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DEVELOPMENTAL AND OTHER CHARACTERISTICS OF LYSINE UPTAKE BY RAT BRAIN SYNAPTOSOMES

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Summary

Synaptosomes isolated from adult or newborn rat cerebrum take up L-lysine by two saturable systems, one with a high affinity low capacity and the other with a low affinity high capacity. Initial rate of uptake for low lysine concentrations is more rapid in newborn, but for high concentrations the rate is greater in adult tissue. Analysis of kinetic data indicates that synaptosomes of the newborn have a higher V_{max} than those of the adult for high affinity system but adult synaptosomes have a higher V_{max} than newborn for low affinity system. At a physiological lysine concentration of 0.5 mM, the calculated contributions of two systems indicate that the adult uptake occurs for about 71% by low affinity system but the newborn utilizes both systems to the same extent. The uptake is sodium independent but pH dependent. Lysine uptake is inhibited by other dibasic amino acids, arginine and ornithine but not cystine. Kinetic analysis indicates that arginine specifically inhibits the high affinity, low K_m system for lysine uptake.

Introduction

Lysine is an essential amino acid whose transport into renal tubules and intestinal mucosal cells has been shown to be genetically defective in human cystinuria [1—3]. Interest in this disorder has prompted intensive investigation of the characteristics of dibasic amino acid transport by normal rat kidney cortex and intestinal mucosa [4,5]. The suggestion that mental dysfunction [6] and neurological disorders [7] occur in patients with cystinuria leads to the possibility that a compromise in dibasic amino acid transport in nerve tissue may exist in some patients. A detailed knowledge of dibasic amino acid transport in normal nerve tissue is thus of considerable import.

The characterization of amino acid transport in brain slices [8—12] and isolated brain synaptosomes [13—17] has included some data on the nature of dibasic amino acid transport. Peterson and Raghupathy [15] have described developmental transitions in the synaptosomal uptake of several amino acids including arginine. We have examined the nature of lysine uptake by rat brain synaptosomes with emphasis on concentration dependence, sodium and pH requirements, interaction with other amino acids and developmental aspects. Our results form the basis of this report.

Materials and Methods

Male Sprague-Dawley rats (35—45 days old) were obtained from Charles River (Wilmington, MA). The animals were decapitated and the cerebral hemispheres removed and placed in 3 mM *N*-2-hydroxyethylpiperazine-2-ethanesulfonic acid (HEPES) buffer containing 0.32 M sucrose and 10 μM $CaCl_2 \cdot 2H_2O$, at pH 7.4. The preparative method was that of Whittaker et al. [18], as modified by Warfield and Segal [19]. Following Ficoll density gradient centrifugation, the material banding between 10 and 13% was recovered. When synaptosomes were prepared from newborn rats material banding between 5 and 13% Ficoll was used. The pooled synaptosomal fractions from the Ficoll gradients were prepared for transport study by a 4-fold dilution with ice-cold Krebs-Ringer bicarbonate buffer 309 mosM), pH 7.4. This suspension was centrifuged at 35 000 $\times g$ at 2°C for 20 min in a Beckman model J-21B refrigerated centrifuge. The supernatant was discarded and the pellet was resuspended in the bicarbonate buffer with a Dounce homogenizer (pestle A) to a final concentration of approximately 5—10 mg synaptosomal tissue (wet weight) per ml of suspension. In studies of sodium dependence, choline chloride or Tris buffer were substituted for sodium in Krebs-Ringer bicarbonate buffer, and pH dependence was carried out in modified Krebs-Ringer phosphate buffers, pH 5.7—7.4; Krebs-Ringer bicarbonate buffer, pH 7.4; and Krebs-Ringer Tris buffer, pH 7.4 to 8.35 [20].

Incubation of the synaptosomal suspension was carried out by the recently described method of Hwang et al. [21] in the flask described by Burg and Orloff [22] at 37°C with continuous bubbling 95% O_2/5% CO_2. When anoxic conditions were imposed, 95% N_2/5% CO_2 was used, and 100% O_2 was employed when using Tris buffer. For incubation times longer than 5 min, the uptake was initiated by addition of [^{14}C]lysine directly to the flask and terminated by removing duplicate samples into tared tubes which were rapidly chilled in ice water and centrifuged at 2°C for 10 min at 33 000 $\times g$. The supernatants were removed for counting. The pellet surface and test tube walls were washed once with ice-cold Krebs-Ringer bicarbonate buffer and dried by suction. The tubes were then weighed, the pellets resuspended by the addition of 1 ml distilled water and the suspended pellets placed in a boiling water bath for 3 min. The tubes were then centrifuged at 33 000 $\times g$ for 10 min. Aliquots (0.2 ml) of the original supernatants and the water extracts of the synaptosomal pellets were added to 10 ml of scintillation cocktail (2.8 ml absolute alcohol/ 7 ml econofluor (NEN) and counted in a Packard liquid scintillation counter. For short incubation of 5 min, tared tubes containing desired concentrations

of substrates were placed in the 37°C water bath. The uptake was initiated by pipetting 1 ml of oxygenated and 37°C equilibrated synaptosomal suspension from flask to each of the tared tubes. After 5 min each tube was chilled and handled as described above. The results of short incubations by both methods were identical.

The 'trapped medium space' was estimated by the addition of inulin[^{14}C]-carboxylic acid to the suspension immediately before centrifugation, the subsequent procedure for handling the samples being identical to that already described [23]. Total tissue water was determined by overnight desiccation of synaptosomal pellets. Uptake of lysine was expressed as a distribution ratio, calculated as previously described by Rosenberg et al. [24] and defined as the ratio of cpm/μl synaptosomal fluid to cpm/μl medium.

The experiments to study the effect of substrate concentration on uptake were analyzed by a Hofstee plot of V vs. V/S drawn by the least squares method with the aid of a Monroe Model 1775 programmable calculator to obtain the best fit. Using the values for K_m and V_{max} from the Hofstee plot as initial estimates a reiterative procedure was carried out using the equation

$$V = \frac{V_{max_1} S}{K_{m_1} + S} + \frac{V_{max_2} S}{K_{m_2} + S}$$

to calculate the parameters which gave the best fit to the observed V at the various concentrations employed.

L-[U-^{14}C]Lysine (286 Ci/mole) and L-[U-^{14}C]arginine (298 Ci/mol) were obtained from New England Nuclear Corp. (Boston, MA). Inulin[^{14}C]carboxylic acid (2.5 mCi/g) was purchased from Amersham Corp. (Arlington Heights, IL). Unlabeled substrates and other amino acids of highest purity were obtained from commercial sources.

Results

Uptake of [^{14}C]lysine

Fig. 1 shows the uptake of lysine by synaptosomes from adult and newborn cerebra for 30 min of incubation. The uptake is expressed as the distribution ratio, the ratio of the cpm per μl synaptosomal fluid to cpm per μl of media. Since our chromatographic analysis of synaptosomal extracts shows the synaptosomal radioactivity to be mostly (80%) lysine throughout the incubation, confirming previous observations [14], the ratios are indicative of concentrative uptake and active transport. At 0.01 mM lysine the early uptake by newborn synaptosomes was significantly higher by about 30% than that of the adult and reached a steady state distribution ratio of about 10 at 15 min. The adult synaptosomes reached the same level by 30 min. With further incubation to 45 min adult tissue was found to maintain the same ratio.

The distribution ratios when 2 mM lysine uptake was measured were much lower than those observed with 0.01 mM indicating that the transport was saturable. There was a significant difference between the uptake of 2 mM lysine by adult and newborn synaptosomes, the distribution ratio at 5 min of the former being 2.7 ± 0.2 and that of the latter 1.7 ± 0.2. Thus, at low concentration the initial uptake by newborn synaptosomes is faster than the adult

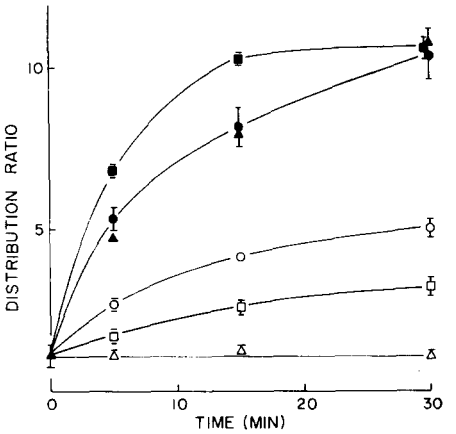

Fig. 1. Synaptosomal uptake of [^{14}C]lysine vs. time. A synaptosomal suspension of 5—10 mg per ml was incubated at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, with [^{14}C]lysine, 0.1 μCi/ml, as described in Methods. Uptake data of 0.01 mM lysine are the averages of 6 determinations for adult (●———●) and 4 determinations for newborn (■———■). Uptake data of 2 mM lysine are the averages of 6 determinations for adult (○———○) and 4 determinations for newborn (□———□). Uptake of 0.01 mM lysine in adult at 0°C is shown as (△———△) (4 determinations) and under anoxic conditions (2 determinations) as (▲———▲).

whereas at high concentration the uptake by the adult synaptosome is more rapid and extensive than the newborn.

Fig. 1 also shows the uptake of 0.01 mM at 0°C. Addition of substrate followed by immediate centrifugation (zero time) resulted in a distribution ratio of about 1 which was maintained with longer incubation times. A distribution ratio of 1 was also seen at 0°C when uptake of 2 mM lysine was examined. When uptake at 0.01 mM was terminated at 5 min or 30 min by the addition of trichloroacetic acid (10% final concentration), the radioactivity associated with the protein precipitate was calculated to be equivalent to a distribution ratio of 1.16. These data suggested a small amount of non-specific uptake or binding which was used as a correction factor in determining kinetic parameters described below. Fig. 1 also indicates that 0.01 mM lysine uptake by adult synaptosomes was not diminished when the incubation was carried out under anaerobic conditions.

Concentration dependence of uptake

Uptake of [^{14}C]lysine at 5 min was studied over a wide range of lysine concentrations. Both at low (0.01—0.1 mM) and high (0.5—5 mM) substrate concentrations the uptake demonstrated saturation kinetics. Fig. 2 shows a Hofstee plot of the results in which a two-limbed curve best fits the data. In all of the studies the velocity of uptake by the newborn synaptosomes at low concentrations was higher than the adult. In the high concentration range the velocities of uptake by the adult tissue were higher than the newborn.

The two limbed curve of concentration dependence is consistent with the presence of two saturable processes whose apparent K_m and V_{max} are shown in Table I. A computer calculation was made of the best fit to the observed velocities assuming the simultaneous function of two uptake systems. These

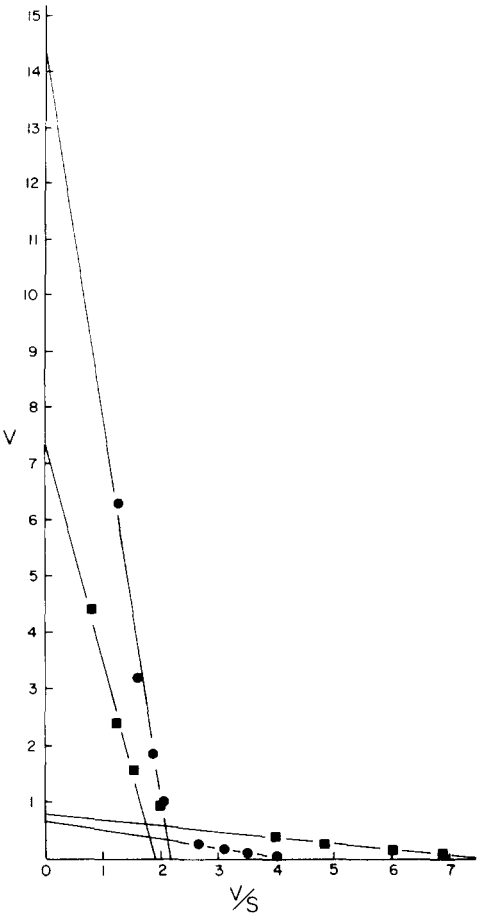

Fig. 2. Hofstee plot of velocity vs. velocity over substrate concentration. Synaptosomes 5—8 mg/ml were incubated for 5 min at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4 with [^{14}C]lysine, 0.1 μCi/ml, and sufficient unlabeled lysine to give the desired concentrations: (●———●) adult and (■———■) newborn. V is expressed as mmol/l per 5 min, S as mM. Each value is an average of 6 to 8 determinations corrected for 0°C uptake.

TABLE I

PARAMETERS OF CONCENTRATION DEPENDENCE OF LYSINE UPTAKE BY SYNAPTOSOMES

Based on data shown in Fig. 2.

	K_{m_1} (mM)	K_{m_2} (mM)	V_{max_1} (mmol/l per 5 min)	V_{max_2} (mmol/l per 5 min)
Observed				
Adult	0.16	6.51	0.67	14.16
Newborn	0.11	3.86	0.81	7.34
Calculated				
Adult	0.16	6.51	0.40	10.56
Newborn	0.12	5.86	0.71	6.44

results are also shown in Table I. The calculated K_m values are very similar for the newborn and adult synaptosomes. The major difference in the kinetic parameter is in the V_{max} values. V_{max} for the low K_m newborn system is about 75% higher than for the adult while the V_{max_2} for the high K_m system is higher in the adult synaptosomes.

Fig. 3 is a plot of the percent of the total uptake mediated by each of the delineated lysine transport systems at various concentrations calculated from kinetic parameters of Table I. It reveals that at low lysine concentrations (0.01 mM), 60% of the uptake by adult synaptosomes is via the low K_m system and 40% by the high K_m system. In newborn tissue 83% of the uptake is mediated by the low K_m system and 17% by the high K_m process. At physiologic plasma lysine levels of about 0.5 mM [25], 71% of uptake by adult synaptosomes takes place via the high K_m process and 29% via the low K_m system. In the newborn preparation at 0.5 mM the uptake is shared equally by the two systems. At 0.5 mM the calculated total velocity is the same in newborn and adult syanaptosomes. Below 0.1 mM total uptake by newborn tissue is about 50% higher than that of adult synaptosomes while at concentrations of 1 mM or higher the total uptake is higher with the adult tissue.

Effect of sodium on uptake by synaptosomes of adult brain

The uptake of lysine at 0.01 mM and 2 mM was found to be independent

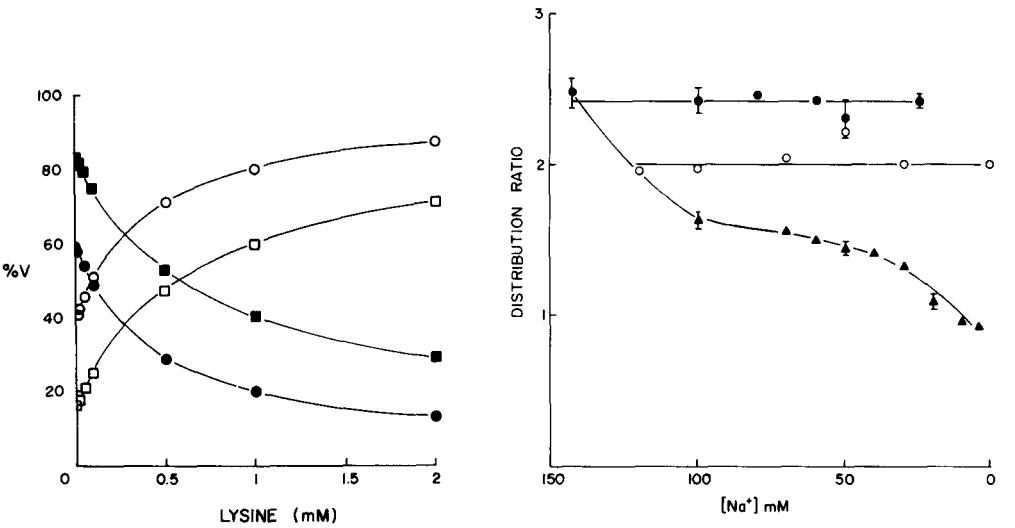

Fig. 3. The relative contribution to total uptake of low and high K_m systems at various lysine concentrations. By using calculated kinetic parameters, the relative contribution of each component was determined from equation of velocity and concentration: low K_m systems, adult (●———●) and newborn (■———■); high K_m systems, adult (○———○) and newborn (□———□).

Fig. 4. Effect of sodium on lysine uptake. 6 mg/ml of synaptosomes were incubated in pH 7.4 buffers with 0.1 μCi/ml, 2 mM [^{14}C]lysine for 5 min at 37°C: (1) Krebs-Ringer bicarbonate or modified Krebs-Ringer bicarbonate (choline chloride was substituted for 43—118 mM of sodium chloride), ●———●; (2) 25 mM Tris replaced 25 mM sodium bicarbonate of Krebs-Ringer bicarbonate and choline chloride was substituted for 20—120 mM of sodium chloride, ○———○; and (3) 25 mM Tris replaced 25 mM sodium bicarbonate of Krebs-Ringer bicarbonate and Tris was further substituted for 20—115 mM of sodium chloride, ▲———▲. Values are the averages of 2 to 14 determinations.

of sodium concentration. This was demonstrated in three types of experiments as shown for 2 mM in Fig. 4. In the first type, sodium chloride of Krebs-Ringer bicarbonate buffer was replaced by choline chloride with buffer maintained at pH 7.35 with 25 mM $NaHCO_3$. The upper curve of Fig. 4 shows no change in the distribution ratio with sodium as low as 25 mequiv./l. In the second group the sodium bicarbonate of the Krebs-Ringer bicarbonate buffer was replaced with 25 mM Tris buffer which resulted in a 20% drop in the distribution ratio. Subsequent replacement of NaCl with choline chloride (middle curve Fig. 4) to achieve sodium free medium revealed no further diminution in distribution ratio beyond that observed when Tris buffer replaced the 25 mM sodium bicarbonate. The third study involved the replacement of sodium by Tris (lower curve Fig. 4) which showed that increasing amounts of Tris inhibited lysine uptake.

In their papers on synaptosomal amino acid transport Peterson and Raghupathy [14,15] used two buffers, one of which, the Tris/sucrose buffer, was sodium free and contained 300 mM sucrose 15 mM $MgCl_2$ and 10 mM Tris-HCl, pH 7.4. In the other 150 mM NaCl replaced the sucrose; it was designated Tris-NaCl buffer. They reported that not only was 0.5 μM lysine uptake sodium independent, but that the addition of sodium inhibited the uptake. This was based on their observation that lysine and arginine uptake was twice as high in Tris-sucrose as in the Tris-NaCl buffer.

We compared lysine uptake by adult synaptosomes in Krebs-Ringer bicarbonate buffer and the Tris/sucrose and Tris-NaCl buffers of Peterson and Raghupathy [14,15]. Since our method involves the determination of synaptosomal water space to assess uptake, these parameters were measured in Tris-sucrose and Tris-NaCl buffers. It was noted that synaptosomal pellets after centrifugation in Tris/sucrose buffer were smaller than the pellets in Tris-NaCl or Krebs-Ringer bicarbonate buffers. The total water content of the synaptosome pellet after incubation in sucrose buffer was 60% of the wet weight, a change from the 80% value of Krebs-Ringer bicarbonate or Tris-NaCl buffers. The extrasynaptosomal inulin space was the same in the pellets derived from incubations in the three buffers. The data in Table II show that at 37 and 0° C

TABLE II

COMPARISON OF LYSINE UPTAKE BY SYNAPTOSOMES INCUBATED IN VARIOUS MEDIA

Synaptosomes from adult cerebrum were incubated with 0.1 μCi/ml lysine for 5 min at 37° C and 0° C in Tris/sucrose, Tris-sodium and Krebs-Ringer bicarbonate. Values for Tris/sucrose and Tris-sodium are the average of 4 determinations and for Krebs-Ringer bicarbonate of 2 determinations.

Medium	Distribution ratio in indicated buffer			
	Distribution ratio in Krebs-Ringer bicarbonate			
	2 mM lysine		0.01 mM lysine	
	37° C	0° C	37° C	0° C
Tris/sucrose	2.3	10.9	2.6	8.0
Tris/sodium	0.6	2.3	0.7	1.2
Krebs-Ringer bicarbonate	1.0	1.0	1.0	1.0

the uptake of lysine in Krebs-Ringer bicarbonate and Tris-NaCl buffers is very similar. The uptake in sucrose buffer without NaCl is over twice as high as in the other buffers with NaCl at 37°C and 8—10-fold higher at 0°C. Since we have shown in our own studies that choline substitution for Na^+ in Krebs-Ringer bicarbonate buffer does not alter lysine uptake, the higher uptake in Tris-sucrose buffer is not due to the absence of NaCl per se. The fact that the uptake at 0°C is so high indicates a much higher binding of lysine to synaptosomes in Tris/sucrose buffer which also is increased at 37°C. Such an increase could be explained by the low ionic strength of the Tris-sucrose buffer.

Dependence on pH

The uptake by adult synaptosomes of 0.01 and 4 mM lysine over the pH range of 6—8.35 is shown in Fig. 5. There was little change in uptake between pH 6 and pH 7 at either substrate level. The uptake of 4 mM lysine which is mediated by over 90% on the high K_m system showed only a small increase in uptake between pH 7.4 and 8.35. On the other hand, uptake of 0.01 mM lysine rose sharply between 7.4 and 8.35 with a doubling of the distribution ratio. Since the uptake at 0.01 mM is mediated about 60% by the low K_m system [8] (Fig. 3) and there is little effect of pH on the high K_m system, it appears that the low K_m system is sensitive to pH above pH 7. It should be noted that the uptake above 7.4 was studied in a Tris Krebs-Ringer bicarbonate buffer and that at 7.4 there is about a 20% lower uptake when Tris is substituted for $NaHCO_3$ (Figs. 4 and 5). The distribution ratios at pH values above 7.4 are probably lower than they should be. It appears also from Fig. 5 that phosphate buffer is not as supportive of lysine transport as Krebs-Ringer bicarbonate buffer.

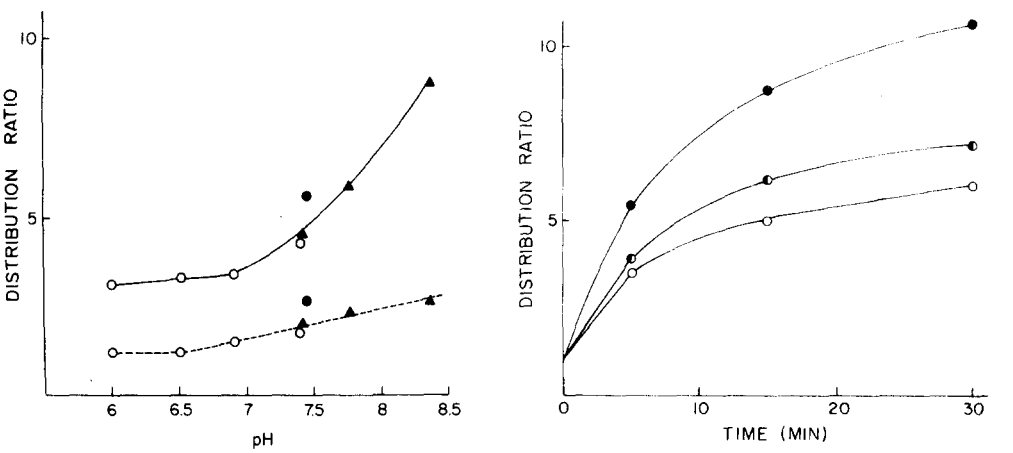

Fig. 5. Variation of lysine uptake with pH. Synaptosomes were incubated with 0.1 μCi/ml, 0.01 mM (———) or 4 mM (- - - - -) [^{14}C]lysine for 5 min in buffers at the pH indicated. Buffers were modified Krebs-Ringer solutions with phosphate at pH 5.7—7.4 (○), bicarbonate (●) at pH 7.4 and Tris at pH 7.4—8.35 (▲).

Fig. 6. Effect of addition of arginine or ornithine on lysine uptake. Synaptosomes, 6 mg/ml were incubated with 0.1 μCi/ml, 0.01 mM [^{14}C]lysine at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4. Control is shown as (●———●) plus 1 mM ornithine (◐———◐) and 1 mM arginine (○———○). Values are two averages agreed within 1%.

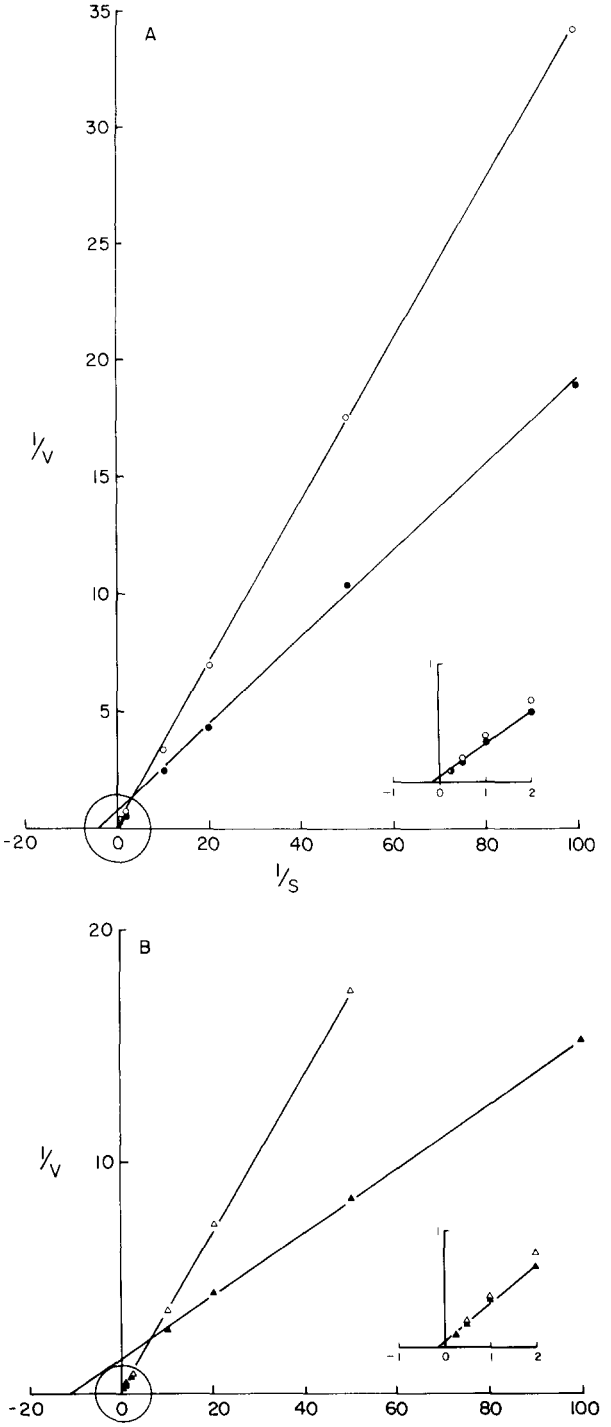

Fig. 7. Uptake interaction of L-lysine and L-arginine. Synaptosomes were incubated for 5 min at 37° C in Krebs-Ringer bicarbonate buffer, pH 7.4 with 0.1 μCi/ml of radioactive substrate at the indicated concentrations. A, lysine uptake: control (●———●) and with addition of 1 mM arginine (○———○); B, arginine uptake: control (▲———▲) and with addition of 1 mM lysine (△———△). Each value is an average of 2 to 4 determinations.

Interaction of lysine uptake with other amino acids

The addition of 1 mM cystine which is at its solubility limit did not affect the uptake of 0.01 mM lysine. The addition, however, of 1 mM arginine or ornithine, the other dibasic amino acids, inhibited lysine uptake as shown in Fig. 6. Neither glycine or taurine at 1 mM significantly inhibited 0.01 mM lysine uptake after 5 min incubations.

The nature of the arginine inhibition of lysine uptake by adult synaptosomes was assessed in concentration dependence experiments and the data are shown as a Lineweaver-Burk plot in Fig. 7A. The addition of 1 mM arginine inhibits the uptake at low concentrations such that the biphasic control curve (see Fig. 7) is converted to a single curve corresponding to that determined by the high concentration and representative of the high K_m system as shown in the inset, Fig. 7A. It thus appears that the addition of 1 mM arginine results in inhibition of the low K_m uptake system with little effect on the high K_m component.

The data of Figure 7A indicate that 1 mM arginine inhibited uptake of low concentrations of lysine about 45%. When 10 mM arginine was used lysine uptake was inhibited by 59% which approximates the inhibition of the high affinity system expected (60%) in the delineation of transport contributions shown in Fig. 3. At higher lysine substrate concentrations (1—5 mM) the inhibition at 10 mM arginine was 17% with a theoretical inhibition expected of 15% from Fig. 3. Also, as seen in Fig. 3, the inhibition of lysine uptake by newborn tissue at low concentration should show greater inhibition by arginine than that observed in the adult. Indeed, this occurred, for inhibition of the newborn by 1 mM arginine was 70% while the adult was inhibited about 45%.

Experiments in which arginine was the substrate and lysine the inhibitor were also performed (shown in Fig. 7B); arginine uptake like lysine uptake was shown to result in a biphasic substrate-velocity curve consistent with the presence of two transport components. The addition of 1 mM lysine resulted in a single curve corresponding to that of the high K_m component (inset Fig. 7B). It thus seems likely that the low K_m transport system is shared by dibasic amino acids and that the high K_m system is unshared and specific for each of them.

Discussion

The uptake of amino acids by mouse brain slices has been extensively examined by Lajtha and his associates [8—12]. Uptake against a concentration gradient indicative of active transport has been demonstrated [8,10]. Substrate specificity [8], kinetic fluxes [26], exchange diffusion [11] and ionic requirements [9,12] have been described. All of these reports have indicated that the long chain dibasic amino acids, lysine and arginine, have transport characteristics which differ from neutral amino acids and segregate them as a group. In general, the work with slices has employed high substrate levels with 2 mM being a standard concentration employed by Lajtha and his colleagues. The recognized complexity of the slice with multiple cellular elements, geometric problems of slice thickness and the desire to focus on the neurotransmission role of amino acids in the central nervous system have lead to the examination

of amino acid uptake by brain synaptosomal preparations. Snyder and his coworkers [13,16] using crude synaptosomal preparations delineated a high and low affinity system for synaptosomal uptake of glutamic and aspartic acid but only a low affinity system for other amino acids including lysine with a K_m of 3.3 mM. Peterson and Raghupathy [14] reported on the synaptosomal uptake of very low concentrations of amino acids (0.5 μM lysine). This led Snyder and his coworkers to the use of purified synaptosomal preparations in which they demonstrated a high affinity system for most amino acids [17]. In general, amino acid transport studies with slices have been carried out with millimolar substrate concentrations while in synaptosomes micromolar levels have been employed.

Our interest in lysine and dibasic amino acid transport stems from concern with inherited disorders of transport involving these amino acids [1]. We have employed the synaptosome as a simplified model system for study of neuronal cell uptake of lysine with an emphasis on physiological aspects other than the neurotransmission function of synapses. Our results indicate that lysine is taken up by synaptosomes against a concentration gradient by two saturable processes, one with high K_m and low affinity and the other with a low K_m and high affinity. The high affinity system is shared by other dibasic amino acids whereas the low affinity system is more specific for lysine. It appears that much of the data obtained with brain slices [8—12] reflects primarily the high K_m system. Since even at 2 mM lysine there is about 20% of total uptake mediated by the low K_m shared system, interactions of lysine with other dibasic amino acids would still be observed. The fact that lysine and arginine demonstrate heteroexchange diffusion in cerebral slices [11] is good evidence for operation of a shared transport system.

An important physiologic aspect of the dual mediation of lysine transport by synaptosomes relates to the changes with development. In vivo injection of small amounts of lysine and arginine in mice has shown that there is a greater accumulation in newborn than in adult brain [27,28]. Peterson and Raghupathy [15] have shown that at very low concentrations arginine is taken up more readily by immature brain synaptosomes than by adult ones. Our own data show that at low concentrations the entry of lysine is faster in newborn synaptosomes but that at high concentrations the reverse is true. With function of two systems whose kinetic parameters change differently with age, one must be circumspect about evaluation of total uptake with age since the changes will depend on the substrate concentration employed. At newborn and adult rat plasma lysine of about 0.5 mM [25] both our observed and total calculated velocity of entry is the same for newborn and adult synaptosomes.

We have found both lysine transport systems to be sodium independent. Margolis and Lajtha [9] reported lysine uptake by mouse brain slices to be totally dependent on Na^+ but subsequently, Lajtha and Sershen [12] reported lysine uptake to be only partially dependent on Na^+. Assuming the cellular elements of the slice responsible for uptake are reflected by the synaptosome, an explanation for the difference must be sought. A likely possibility to explain the discrepancy may be related to the buffers used. In the studies on Na^+ effects by Margolis and Lajtha and in most other experiments reported by the Lajtha group, the buffer used contained 100 mM Tris. We have shown that Tris

can be inhibitory to lysine uptake probably on the basis of its amine structure. It is possible that the slice responds adversely to elimination of Na^+ in the presence of Tris. In the later paper where only partial dependence of lysine on Na^+ was reported, the buffer used by Lajtha and Sershen was altered containing only 25 mM HEPES and no Tris [12]. The report of Peterson and Raghupathy [14] that lysine uptake by synaptosome is maximal in Na^+-free buffer and that uptake is inhibited by Na^+ also differs from our results. As we have shown (Table II) the uptake in their sodium containing buffer is similar to uptake observed by us in Krebs-Ringer bicarbonate buffer. Their use of a standard buffer containing Tris, $MgCl_2$ and sucrose, which is low in ionic strength, permits excessive lysine binding to synaptosomal protein.

The presence of two systems for lysine transport have been shown in renal cortex [4,29]. Most recent data with renal tubule fragments [30] indicate that as in the synaptosome the high affinity system is shared while the low affinity high K_m system is specific for each dibasic amino acid. Unlike the renal cortical cells in which cystine shares the low K_m lysine system, the synaptosomal low K_m system is unreactive with cystine. Cystine uptake by synaptosomes occurs via two systems neither of which is inhibited by lysine [31]. Only one dibasic transport system has been shown in intestinal mucosal cells [5]. The lack of Na^+ dependence of lysine uptake by synaptosomes is seen also with renal cortical cells [4]. The uptake of lysine by intestinal mucosal cells, on the other hand, is greatly dependent on the presence of Na^+ [5].

The implications in the literature that defective dibasic amino acid transport may place some human cystinuric patients at risk for brain [6] or other nerve [7] dysfunction remain to be validated. Characteristics of transport of dibasic amino acids by synaptosomes, however, resemble that of the renal cortex [4,29,30]. Whether the genetic regulation of dibasic amino acid by nerve cell membranes is similar to that of kidney or intestine is an open question. The only tissues studied, skin fibroblasts [32] and circulating leucocytes [33] from human cystinurics, other than kidney or intestine, do not share the defect observed in intestine and kidney.

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