The Journal of Neuroscience, June 1989, 9(6): 2066–2072

High-Affinity Uptake of L-Kynurenine by a Na^+-Independent Transporter of Neutral Amino Acids in Astrocytes

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L-Kynurenine (KYN), an intermediary product in the kynurenine pathway of tryptophan metabolism, is the common precursor from which are formed both quinolinic acid, a potent endogenous "excitotoxin," and kynurenic acid, a nonselective antagonist of excitotoxins. The present work examines ^{3}H-KYN transport in primary astrocyte cultures derived from the cerebra of newborn mice.

Influx and efflux of ^{3}H-KYN were attributable almost entirely to carrier-mediated transport. The tritium recovered in uptake experiments was identifiable as ^{3}H-KYN, indicating a low rate of KYN metabolism during incubations up to 30 min. KYN uptake decreased in the presence of extracellular Na^+, at least in part because KYN efflux was accelerated. Marked *trans* stimulation of KYN efflux by extracellular KYN provided evidence of the exchanging nature of the carrier. Saturation curves for the initial velocity of KYN uptake conformed to a 1-component saturable system with K_m of 32 μM and V_{max} of 2.1 nmol mg^{-1} protein min^{-1}. KYN was notably concentrated by the astrocytes, with an estimated steady-state distribution ratio of 180-fold for 1 μM KYN. Analog inhibition studies showed that the KYN transporter exhibited a clear preference for large neutral amino acids; leucine, tryptophan, and phenylalanine were recognized with relatively higher affinity than KYN.

In summary, KYN is concentratively transported into astrocytes by a Na^+-independent exchanger with high affinity for branched-chain and aromatic neutral amino acids. The substrate specificity and high affinity of this transport system resemble the properties of neutral amino acid transport across the blood–brain barrier in the rat and human.

L-Kynurenine (KYN) is a diamino acid (predominantly noncationic) formed as an intermediary in the "kynurenine pathway" of tryptophan metabolism, the major route by which tryptophan is degraded in the mammalian periphery (Schlossberger et al., 1984). The role of this pathway in the CNS has become a subject of particular interest since one of its products, quinolinic acid, was found to be a potent agonist at *N*-methyl-D-aspartate (NMDA) receptors and a potent neuroexcitatory toxin ("excitotoxin") that putatively could serve as a mediator of cell destruction in a variety of neurodegenerative disorders (Stone and Perkins, 1981; Schwarcz et al., 1983). An alternate path of KYN metabolism in the CNS is transamination to yield kynurenic acid (Minatogawa et al., 1974), a neuroinhibitory metabolite that protects neurons from the excitotoxic action of quinolinic acid by apparently nonselective antagonism at receptors for excitatory amino acids (Foster et al., 1984; but see Kempe et al., 1988). Progress in demonstrating the occurrence and distribution in the CNS of the enzyme activities and products of the kynurenine pathway has been reviewed recently (Schwarcz et al., 1989).

The KYN content of rat whole brain is approximately 5% that of tryptophan and is nonuniformly distributed, ranging from 0.03 pmol/mg tissue in cerebellum to 1.05 pmol/mg tissue in putamen (Joseph, 1978; Gàl and Sherman, 1980). It has been estimated that about 60% of the cerebral pool of KYN enters the brain from plasma (Gàl and Sherman, 1980). Preliminary evidence suggests that KYN crosses the blood–brain barrier in competition with other substrates for a high-affinity, Na^+-independent transporter of large neutral amino acids (Smith et al., 1987; Fukui et al., 1988). The unique position of KYN as a precursor of metabolites with opposing neuroactivities focuses attention on the mechanisms governing its access to individual CNS cell types. Experiments with rat brain slices revealed evidence of 2 saturable components of KYN uptake: Na^+-dependent neuronal uptake and Na^+-independent glial uptake (Speciale et al., 1987; C. Speciale and R. Schwarcz, unpublished observations). The present study pursues this analysis to the cellular level by examining KYN uptake in cell cultures of astrocytes derived from the cerebra of newborn mice. These experiments were conducted also in the context of an ongoing study of neutral amino acid transport in mouse astrocytes.

In characterizing KYN transport in astrocytes, we have made use of criteria developed largely by Christensen and colleagues in their extensive studies of amino acid transport in peripheral cells (Christensen, 1984, 1985). The structure of KYN admits the possibility of transport with or without net positive charge. Ubiquitous, usually low-affinity transport systems with overlapping substrate specificities have been described, including system y^+ for Na^+-independent transport of cationic amino acids, systems A and ASC for Na^+-dependent transport of most neutral amino acids, and system L, which is a Na^+-independent exchanger that prefers branched-chain or aromatic neutral amino acids (Christensen, 1984, 1985). Synthetic model substrates useful in differentiating these systems include α-aminoisobutyric acid (AIB) and α-methylaminoisobutyric acid (MeAIB), which are preferred by system A, and 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH), which is preferred by system L.

Received July 25, 1988; revised Nov. 14, 1988; accepted Nov. 19, 1988.

This work was supported by a Fogarty Fellowship (to C.S.), NIH Grants NS 16102 (to R.S.), and ES 03928 (to N.B.), and by institutional Special Research Initiative Support. We wish to thank Yvonne Logan for preparation of cell cultures, Scott Lesser for technical assistance, and Dr. H. R. Zielke for the glutamine assays.

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A recent study of AIB transport in mouse brain astrocytes revealed characteristic system A transport and provided evidence suggestive of a system L variant of unusually high affinity (Brookes, 1988). The present study shows that KYN is concentratively transported into astrocytes by a Na^+-independent exchanger with high affinity for branched-chain and aromatic neutral amino acids. The substrate specificity and high affinity of this transport system resemble the properties of neutral amino acid transport across the blood–brain barrier in the rat (Smith et al., 1987) and in the human (Choi and Pardridge, 1986).

Parts of this study were presented in abstract at the conference "Excitatory Amino Acids '88" (Speciale et al., 1988), and were communicated at the Annual Meeting of the Society for Neuroscience (Hares et al., 1988).

Materials and Methods

Cell culture. The preparation of astrocyte cell cultures from the cerebral hemispheres of 1-d-old outbred mice (CD-1, Charles River), and the criteria for cell identification, were as previously described in detail (Brookes and Yarowsky, 1985; Yarowsky et al., 1986). Briefly, aliquots of a screened cell suspension of triturated cerebra were plated in collagen-coated 35 mm dishes (Nunc) or 12 × 24 mm multiwell plates (Linbro). The cultures were maintained in modified Eagle medium (MEM #82-0234, GIBCO) supplemented with 15% fetal calf serum (Hazleton/KC) and were grown to confluence (12–15 d) at 35.5°C in a water-saturated atmosphere of 10% CO_2/90% air. Confluent cultures 12–28 d old were used for the uptake experiments.

Purification and assay of KYN by HPLC. Samples for purification or assay by high-performance liquid chromatography (HPLC) were injected into a C_{18} reversed-phase column (100 × 3.2 mm i.d., 3 μm particle size; Bioanalytical Systems). KYN was eluted isocratically with a mobile phase consisting of 50 mM ammonium acetate and 5% methanol at a flow rate of 0.5 ml/min. Under these conditions, KYN (retention time of approximately 3.5 min) was discriminated from metabolites 3-hydroxykynurenine, anthranilic acid, kynurenic acid, and quinolinic acid. KYN was detected spectrophotometrically to a sensitivity limit of approximately 1 pmol using an ultraviolet absorbance detector (Beckman 160) set at 340 nm and an integrator (Hewlett Packard 3390A).

^{3}H-L-kynurenine (^{3}H-KYN, 8.9 Ci/mmol, custom-prepared by tritium exchange at Amersham Corp.) was purified by HPLC before use in uptake experiments. The radioactive material in elution fractions corresponding to the KYN peak was found to be stable for at least 2 weeks when lyophilized in batches of 20 μCi and stored at −20°C.

A chiral HPLC column (Alltech, Resolvosil-BAS-7) was used to examine the enantiomeric composition of samples of purified ^{3}H-KYN. D-Kynurenine was kindly provided as sulfate by Drs. W. P. Todd and B. K. Carpenter (Department of Chemistry, Cornell University). The retention times of D-kynurenine and L-kynurenine standards were 3.5 and 18.5 min, respectively, using a mobile phase consisting of 50 mM phosphate buffer (pH 8.1) and 2% *n*-propanol, with a flow rate of 1 ml/min, and ultraviolet detection at 264 nm. By this method, repurified ^{3}H-KYN was found to contain 82% L-enantiomer. Assumption of absolute stereospecificity of KYN transport would require upward revision of rates of uptake reported in this study by a factor of 1.22. However, the inhibition studies reported here suggest the possibility of only relative stereospecificity, in which case the correction factor would be variably smaller.

HPLC was used to assay for endogenous KYN content in astrocyte cultures and to identify tritiated or unlabeled KYN in some uptake experiments (see below). Cultures were washed with ice-cold buffer and harvested into 1 ml volumes of distilled water. Following sonication and centrifugation, 100–200 μl samples of the supernatant were applied to the C_{18} column system described above. Radioactivity recovered in fractions corresponding to the KYN peak was measured by liquid-scintillation counting.

KYN uptake and exodus. Immediately before measurement of uptake the cultures were equilibrated for 30 min at 34.5°C in a buffered salts solution (buffer) containing (mM) NaCl, 150; KCl, 3; $CaCl_2$, 2; $MgCl_2$, 0.8; dextrose, 5; and HEPES acid, 10. The pH of the buffer was adjusted to 7.4, or other required value in the range pH 7–9, by addition of Tris base. For pH adjustment in the range pH 5–7, HEPES-Tris (10 mM) was replaced with Na acetate-acetic acid. The osmolarity was adjusted to 320 mOsmol (the osmolarity of serum-supplemented MEM) by addition of sucrose. Na^+-free buffer was prepared by isosmotic substitution of choline chloride for NaCl. K^+ concentration was varied by isosmotic substitution of KCl for NaCl.

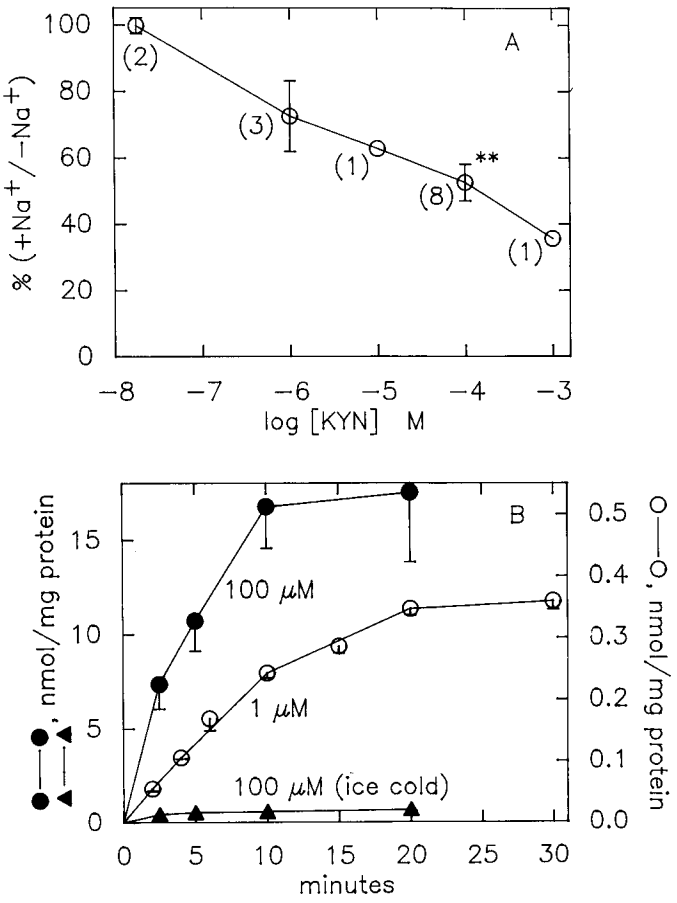

Figure 1. *A*, KYN uptake in the presence of Na^+, expressed as a percentage of uptake in the absence of Na^+. The cumulative data are shown for 15 experiments in which 10 min uptake was measured with and without Na^+ at various concentrations of KYN. The plotted values are means ± SEM for the number of experiments indicated in parentheses, each experiment employing 2–3 replicate culture wells. At 100 μM, KYN uptake was significantly less in the presence of Na^+ (** $p < 0.005$, paired *t* test) than in its absence. *B*, Time course of KYN uptake in the absence of Na^+. The concentration of KYN is indicated for each plot. The plotted values are means ± SD for triplicate cultures.

Uptake was initiated by draining the cultures and adding 1 ml (or in some experiments, 0.5 ml in 24 mm wells) of prewarmed buffer containing 1–100 μM KYN with approximately 0.15 μCi ^{3}H-KYN tracer. Following an incubation of 1–30 min, uptake was terminated by washing the cultures with 4 × 2 ml vol of ice-cold buffer. The cells were lysed with ice-cold 0.3 M perchloric acid, and the ^{3}H content of the lysate was measured by liquid-scintillation counting at an efficiency of about 40%. After further washing with ice-cold 0.3 M perchloric acid, the culture residues were dispersed in 0.2 M NaOH/1% sodium lauryl sulfate and their protein content determined by the method of Lowry et al. (1951), using BSA as the protein standard. Measurements of KYN uptake with 10 min incubations are likely to reflect changes in either initial velocity of KYN entry or in steady-state distribution (Figs. 1, 4, 5; Table 1), whereas 1–2 min incubations estimate primarily initial velocity (Fig. 3, Table 2). The clearance of tracer from the incubation solution during uptake did not exceed 5%.

In experiments to measure net exodus of KYN, cultures were reincubated in the absence of ^{3}H-KYN following the above loading and washing steps. After further cold washes, residual ^{3}H content in the cultures was measured in the lysate. Na^+-independent uptake of α-1-^{14}C-aminoisobutyric acid (50 mCi/mmol, Dupont NEN) was mea-

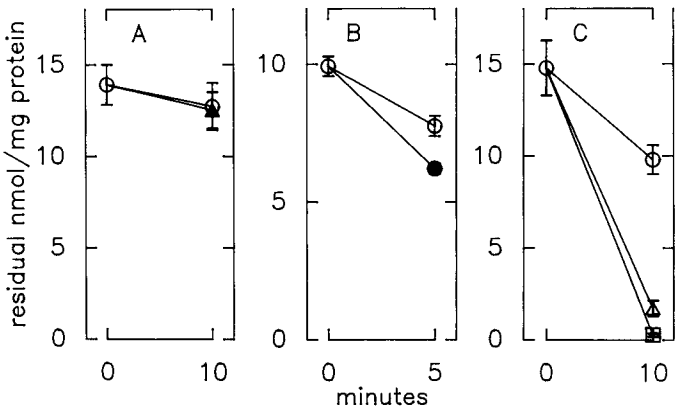

Figure 2. Effect of: *A,* cold; *B,* extracellular Na^+ ions; and *C,* extracellular KYN, on ^{3}H-KYN exodus. Cultures were loaded by incubation with 100 μM ^{3}H-KYN in Na^+-free buffer for 10 min. The ^{3}H-KYN content of the loaded cultures is shown at time zero. Residual ^{3}H-KYN content was measured after a further period of exposure to: *A,* ice-cold Na^+-free buffer alone (*open circle*) or containing 1 mM KYN (*filled triangle*); *B,* Na^+-free buffer at 34.5°C (*open circle*) compared with regular buffer (*filled circle*; significantly different, see text); and *C,* Na^+-free buffer control at 34.5°C (*open circle*) compared with 100 μM KYN (*open triangle*) and 1 mM KYN (*open square*) in Na^+-free buffer. Plotted values are means ± SD for 3–4 replicate cultures (*A, C*) or means ± SEM for 3 experiments each employing triplicate cultures (*B*).

sured as above in experiments to determine the inhibitory effect of KYN. SD is used to indicate the scatter of values determined in individual cultures, whereas SEM indicates the scatter of means obtained in replicate experiments. L-Kynurenine and amino acids used for analog inhibition experiments were obtained from Sigma Chemical Co.

Results

Effect of Na^+ and time course of KYN uptake

KYN uptake (10 min incubation) was compared in regular buffer and in Na^+-free buffer for a range of KYN concentrations. In several of these experiments, the amount of KYN taken up was less in the presence of Na^+ than in its absence. On plotting the cumulative results of 15 such experiments (Fig. 1*A*), it became apparent that KYN uptake in the presence of Na^+, expressed as a fraction of uptake in Na^+-free buffer, decreased as KYN concentration increased. Because the initial velocity of uptake was not sustained for 10 min at KYN concentrations above 1 μM (Figs. 1*B,* 3*A*), the effect of Na^+ depicted in Figure 1*A* could be caused either by decreased influx, by increased efflux, or both. Nevertheless, KYN uptake may be described as Na^+ independent in the conventional sense of not requiring Na^+.

The time course of uptake of KYN (1 and 100 μM) to steady state in Na^+-free buffer is shown in Figure 1*B,* which also shows that uptake of 100 μM KYN was almost completely inhibited when the cultures were cooled over ice. In 3 such experiments, the uptake of KYN (1 and 100 μM, for incubation times of 2.5–20 min) was inhibited 94–97% by cooling, which indicates that the contribution of influx by nonfacilitated aqueous diffusion was not large.

The near steady-state levels of KYN shown in Figure 1*B,* 0.36 nmol/mg protein (1 μM KYN) and 17.5 nmol/mg protein (100 μM KYN), suggest a remarkable degree of concentration of KYN by the astrocytes (see "Identity of tritiated species" below). A "solute accessible" cell water content of 2 μl/mg protein was measured in astrocyte cultures using urea as a passively distributed solute (Brookes, 1988). Based on this estimate, which is similar to values published for several cell types (Kimmich,

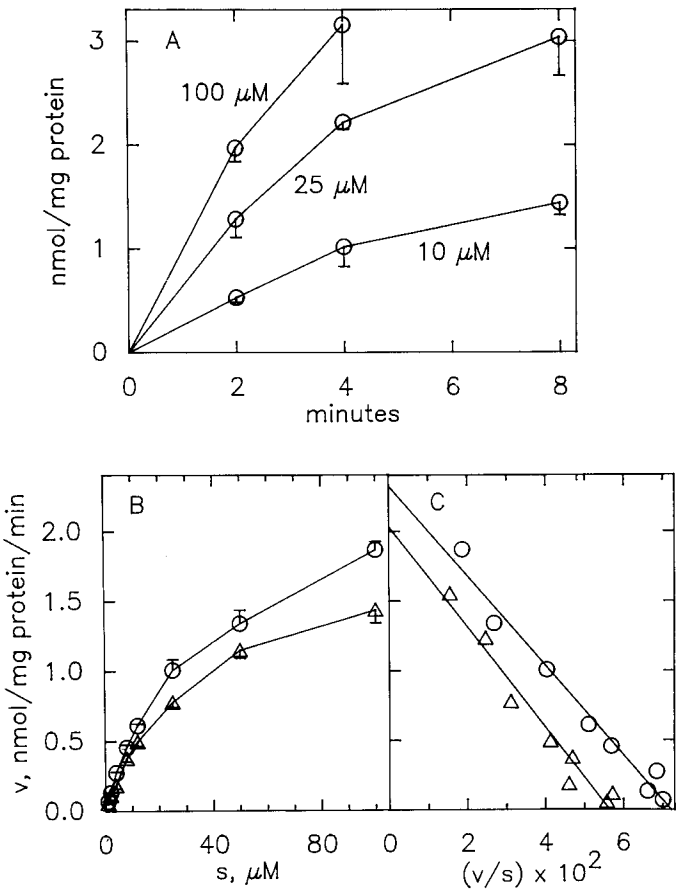

Figure 3. Initial velocity (*v*) of KYN uptake as a function of KYN concentration (*s*) in regular buffer. *A,* Initial time course of KYN uptake at concentrations of 10, 25, and 100 μM. Plotted values are means ± SD for triplicate cultures. *B,* Direct plots of initial velocities determined by 2 min incubations (*open circles*) and by 1 min incubations (*open triangles*). Plotted values are means ± SD for 3–6 replicate cultures. *C,* Eadie-Hofstee transformations of the same data, showing unweighted linear regression, indicate similar kinetic constants for both incubation times.

1975; Betz et al., 1979; Martin and Shain, 1979; Weissbach et al., 1982), intracellular KYN concentrations of 0.18 and 8.8 mM were obtained, corresponding to distribution ratios of 180 for 1 μM KYN and 88 for 100 μM KYN, respectively.

Endogenous kynurenine in the astrocytes, before or after 30 min incubation in buffer, was not detectable by HPLC when the entire culture lysate was reduced in volume and injected as a single sample. Based upon a detection sensitivity of 1 pmol and cell water content of 2 μl/mg protein, this places an upper limit of 2.5 μM on the intracellular concentration of endogenous kynurenine. Thus, the initial velocities of KYN uptake observed in these experiments do not reflect homoexchange, although they may reflect exchange with other endogenous amino acids, as discussed below.

Identity of tritiated species

The uptake of KYN (10 μM in Na^+-free buffer) was determined by tracer and HPLC methods comparatively in the same batch of cultures. Tracer uptake yielded 2.39 ± 0.19 nmol/mg protein (±SD, n = 4) at 10 min and 2.75 ± 0.69 nmol/mg protein at 30 min, compared with HPLC values of 2.71 ± 0.05 nmol/mg protein at 10 min and 3.66 ± 1.20 nmol/mg protein at 30 min.

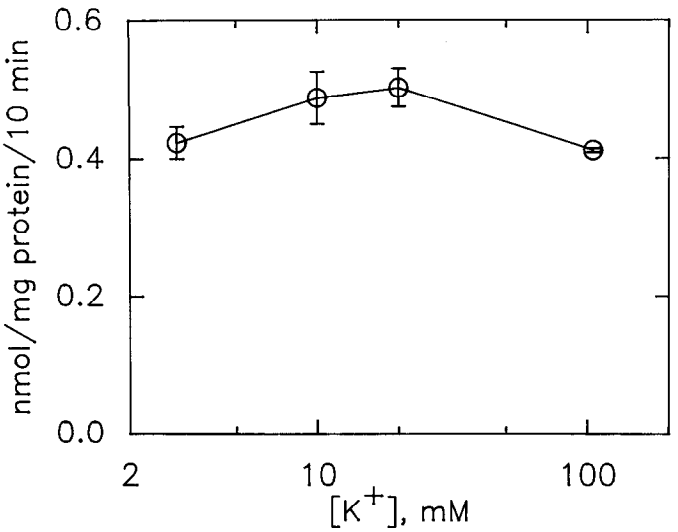

Figure 4. Effect of K^+ concentration on uptake of KYN (1 μM, 10 min). Plotted values are means ± SD for triplicate cultures.

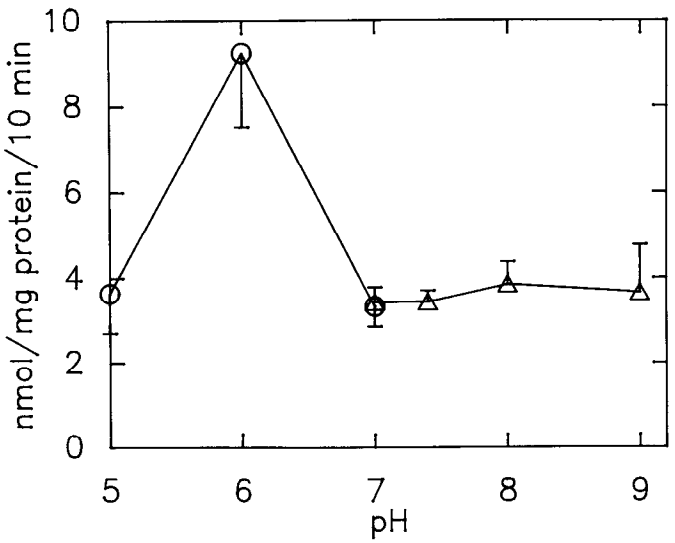

Figure 5. Effect of pH on uptake of KYN (30 μM, 10 min). Solutions were buffered with Tris-HEPES (pH 7–9, *open triangles,* upward error bars) or sodium acetate (pH 5–7, *open circles,* downward error bars). Plotted values are means ± SD for 12 replicate cultures.

Corresponding values for uptake in ice-cold buffer at 30 min were 0.13 ± 0.01 nmol/mg protein using tracer and 0.10 ± 0.01 nmol/mg protein by HPLC. In addition, total dpm recovered in the lysate did not differ from dpm recovered in the "kynurenine fraction" eluted from the HPLC column. It is assumed, therefore, that the tritium recovered and counted in this study represented substantially unmetabolized KYN.

Rates of KYN exodus

There was no significant loss of ^{3}H-KYN into ice-cold buffer, with or without addition of 1 mM unlabeled KYN, during 10 min following loading of cultures under the usual uptake conditions (Fig. 2*A*). Thus, extracellular uptake may be considered negligible, and the appreciable exodus observed at 34.5°C (Fig. 2, *B, C*) is assumed to be carrier-mediated.

The experiment shown in Figure 2*B* supports the possibility that reduced uptake of KYN in the presence of Na^+ (Fig. 1*A* above) was related to increased efflux. The fraction of KYN leaving the cultures after 5 min at 34.5°C was 37% in regular buffer compared with 22% in Na^+-free buffer ($p < 0.02$, paired t test, $n = 3$).

Inclusion of 0.1 or 1 mM unlabeled KYN in Na^+-free buffer greatly accelerated the exodus of ^{3}H-KYN (Fig. 2*C*). Such *trans* stimulation of efflux is characteristic of the exchanger properties of Na^+-independent transport of large neutral amino acids (system L).

Initial velocity and kinetic parameters of uptake

Figure 3*A* shows rates of uptake of KYN (10–100 μM) at short times. The use of uptake times down to 2 min resolved the initial velocity of influx unequivocally only for KYN concentrations of 10 μM or below. Therefore, initial velocities of uptake were estimated for KYN concentrations in the range 1–100 μM using uptake times of both 1 and 2 min. The saturation curves obtained are shown in Figure 3*B*. Mixing and diffusion delays may introduce significant error when the uptake time is reduced to 1 min, whereas it becomes uncertain that the initial velocity is sustained for 2 min as concentration increases (Fig. 3*A*). Thus, mutual corroboration of the kinetic parameters determined using both uptake times is useful to support their validity. Figure 3*C* shows that both saturation curves were linearized by Eadie-Hofstee transformation, in conformity with a single saturable uptake process, and that the regression lines exhibited similar slopes and intercepts. The apparent K_m was 32 ± 3 μM (SEM, 3 experiments) and V_{max} was 2.1 ± 0.2 nmol mg^{-1} protein min^{-1} estimated by linear regression of Eadie-Hofstee plots, with bias correction according to Zivin and Waud (1986). K_m values for Na^+-independent transport of large neutral amino acids in cells derived from peripheral tissues are generally 1–2 orders of magnitude greater than observed here for KYN (Oxender and Christensen, 1963).

Effect of K^+ and H^+ concentrations

Protonation of the distal (aromatic) amino group endows KYN with net positive charge. By analogy with other ortho-substituted anilines, it can be estimated that this amino group is only 1–2% ionized at neutral pH. It is conceivable, though unlikely, that this degree of ionization could enable KYN uptake to be driven by the gradient of membrane potential or by a gradient of pH. To examine the effect of membrane potential on KYN uptake, the K^+ concentration in the buffer was varied between 3 and 100 mM (Fig. 4). The membrane potential of glia responds to extracellular K^+ concentration in approximate accord with the Nernst relationship for a K^+-selective electrode (Kuffler and Nicholls, 1966). However, changing the K^+ concentration did not affect the initial velocity of uptake of 1 μM KYN (Fig. 4).

The effect of pH change in the buffer (Fig. 5) was examined on 10 min uptake of 30 μM KYN, reflecting net effect on both influx and efflux near steady state. KYN uptake increased at pH 6, as is characteristic for Na^+-independent transport of large neutral amino acids. However, KYN uptake decreased markedly at pH 5, perhaps because the cationic form of KYN is less effectively transported (Im and Christensen, 1976), but also possibly because of increased efflux.

Analog inhibition

Table 1 shows the extent of inhibition of KYN uptake by amino acids present in excess concentration (10 mM). The large neutral

Table 1. Inhibition of L-kynurenine uptake by excess amino acids

Amino acid (10 mM)	Percentage of control uptake of ^{3}H-L-kynurenine (30 μM, 10 min)
Alanine[a]	15.5 ± 1.8
Arginine[a]	76.4 ± 4.4
Aspartate[a]	130.6 ± 4.4
BCH[a]	6.2 ± 0.9
GABA	134.0 ± 1.3
Glutamate	81.7 ± 3.9
Glutamine[a]	13.8 ± 0.05
Glycine[a]	28.1 ± 0.8
Histidine	6.4 ± 0.4
D-Kynurenine	26.7 ± 2.7
Leucine[a]	5.9 ± 0.2
Lysine	14.2 ± 0.5
MeAIB[a]	105.5 ± 0.2
Phenylalanine[a]	5.9 ± 1.4
Serine	13.5 ± 1.0
Taurine	110.4 ± 0.5
Threonine[a]	11.8 ± 1.9
D-Tryptophan[a]	5.3 ± 0.05
L-Tryptophan[a]	4.7 ± 0.01

Values are means ± SEM for 2–3 experiments, each employing 3–4 replicate cultures.

[a] Amino acid inhibitors correlated in Figure 6.

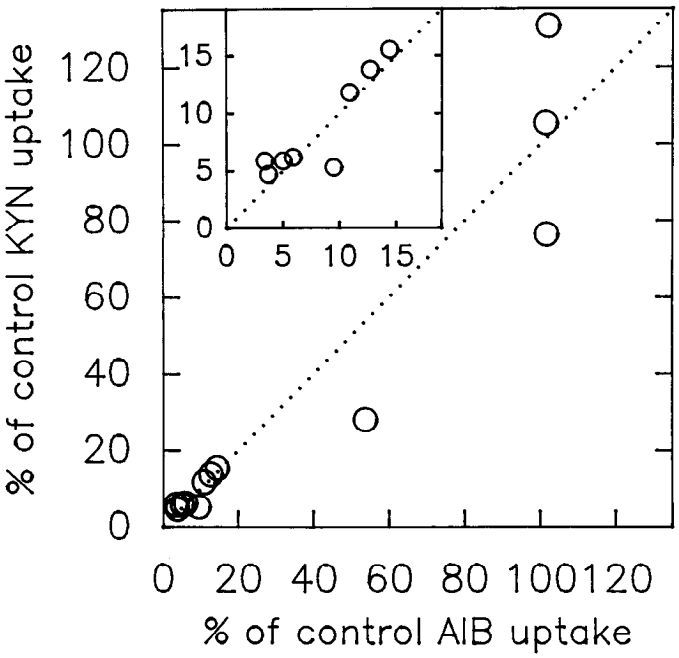

Figure 6. Correlation of the inhibitory potency of 12 amino acids (10 mM; identities indicated in Table 1) on the uptake of AIB versus KYN. This figure was generated from data shown in Table 1 of this manuscript and Table 1 of Brookes (1988). *Inset,* Expansion of the same plot for points <20% of control. The *dotted lines* are diagonals.

amino acids associated with Na^+-independent system L (leucine, tryptophan, phenylalanine, and the model substrate, BCH) almost completely inhibited KYN uptake. D-Tryptophan was unexpectedly potent, but D-kynurenine was less so, indicating stereoselectivity in the recognition of KYN (see also Table 2). Alanine, glycine, serine, and threonine, which are transported preferentially by Na^+-dependent systems A and ASC, and less efficiently by system L, were moderately effective inhibitors. However, MeAIB, an N-methylated model substrate for system A, was ineffective, as were aspartate, glutamate, GABA, and taurine, which are transported by substrate-specific, high-affinity systems in astrocytes (Schousboe et al., 1976, 1977a, b). The diamino acids (arginine, glutamine, histidine, and lysine) varied widely in inhibitory potency: System y^+ substrate arginine was weakly inhibitory, implying that KYN is not transported significantly as a cation at pH 7.4, whereas histidine was as potent as other system L-preferring substrates.

Table 2. Inhibition of the initial velocity of uptake of L-kynurenine (30 μM) by equal concentrations of amino acids

Amino acid (30 μM)	Percentage of control uptake of ^{3}H-L-Kynurenine (30 μM, 2 min)
BCH	64.6 ± 3.0
D-Kynurenine	112.3 ± 3.2
L-Kynurenine	68.4 ± 3.1
Leucine	51.2 ± 4.9
Phenylalanine	45.7 ± 5.9
D-Tryptophan	89.2 ± 4.0
L-Tryptophan	60.5 ± 4.1
Tyrosine	78.6 ± 8.1

Values are means ± SEM for 2–4 experiments, each employing 3–4 replicate cultures.

Because recent evidence suggests that AIB, a nonmetabolized model substrate for system A, is transported also by a high-affinity variant of Na^+-independent system L in astrocytes (Brookes, 1988), the inhibitory effect of 10 mM KYN was examined on Na^+-independent uptake of ^{14}C-AIB (0.2 mM, 20 min). In 2 experiments ^{14}C-AIB uptake was reduced to 5.4 and 7.0% of control values. In Figure 6, analog inhibition data for AIB from the previous study show a good correlation with the corresponding data for inhibition of KYN uptake by 12 amino acids from Table 1, suggesting that KYN and AIB share a common Na^+-independent transport mechanism (Christensen, 1985) despite marked differences in structure and affinity for the carrier.

A more accurate (though still tentative) measure of the relative affinity of substrates for the KYN transporter was obtained by employing amino acids at a concentration close to the K_m value for KYN and abbreviating the uptake time to measure initial velocity of KYN uptake (Table 2). Allowing that inhibitory potency does not necessarily correspond to relative substrate affinity, it appears that leucine and phenylalanine were recognized with highest affinity by the transporter, yielding anticipated K_m values about half of the K_m for KYN.

Discussion

We show that KYN is transported with high affinity and stereospecificity in mouse cerebral astrocytes by a Na^+-independent carrier that prefers branched-chain and aromatic neutral amino acids. Further, the analog inhibition data suggest that the transporter of these large neutral amino acids in astrocytes resembles the high-affinity L1 system described for cultured rat hepato-

cytes rather than the widespread low-affinity L system (Weissbach et al., 1982). In this respect the astrocytes behave similarly to the blood–brain barrier in the rat and human (Choi and Pardridge, 1986; Smith et al., 1987).

The KYN carrier functioned as an exchanger (intracellular ^{3}H-KYN was rapidly displaced by unlabeled extracellular KYN) and yet was able to accumulate KYN against a large concentration gradient. Similar observations were made for Na^+-independent uptake of AIB in astrocytes (Brookes, 1988). Unusually concentrative uptake of amino acids by L system transport has been noted and investigated previously (Christensen, 1972). The accumulation of L system substrates generally results from asymmetric recognition by the transporter at the inner and outer faces of the membrane, combined with a driving force such as a proton gradient. Indifference to external K^+ and weak inhibition by the basic diamino acid arginine make it clear that KYN is not transported by system y^+ as a cation driven by transmembrane potential. Although there is some evidence for comigration of H^+ and L system substrates (Christensen et al., 1974), the buffering of cytoplasmic pH (Madshus, 1988) prevents formation of an H^+ gradient sufficient to drive the KYN distribution ratios of 90 and above observed here for astrocytes in pH 7.4 buffer.

Countertransport of endogenous free amino acids offers another possible driving force for concentrative transport of KYN. For instance, Cangiano et al. (1983) observed that Na^+-dependent accumulation of glutamine in brain microvessels *trans* stimulated Na^+-independent uptake of large neutral amino acids. Astrocytes are distinguished by a remarkably high content of free glutamine (for example, Patel and Hunt, 1985). The free glutamine content of the mouse astrocyte cultures used in this study was measured in the laboratory of Dr. H. R. Zielke by HPLC assay (Zielke, 1985). Cultures contained 190 ± 7 nmol glutamine/mg protein (SEM, $n = 3$) 1 d after medium change. This concentration declined to 10% of control after 30 min equilibration in regular buffer, but only to 44% in Na^+-free buffer. Glutamine was moderately effective as an inhibitor of KYN transport, which suggests that it is accepted by the KYN carrier. Other available evidence concerning glutamine uptake in astrocytes is less supportive of a countertransport role for glutamine. Notably, intracellular glutamine tracer does not exchange with extracellular unlabeled glutamine either in mouse astrocytes (Schousboe et al., 1979) or in rat astrocytes (Ramaharobandro et al., 1982). However, this result does not exclude the possibility of exchange of KYN with intracellular glutamine or with other endogenous L system substrates (Christensen, 1972).

Although Na^+-independent uptake of KYN was observed in rat brain slices (Speciale et al., 1987), the low affinity of that process ($K_m = 1.0$ mM; C. Speciale and R. Schwarcz, unpublished observations) raises the question of whether the high-affinity transport reported here is peculiar to the mouse or perhaps to the conditions of cell culture. Efflux of endogenous free amino acids into the confines of the interstitial space conceivably could account for the observation of apparent low affinity in the slice, as substrate competition is pronounced, Emergence of high-affinity transport in mouse astrocytes during cell culture, as originally described for the L1 system in rat hepatocytes (Weissbach et al., 1982), is a further possibility that cannot be discounted.

It may be inferred from this study that KYN is transported into mouse astrocytes, as across the blood–brain barrier of the rat, in competition with large neutral amino acids such as leucine, phenylalanine, and tryptophan. KYN was not rapidly metabolized within the astrocytes but does exchange freely across the astrocyte membrane for metabolism at other sites. Kynurenine hydroxylase activity (which yields 3-hydroxykynurenine) and kynurenine transaminase activity (which mediates formation of kynurenic acid) are both present in rat brain (Minatogawa et al., 1974; Battie and Verity, 1981). On the basis of the present evidence, these enzyme activities are very weak or suppressed in mouse astrocytes in culture.

The implications of the high-affinity Na^+-independent transport system described here, if it is representative of *in vivo* function, extend beyond the kynurenine pathway of tryptophan metabolism to the general economy of large neutral amino acids in the CNS. Such a transport system would play an important role in maintaining low interstitial concentrations of large neutral amino acids, and, given the close apposition of astroglia to cerebrovascular endothelium, would exert a significant influence on blood–brain barrier function. It remains to be determined whether substrates other than KYN and AIB exhibit similarly concentrative transport and what is the nature of the driving force that generates large concentration gradients of amino acids transported by this Na^+-independent exchanger.

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