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Changes in the activities of amino acid transport systems $b^{0,+}$ and L during development of preimplantation mouse conceptuses

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Uptake of leucine, lysine, and arginine was predominantly Na^+-independent in mouse conceptuses through the 8-cell stage of development, and two components of saturable transport were detected for each of these amino acids. Uptake of cationic substrates from solutions near 1 μM was inhibited most strongly by bulky cationic and zwitterionic amino acids whose carbon skeletons do not branch at the α or β positions. By this criterion, system $b^{0,+}$ accounted for most of the Na^+-independent arginine and lysine transport in eggs and conceptuses throughout preimplantation development. A small, leucine-resistant, cation-preferring component of amino acid transport was also detected in these cells. Leucine uptake was inhibited most strongly by bicyclic, branched-chain or benzenoid, zwitterionic amino acids in eggs and conceptuses prior to formation of blastocysts. Therefore, it appeared to be taken up mainly by system L, while system $b^{0,+}$ accounted for a smaller portion of leucine uptake during this developmental period. In blastocysts, in contrast, system L was less conspicuous, and system $b^{0,+}$ was primarily responsible for Na^+-independent leucine uptake. The V_{max} values for transport of amino acids by system $b^{0,+}$ increased by up to 30-fold in conceptuses between the 1-cell and blastocyst stages. In contrast, the V_{max} value for leucine transport via system L decreased while the K_m value increased between these two developmental stages. Although several explanations for these changes are possible, we favor the hypothesis that the density of system L transport sites in plasma membranes decreases while the number of system $b^{0,+}$ sites increases during development of blastocysts from 1-cell conceptuses.

Introduction

Several amino acid transport systems in mammalian cell membranes have been characterized over the past 30 years [1–4]. These better-known systems are divided into two broad categories based on whether they are Na^+-dependent or Na^+-independent, and each of these categories is subdivided into three groups depending on whether the systems prefer cationic, zwitterionic or anionic substrates. Examples of zwitterion-preferring transport processes include the Na^+-dependent systems A, ASC, and Gly and the Na^+-independent systems L, T and asc [1–4]. System y^+ is the most thoroughly studied and best-known example of a Na^+-independent transporter of cationic amino acids in the mammalian plasma membrane [1–4].

Five distinct amino acid transport processes have been detected in preimplantation mouse blastocysts [4–9]. Some of these processes accept *both* cationic and zwitterionic amino acids as substrates, however, and at least one is Na^+-inhibited. Thus, they do not all fit well into the classification scheme outlined above. The processes in blastocysts include the broad scope, Na^+-dependent system for both cationic and zwitterionic substrates, provisionally designated $B^{0,+}$ [5–7], and a less conspicuous, zwitterion-preferring Na^+-dependent transport activity [7]. Bulky cationic and zwitterionic amino acids whose carbon skeletons do not branch at the α or β positions are the best substrates of system $b^{0,+}$, which is the major Na^+-independent system in blastocysts [8]. Although it is not Na^+-stimulated, Na^+ and other cations strongly inhibit lysine but not leucine uptake via system $b^{0,+}$ [9]. Smaller amounts of two other Na^+-independent systems are also present in blastocysts. One of the latter transport activities resembles the

Abbreviations, BCH, 2-amino-*endo*-bicyclo[2.2.1]heptane-2-carboxylic acid; BCO, 3-amino-*endo*-bicyclo[3.2.1]octane-3-carboxylic acid; HCG, human chorionic gonadotropin; MeAIB, 2-(methylamino)isobutyrate.

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better-known system L and was referred to previously as a zwitterion-preferring transporter of bulky amino acids [8]. The other system prefers cationic amino acids as substrates, but it may be novel [8]; its characteristics will be described more thoroughly in another report. Although other amino acid transport systems are probably present in blastocysts, especially for the transport of anionic amino acids, it seems likely that many of the more conspicuous transporters of cationic and zwitterionic α-amino acids in these conceptuses have now been delineated.

In contrast to blastocysts, most of the amino acid transport processes present in conceptuses at earlier stages of development have not been studied thoroughly. System Gly has been characterized in eggs and cleavage-stage conceptuses [7,10], and saturable Na^+-independent uptake of leucine [11,12], phenylalanine [13], and methionine [12,14,15] by these cells has been attributed, tentatively, to system L [11,13–15]. The substrate selectivities of the system or systems responsible for uptake of the latter amino acids have not been examined in detail, however. Similarly, the transport processes responsible for uptake of basic amino acids have not been characterized in conceptuses prior to blastocyst formation. For these reasons, we studied the characteristics of systems responsible for uptake of leucine and cationic amino acids in mouse eggs and cleavage-stage conceptuses.

Materials and Methods

Several descriptions of the methods for obtaining eggs and conceptuses and measuring their abilities to take up amino acids have been published recently [5–8]. In brief, sexually mature, 8–11-week-old Swiss ICR mice (Harlan Sprague Dawley, Inc.), that had been acclimated to a 14 h light: 10 h dark cycle for at least two weeks in our animal facility, were treated with gonadotropins to induce them to ovulate [16]. In most experiments, unfertilized eggs were removed from oviducts in Brinster's medium [17] approximately 17 h after administration of human chorionic gonadotropin (HCG) at about 1600 h the preceeding day. Eggs were freed from cumulus cells by exposing them to 145 IU of hyaluronidase (Sigma Chemical Co.) in 1.0 ml of Brinster's medium for less than 5 min. In a few experiments unfertilized and fertilized eggs were isolated and used in experiments within less than 16 h after administration of HCG, but the results of these studies were indistinguishable from results obtained with eggs obtained about 17 h after injection of HCG (data not shown). Conceptuses were removed from oviducts about 17 (1-cell stage), 41 (2-cell stage), and 66 (8-cell stage) h after HCG administration or from uteri about 94 h after administration of this hormone (blastocysts). No attempt was made in the studies reported here to distinguish between 8-cell conceptuses before and after compaction, although we are planning to study in detail the relationship between compaction and amino acid transport in conceptuses after the amino acid transport systems in conceptuses have been more completely delineated. Detection of a copulatory plus the morning after injection of HCG and observation of sperm were the only critera used to designate eggs as fertilized, so some eggs that we assumed to be fertilized probably were not. Nevertheless, since most such eggs develop in situ (unpublished observation), most eggs were probably fertilized after mice mated. Eggs and conceptuses were washed and stored for less than 6 h in Brinster's medium in a humidified atmosphere of 5% CO_2 in air at 37°C (pH 7.4). Transport was not observed to change in eggs or conceptuses during incubation for 6 h in vitro.

Eggs or conceptuses were incubated with a ^3H-labeled form of L-arginine, L-leucine, or L-lysine (20–60 Ci/mmol; ICN Pharmaceuticals or Amersham) and various concentrations of nonradioactive amino acids for 5 min as indicated in figures and tables. Amino acids were dissolved in a modification of Brinster's medium ($NaHCO_3$ replaced with $KHCO_3$ and sodium salts of pyruvate and lactate replaced with NaCl) or in phosphate-buffered NaCl (pH 7.1; Refs. 5–8). Amino acid uptake by eggs and conceptuses appears to be nearly linear with time for at least 5 min and virtually the same when it is measured in Brinster's medium, modified Brinster's medium or phosphate-buffered NaCl (Refs. 5–8 and unpublished data), but phosphate buffer allows us to maintain more easily the desired pH during relatively short (e.g., 5-min) assay periods [7]. In some cases Na^+ in the medium was replaced with choline or Li^+ during labeling, while in other cases the total ion concentration of the phosphate buffer was reduced to less than 4 mM (pH 7.4) by replacing the ions with sucrose or mannitol [8,9]. The concentrations of amino acids in the medium did not change significantly in the presence of eggs and conceptuses as discussed previously [7,8]. 2-(Methylamino)isobutyrate (MeAIB), L-glutamate, L-serine, L-lysine, glycine, L-pipecolate, L-proline, L-leucine, L-homoarginine, L-isoleucine, L-valine, L-tryptophan, L-alanine, cycloleucine, L-arginine, and L-homoserine were purchased from Sigma, and 2-amino-*endo*-bicyclo[2.2.1]heptane-2-carboxylic acid (BCH) was purchased from Behring Diagnostics. 3-Amino-*endo*-bicyclo[3.2.1]octane-3-carboxylic acid (BCO) was a gift from Professor Carmen Avendaño [18]. After incubation with amino acids, eggs or conceptuses were processed [5–8] to determine how much of the substrate they had taken up. Parametric (e.g., analysis of variance; Refs. 19, 20) and non-parametric [21–24] statistical methods were used to assess the data as indicated in the figures and tables. We conclude that the values of kinetic parameters differ significantly if their 92–94% confidence intervals do not overlap, as

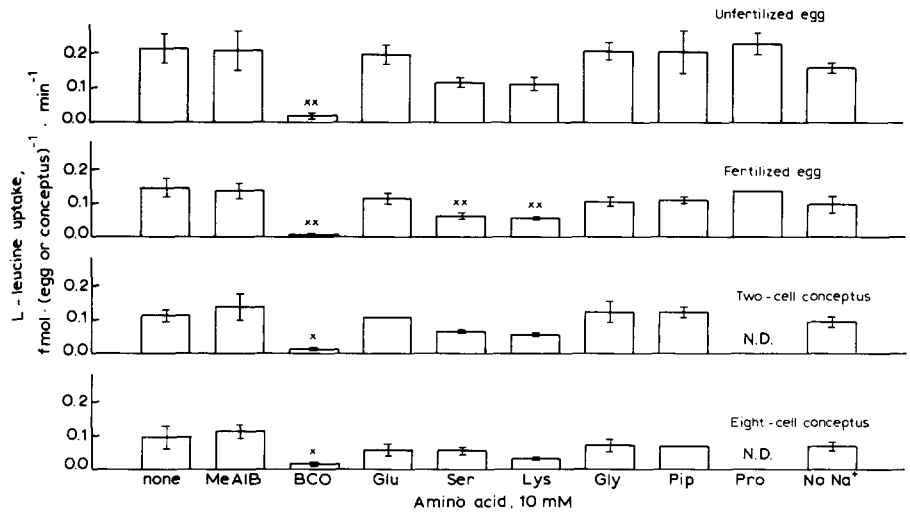

Fig. 1. Effect of various amino acids on uptake of L-leucine by eggs and cleavage-stage conceptuses. Eggs or conceptuses were incubated with 0.40 μM [^{3}H]leucine for 5 min in modified Brinster's medium or this medium in which choline was substituted for Na^+. The mean uptake $\pm$ S.E. was calculated from two to six (usually four) replicate determinations (approximately six eggs or conceptuses/determination) obtained in two or three independent experiments. Statistically significant inhibition is indicated with single ($P < 0.05$) or double ($P < 0.01$) asterisks as determined with analysis of variance. No S.E. is reported when only two replicate determinations were obtained and these groups were not included in the statistical analyses. Similar results (not shown) were obtained when these studies were performed in medium that contained no added Na^+. Pip, L-pipecolate; N.D., not determined.

determined by non-parametric statistical methods [21,22], because even the 90% confidence intervals overlap somewhat when $P = 0.05$ in a 't'-test [19].

Results

L-Leucine transport in eggs and cleavage-stage conceptuses

Leucine uptake was solely or predominantly Na^+-independent and inhibited almost completely by bulky zwitterionic amino acids, such as BCO, but less completely or not at all by a wide variety of other zwitterionic, cationic, or anionic amino acid structures ((Fig. 1). The range of structures that strongly inhibited the predominant transporter of leucine included branched chain, benzenoid or bicyclic amino acids (Fig. 2). Bicyclic amino acids (e.g., BCH and BCO) did not inhibit all saturable leucine transport, however, (Figs. 3 and 4). The BCH-resistant component of transport in 2-cell conceptuses is difficult to discern visually in Fig. 3 due

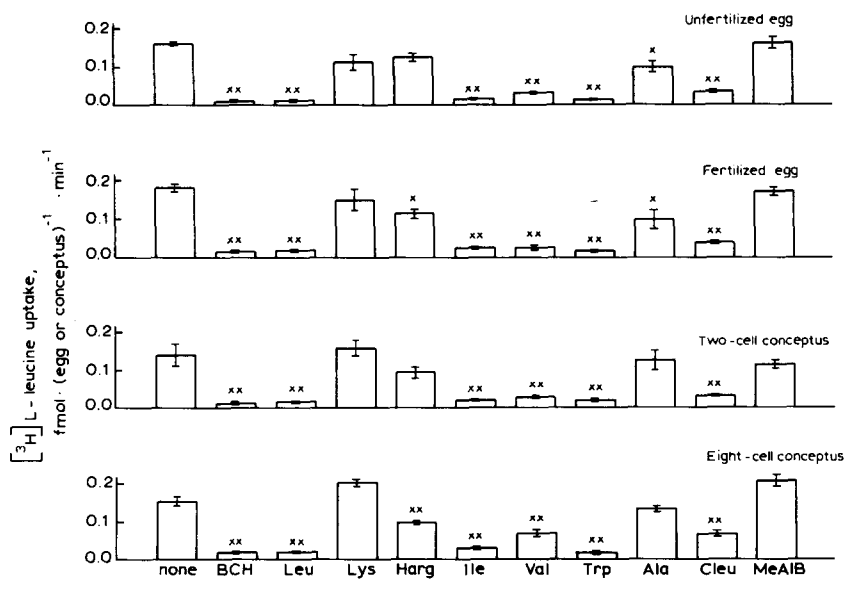

Fig. 2. Effect of various bulky amino acids on uptake of L-[^{3}H]leucine by eggs and cleavage-stage conceptuses. Eggs or conceptuses were incubated with 0.40 μM [^{3}H]leucine for 5 min in phosphate-buffered LiCl. The mean uptake $\pm$ S.E. was calculated from four to six replicate determinations (approximately six eggs or conceptuses/determination) obtained in two or three independent experiments. Statistically significant inhibition is indicated with single ($P < 0.05$) or double ($P < 0.01$) asterisks as determined with analysis of variance. Cleu, cycloleucine; Harg, L-homoarginine.

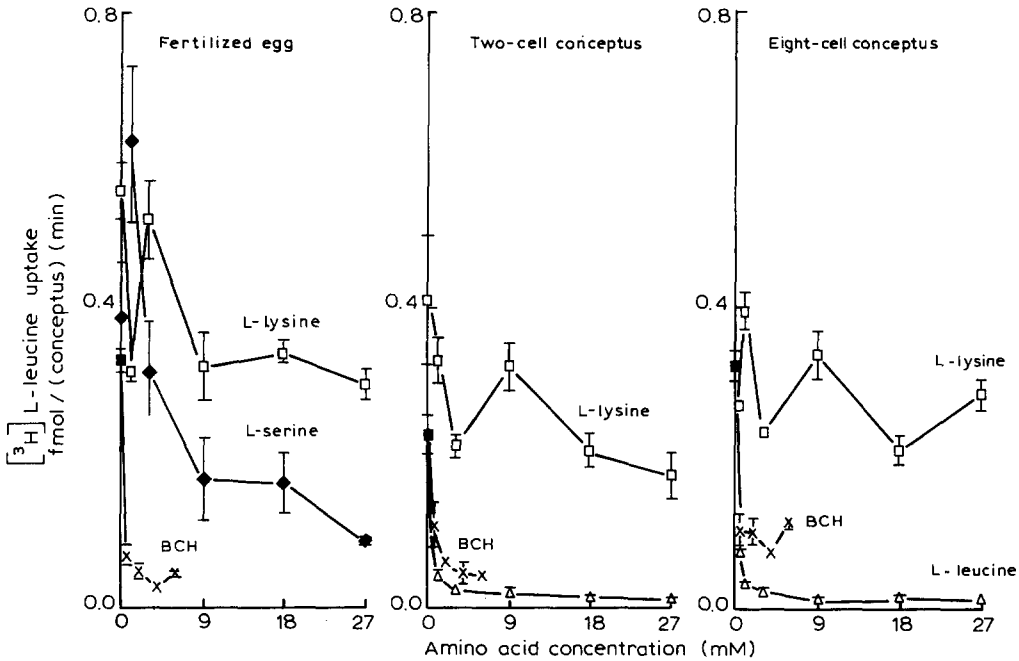

Fig. 3. A BCH-sensitive portion and a component of saturable L-leucine transport that is relatively insensitive to inhibition by BCH in cleavage-stage conceptuses. Fertilized eggs, 2-cell conceptuses, or 8-cell conceptuses were incubated with 1.0 μM [^{3}H]leucine and the indicated concentrations of nonradioactive amino acids for 5 min in phosphate-buffered LiCl. The mean uptake $\pm$ S.E. was calculated from four to six replicate determinations (approximately six conceptuses/determination) obtained in two or three independent experiments. When error bars are not shown they fall within the symbol. The lines connect the means for different concentrations of each amino acid. The component of mediated leucine transport in eggs which resisted inhibition by bicyclic amino acids is not delineated in this figure but it is shown in Fig. 4.

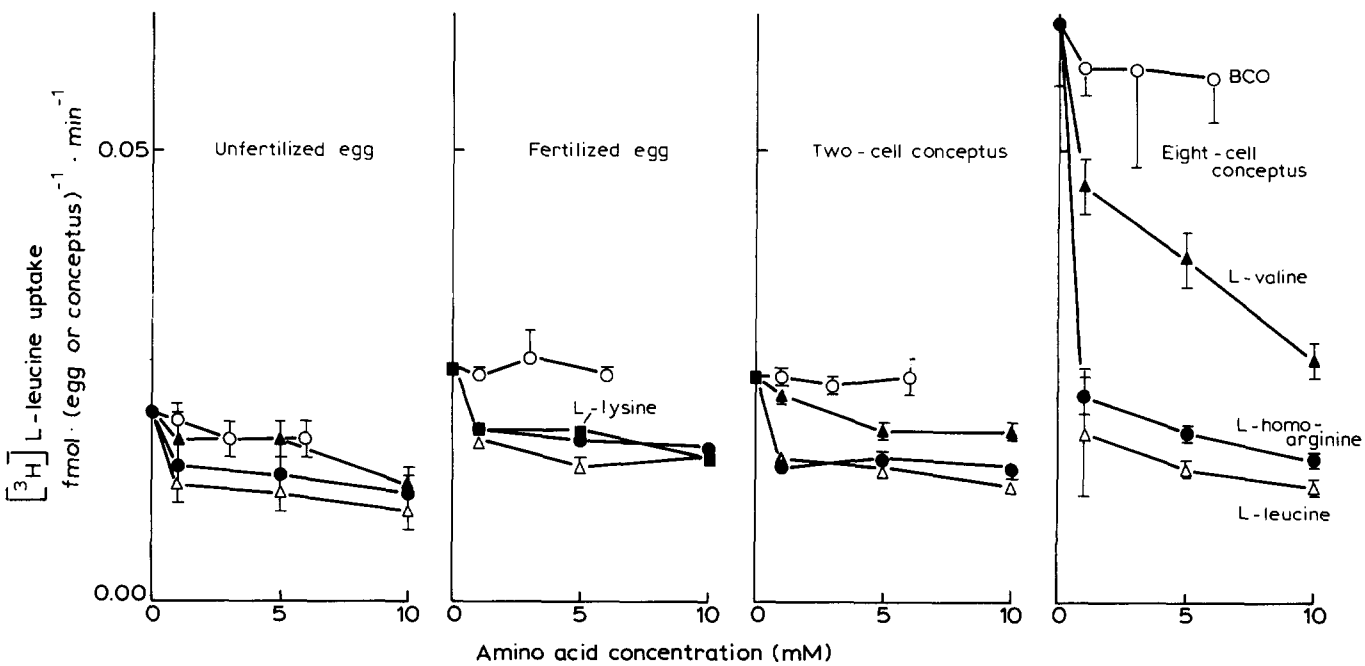

Fig. 4. Effect of various amino acids on BCH-resistant L-[^{3}H]leucine transport in eggs and cleavage-stage conceptuses. Eggs or conceptuses were incubated with 1.0 μM [^{3}H]leucine and the indicated concentrations of nonradioactive amino acids plus 6 mM BCH for 5 min in phosphate-buffered LiCl. The mean uptake $\pm$ S.E. was calculated from five to eight replicate determinations (approximately nine eggs or conceptuses/determination) obtained in three independent experiments (four determinations and two experiments at the two-cell stage). Statistically significant inhibition beyond that provided by 6 mM BCH was detected for each amino acid ($P < 0.01$) except BCO.

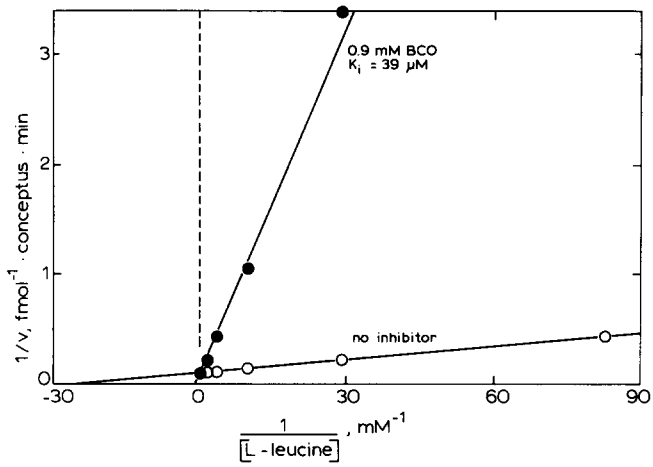

Fig. 5. Effect of 0.9 mM BCO on the values of the kinetic parameters for L-leucine uptake via the L-lysine-resistant component of its transport in fertilized eggs. Eggs were incubated with the indicated concentrations of substrate (1.0 μM [^{3}H]leucine) for 5 min in phosphate-buffered LiCl to determine the mean uptake of four replicate determinations (approximately 11 eggs/determination) obtained in two independent experiments. Experiments were performed in the presence of 20 mM lysine to inhibit most of the leucine uptake that is resistant to inhibition by bicyclic amino acids (Figs. 3 and 4), and nonsaturable uptake was also subtracted from total uptake to produce the data presented. One point in the presence of 0.9 mM BCO at 82.6 mM^{-1} and 8.64 fmol^{-1}·egg·min is not shown. The V_{max} and K_m values derived from the double reciprocal relationship between the velocity of leucine uptake and the substrate concentration were 9.8 fmol·egg^{-1}·min^{-1} and 40 μM, respectively, in the absence of BCO and 9.3 fmol·egg^{-1}min^{-1} and 962 μM in the presence of BCO (correlation coefficients were greater than 0.997). The medians and 94% confidence intervals for these parameters calculated utilizing a nonparametric statistical method (Refs. 21, 22) were 9.8 (9.3–11.3) fmol·egg^{-1}·min^{-1} and 43 (37–67) μM in the absence of BCO and 15.0 (7.8–25.4) fmol·egg^{-1}·min^{-1} and 1600 (716–4412) μM in the presence of BCO.

partly to the large size of the symbols which obscure some of the error bars. Nevertheless, it is clear from the data in Fig. 4 (which was gathered in the presence of 6 mM BCH) that a component of leucine uptake in both eggs and 2-cell conceptuses, as well as a component in eight-cell conceptuses, resisted inhibition by bicyclic amino acids. When [^{3}H]leucine uptake was measured in the presence of 6 mM BCH, the remaining saturable [^{3}H]leucine transport was inhibited strongly by nonradioactive leucine, lysine, and homoarginine, weakly by valine, and very weakly or not at all by BCO (Fig. 4). Therefore, the latter component of leucine transport was more susceptible to inhibition by cationic than bicyclic amino acids (Fig. 4), whereas the reverse was true for the predominant component of leucine transport (Figs. 1–3). In the presence of 20 mM lysine to inhibit the smaller component of leucine transport, 0.9 mM BCO raised the K_m value for leucine uptake about 20-fold, while it did not affect the value of V_{max} (Fig. 5), so BCO is a competitive inhibitor of the major component of leucine transport in fertilized eggs. The values of the kinetic parameters for leucine uptake that were calculated from double-reciprocal relationships were well within the range of values that were calculated utilizing a nonparametric statistical method (Fig. 5; Table I).

Transport of cationic substrates

Out of the broad scope of amino acid structures tested initially, only L-leucine and L-arginine consistently inhibited L-lysine uptake in eggs and cleavage-stage conceptuses, and this process was solely or primarily Na^+-independent (Fig. 6). Leucine-sensitive and leucine-resistant components of mediated L-arginine transport were present in unfertilized and fertilized eggs and 2-cell conceptuses (Fig. 7, and similar results were obtained for L-lysine uptake by 2-cell and 8-cell conceptuses when either Li^+ or choline was substituted for Na^+ in the medium (data not shown). The smaller,

TABLE I

Kinetic parameters for uptake of L-leucine, L-lysine, and L-arginine by systems L and $b^{0,+}$ in fertilized eggs and blastocysts

A nonparametric statistical method was used to estimate the median values of the kinetic parameters and their 92–94% confidence intervals (Refs. 21, 22). Data were obtained in a manner similar in general to that described in the legend of Fig. 5, and data reported previously (Ref. 8) were used to calculate the values of kinetic parameters reported for blastocysts. Arginine and lysine transport that resisted inhibition by 20 mM L-leucine (e.g., Fig. 7 and Ref. 8) was subtracted from total uptake to yield uptake that was primarily via system $b^{0,+}$. For kinetic studies, we determine uptake at several substrate concentrations, and we try to select these concentrations so that most of them will not differ from the likely K_m value by more than a factor of about ten (Ref. 27), as determined in preliminary experiments.

Stage of development	Transport system	Substrate	Median (92–94% confidence interval)	
			K_m value [1]	V_{max} value [2]
Fertilized egg	L [3]	L-leucine	43 (37–67)	9.8 (9.3–11.3)
	$b^{0,+}$	L-arginine	1.6 (1.1–2.4)	1.0 (0.9–1.1)
		L-lysine	60 (54–70)	1.36 (1.24–1.41)
Blastocyst [4]	L [3]	L-leucine	126 (74–156)	6.3 (4.8–7.1)
	$b^{0,+}$	L-arginine	[5]	>19 [5]
		L-lysine	59 (43–72)	42 (39–51)
		L-leucine	138 (118–153)	39 (37–42)

[1] μM.
[2] fmol·conceptus^{-1}·min^{-1}.
[3] Zwitterion-preferring transport process in Ref. 8.
[4] Blastocysts were obtained approx. 94 h after administration of HCG to mice.
[5] Values could not be determined precisely because of unusual kinetics resembling product inhibition.

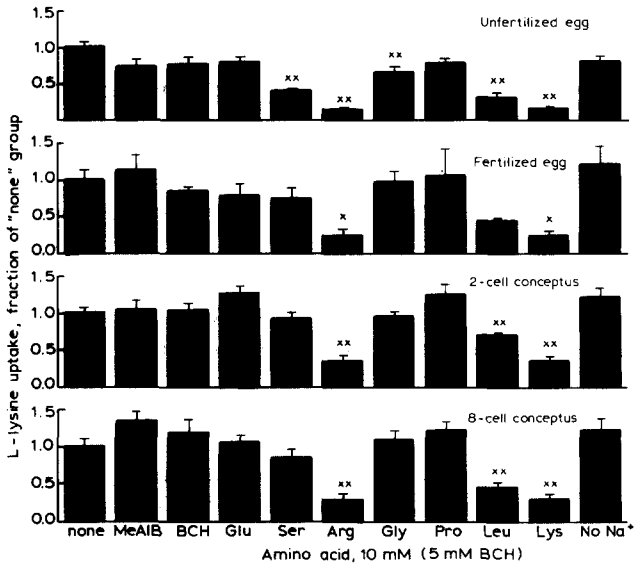

Fig. 6. Effect of various amino acids on uptake of L-[^{3}H]lysine by eggs and cleavage-stage conceptuses. Unfertilized or fertilized eggs were incubated with 2.0 μM [^{3}H]lysine and 2-cell or 8-cell conceptuses were incubated with 0.40 μM [^{3}H]lysine for 5 min in phosphate-buffered NaCl (eggs), modified Brinster's medium (2-cell and 8-cell conceptuses), or these media in which Li^+ was substituted for Na^+. The mean uptake $\pm$ S.E. was calculated from six to eight replicate determinations (approximately six eggs or conceptuses/determination) obtained in three or four independent experiments. Statistically significant inhibition is indicated with single ($P < 0.05$) or double ($P < 0.01$) asterisks as determined with analysis of variance. The uninhibited rates of lysine uptake from a 2.0 μM solution were 0.081 ± 0.004 and 0.083 ± 0.010 fmol·egg^{-1}·min^{-1} for unfertilized and fertilized eggs, respectively, and from a 0.40 μM solution they were 0.0322 ± 0.0018 and 0.105 ± 0.011 fmol·conceptus^{-1}·min^{-1} for 2-cell and 8-cell conceptuses, respectively. Similar results (not shown) were obtained when these studies were performed in medium that contained no added Na^+.

TABLE II

Inhibition of L-lysine uptake in 1-cell conceptuses by ionic osmolites

Conceptuses were incubated for 5 min with [^{3}H]lysine (2.5 μM) in each isotonic solution (300 mosmolar of each substance) buffered with 0.75 mM phosphate (pH 7.4). The mean $\pm$ S.E. uptake was calculated from six replicate determinations and there were 12 conceptuses per determination. Uptake by conceptuses in solutions of nonelectrolytes was significantly faster than by conceptuses in solutions of electrolytes ($P < 0.01$).

Major osmolite	Mean $\pm$ S.E. lysine uptake (fmol·conceptus^{-1}·min^{-1})
Sucrose	0.927 ± 0.065
Mannitol	0.876 ± 0.104
NaCl	0.156 ± 0.008
Choline chloride	0.132 ± 0.004

cation-preferring (i.e., leucine-resistant) component of arginine transport is obscured somewhat in the first two panels of Fig. 7 by the large size of the symbols within which fall many of the relevant error bars. The characteristics of the cation-preferring component have been studied in detail, however, and will be the subject of a later report. The more conspicuous, leucine-sensitive component of arginine uptake was inhibited best by bulky cationic and zwitterionic amino acids whose carbon skeletons do not branch at the α or β positions, whereas amino acids that branch at the β position inhibited uptake weakly (Figs. 7 and 8). Lysine uptake by 1-cell conceptuses was about 5-fold more rapid in isotonic solutions of sucrose or mannitol than in similar solutions of NaCl or choline chloride (Table II), as has been observed for lysine transport in mouse blastocysts [9]. The medians and 92–94% confidence intervals of

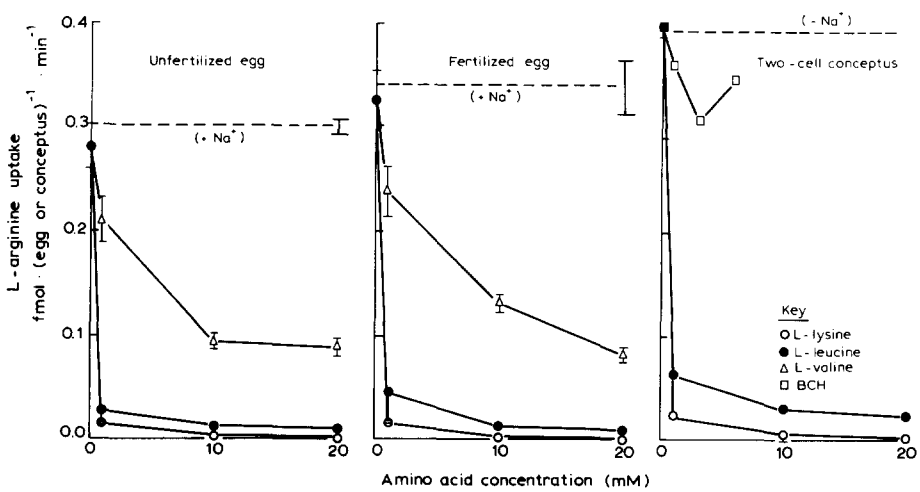

Fig. 7. An L-leucine sensitive portion and a component of saturable L-arginine transport that is relatively insensitive to inhibition by leucine in eggs and 2-cell conceptuses. Eggs or conceptuses were incubated with 0.67 (eggs) or 1.7 (2-cell conceptuses) μM [^{3}H]arginine and the indicated concentrations of nonradioactive amino acids for 5 min in phosphate-buffered LiCl (eggs) or NaCl (2-cell conceptuses). The mean uptake $\pm$ S.E. was calculated from five replicate determination (approximately 10 eggs or conceptuses/determination) obtained in two independent experiments (two determinations and one experiment for 2-cell conceptuses). Uptake by eggs in phosphate-buffered NaCl or by 2-cell conceptuses in phosphate-buffered LiCl is indicated by the dashed lines. When error bars are not shown for eggs they fall within the symbol.

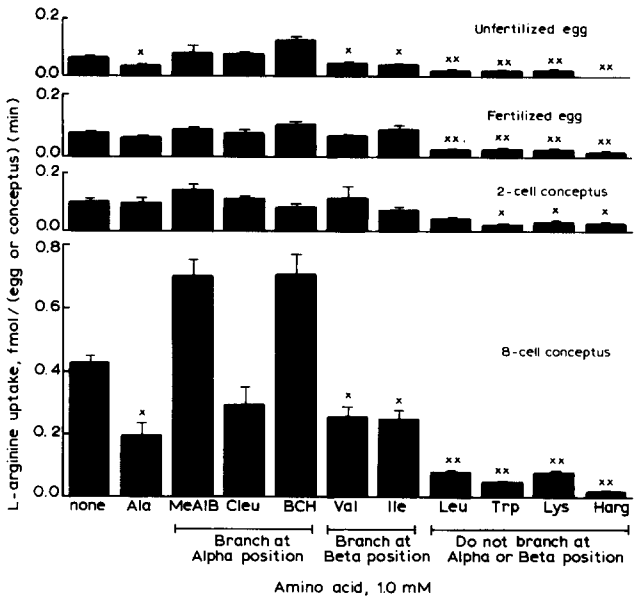

Fig. 8. Effect of amino acids whose carbon skeletons branch at the α, β, or neither position on L-arginine uptake by eggs and cleavage-stage conceptuses. Eggs or conceptuses were incubated with 0.35 μM [^{3}H]arginine for 5 min in phosphate-buffered LiCl to determine the mean $\pm$ S.E. uptake of four to six replicate determinations (approximately 5 conceptuses/determination) obtained in two or three independent experiments. Statistically significant inhibition is indicated with single ($P < 0.05$) or double ($P < 0.01$) asterisks as determined with analysis of variance. (Some groups with low means, each of which was also clearly below the 'none' group, were not included in the analyses because their variances were significantly smaller than the variances of the other groups.) Cleu, cycloleucine; Harg, L-homoarginine.

the values of the kinetic parameters for lysine and arginine transport by the more conspicuous, leucine-sensitive system in fertilized eggs are presented in Table I. Five-hundred μM L-leucine raised by a factor of 4.6 the K_m value for arginine uptake by the latter system (data not shown).

Discussion

Predominance of system L in eggs and cleavage-stage conceptuses

Leucine uptake into eggs and cleavage-stage conceptuses was inhibited most strongly by branched chain, bicyclic or benzenoid, zwitterionic amino acids (Figs. 1–3 and 5). Thus, its most conspicuous transporter resembles an inconspicuous system for leucine uptake in blastocysts as well as the various forms of system L and the somewhat more selective system T in other types of cells [2,3,8]. We suggested that perhaps all of these transport activities could be considered variants of an extended system L family [8]. Since several of these Na^+-independent transporters of bulky zwitterionic amino acids appear to exist, it is possible that different forms of them are expressed in eggs or in conceptuses at various times prior to implantation, although we have no unequivocal evidence for such developmental discontinuities. The V_{max} value for transport of leucine by one or more of the system L family decreases by about one-third while the K_m value increases nearly 3-fold during development of blastocysts from fertilized eggs (Table I). Since the diameters of fertilized eggs and blastocysts not including zona pellucidae are about 72.8 ± 0.7 and 105.0 ± 1.5 μm, respectively, 1-cell conceptuses appear to have less than half as much external surface area as blastocysts do. Therefore, the differences in the values of the kinetic parameters for leucine uptake by system L in blastocysts and 1 cell conceptuses reflect a decrease during development in the capacity of preimplantation conceptuses to take up amino acids via this family of systems.

System $b^{0,+}$ is the most conspicuous transporter of cationic amino acids in eggs and cleavage-stage conceptuses

Bulky cationic and zwitterionic α-amino acids whose carbon skeletons do not branch at the α or β positions interact strongly with the most conspicuous transporter of arginine and lysine in eggs and cleavage-stage conceptuses (Fig.s 6–8). Amino acids that branch at the β position interact weakly with this system, whereas those without an α hydrogen appear to be excluded by it. Moreover, lysine uptake by 1-cell conceptuses was about 5-fold more rapid in isotonic solutions of nonelectrolytes than in solutions of electrolytes (Table II). By these criteria, the most conspicuous transporter of cationic amino acids in eggs and cleavage-stage conceptuses appears to be system $b^{0,+}$ [4,8,9]. Weak inhibition by small amino acids, such as alanine (Fig. 8), serine and glycine (first panel in Fig. 6), is also consistent with the conclusion that system $b^{0,+}$ is present in the latter cells, since this system in blastocysts does not completely exclude such substrates [8]. In contrast to cationic amino acids, system $b^{0,+}$ catalyzes a relatively small component of leucine transport in conceptuses prior to formation of blastocysts (Figs. 1–5). We attribute the incomplete but in some cases statistically significant inhibition of total leucine uptake by lysine and homoarginine (Figs. 1 and 2) to their strong inhibition of leucine uptake via system $b^{0,+}$ (Fig. 4) and to weak interaction of basic amino acids with system L at neural pH (3). The velocity of leucine uptake via system L in fertilized eggs was rapid enough to be detected accurately with our methods [5–8] and to determine a V_{max} value with a 94% confidence interval that is only about 20% of the median (Table I). In contrast, uptake of leucine by system $b^{0,+}$ in 1-cell conceptuses was too slow to determine reliably K_m and V_{max} values. Nevertheless, the K_m value for arginine uptake via the latter transport activity was higher ($P < 0.05$) by about 4.6-fold in the presence of 500 μM leucine, and this finding is consistent with the interpretation that the inhibition is competitive ($K_i \cong 138$ μM). The K_m value for arginine

transport by system $b^{0,+}$ in fertilized eggs is only about 1.6 μM while this parameter is apparently about 100 μM for both lysine (Table I) and leucine ($K_m = K_i$). Therefore, this system may catalyze mainly arginine uptake and exodus in situ depending on the concentrations of the various substrates of system $b^{0,+}$ in 1-cell conceptuses and in oviductal fluid. The same conclusion may apply as well to blastocysts and uterine secretions, although unusual kinetics resembling substrate inhibition have so far precluded estimation of a K_m value for arginine uptake via system $b^{0,+}$ in these conceptuses.

Increase in system $b^{0,+}$ activity during preimplantation development

Inhibition studies indicate that leucine uptake by system $b^{0,+}$ is much lower than transport via system L in eggs and cleavage-stage conceptuses (Figs. 1–4), whereas these relative levels of uptake are reversed in blastocysts [8]. Since the K_m and V_{max} values for leucine transport by system L do not appear to change enough during development to account for this reversal (Table I), the most likely explanation for it is that the amount of system $b^{0,+}$ activity increases greatly between the two-cell and blastocyst stages (Figs. 2, 4, 6; Ref. 8). Moreover, the V_{max} values for amino acid uptake via system $b^{0,+}$ are up to about 30-fold higher in blastocysts than they are in 1-cell conceptuses (Table I). The external surface area of blastocysts appears to be more than twice the surface area of fertilized eggs even if microvilli are assumed to be equally abundant on the surfaces of both types of conceptuses (see estimates of conceptuses' diameters above). Therefore, a substantial portion of the increase in system $b^{0,+}$ activity per conceptus may occur in association with elaboration of new plasma membrane. Additional plasma membrane is also needed to form the membrane of the trophoblast next to the blastocyst cavity, and Na^+/K^+-ATPase has been shown by immunological means to be located primarily in the latter membrane of blastocysts [25]. If this Na^+/K^+-ATPase is fully active, it could substantially increase the magnitude of the conceptus' plasma membrane potential; the velocity of arginine uptake via system y^+ in human fibroblasts correlates directly with the magnitude of the membrane potential [26]. It seems unlikely, however, that a change in membrane potential can account for the increase in system $b^{0,+}$ activity between the two-cell and blastocyst stages. Leucine as well as arginine and lysine uptake increases during this period of development, and leucine transport by system $b^{0,+}$ is probably electrically neutral. (Leucine transport via system $b^{0,+}$ does not appear to be affected by ouabain, other ions in the medium, or changes in pH between 6.3 and 8.0 [7–9].) Therefore, although it remains to be determined precisely how system $b^{0,+}$ activity increases in conceptuses, we favor the hypothesis that new sites for transport are added to their plasma membranes during preimplantation development.

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