Biochimica et Biophysica Acta, 1028 (1990) 165–173
Elsevier

BBAMEM 74983

Functional changes in cation-preferring amino acid transport during development of preimplantation mouse conceptuses

Lon J. Van Winkle and Allan L. Campione

Department of Biochemistry, Chicago College of Osteopathic Medicine, Downers Grove, IL (U.S.A.)

(Received 24 January 1990)
(Revised manuscript received 18 June 1990)

Key words: Amino acid transport; Cationic amino acid; Development; Preimplantation conceptus; (Mouse egg); (Mouse embryo)

In a previous study, a Na^+-independent, cation-preferring amino acid transport system was detected in preimplantation mouse blastocysts. The system resisted Na^+-dependent inhibition by homoserine and so resembled the lysosomal system c more than it resembled the plasmalemmal system y^+. We now report the presence of a cation-preferring system in unfertilized and fertilized eggs and cleavage-state conceptuses which also resists Na^+-dependent inhibition by homoserine. The systems in 1-cell conceptuses and blastocysts are, however, insensitive to changes in pH in the interval of 6.0 to 8.0 and, thus, different from the pH-sensitive system c. Moreover, the relative strengths of the interactions of a variety of basic amino acids with the systems in conceptuses do not correspond well with the relative strengths of their interactions with either system c or system y^+. Similarly, the system in 1-cell conceptuses can be distinguished from the system in blastocysts because L-arginine interacts about equally well with each of these systems, whereas the system in 1-cell conceptuses is inhibited more strongly than the system in blastocysts by most other basic amino acids. In addition, inhibition of the system in 1-cell conceptuses by some basic amino acids is Na^+-stimulated, whereas Na^+ does not affect inhibition of the system in blastocysts. Finally, L-tryptophan inhibits the system in blastocysts better than L-histidine or D-arginine do, but the reverse is true for the system in 1-cell conceptuses. Therefore, the relative activities of at least two forms of a novel, cation preferring amino acid transport process change during development of blastocysts from fertilized eggs. For convenience, the forms of the cation-preferring transport processes that seem to predominate at the 1-cell and blastocysts stages are provisionally designated systems b_1^+ and b_2^+, respectively, although these two systems need not represent entirely different gene products.

Introduction

System y^+ is the only cation-preferring amino acid transport process in mammalian cell membranes that has been characterized well *. The reactivity of cationic amino acids with this Na^+-independent system increases as the distance between the α carbon and the positive charge on the side chain increases. For example, the K_m (or K_i) values of substrates of system y^+ increase in the series, L-homoarginine < L-arginine < L-2-amino-4-guanidinobutyric acid, and these relative reactivities apparently cannot be accounted for solely by variations in the characteristics of dissociable groups on the substrates [7,8]. Moreover, α-N-methylation of cationic amino acids greatly reduces or eliminates their reactivity with system y^+ [7,8]. Interestingly, system y^+ accepts some zwitterionic amino acids, such as L-glutamine and L-homoserine, as weak substrates in the presence, but not in the absence, of Na^+ [7]. The latter characteristic was used to draw a parallel between the substrate receptor sites of system y^+ and the zwitterion-preferring, Na^+-dependent system ASC, because system ASC also interacts weakly with cationic amino acids regardless of the Na^+ concentration [9]. We [10] and others [11] extended the latter parallel to

* The amino acid transport systems discussed in this paper can be defined briefly as follows. Systems ASC and $B^{0,+}$ are Na^+-dependent and prefer as substrates zwitterionic amino acids with 3–5 carbon atoms in a chain (ASC) or most cationic and zwitterionic amino acids ($B^{0,+}$), respectively. Systems asc and $b^{0,+}$ are Na^+-independent and prefer as substrates small zwitterionic amino acids (asc) or bulky cationic and zwitterionic amino acids that do not branch at the α or β positions ($b^{0,+}$), respectively [1–6]. Systems y^+, b^+ and c are Na^+-independent systems each of which prefers cationic amino acids as substrates, although the details of their substrate selectivities differ significantly as discussed in the text.

Correspondence: L.J. Van Winkle, Department of Biochemistry, Chicago College of Osteopathic Medicine, 55531st Street, Downers Grove, IL 60515, U.S.A.

include system $b^{0,+}$ in mouse blastocysts and system asc_1 in horse erythrocytes.

In sharp contrast, a Na^+-independent, cation-preferring transport process in mouse blastocysts does not appear to interact with L-homoserine or most other zwitterionic amino acids even when Na^+ is present [6]. Thus, the system in blastocysts resembles system c in lysosomes from human fibroblasts more than it resembles system y^+ [12]. Little else is known about the system in blastocysts, however, so it is difficult to conclude whether it is a variant of system c or, perhaps, system y^+, or unlike either of these distinct transport processes. Moreover, it has not been determined whether a cation-preferring system is also present in preimplantation mouse conceptuses prior to formation of blastocysts. Therefore, we have further characterized the cation-preferring transport activity in blastocysts, and we have examined unfertilized and fertilized eggs and cleavage-stage conceptuses for the presence of a cation-preferring amino acid transport process.

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 [5,6,13,14]. Sexually mature, 8–11-week-old Swiss ICR mice (Harlan Sprague Dawley, Inc.) were treated with gonadotropins to induce them to ovulate [15]. In most experiments, unfertilized eggs were removed from oviducts in Brinster's medium [16] or Spindle's flushing medium-I [17] approx. 17 h after administration of human chorionic gonadotropin (HCG), and they 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 five minutes. 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 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). Detection of a copulatory plug and observation of sperm were the only criteria 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 or L-lysine (18–60 Ci/mmol; ICN Pharmaceuticals or Amersham) and various concentrations of nonradioactive amino acids in phosphate-buffered NaCl or LiCl (pH 7.1; Refs. 5, 6, 14) or modified Spindle's flushing medium-I (Na_2HPO_4 replaced with K_2HPO_4 and other Na^+ salts replaced with LiCl; pH 7.1). Amino acid uptake frequently was studied at substrate concentrations near 1 μM, and the efficacy

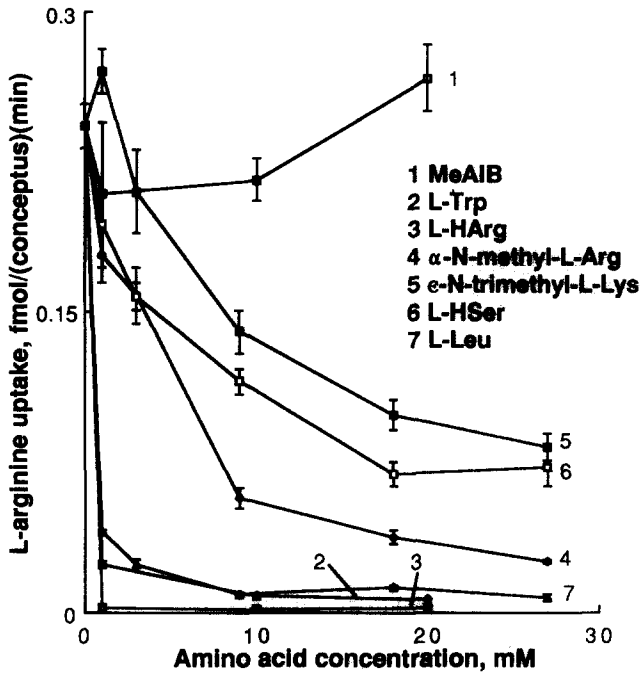

Fig. 1. An L-leucine-sensitive portion and a component of saturable L-arginine transport that is relatively insensitive to inhibition by leucine in fertilized eggs. Eggs were incubated with 0.67 μM [^3H]arginine and the indicated concentrations of nonradioactive amino acids for 5 min in phosphate-buffered LiCl. The mean uptake ± S.E. was calculated from four replicate determinations (approx. nine conceptuses per determination) obtained in two independent experiments. When error bars are not shown they were within the symbols. Because the symbols are relatively large, the leucine-resistant component of arginine transport is easier to visualize in Fig. 2. Based on the K_i value of leucine and the K_m value of arginine, which are about 138 and 1.6 μM, respectively, for system $b^{0,+}$ (Ref. 20), the total leucine-resistant component of arginine uptake at infinite leucine concentration was calculated to be 2.9 ± 0.3% of the total arginine uptake in the absence of nonradioactive amino acids. Uptake in the presence of 20 mM L-homoarginine was 0.9 ± 0.2% of the total uptake and was statistically indistinguishable from the nonsaturable uptake which is reported in the legend of Fig. 6. Therefore, mediated, leucine-insensitive uptake is about 2% of the total uptake represented in the figure. (The proportion of mediated, leucine-insensitive uptake is higher at higher substrate concentrations; e.g., Fig. 6.) Similar results were obtained for blastocysts, and both mediated, Na^+-independent components of arginine transport also have been detected in conceptuses throughout preimplantation development and in the presence as well as the absence of Na^+ (data not shown and Refs. 6, 20). Moreover, both mediated components were detected when the osmolarity was held constant by removing LiCl to compensate for added amino acids (data not shown). MeAIB, 2-(methylamino)isobutyrate; HArg, homoarginine; HSer, homoserine.

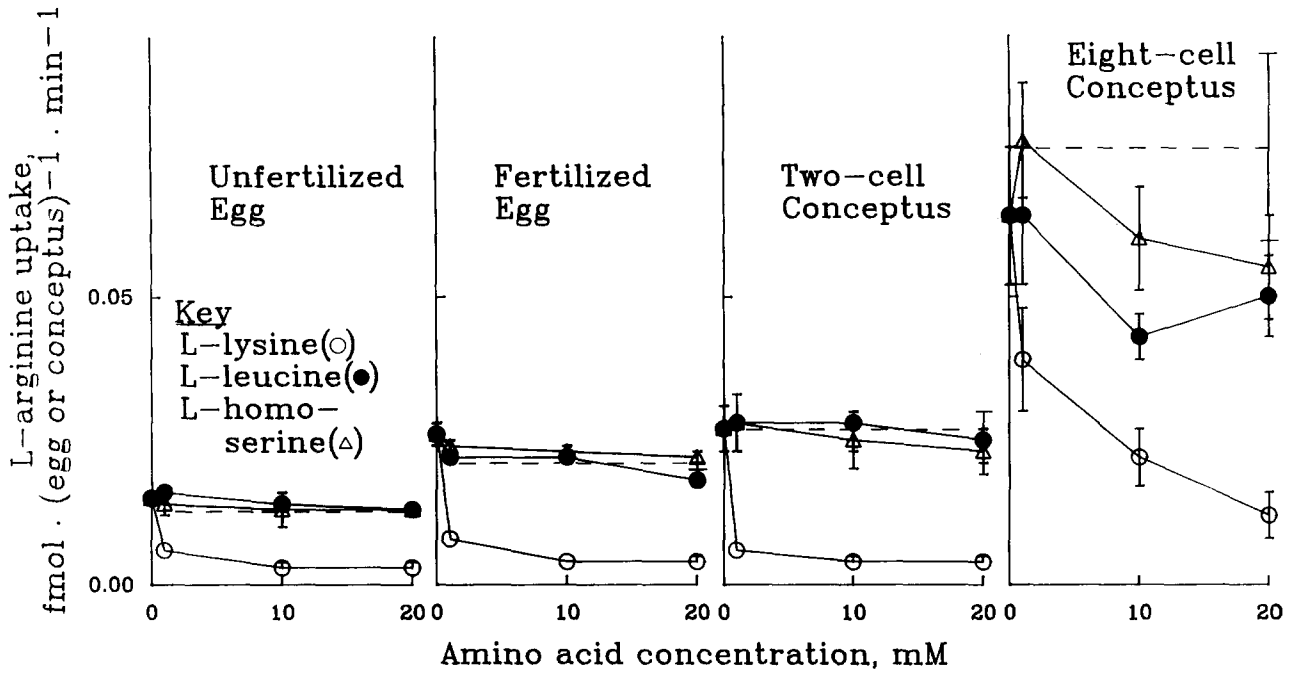

Fig. 2. Efect of L-homoserine on L-leucine-resistant L-arginine uptake by eggs and cleavage-stage conceptuses. Eggs or conceptuses were incubated with 1.7 μM [^{3}H]arginine and the indicated concentrations of nonradioactive amino acids plus 20 mM leucine for 5 min in phosphate-buffered NaCl. A higher concentration of [^{3}H]arginine was used here than in the studies reported in Fig. 1 in order to increase the amount of uptake via the L-leucine-resistant component of transport. The mean uptake ± S.E. was calculated from 4–7 determinations (approx. 10 eggs or conceptuses per determination) obtained in two or three independent experiments. When error bars are not shown they were within the symbols. (Uptake in phosphate-buffered LiCl is indicated by the dashed lines.) Statistically significant inhibition beyond that provided by 20 mM leucine was detected for L-lysine ($P < 0.01$) but not for the other amino acids at each stage of development. A mixture of compacted and uncompacted conceptuses were studied at the 8-cell stage. Similar results (not shown) were obtained for 2-cell conceptuses when Na^+ was replaced with Li^+.

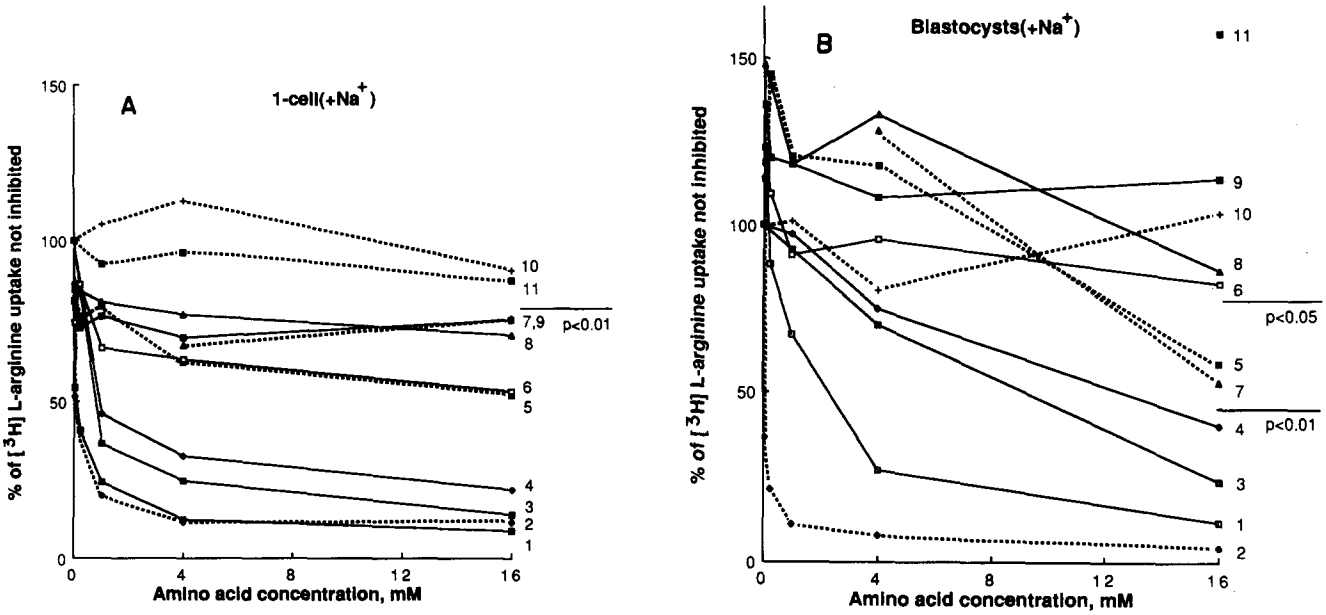

Fig. 3. Effect of various amino acids on L-leucine-resistant L-[^{3}H]arginine uptake by fertilized eggs (A) and blastocysts (B). Conceptuses were incubated with 1.9 (blastocysts) or 3.8 (eggs) μM [^{3}H]arginine and the indicated concentrations of nonradioactive amino acids plus 20 mM leucine for 5 min in phosphate-buffered NaCl. In most cases, the results were similar in phosphate-buffered LiCl (data not shown), although inhibition by the amino acids numbered 7, 8 and 9 was Na^+-dependent in 1-cell conceptuses ($P < 0.01$). The mean uptake ± S.E. was calculated from 6–12 determinations (approx. ten eggs or six blastocysts per determination) obtained in 3–6 independent experiments. Error bars are not shown but the S.E. values averaged about 1/10 of the mean values. Statistically significant inhibition is indicated with P values next to groups of amino acids (inhibition by about 20% or less was not statistically significant). Values were converted to percentages to make comparisons between 1-cell conceptuses and blastocysts easier. The 100% values correspond to uptake of [^{3}H]arginine of about 0.0385 and 0.136 fmol · conceptuses^{-1} · min^{-1} in eggs and blastocysts, respectively. The numbers correspond to the following amino acids: 1, L-homoarginine; 2, L-arginine; 3, *S*-2-aminoethyl-L-cysteine; 4, L-lysine; 5, D-arginine; 6, *ε*-*N*-methyl-L-lysine; 7, *α*-*N*-methyl-L-arginine; 8, L-2-amino-3-guanidinopropionic acid; 9, 1-methylpiperidine-4-amino-4-carboxylic acid (MPA); 10, *ε*-*N*-trimethyl-L-lysine; 11, L-alanine.

of this experimental approach is discussed more completely elsewhere [10,18]. Briefly, this approach improves the chances of detecting low-K_m, low-capacity transport systems, usually without decreasing the ability to detect higher-K_m, higher-capacity systems [19], and some systems in conceptuses have K_m values for lysine and arginine transport near 1 μM [10,20]. Incubations were short enough to estimate initial velocities of amino acid uptake (i.e., 5 min or less), and no binding of cationic or other amino acids to plasma membranes has been detected in preimplantation conceptuses (Refs. 10, 18 and data not shown). In some cases, the pH was adjusted to a specific pH between 6.0 and 8.0 with concentrated HCl or KOH [5]. The concentrations of amino acids in the solutions are unlikely to have changed significantly during these experiments as discussed previously [6,14]. ε-*N*-Methyl-L-lysine, D-arginine, L-2-amino-3-guanidinopropionic acid, L-lysine, L-leucine, L-homoarginine, *S*-2-aminoethyl-L-cysteine, L-tryptophan, L-alanine, L-arginine, 2-(methylamino)isobutyrate, L-histidine and L-homoserine were purchased from Sigma, ε-*N*-trimethyl-L-lysine and 2-amino*endo*bicyclo[2.2.1]heptane-2-carboxylic acid (BCH) were purchased from Behring Diagnostics, and α-*N*-methyl-L-arginine was purchased from Vega Biotechnologies, Inc. 1-Methylpiperidine-4-amino-4-carboxylic acid (MPA) was a gift from Dr. Halvor N. Christensen [21]. After incubation with a labeled substrate, eggs or conceptuses were processed [5] to determine how much of the substrate they had taken up. Parametric (e.g., analysis of variance) and nonparametric (e.g., Kruskal-Wallis *H* test) statistical methods [22,23] were used to assess the data.

Results

Presence of a Na^+-independent, cation-preferring amino acid transport process in eggs and cleavage-stage conceptuses

An inconspicuous but consistently detected portion of mediated L-arginine uptake by unfertilized and fertilized eggs and cleavage-stage conceptuses resisted inhibition by L-leucine (Fig. 1 and data not shown). (Because many of the error bars are well within the symbols in Fig. 1, the L-leucine-resistant component of L-arginine uptake is easier to visualize in Fig. 2.) Based on the K_m and K_i values for the competitive interactions of L-arginine and L-leucine with the more conspicuous component of L-arginine transport in 1-cell conceptuses (i.e., system $b^{0,+}$, Ref. 20), it was calculated

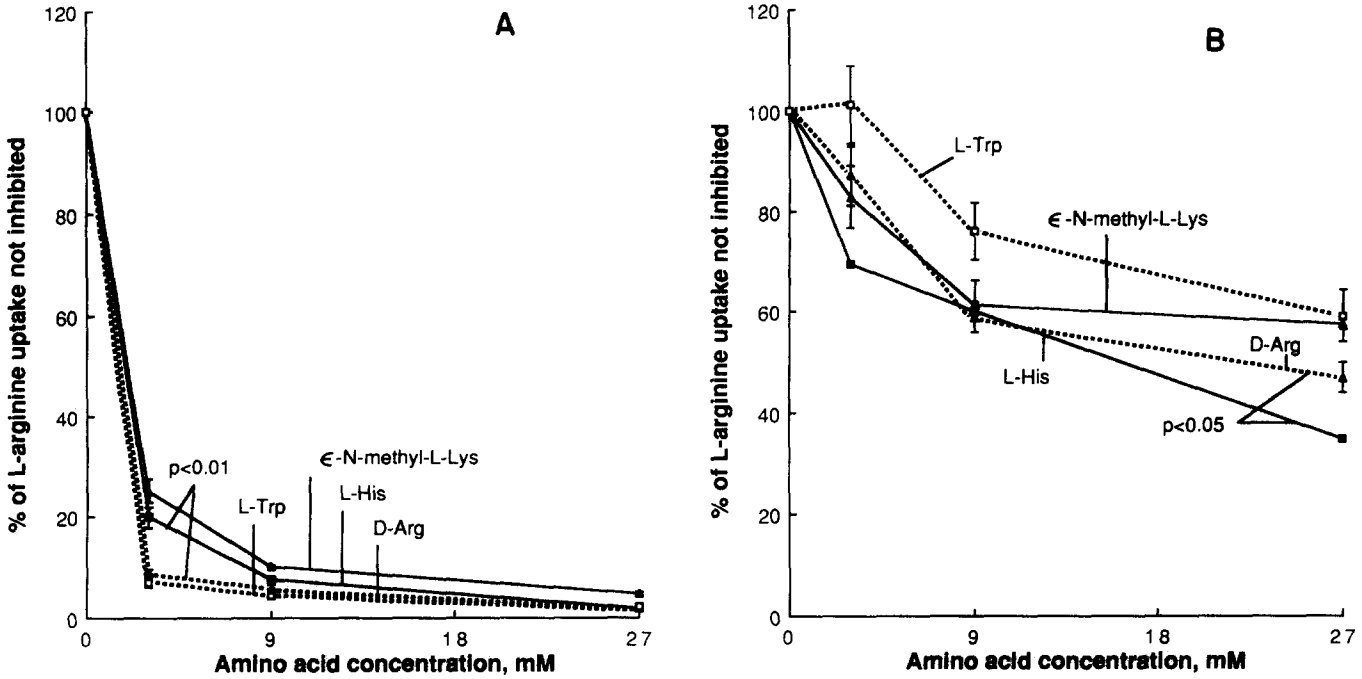

Fig. 4. Different relative levels of inhibition of total (mainly system $b^{0,+}$, A) and L-leucine-resistant (mainly systems b_1^+, B) L-arginine uptake by L-histidine, D-arginine, ε-*N*-methyl-L-lysine and L-tryptophan in 1-cell conceptuses. Conceptuses were incubated with 1.1 μM [^{3}H]arginine (A) or 5.6 μM [^{3}H]arginine plus 40 mM L-leucine (B) and the indicated concentrations of other amino acids for 5 min in phosphate-buffered LiCl. When 40 mM leucine was present, the LiCl concentration was reduced by 20 mM. The mean uptake ± S.E. was calculated from four or five replicate determinations (approx. 8 conceptuses per determination) obtained in two independent experiments. When error bars are not shown they were within the symbols. D-Arginine and L-tryptophan were better inhibitors of total Na^+-independent arginine uptake (A) than L-histidine or ε-*N*-methyl-L-lysine were ($P < 0.01$; analysis of variance), whereas histidine was a better inhibitor of leucine-resistant arginine uptake (B) than each of the other amino acids were ($P < 0.05$ for D-arginine and $P < 0.01$ for L-tryptophan and ε-*N*-methyl-L-lysine). Moreover, D-arginine inhibited system b_1^+ (B) better than L-tryptophan or ε-*N*-methyl-L-lysine did ($P < 0.05$).

that about 2% of the mediated uptake in Fig. 1 resisted inhibition by L-leucine. At higher L-arginine concentrations, however, a greater proportion of its mediated transport occurred via the leucine-resistant component (see below). Similarly, a portion of mediated L-lysine uptake by 2-cell and 8-cell conceptuses resisted L-leucine inhibition (data now shown). This L-leucine-resistant, cation-preferring transport process also was not inhibited by L-homoserine even in the presence of Na^+ (Fig. 2). By this criterion, the transport process resembled the cation-preferring transport system in blastocysts [6]. The transport system in 1-cell conceptuses also resembled the system in blastocysts because both were insensitive to changes in pH in the interval of 6.0 to 8.0 (data not shown). Nevertheless, the cation-preferring transport processes in 1-cell conceptuses and blastocysts had different abilities to interact with various basic amino acids.

Lower or absent inhibition of the cation-preferring transport process in blastocysts by most basic amino acids that interact with the cation-preferring system in 1-cell conceptuses

Inhibition of leucine-resistant L-[^{3}H]arginine uptake by unlabeled L-arginine was at least as strong in blastocysts as in 1-cell conceptuses (amino acid No. 2 in Fig. 3). In contrast, inhibition of arginine uptake by L-homoarginine (No. 1), L-lysine (No. 4) and *S*-2-aminoethyl-L-cysteine (No. 3) was considerably weaker in blastocysts than in 1-cell conceptuses (Fig. 3 and Table I). Moreover, L-2-amino-3-guanidinopropionic acid (No. 8), ε-*N*-methyl-L-lysine (No. 6) and 1-methylpiperidine-4-amino-4-carboxylic acid (MPA, No. 9) were weak inhibitors of the cation-preferring transport process in 1-cell conceptuses, but they did not inhibit the cation-preferring system in blastocysts (Fig. 3). Interestingly, inhibition of the cation-preferring process by MPA, α-*N*-methyl-L-arginine, L-2-amino-3-guanidinopropionic acid, and L-tryptophan was Na^+-stimulated in 1-cell conceptuses ($P < 0.01$), whereas Na^+ did not seem to affect interaction of these or other amino acids with the cation-preferring process in blastocysts (data not shown).

Further evidence that the cation-preferring transport processes in preimplantation conceptuses are distinct from system $b^{0,+}$

The K_m value for L-arginine transport via system $b^{0,+}$ is much lower than the K_m value for L-leucine transport by this system [6,20]. For this reason, we performed further studies to verify that the 'leucine-resistant', cation-preferring transport activities could not

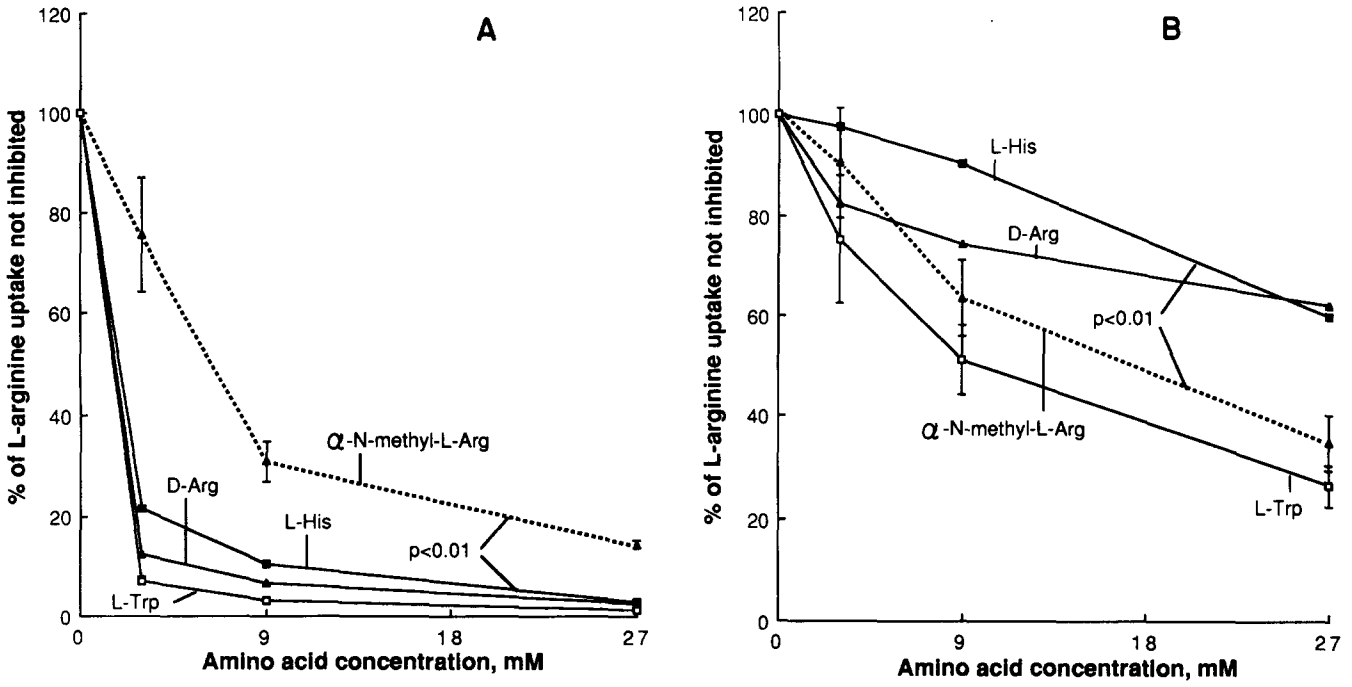

Fig. 5. Different relative levels of inhibition of total (mainly system $b^{0,+}$, A) and L-leucine-resistant (mainly system b_2^+, B) L-arginine uptake by L-histidine, D-arginine, α-*N*-methyl-L-arginine and L-tryptophan in blastocysts. Conceptuses were incubated with 1.1 μM [^{3}H]arginine (A) or 2.8 μM [^{3}H]arginine plus 20 mM L-leucine (B) and the indicated concentrations of other amino acids for 5 min in phosphate-buffered LiCl. When 20 mM leucine was present, the LiCl concentration was reduced by 10 mM. The mean uptake ± S.E. was calculated from six replicate determinations (five or six blastocysts per determination) obtained in two or three independent experiments. When error bars are not shown they were within the symbols. L-Histidine, D-arginine and L-tryptophan were better inhibitors of total Na^+-independent arginine uptake (A) than α-*N*-methyl-L-arginine was ($P < 0.01$), whereas α-*N*-methyl-L-arginine and L-tryptophan were better inhibitors of leucine-resistant arginine uptake (B) than L-histidine ($P < 0.01$) or D-arginine ($P < 0.05$) were (pairing design *t*-tests).

be attributed to residual system $b^{0,+}$ activity. In 1-cell conceptuses, L-tryptophan and D-arginine inhibited system $b^{0,+}$ better than L-histidine did, whereas histidine was the better inhibitor of the leucine resistant, cation-preferring component of transport (Fig. 4). Similarly, system $b^{0,+}$ in blastocysts was inhibited more strongly by L-histidine and D-arginine than by α-*N*-methyl-L-arginine, whereas the latter amino acid was the better inhibitor of the cation-preferring transport process in blastocysts (Fig. 5). Moreover, Hofstee plots of substrate-saturable, L-arginine uptake were consistent with the interpretation that at least two Na^+-independent components of mediated arginine transport are present in both 1- and 2-cell conceptuses (Fig. 6).

We did not obtain a Hofstee plot for arginine uptake in blastocysts because we could not reliably measure the initial velocities of arginine uptake in these conceptuses at total arginine concentrations above about 10 μM (data not shown). Such was not the case, however, when system $b^{0,+}$ was inhibited with an excess of L-leucine, or when uptake of [^{3}H]arginine from solutions below 10 μM was measured at various concentrations of nonradioactive L-lysine. On the other hand, although initial rates of L-lysine uptake could be measured at all of the necessary substrate concentrations, uptake of lysine via the cation-preferring transport process in blastocysts probably occurs with a K_m value nearly 100-times higher than for arginine uptake (Table I). Therefore, uptake of [^{3}H]lysine at mM concentrations of nonradioactive lysine is technically more difficult and much more expensive to measure than uptake of [^{3}H]arginine at mM concentrations of lysine. For these reasons, we measured apparent lysine transport indirectly by determining its effect on [^{3}H]arginine uptake. The Hofstee plot derived from such measurements was consistent with the conclusion that blastocysts contain at least two components of mediated, Na^+-independent lysine and arginine transport (Fig. 7). Moreover, in the presence of 92 mM L-leucine to inhibit system $b^{0,+}$ (46 mM LiCl was deleted), we determined the K_m value for L-arginine uptake via the cation-preferring component of transport in blastocysts (≅ 82 μM; data not shown) to be about half the value in 1-cell conceptuses (≅ 190 μM; Fig. 6). In the same experiment, L-homoarginine was found to competitively inhibit arginine transport by the cation-preferring system in blastocysts (K_i ≅ 1.2 mM; data not shown).

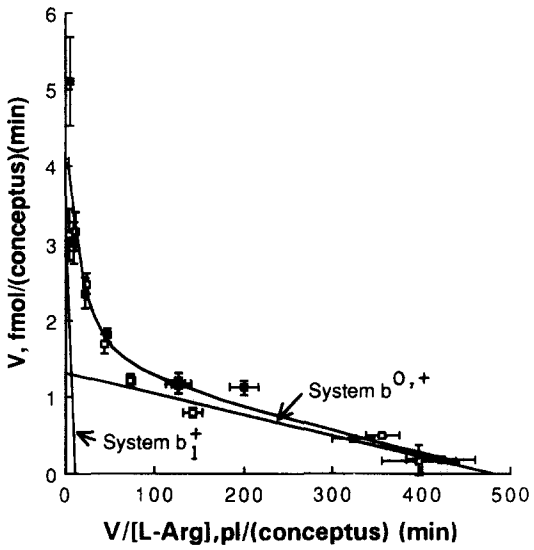

Fig. 6. Hofstee plots of mediated, Na^+-independent L-arginine uptake by 1-cell and 2-cell conceptuses. Conceptuses were incubated with the indicated concentrations of arginine and 10 mM BCH for 2 min in Na^+-free, modified Spindle's flushing medium-I (see Materials and Methods) to determine the mean uptake of eight (filled squares, 1 cell) or ten (open squares, 2-cell) replicate determinations (approx. five conceptuses per determination) obtained in three independent experiments. Nonsaturable uptake (4.8 ± 0.5 pl·conceptuses^{-1}·min^{-1}) was subtracted from total uptake to produce the data presented. Since the data for 1-cell and 2-cell conceptuses were nearly indistinguishable, they were combined for analysis by the method of Spears et al. [27] assuming two transport systems were active. The values of the kinetic parameters derived in this way were $K_m \cong 2.7$ μM and $V_{max} \cong 1.3$ fmol·conceptus^{-1}·min^{-1} for systems $b^{0,+}$ and $K_m \cong 190$ μM and $V_{max} \cong 3.2$ fmol·conceptus^{-1}·min^{-1} for system b_1^+. The curved line represents the combination of the two straight lines.

TABLE I

K_i values for the interactions of basic amino acids with the cation-preferring transport systems in 1-cell conceptuses and blastocysts

After subtracting nonsaturable uptake from the data presented in Fig. 3 and from similar data obtained in the absence of Na^+ (not shown), the mean ± S.E. of the K_i value for each amino acid was calculated utilizing the formula (Ref. 13);

$$K_i = (v_i/(v - v_i)) \cdot (K_m \cdot [\text{inhibitor}]/([[^3\text{H}]\text{arginine}] + K_m))$$

where v_i is the rate of L-[^{3}H]arginine uptake in the presence of the inhibitor and v is the rate of [^{3}H]arginine transport in the absence of the inhibitor. As for the K_i values of L-arginine in the table, the K_m values for L-arginine uptake were not greatly different in 1-cell conceptuses (≅ 190 μM, Fig. 6) and blastocysts (≅ 82 μM, see text). Moreover, at the substrate concentrations utilized for experiments reported in Fig. 3, such a difference in K_m values had almost no effect on the calculated values of K_i. The differences between the K_i values in 1-cell conceptuses and blastocysts were statistically significant ($P < 0.01$; Kruskal-Wallis H test) for each amino acid listed except nonradioactive L-arginine.

Stage of development	Amino acid	K_i value (μM)
1-cell	L-arginine	133 ± 45
	L-homoarginine	156 ± 43
	S-2-aminoethyl-L-cysteine	624 ± 92
	L-lysine	1250 ± 180
Blastocyst	L-arginine	84 ± 21
	L-homoarginine	1160 ± 240
	S-2-aminoethyl-L-cysteine	6800 ± 1200
	L-lysine	8100 ± 1000

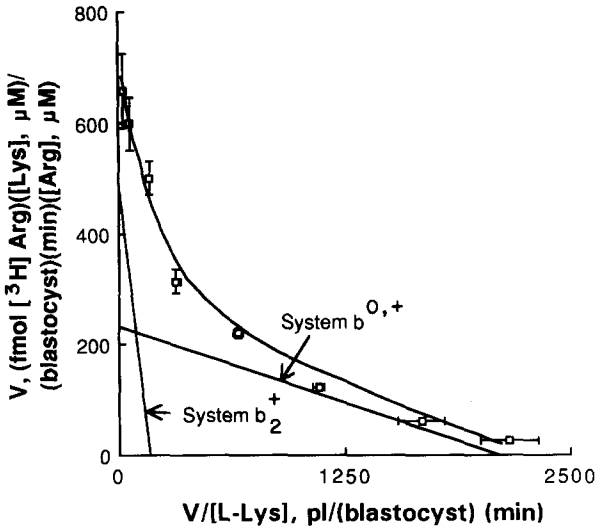

Fig. 7. Hofstee plot of mediated Na^+-independent L-lysine utpake by blastocysts measured indirectly in the presence of 2.78 μM L-[^{3}H]arginine. Conceptuses were incubated with the indicated concentrations of lysine, 2.78 μM [^{3}H]arginine, and 10 mM BCH for 2 min in phosphate-buffered LiCl to determine the mean uptake of six replicate determinations (approx. five blastocysts per determination) obtained in two independent experiments. Nonsaturable uptake was subtracted from total uptake to produce the data presented. The K_m values for systems $b^{0,+}$ and b_2^+ were estimated to be about 110 μM and 2.8 mM, respectively, utilizing the method of Spears et al. [27] to generate the two straight lines. The curved line represents the combination of these two lines. The experiments presented here were performed in the presence of 2.78 μM arginine, and the K_m (and K_i) value for arginine uptake via system $b^{0,+}$, although difficult to determine precisely in blastocysts (see Results), appears to be about the same as in 1- and 2-cell conceptuses (i.e., ≅ 2.7 μM; Fig. 6, Ref. 20 and data not shown). Therefore, the apparent K_m value for lysine uptake via system $b^{0,+}$, determined here to be about 110 μM in the presence of 2.78 μM arginine, is about twice its value of 48 μM determined in the absence of arginine (Ref. 6). See Results for justification of the use of a Hofstee plot.

In some ways, a Hanes plot (i.e., $[S]/v$ versus $[S]$) would have been more desirable than a Hofstee plot in Fig. 7. A Hanes plot would not have required the unconventional velocity construct of [^{3}H]arginine clearance multiplied by lysine concentration which we had to use for units on the y-axis (Fig. 7). Our main purpose, however, was to show kinetic evidence for the presence of at least two mediated Na^+-independent transport processes for cationic amino acids in blastocysts. Kinetic evidence for the presence of more than one transport process is usually easier to visualize when data points are more equally spaced and weighted as in Hofstee plots. In a Hanes plot of the data represented in Fig. 7, points at higher lysine concentrations are more widely spaced and thus visually more obvious and more heavily weighted in simple linear regression analysis than are points at lower concentrations.

Contamination of our preparations of 1-cell conceptuses with a few unfertilized eggs could conceivably produce a curved Hofstee plot if the same system has different K_m values in fertilized and unfertilized eggs. Heterogeneous populations of conceptuses are, however, unlikely to account for the two components of transport detected kinetically in 2-cell conceptuses and blastocysts (Figs. 6 and 7). Moreover, the kinetic experiments reported in Figs. 6 and 7 were performed in the presence of 10 mM BCH, which inhibits systems L and $B^{0,+}$ in conceptuses [4–6,18,20]. For this reason, neither component of Na^+-independent arginine or lysine transport can be attributed to weak interaction of these amino acids with the zwitterion-preferring system L or the normally Na^+-dependent system $B^{0,+}$. The lower-K_m component of cationic amino acid transport (i.e., system $b^{0,+}$) is competitively inhibited by L-leucine in both 1-cell conceptuses and blastocysts [6,20]. Therefore, we attribute the components of transport with higher K_m values in Figs. 6 and 7 to the leucine-resistant, cation-preferring transport processes. Since neither of the latter, cation-preferring components was susceptible to inhibition by all cationic amino acids equally (e.g., compare inhibition by D-arginine to that of non-radioactive L-arginine in Fig. 3), inhibition of the transport processes cannot be attributed to collapse of the membrane potential during nonsaturable uptake of cationic amino acids rather than to inhibition of mediated transport.

Discussion

Differences between the cation-preferring amino acid transport processes in preimplantation mouse conceptuses and system y^+ and c

The cation-preferring transport processes in mouse eggs and preimplantation conceptuses (Fig. 2 and Ref. 6) and system c [24] resist Na^+-dependent inhibition by L-homoserine. Thus, they differ from system y^+ by a criterion that has been used to help describe the substrate receptor site of system y^+ [9]. On the other hand, the activities of the systems in 1-cell conceptuses and blastocysts and system y^+ [7] are independent of pH in the interval 6.0 to 8.0, whereas the activity of system c changes dramatically over this range [24]. Similarly, the relative abilities of basic amino acids to interact with the systems in 1-cell conceptuses and blastocysts distinguishes both of these transport processes from systems y^+ and c.

The cation-preferring system in 1-cell conceptuses is inhibited by MPA (Fig. 3), whereas system y^+ resists inhibition by the concentrations of this substance we used [2,5,26]. In contrast, the systems in 1-cell conceptuses and blastocysts interact more weakly with ε-*N*-methyl-L-lysine, D-arginine and L-2-amino-3-guanidinopropionic acid (Fig. 3) than system y^+ does [7,8]. Moreover, the system in blastocysts interacts more strongly with L-arginine than with L-homoarginine, whereas the reverse is true for system y^+ [7,8]. A final distinction between system y^+ and the systems in 1-cell

conceptuses and blastocysts is that the systems in conceptuses both interact more strongly with L-arginine than with L-lysine (Fig. 3), but these amino acids are accepted equally well by system y^+ in fibroblasts and hepatoma cells [7,8]. The systems in 1-cell conceptuses and blastocysts are unlike system c because they do not interact with ε-*N*-trimethyl-L-lysine at concentrations that inhibit system c (Fig. 3), although interaction of L-arginine with the systems in conceptuses (Table I) is as strong as or somewhat stronger than the interaction of L-arginine with system c [24]. D-Arginine also interacts more strongly with system c [24] than with the systems in conceptuses (Fig. 3). In addition, inhibition of the systems in 1-cell conceptuses and blastocysts by α-*N*-methyl-L-arginine is considerably weaker than inhibition by L-lysine, L-homoarginine or *S*-2-aminoethyl-L-cysteine (Fig. 3), whereas α-*N*-methyl-L-arginine inhibits system c more strongly than any of these other three amino acids [24]. For all of these reasons, the cation-preferring amino acid transport processes in mouse eggs and preimplantation conceptuses may be as distinct from systems y^+ and c as systems y^+ and c are distinct from each other. We propose that the processes in eggs and conceptuses be designated, provisionally, system b^+. For convenience, we refer to the different forms of system b^+ that seem to predominate in 1 cell conceptuses and blastocysts, as systems b_1^+ and b_2^+, respectively, although undetected heterogeneity within each of these cation-preferring components cannot be ruled out.

Changes in the characteristics of system b^+ during preimplantation development

Each of the basic amino acids, ε-*N*-methyl-L-lysine, L-2-amino-3-guanidinopropionic acid and MPA interacts with system b_1^+ in 1-cell conceptuses, whereas these amino acids, and in particular MPA, do not inhibit system b_2^+ in blastocysts (Fig. 3). Similarly, although L-arginine interacts at least as strongly with system b_2^+ as with system b_1^+, each of the cationic amino acids, L-homoarginine, L-lysine and *S*-2-aminoethyl-L-cysteine, interacts more strongly with system b_1^+ than with system b_2^+ (Fig. 3 and Table I). Therefore, it is possible that system b_2^+ simply has higher K_i (and probably K_m) values than system b_1^+ for most basic amino acids. It is, however, somewhat difficult to imagine how L-

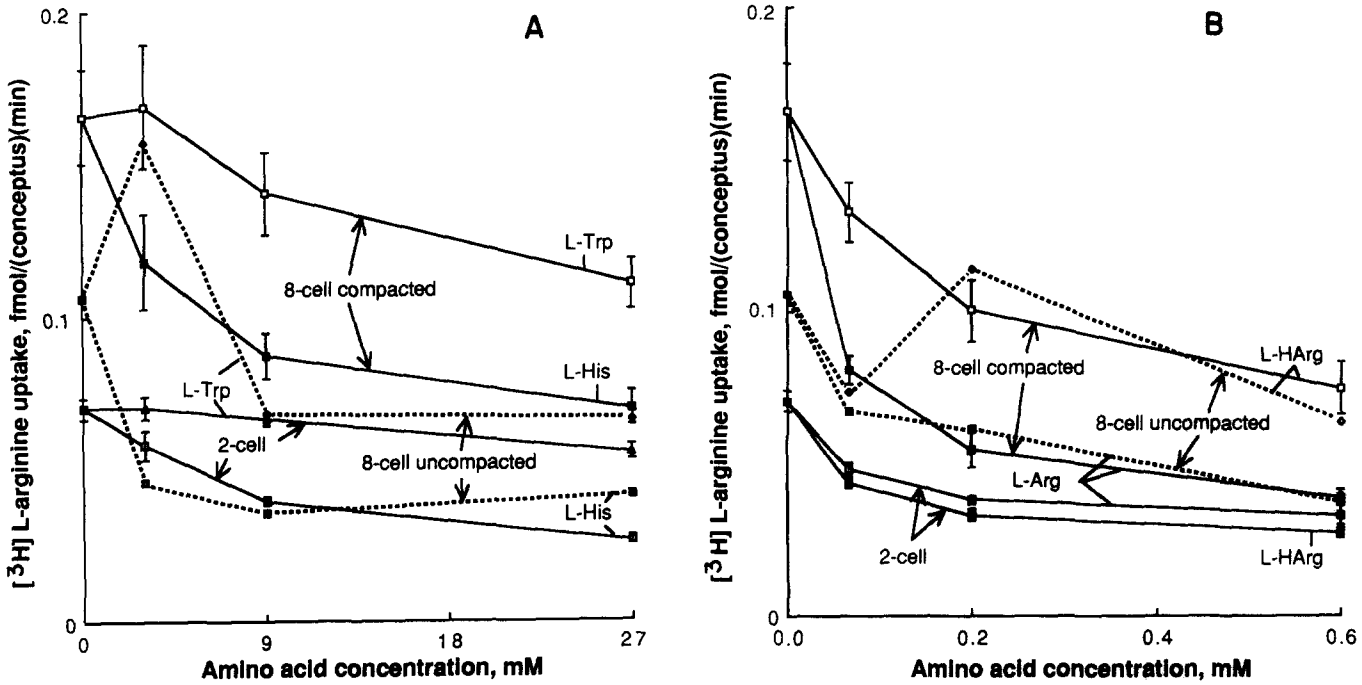

Fig. 8. Effect of various amino acids on L-leucine-resistant L-[^{3}H]arginine uptake by 2-cell conceptuses and compacted and uncompacted 8-cell conceptuses. Conceptuses were incubated with 5.6 μM [^{3}H]arginine, 20 or 55 mM L-leucine and the indicated concentrations of nonradioactive amino acids for 5 min in phosphate buffered LiCl or modified Spindle's flushing medium-I (see Materials and Methods). Data obtained for compacted 8-cell conceptuses in each of these media and in the presence of 20 or 55 mM L-leucine were indistinguishable, so they were pooled. The LiCl concentration was reduced in the media to compensate for the osmotic effect of 20 or 55 mM leucine. The mean uptake ± S.E. was calculated from 2–7 replicate determinations (3–7 conceptuses per determination) obtained in 2–4 independent experiments. When error bars are not shown they were within the symbol. (The S.E. was not calculated for points representing uptake by uncompacted 8-cell conceptuses since several of these values were calculated from only two determinations.) Each amino acid inhibited uptake in both 2-cell and compacted 8-cell conceptuses ($P < 0.01$, analysis of variance). Moreover, L-histidine was a better inhibitor than L-tryptophan at each of the three stages of development (A; $P < 0.01$). In addition, nonradioactive L-arginine inhibited [^{3}H]arginine uptake more strongly than L-homoarginine did in both types of 8-cell conceptuses (B; $P < 0.05$ for uncompacted and $P < 0.01$ for compacted conceptuses), but the reverse was the case in 2-cell conceptuses ($P < 0.01$; pairing design *t*-test). HArg, homoarginine.

arginine could escape an otherwise general transition to higher values of K_i and K_m. Moreover, L-histidine and D-arginine inhibit system b_1^+ more strongly than L-tryptophan does (Fig. 4B), whereas the latter amino acid inhibits system b_2^+ better than the former amino acids (Fig. 5B). In fact, early attempts to study system b_2^+ kinetically failed because they were performed in the presence of 40 mM tryptophan which almost completely inhibited all mediated arginine uptake (data not shown). Finally, inhibition of system b_1^+ by MPA, L-tryptophan, α-*N*-methyl-L-arginine, and L-2-amino-3-guanidinopropionic acid is stimulated by Na^+ ($P < 0.01$), whereas the effect of these and other amino acids on system b_2^+ is not influenced by Na^+ (data not shown). Therefore, system b^+ changes at least functionally as preimplantation development proceeds.

Experiments were designed to determine more precisely when during development system b_2^+ replaces system b_1^+ as the apparently predominant, cation-preferring transport process in preimplantation mouse conceptuses. L-Histidine inhibits the cation-preferring system in 2-cell conceptuses more strongly than L-tryptophan does, and L-homoarginine inhibits [^{3}H]arginine uptake by this same system a little better than nonradioactive L-arginine does (Fig. 8). By these criteria, the system in 2-cell conceptuses resembles system b_1^+ in 1-cell conceptuses (Figs. 3 and 4). In contrast, the cation-preferring systems in both compacted and uncompacted 8-cell conceptuses interact more strongly with L-arginine than with L-homoarginine (Fig. 8B) so they resemble system b_2^+ in blastocysts (Fig. 3). In regard to relative inhibition by L-histidine and L-tryptophan, however, the systems in 8-cell conceptuses resemble system b_1^+ more than they resemble system b_2^+ (Figs. 4, 5 and 8A). Therefore, conversion from system b_1^+ to system b_2^+ during development of blastocysts from 2-cell conceptuses appears to occur in a stepwise or gradual manner. Further studies are needed, however, to determine whether this conversion represents expression of separate genes. Although the mechanism and physiological significance of the change from system b_1^+ to system b_2^+ remain to be determined, the discovery of these and other novel transport systems in preimplantation mouse conceptuses (Refs. 4–6, 10, 13, 14, 18, 20, and this report) has already expanded our knowledge of the range of different characteristics that amino acid transport systems may have. This knowledge should not only help us to perceive more accurately the complexity of amino acid homeostasis in multicellular animals, but also dispel the notion that the transport systems in unexamined tissues can be assumed to be the same as those few systems that have already been well characterized.

Acknowledgements

The authors thank Barbara Le Breton, Mr. Howard Wasserlauf and Mr. Brian Weimer for their help in preparing the manuscript. This work was supported by USPHS Grant HD21801.

References

1 Christensen, H.N. (1966) Fed. Proc. 25, 850–853.
2 Christensen, H.N. (1985) J. Membr. Biol. 84, 97–103.
3 Christensen, H.N. (1989) Methods Enzymol. 173, 576–616.
4 Van Winkle, L.J. (1988) Biochim. Biophys. Acta 947, 173–208.
5 Van Winkle, L.J., Christensen, H.N. and Campione, A.L. (1985) J. Biol. Chem. 260, 12118–12123.
6 Van Winke, L.J., Campione, A.L. and Gorman, J.M. (1988) J. Biol. Chem. 263, 3150–3163.
7 White, M.F., Gazzola, G.C. and Christensen, H.N. (1982) J. Biol. Chem. 257, 4443–4449.
8 White, M.F. and Christensen, H.N. (1982) J. Biol. Chem. 257, 4450–4457.
9 Thomas, E.L. and Christensen, H.N. (1970) Biochem. Biophys. Res. Commun. 40, 277–283.
10 Van Winkle, L.J., Campione, A.L. and Gorman, J.M. (1990) Biochim. Biophys. Acta 1025, 215–224.
11 Young, J.D., Mason, D.K. and Ficham, D.A. (1988) J. Biol. Chem. 263, 140–143.
12 Christensen, H.N. (1988) Biosci. Rep. 8, 121–129.
13 Van Winkle, L.J. and Campione, A.L. (1987) Biochim. Biophys. Acta 925, 164–174.
14 Van Winkle, L.J., Haghighat, N., Campione, A.L. and Gorman J.M. (1988) Biochim. Biophys. Acta 941, 241–256.
15 Fowler, R.E. and Edwards, R.G. (1957) J. Endocrinol. 15, 374–384.
16 Brinster, R.L. (1971) in Pathways to Conception: The Role of the Cervix and the Oviduct in Reproduction (Sherman, A.I., ed.), Charles C. Thomas, Springfield, IL.
17 Spindle, A. (1980) In Vitro 16, 669–674.
18 Van Winkle, L.J., Campione, A.L. and Farrington, B.H. (1990) Biochim. Biophys. Acta 1025, 225–233.
19 Christensen, H.N. (1975) Biological Transport, 2nd Edn., pp. 63–213, W.A. Benjamin, Inc., Reading, MA.
20 Van Winkle, L.J., Campione, A.L., Gorman, J.M. and Weimer, B.D. (1990) Biochim. Biophys. Acta 1021, 77–84.
21 Christensen, H.N. and Cullen, A.M. (1973) Biochim. Biophys. Acta 298, 932–950.
22 Woolf, C.M. (1968) Principles of Biometry, 1st Edn., D. Van Nostrand, London.
23 Kramer, C.Y. (1956) Biometrics 12, 307–310.
24 Pisoni, R.L., Theone, J.G., Lemons, R.M. and Christensen, H.N. (1987) J. Biol. Chem. 262, 15011–15018.
25 Christensen, H.N. (1972) Bioenergetics 4, 233–263.
26 Christensen, H.N. (1975) Curr. Top. Membr. Transp. 6, 227–258.
27 Spears, G., Sneyd, J.G.T. and Loten, E.G. (1971) Biochem. J. 125, 1149–1151.