experiments, each containing 6–12 inner cell masses.

at 37°C was soluble in $10\% \text{Cl}_3\text{C}_2\text{O}_2\text{H}$. There was no significant loss of preaccumulated [^3H]glutamine from inner cell masses into amino acid-free medium at 37°C after preloading with $100\ \mu\text{mol}$ or $1\ \text{mmol}$ [^3H]glutamine l^{-1} medium (Fig. 1). The rates of uptake of $13\ \mu\text{mol}$ [^3H]glutamine l^{-1} by blastocysts and inner cell masses were compared in parallel experiments on blastocysts and inner cell masses collected from the same group of superovulated mice. The rates determined by least squares linear regression were $2.26 \pm 0.05\ \text{fmol per blastocyst min}^{-1}$ and $0.72 \pm 0.06\ \text{fmol per inner cell mass min}^{-1}$ (Fig. 2).

Competition by other amino acids

In blastocysts, $14\ \mu\text{mol}$ [^3H]glutamine l^{-1} uptake was inhibited 60% by $1\ \text{mmol Trp l}^{-1}$ ($P < 0.01$) and about 25% by BCH ($P < 0.01$, Fig. 3). Multiple range testing of the differences between the means showed that the 10% inhibition by MeAIB was not significant at $P < 0.05$, but that the 25% inhibition by BCH was significant at $P < 0.01$.

In inner cell masses, both MeAIB and BCH significantly inhibited glutamine uptake by 25–30% ($P < 0.01$). As in blastocysts, Trp caused a 60% inhibition ($P < 0.01$).

Uptake of other amino acids in the presence of MeAIB

Preliminary investigations of the uptake of $15.6\ \mu\text{mol}$ [^3H]glutamine l^{-1} and $6.3\ \mu\text{mol}$ [^3H]leucine l^{-1} in the presence of $6.5\ \text{mmol MeAIB l}^{-1}$ also indicated a 27–29% inhibition (Fig. 4).

Na^+ dependence

About 75% of the uptake of $13\ \mu\text{mol}$ glutamine l^{-1} by inner cell masses required the presence of Na^+ (Fig. 5). The

Fig. 2. Uptake of $13\ \mu\text{mol}$ ($0.5\ \text{Ci l}^{-1}$) [^3H]glutamine l^{-1} into mouse blastocysts (■) and inner cell masses (□). Values are means $\pm$ SEM of three experiments, each containing 4–6 embryos or inner cell masses per time point. Lines fitted by least squares linear regression.

Na^+ -independent uptake was inhibited strongly (50%, $P < 0.01$) by $1\ \text{mmol Trp l}^{-1}$, moderately (20%, $0.01 < P < 0.05$) by $1\ \text{mmol Lys l}^{-1}$, but not at all by $1\ \text{mmol MeAIB l}^{-1}$. In contrast, the Na^+ -dependent component was inhibited strongly (68%, $P < 0.01$) by $1\ \text{mmol Trp l}^{-1}$, (50%, $P < 0.01$) by $1\ \text{mmol Lys l}^{-1}$, and moderately (20%, $0.01 < P < 0.05$) by $1\ \text{mmol MeAIB l}^{-1}$.

Kinetics

MeAIB-resistant uptake. The contribution to Na^+ -dependent glutamine uptake by the MeAIB-sensitive system was blocked by inclusion of $2\ \text{mmol MeAIB l}^{-1}$. In the absence of Na^+ , the rate of glutamine uptake appeared to increase linearly with glutamine concentration to $5\ \text{mmol l}^{-1}$ (Fig. 6). In the presence of Na^+ , above approximately $500\ \mu\text{mol glutamine l}^{-1}$, the total rate of uptake appeared to increase proportionally to the increase in glutamine concentrations. The slope in this portion of the curve was similar to that observed in the absence of Na^+ . This finding suggested that a combination of Na^+ -independent nonsaturable uptake and a Na^+ -dependent saturable system was operating in inner cell masses to take up glutamine.

The apparent combination of saturable and nonsaturable uptake could be described by the equation

$$v = K_0[\text{Gln}] + \left\{ \frac{V_{\max}[\text{Gln}]}{K_m + [\text{Gln}]} \right\}^{-1}.$$

The value of K_0 was assumed to approximate to that of the Na^+ -dependent uptake, that is, $66 \pm 2\ \text{pl per inner cell mass per 10 min}$, calculated by linear regression (Fig. 6; $r = 0.996$; $n = 5$).

Fig. 3. Percentage inhibition of [^3H]glutamine uptake by mouse blastocysts and inner cell masses in the presence of tryptophan (Trp), 2-amino-2-norbornanecarboxylic acid (BCH) or methyl(amino)isobutyric acid (MeAIB). Inhibition is calculated from the means as [(control - inhibitor) control $^{-1}$] %. $^{**}P < 0.01$ when tested by multiple-range test. Control values for blastocysts and inner cell masses were 23 fmol per blastocyst per 10 min and 10 fmol per inner cell mass per 10 min, respectively.

This value was then used to fit the curve for uptake in the presence of Na^+ to the equation above. This procedure reduced the total variance of the fit and yielded estimates of the parameters for the saturable system of $V_{\max} = 237 \pm 38$ fmol per inner cell mass per 10 min and $K_m = 434 \pm 72 \mu\text{mol l}^{-1}$ (Fig. 6).

Lysine-resistant uptake. To examine the kinetics of the major Na^+ -dependent Lys-resistant system, glutamine uptake was analysed in the presence of 20 mmol Lys l^{-1} . The possibility that the expression of MeAIB-resistant uptake was affected by developmental conditions was examined by conducting separate experiments on inner cell masses isolated from blastocysts which had developed *in vivo* and *in vitro*. The uptake rates for inner cell masses in the presence of Na^+ were not significantly different whether they were isolated from blastocysts that had developed *in vivo* or *in vitro*. The results for inner cell masses from both sources were combined. The K_0 was determined from Na^+ -independent uptake to be 77 ± 3 pl per inner cell mass per 10 min by linear regression (Fig. 7; $r = 0.998$; $n = 8$) and the Na^+ -dependent component yielded the following kinetic estimates: $K_m = 4.3 \pm 1.7$ mmol l^{-1} and $V_{\max} = 451 \pm 119$ fmol per inner cell mass per 10 min by non-linear regression (Fig. 7).

Fig. 4. Percentage inhibition of uptake of $15.6 \mu\text{mol } [^3\text{H}]\text{glycine l}^{-1}$, $6.3 \mu\text{mol } [^3\text{H}]\text{leucine l}^{-1}$ or $14 \mu\text{mol } [^3\text{H}]\text{glutamine l}^{-1}$ by mouse inner cell masses in the presence of 5 mmol glutamine l^{-1} or 6.5 mmol methylaminoisobutyric acid l^{-1} . Inhibition was calculated from the means as [(control - inhibitor) control $^{-1}$] %. Bars are means from 3-5 experiments, each containing 4-10 inner cell masses. $^{***}P < 0.01$, when tested by multiple range test. Control uptake values were 316 fmol per inner cell mass per 10 min for [^3H]glycine, 10.6 fmol per inner cell mass per 10 min for [^3H]glutamine and 454 fmol per inner cell mass per 10 min for [^3H]leucine.

Discussion

Tests assessing the fixation of [^3H]glutamine into acid-insoluble material (mainly protein) during the incubation showed that 95% of the glutamine remained in the inner cell masses as acid-soluble material. Thus [^3H]glutamine uptake was accurately reflecting the simple uptake of glutamine by the inner cell masses, which is similar to the situation for blastocysts (Lewis and Kaye, 1992).

Kinetic studies require estimation of an initial rate. Parallel studies of glutamine uptake in blastocysts and inner cell masses were made to enable accurate comparisons. The results for blastocysts reflected those reported earlier (Lewis and Kaye, 1992).

In inner cell masses at $13 \mu\text{mol glutamine l}^{-1}$, the rate was only about 30% (0.7 fmol per inner cell mass min^{-1}) of that in blastocysts, with no indication of equilibrium even after 40 min of uptake, supporting predictions of very little efflux of glutamine from the inner cell mass.

In the presence of Trp or BCH, significant inhibition of glutamine uptake into inner cell masses was apparent, as in blastocysts. This inhibition suggested a contribution of system B^0+ to glutamine uptake by inner cell masses, as was concluded earlier for blastocysts (Lewis and Kaye, 1992).

Other identifying data came from the kinetic studies. The inclusion of MeAIB was designed to block the contribution of the other major Na^+ -dependent saturable system indicated by the inhibition studies. The inner cell mass K_m value of $434 \pm 72 \mu\text{mol l}^{-1}$ for glutamine was not significantly different by *t* test from the $524 \pm 75 \mu\text{mol l}^{-1}$ reported for Na^+ -dependent uptake of glutamine by whole blastocysts (Lewis and Kaye, 1992). The equivalent K_m values suggest identity

Fig. 5. Uptake of $13 \mu\text{mol } [^3\text{H}]\text{glutamine l}^{-1}$ by mouse inner cell masses in (a) presence or (b) absence of Na^+ and in the presence of 1 mmol lysine (Lys) l^{-1} , methyl(amino)isobutyric acid (MeAIB) or tryptophan (Trp). Means with identical superscripts are significantly different by multiple range test; $^{ij}0.01 < P < 0.05$; $^{a,b,c,d,e,g,h,i,j}P < 0.01$. Values are means $\pm$ SEM from three experiments, each containing 5–10 inner cell masses per treatment.

between the MeAIB-resistant, Na^+ -dependent system in inner cell masses and the major Na^+ -dependent system in blastocysts, which was concluded to be $\text{B}^{0,+}$ (Lewis and Kaye, 1992). The strong inhibitions by Trp and BCH support this conclusion, as both are strong competitors for $\text{B}^{0,+}$ uptake (Van Winkle, 1988). The V_{max} value of $237 \pm 38 \text{ fmol}$ per inner cell mass per 10 min is close to that for methionine uptake by inner cell masses (280 fmol per inner cell mass per 10 min; Miller and Schultz, 1985) and this similarity suggests the major Na^+ -dependent system transporting these two amino acids in inner cell masses is indeed $\text{B}^{0,+}$.

The estimated diffusion rate in inner cell masses was $66\text{--}77 \text{ pl}$ per inner cell mass per 10 min. This is 50% greater than the value in blastocysts (40 pl per embryo per 10 min; Hobbs and Kaye, 1985). However, since uptake over these short periods probably reflects trophectoderm activity, it may be concluded that the tight junctions between these epithelial cells present a significant barrier to diffusion of glutamine in whole blastocysts that is not present in inner cell masses. Thus blastocysts revealed a lower diffusion rate than did inner cell masses, because of the resistance that these tight junctions present to paracellular diffusion. This conclusion is further supported by the fact that the high concentration of amino acids in the uterus of rabbits (25.5 mmol l^{-1}) is not reflected within the blastocoel (12.1 mmol l^{-1} ; Miller and Schultz, 1987).

Another finding of this study was the demonstrated appearance of the MeAIB-sensitive system. As MeAIB is exclusively

Fig. 6. Kinetics of glutamine uptake by mouse inner cell masses at 37°C in the presence of 5 mmol methyl(amino)isobutyric acid $\text{l}^{-1} \pm \text{Na}^+$. Inner cell masses were collected from expanded blastocysts cultured for 48–52 h from two-cell stage embryos in BMOC2 medium. The curve for total uptake data ($\square$) is fitted to the equation $v = K_0[\text{Gln}] + \{V_{\text{max}}[\text{Gln}]\{K_m + [\text{Gln}]\}^{-1}\}$, with K_0 set to 66 pl per inner cell mass per 10 min obtained from Na^+ -independent uptake data by linear regression ($\blacksquare$). The Na^+ -dependent curve is fitted to the equation $v = V_{\text{max}}[\text{Gln}]\{K_m + [\text{Gln}]\}^{-1}$ and indicates the calculated activity due only to a system with these kinetic parameters. Each value represents the mean $\pm$ SEM of 6–14 inner cell masses per experiment, from three experiments for both Na^+ -dependent and Na^+ -independent uptake.

transported by system A, its inhibition of glutamine uptake by inner cell masses suggests the presence of system A activity in these cells. Thus this system, previously shown to be absent from preimplantation mouse embryos (Van Winkle *et al.*, 1985), has now been located in inner cell masses. Furthermore, uptake of both glycine and leucine was inhibited to the same extent by MeAIB as was glutamine uptake. Both of these amino acids have been shown to react with system A (Oxender and Christensen, 1963; Bracy *et al.*, 1986). A further observation was that development *in vitro* did not appear to affect the expression or activity of this system A in inner cell masses, within the precision of these studies. As *in vitro* culture has been observed to affect amino acid transport systems (Van Winkle, 1988), this observation suggests that the inner cell mass cells are protected from these environmental effects. However, the variances in these data require further investigation before this observation can be confirmed.

Kaye *et al.* (1982) showed a very low uptake of $[^{14}\text{C}]\text{MeAIB}$ by blastocysts during incubation for 2 h. But later inhibition studies with MeAIB failed to indicate the presence of system A in whole blastocysts (Van Winkle *et al.*, 1985). The present results suggest the long incubation period used by Kaye *et al.* (1982) may have allowed uptake by inner cell masses to contribute to total uptake in blastocysts; this may have been insignificant over the 5–10 min uptake typical of kinetic studies. Thus the location of system A in the inner cell mass may have made it difficult for earlier investigators to reveal its presence in whole blastocysts.

Fig. 7. Kinetics of glutamine uptake by mouse inner cell mass cells at 37°C in the presence of 20 mmol Lys l⁻¹ ± Na⁺. Inner cell masses were collected from expanded blastocysts (96 h after hCG treatment) developed *in vivo* or during culture for 48–52 h, from the two-cell stage in BMOC2, or from blastocysts. The curve for total uptake (○) is fitted to the equation $v = K_0[\text{Gln}] + \{V_{\text{max}}[\text{Gln}]\{K_m + [\text{Gln}]\}^{-1}$, with K_0 set to 77 pl per inner cell mass per 10 min obtained from Na⁺-independent uptake data by linear regression (●). The curve for Na⁺-dependent uptake was fitted to the equation $v = V_{\text{max}}[\text{Gln}]\{K_m + [\text{Gln}]\}^{-1}$, and indicates the calculated activity due only to a system with these kinetic parameters. Each point represents the mean ± SEM from two experiments, each using *in vitro* or *in vivo* developed blastocysts containing 5–10 embryos per treatment per experiment.

At 13 μmol l⁻¹ glutamine uptake in cell masses was about 75% Na⁺-dependent. The inhibition of Na⁺-independent glutamine uptake by Lys may indicate a contribution by system b⁰⁺, which is Na⁺-independent and transports both zwitterionic and cationic amino acids such as Lys. Further studies of this system in inner cell masses are underway. A contribution by system L is also suggested from the BCH inhibition of Na⁺-independent glutamine uptake (M. B. Jamshidi and P. L. Kaye, unpublished data).

In the methionine studies, the presence of systems L and A in inner cell masses was implied from inhibition profiles, providing some support for the presence of these systems in inner cell masses (Miller and Schultz, 1985). However this study was performed before characterization of systems B⁰⁺ and b⁰⁺ (Van Winkle *et al.*, 1985).

In conclusion, the results indicate that inner cell masses take up glutamine by a combination of the activity of several systems. The predominant Na⁺-dependent system is B⁰⁺, which is first expressed in eight-cell mouse embryos and is present in whole blastocysts. These observations therefore suggest that B⁰⁺ is expressed in both cell lines of the blastocyst established at compaction and that expression continues throughout the establishment of the inner cell mass. A second important Na⁺-dependent system is expressed in inner cell masses, and has not been reported previously in blastocysts or embryos at earlier stages. This system has the identifying characteristic of sensitivity to inhibition by MeAIB and is thus

concluded to be system A. Similar inhibition of uptake of glycine and leucine by MeAIB suggests that system A also contributes to the uptake of these amino acids in inner cell masses. Na⁺-independent uptake contributes only about 20% to the total uptake of 13 μmol glutamine l⁻¹, and inhibition by Lys, Trp and BCH suggests contributions by systems b⁰⁺ and L.

The cells of the blastocyst thus express the amino acid transport activities necessary to gain access to significant supplies of uterine amino acids. The tight junctions and relative impermeability of the trophectoderm to amino acids would otherwise limit this access to the abundant sources in the uterine fluids. Since in other cells, system A is subject to substrate and other regulatory factors (Kilberg, 1986), its expression in inner cell masses implies the need for more complex regulation of this supply to the inner cell mass. The recent isolation of the DNA sequence for this transporter system (Kong *et al.*, 1993) provides a tool to demonstrate molecular differentiation of the inner cell mass from the trophectoderm.

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