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Biochimica et Biophysica Acta 1192 (1994) 95–100

BBA Biochimica et Biophysica Acta

Expression of Na^+-independent isoleucine transport activity from rat brain in *Xenopus laevis* oocytes

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(Received 19 January 1994)

Abstract

Poly(A)$^+$ RNA from C6-BU-1 rat glioma cells and rat astroglial cells induced isoleucine transport activity when injected into *Xenopus laevis* oocytes. The Na^+-independent component of isoleucine transport was inhibited by leucine, phenylalanine and 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH) but neither by methylaminoisobutyric acid (MeAIB) nor lysine. A K_m value of approx. 100 μM was determined for the Na^+-independent transport of isoleucine. These data are in accordance with expression of a system L like transporter. By injection of size fractionated poly(A)$^+$ RNA a length of approx. 1.9 kb was determined for the pertinent mRNA.

Key words: Amino acid transporter; Oocyte; Expression; C6-BU-1 cell; Astroglial cell

1. Introduction

It is now recognized that astroglial cells serve an important function in energy metabolism of the brain. The endfeet of astroglial cells cover the brain capillaries, and astroglial lamellae are in contact with practically all neurons. It is tempting to speculate that most substrates pass through astroglial cells after having passed the blood brain barrier formed by the capillary endothelial cells. Of all amino acids, the brain consumes considerable amounts only of branched chain amino acids [1]. These amino acids pass through the blood brain barrier by means of the system L amino acid transporter [2]. For uptake astroglial cells possess the same system [3]. Transport of neutral amino acids by systems A and L was described for the first time by Oxender and Christensen in 1963 [4]. Later additional transport systems for neutral amino acids, namely systems ASC, asc, T, $B^{0,+}$ and $b^{0,+}$ [5–7] were identified. Two criteria are mainly used for the discrimination of amino acid transport systems: first the dependence on sodium ions and second inhibition by other amino acids and analogues. This approach has been reviewed and updated recently [5,8]. The molecular nature of the system L transporter has not been resolved.

Expression cloning by using *Xenopus laevis* oocytes is one of the methods which have been used successfully in cloning of several transport proteins. The first step in this procedure is the identification of an mRNA fraction which codes for the desired transporter. Induction of Na^+-independent neutral amino acid transport activity was recently detected after injection into oocytes of mRNA from rabbit kidney cortex [9,10], rat kidney [11,12], Chinese hamster ovary (CHO) cells [13], human lymphoid cells [11] and rabbit intestine [14–16]. So far characterized these transporters showed features not expected for a system L transporter but typical for system $b^{0,+}$ [17], in particular inhibition by cationic amino acids.

Several amino acid transport systems or proteins stimulating these transport activities have been cloned recently. System $b^{0,+}$ was cloned from human [18], rabbit [19] and rat kidney [20], system A from LLC-PK_1 cells [21], a system similar to ASC from whole brain [22] and hippocampus [23], and system y^+ from mouse [24,25]. A protein stimulating y^+ activity was identified as a human cell surface antigen [26]. Cloning of the system L transporter was claimed recently [27]. Se-

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Abbreviations: BCH, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid; AIB, aminoisobutyric acid; MeAIB, methylaminoisobutyric acid; Hepes, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid.

SSDI 0005-2736(94)00043-O

quence comparison with other amino acid transport systems which were cloned later, however, led to a reassignment to system $b^{0,+}$ [28]. A different group of amino acid transporters was cloned by searching for neurotransmitter transporters. These include now glutamate, proline, GABA, β-alanine, betaine, taurine and the amines, serotonin, adrenaline, dopamine (see Ref. [29] for a review).

In this report we demonstrate induction of a system L like transport activity after injection of mRNA from rat C6-BU-1 glioma or rat astroglial cells. We also found system ASC like transport activity which is in accordance with the recent cloning of system ASC from human brain and hippocampus [22,23]. Expression of system L like transport activity in oocytes is the first step in cloning this transporter from rat brain astroglial cells.

2. Materials and methods

2.1. Materials

L-[U-^{14}C]Isoleucine, L-[U-^{14}C]valine, L-[U-^{14}C]alanine and L-[2,3,4,5,6-^{3}H]phenylalanine were purchased from Amersham Buchler, Braunschweig (Germany). Oligo(dT)cellulose was purchased from New England Biolabs, Schwalbach (Germany); BCH, MeAIB and Sephacryl S-400 HR from Sigma, Deisenhofen (Germany), silica gel thin-layer chromatography plates (DC Karte SI) from Riedel de Haen, Seelze (Germany), collagenase (EC 3.4.24.3; 0.6–0.8 U/mg from *Clostridium histolyticum*) from Serva, Heidelberg (Germany), gentamycin from Boehringer, Mannheim (Germany), Ultima Gold scintillation cocktail from Canberra Packard, Frankfurt (Germany). Guanidinium thiocyanate was supplied by Roth, Karlsruhe (Germany); all solutions containing this compound had to be filtered (Millipore 0.22 μm) before use. All other chemicals were of analytical grade and supplied by E. Merck, Darmstadt (Germany).

2.2. Cell culture

Astroglia-rich primary cultures, derived from brains of neonatal Wistar rats, were prepared and cultured as described by Hamprecht and Löffler [30]. Rat glioma cells C6-BU-1 were cultured according to Amano et al. [31].

2.3. Isolation and fractionation of poly(A)$^+$ RNA

Total RNA was isolated from astroglia-rich primary cultures or C6-BU-1 cells by the 'acid-guanidinium-thiocyanate-phenol-chloroform extraction' method of Chomczynski and Sacchi [32]. Poly(A)$^+$ RNA was than isolated by two passages over an oligo(dT)cellulose column [33]. Poly(A)$^+$ RNA generally had a ratio of A_{260}/A_{280} of 2.0. Purification against ribosomal RNA and chromosomal DNA was controlled by agarose gel electrophoresis under denaturing conditions [33]. Poly(A)$^+$ RNA was size-fractionated by gel filtration on Sephacryl S-400 HR. A 5 ml column was poured and equilibrated in TE-buffer (10 mM Tris-HCl, 1 mM EDTA (pH 7.5)). Next a mixture of 150 μl poly(A)$^+$ RNA (1 mg/ml) and 150 μl formamide was applied to the column. The column was eluted with TE-buffer and 250 μl fractions were collected. RNA was precipitated by addition of 25 μl 2 M sodium acetate (pH 4) and 250 μl isopropanol.

2.4. Oocytes and injections

Xenopus laevis females were obtained from H. Kähler (Institut für Entwicklungsbiologie, Hamburg). The animals were anesthesized and ovarian lobes surgically removed using standard procedures [34]. Oocytes were defolliculated by collagenase treatment (2 mg/ml) in OR2$^-$ buffer (82.5 mM NaCl, 2.5 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM Na_2HPO_4, 5 mM Hepes (pH 7.8)) [34]. Collagenase was removed carefully by washing cells several times with OR2$^-$. Oocytes were incubated at 18°C overnight in OR2$^+$ buffer (OR2$^-$ supplemented with 1.0 mM $CaCl_2$). Healthy looking stage V/VI oocytes (animal pole evenly coloured, sharp border between both poles, see Ref. [34]) were selected on the following day for injection. 50 nl of mRNA (approx. 1 ng/nl) or H_2O were injected by a microinjection device (Bachhofer, Reutlingen, Germany). Oocytes were incubated for 1–6 days at 18°C in OR2$^+$ containing gentamycin (10 mg/l).

2.5. Uptake measurements

For each point in figures and tables, groups of 7 healthy looking oocytes (mRNA or H_2O injected) were washed with OR2$^-$ buffer and then incubated in a 5 ml polypropylene tube containing 70 μl of the same buffer supplemented with 7 kBq radioactive substrate (e.g., isoleucine, valine, alanine, phenylalanine) and different amounts of unlabelled substrate. In Na^+-free buffer NaCl was replaced by choline chloride or LiCl. Generally, transport experiments proceeded for 1 h at 25°C, if not indicated otherwise. Transport was stopped by washing cells three times with 5 ml ice-cold OR2$^-$ buffer. The seven oocytes of each determination were placed seperately into scintillation vials and lysed by addition of 200 μl 10% SDS. After lysis 3 ml scintillation fluid was added and radioactivity determined in a liquid scintillation counter (LKB, Freiburg, Germany).

2.6. Thin-layer chromatography (TLC)

Samples for TLC were prepared from transport assays as described above, but with four oocytes. After

aspiration of the last washing buffer 100 μl water was added, and the oocytes were homogenized by several passages through a 200 μl pipet tip. The homogenate was boiled for 10 min and then centrifuged for 5 min in a table top centrifuge. 20 μl of the supernatant was applied to a silica gel TLC plate. Standard solutions (e.g., isoleucine, α-keto-β-methylvalerate) were applied to the same plate. The mobile phase was composed of butanol/acetic acid/water (67:17:17, v/v). Chromatography ran for 6 h at room temperature. Silica gel was scraped off the plate at spots corresponding to the R_f values of the standards and subjected to liquid scintillation spectrometry. The pellet which remained after boiling of the homogenate was washed once with 1 ml 5% trichloroacetic acid before its radioactivity was measured.

2.7. Calculations

Standard deviations are given for all values. Gauss' law of error propagation was applied when values had to be subtracted. Each experiment presented, was performed at least twice with similar results.

3. Results

Poly(A)$^+$ RNA isolated from C6-BU-1 rat glioma cells induced elevated isoleucine transport activity when injected into *Xenopus laevis* oocytes (Fig. 1 and Table 1). Transport of alanine, valine and phenylalanine also increased after injection of mRNA (Table 1). We found uptake ratios between mRNA and H_2O injected oocytes of two to six. The ratio and absolute values depended on expression time (Fig. 2A) and varied with different poly(A)$^+$ RNA and oocyte preparations. Transport activity was positively correlated with the amount of injected poly(A)$^+$ RNA (Fig. 2B). For the determination of transport parameters only preparations with a more than three-fold stimulation were used.

Before the assessment of kinetic parameters we determined, if metabolism of isoleucine could influence uptake of radioactivity. For this we examined oocyte extracts by thin-layer chromatography. From mRNA-injected oocytes 92% of the radioactivity, which remained in the supernatant after boiling and centrifugation, was found at an R_f value of 0.48. This corresponded to an isoleucine standard applied to the same plate. 3% of the radioactivity was found at $R_f = 0.69$ which corresponded to α-keto-β-methylvalerate; 2% remained at the origin. In water-injected oocytes 45% of the radioactivity was found in the isoleucine spot. 10% was found at $R_f = 0.14$ (unidentified), 15% remained at the start and the rest was evenly distributed. Poly(A)$^+$ RNA-injected oocytes contained 109 pmol and water injected oocytes 17 pmol isoleucine. From poly(A)$^+$ RNA-injected oocytes 87% of the radioactivity remained in the supernatant after boiling and centrifugation; 13% remained in the pellet. From water injected oocytes 66% was soluble and 34% remained in the pellet.

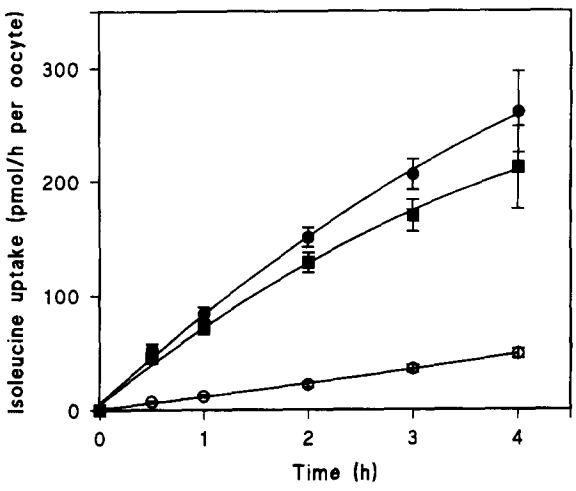

Fig. 1. Uptake of labelled isoleucine by *Xenopus laevis* oocytes. 50 ng of poly(A)$^+$ RNA (1 μg/μl) or 50 nl water were injected into each oocyte. Each point represents the mean of seven oocytes. After incubation for five days, oocytes were washed and incubated for 1 h in medium containing [^{14}C]isoleucine and unlabelled isoleucine at a final concentration of 100 μM. The transport buffer contained 82.5 mM NaCl. Transport was stopped by three washing steps with ice-cold buffer. Uptake induced by poly(A)$^+$ RNA (■) was calculated by subtraction of the transport rate of water injected oocytes (○) from the values of poly(A)$^+$ RNA injected oocytes (●).

Isoleucine transport was mediated by Na^+-dependent as well as by Na^+-independent transporters (Table 2). In the latter case sodium chloride was replaced by choline chloride in the transport buffer. Lithium chloride could also replace sodium chloride but the transport activity was only 66% of the transport activity measured in choline chloride. In competition studies it

Table 1
Poly(A)$^+$ RNA from C6-BU-1 rat glioma cells induced neutral amino acid transport in *Xenopus laevis* oocytes

Amino acid	Expression (days)	Transport activity (pmol/h per oocyte)	
		H_2O injected	mRNA injected
Isoleucine	4	21±2.8	60±6.0
Valine	6	11±0.6	56±4.4
Alanine	6	17±1.5	58±6.2
Phenylalanine	6	31±3.2	69±8.6

Oocytes were injected with approx. 50 ng of poly(A)$^+$ RNA. After incubation for the indicated number of days, oocytes were washed and incubated in fresh buffer. Transport was initiated by addition of transport buffer (OR2$^-$) containing 82.5 mM NaCl and the labelled amino acid and stopped by three washing steps with ice-cold buffer. Each value represents the mean of seven oocytes. Amino acids were present at a concentration of 100 μM in the transport assay.

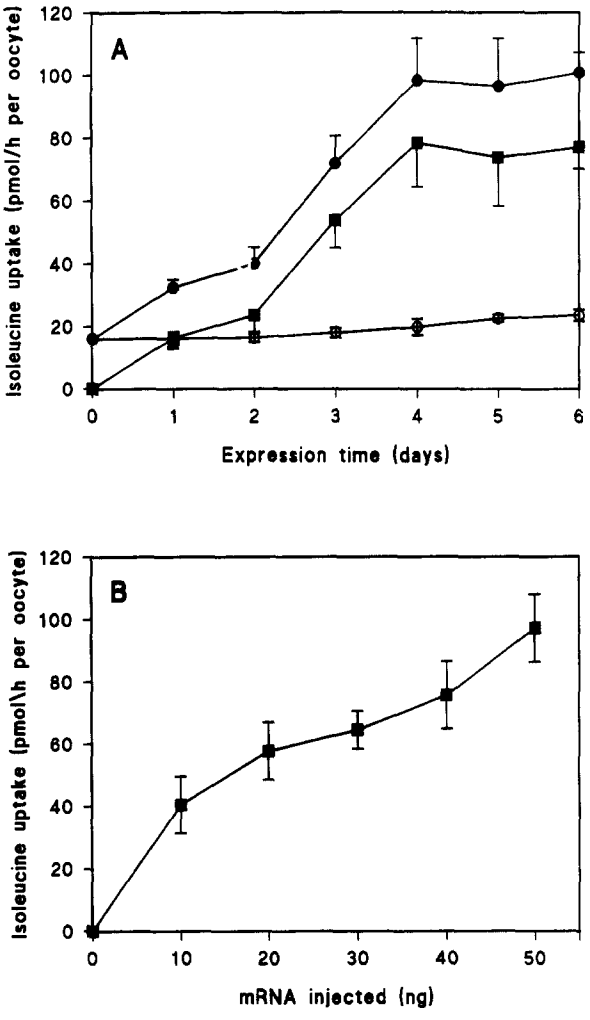

Fig. 2. Dependence of isoleucine transport on the expression time (A) and the amount of injected poly(A)$^+$ RNA (B). Transport was measured as described in Fig. 1, but with variations in the amount of injected RNA or the number of expression days. The transport buffer (OR2$^-$) contained 82.5 mM NaCl. (●) poly(A)$^+$ RNA injected oocytes, (○) water-injected oocytes, (■) difference between poly(A)$^+$ RNA-injected and water-injected oocytes.

was found that the Na$^+$-independent part of isoleucine transport resembled system L amino acid transporter activity: transport of isoleucine was inhibited by BCH, leucine, and phenylalanine, but not by MeAIB or lysine (Table 2). The Na$^+$-dependent part of isoleucine transport showed the same inhibition pattern as expected for a system ASC amino acid transporter: transport was not inhibited by MeAIB or BCH; leucine and to a lesser extent phenylalanine were effective inhibitors (Table 2). Presence of MeAIB even stimulated isoleucine transport in all experiments.

The mixed K_m value of Na$^+$-dependent plus Na$^+$-independent isoleucine uptake was determined to be about 0.8 mM (data not shown). The Na$^+$-independent part of isoleucine transport had a higher affinity for isoleucine, as indicated by a lower K_m value of 119

Table 2
Specificty of Na$^+$-dependent and Na$^+$-independent isoleucine transport in oocytes injected with poly(A)$^+$ RNA from C6-BU-1 cells

Addition	Transport activity (pmol/h per oocyte)		
	Na$^+$-dependent + independent transport	Na$^+$-independent transport	Na$^+$-dependent transport
None	72.0± 4.7	39.0±6.2	33.0± 7.8
+10 mM Leu	1.4± 0.3	1.0±0.5	0.4± 0.6
+10 mM BCH	48.0± 7.7	2.7±0.3	45.3± 7.7
+10 mM MeAIB	102.0± 8.0	42.5±9.4	59.5±12.3
+10 mM Phe	8.8± 0.9	0.5±1.6	8.3± 1.8
+10 mM Lys	59.7±10.2	30.3±5.9	29.4±11.8

Experiments were performed as described with Table 1. Water injected controls were already subtracted. Isoleucine was added at a final concentration of 100 μM. The transport buffer contained either NaCl (Na$^+$-dependent + independent transport) or choline chloride (Na$^+$-independent transport). Na$^+$-dependent transport is the difference between the results of the two assays.

μM (Fig. 3). In a second determination the substrate concentration was varied up to 10 mM and a K_m value of 80 μM was found (data not shown). The transport velocity at an isoleucine concentration of 100 μM varied between 50 and 90 pmol/h per oocyte in the presence of sodium (Figs. 1 and 2, Table 1). In the absence of sodium we found transport velocities between 7 and 39 pmol/h per oocyte (Figs. 3 and 4, Table 2).

Poly(A)$^+$ RNA was fractionated according to size by gel filtration on a Sephacryl S-400 HR column. All fractions were tested for expression of Na$^+$-independ-

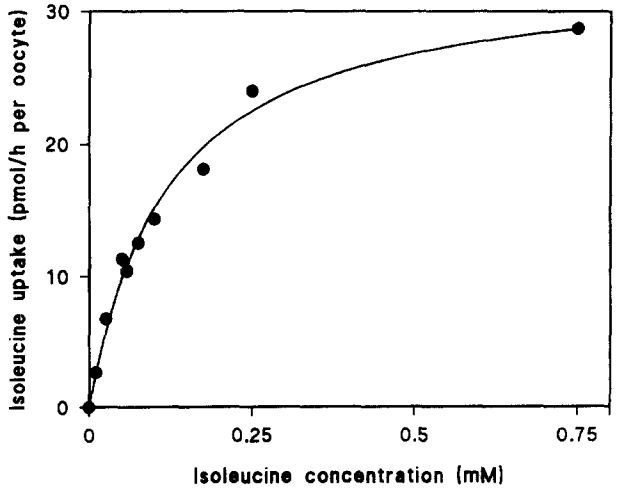

Fig. 3. Dependence of the Na$^+$-independent isoleucine transport rate on substrate concentration. Transport was measured as described in Fig. 1, however, with varied isoleucine concentrations and in sodium-free OR2$^-$ buffer. The transport rate of water-injected oocytes is already subtracted from the values. The K_m value was determined by non-linear regression analysis. The curve is the best fit of the data.

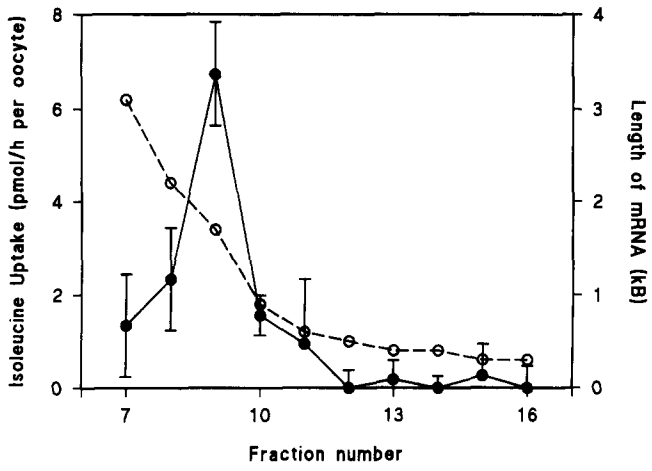

Fig. 4. Induction of Na^+-independent isoleucine transport by size-fractionated poly(A)$^+$ RNA. Poly(A)$^+$ RNA was size-fractionated by gel filtration and the size was determined by gel electrophoresis under denaturing conditions (○). Transport was measured as described in Fig. 1 (●). The transport buffer OR2- contained choline chloride instead of sodium chloride.

ent isoleucine transport. We found the highest transport activity in a fraction of poly(A)$^+$ RNA of 1.7–2.2 kb length (Fig. 4).

The cell line C6-BU-1 is more easily cultivated than primary astroglial cells. To find out whether C6-BU-1 cells are a good model for astroglial cells in respect to amino acid transport we determined the basic transport parameters also with poly(A)$^+$ RNA isolated from astroglia rich primary cultures. Poly(A)$^+$ RNA derived from this source also induced isoleucine transport activity when injected into *Xenopus laevis* oocytes. After a 4 days' expression uptake rates were determined to amount to 72 ± 8.7 pmol/h per oocyte for mRNA-injected oocytes and 22 ± 2.1 pmol/h per oocyte for water-injected oocytes. The Na^+-independent part of isoleucine uptake induced by mRNA from primary astroglial cultures showed the same pattern of inhibition as the transport activity demonstrated with mRNA from C6-BU-1 cells (Table 3).

Table 3
Specificity of isoleucine transport, induced by injection of poly(A)$^+$ RNA from rat astroglial cells

Addition	Salt	Transport activity (pmol/h per oocyte)	%
None	NaCl	18.7 ± 2.3	–
None	ChoCl	3.8 ± 0.9	100
+10 mM Leu	ChoCl	0.2 ± 0.03	5
+10 mM BCH	ChoCl	0.7 ± 0.1	18
+10 mM MeAIB	ChoCl	4.5 ± 1.5	118
+10 mM Phe	ChoCl	0.4 ± 0.1	10
+10 mM Lys	ChoCl	4.2 ± 1.1	111

Experiments were performed as described in Table 1.The values for water injected controls were already subtracted. Isoleucine was added at a final concentration of 100 μM. The transport buffer (OR2-) contained either NaCl or choline chloride.

4. Discussion

The experiments presented here, are the first step in cloning of a system L amino acid transporter from rat brain. A mRNA in the range of 1.7–2.2 kb was responsible for the induction of Na^+-independent transport activity in the oocytes. The mRNA could also be a little larger because Sephacryl S-400 does not separate RNA very well above 2 kb. This fraction can now be used for construction of a cDNA library which should contain the information for the transporter.

Induction of isoleucine transport led to an increased internal isoleucine concentration within 1 h. Isoleucine was metabolized only to a small extent. Poly(A)$^+$ RNA-injected oocytes showed even less metabolism than water injected cells. Therefore, uptake of radioactivity was identical with accumulation of isoleucine. All kinetic parameters determined were those of the transport process.

In the identification of the amino acid transport system, a scheme was followed similar to the one exemplified with Chinese hamster ovary cells [35]. The portion of Na^+-independent isoleucine uptake, which was inhibited by BCH, leucine and phenylalanine was assigned to system L. The small portion of uptake, which was not inhibited by BCH, but by phenylalanine and leucine, was not characterized further. Recently, system L transport was subdivided into L_1 and L_2 transport [36]. The difference between the two is mainly due to the K_m values. With a K_m value about 100 μM the expressed system is more similar to the L_1 type. However, it is not clear whether these two subtypes are represented in all tissues. Transport was not inhibited by lysine, excluding expression of a system similar to $b^{0,+}$. The Na^+-dependent component was not inhibited by MeAIB. It was tentatively assigned to system ASC activity. These results were in accordance with data on AIB and citrulline transport in astroglial [3,37,38] and other brain cells [39], and the strong appearance of mRNA for system ASC in Northern blots from whole brain [22]. Isoleucine transport induced by injection of poly(A)$^+$ RNA from astroglial cells showed the same inhibition pattern as transport resulting from injection of poly(A)$^+$ RNA from C6-BU-1 cells. The low transport activity of the experiment shown in Table 3 may have resulted from the fact that the mRNA was purified only by a single passage over an oligo(dT)-column. Ribosomal RNA was still present in considerable amounts after the first passage. Absolute velocities are difficult to compare between different experiments, since large deviations between different oocyte preparations were found also by other investigators [40]. The ratio between Na^+-dependent and Na^+-independent

transport also differed between C6-BU-1 cells and astroglial cells.

Tate et al. [9] suggested system L activity to be induced by injection of mRNA from rabbit kidney cortex. However, subsequent characterization revealed inhibition by cationic amino acids and cloning led to a reassignment as system $b^{0,+}$. Coady et al. [10] also suggested system L activity after injection of poly(A)$^+$ RNA from rabit renal cortex. No data on inhibition by other amino acids and analogues were presented. Since system $b^{0,+}$ shows strong activity in rabbit renal cortex [41] and two other groups cloned $b^{0,+}$ from renal cells [19,20] it is probable that also in this case $b^{0,+}$ activity was measured.

To the best of our knowledge this is the first report on expression of a Na^+-independent neutral amino acid transporter which is not inhibited by cationic amino acids (for a recent compilation on expressed amino acid transporters, see Ref. [42]). The kinetic parameters and inhibition pattern are consistent with expression of a system L like transporter.

Acknowledgement

The authors thank Daniel Markovich (University of Zürich) for his generous help with many technical problems which we had to master at the beginning of our work with oocytes.

References

[1] Betz, A.L. and Gilboe, D.D. (1973) Am. J. Physiol. 224, 580–587.
[2] Pardridge, W.M. and Oldendorf, W.H. (1977) J. Neurochem. 28, 5–12.
[3] Brookes, N. (1988) J. Neurochem. 51, 1913–1918.
[4] Oxender, D.L. and Christensen, H.N. (1963) J. Biol. Chem. 238, 3686–3699.
[5] Christensen, H.N. (1990) Physiol. Rev. 70, 43–77.
[6] Shotwell, M.A., Kilberg, M.S. and Oxender, D.L. (1983) Biochim. Biophys. Acta 737, 267–284.
[7] Van Winkle, L.J. (1988) Biochim. Biophys. Acta 947, 173–208.
[8] Christensen, H.N. (1985) J. Membr. Biol. 84, 97–103.
[9] Bertran, J. Werner, A., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M. and Murer, H. (1992) Biochem. J. 281, 717–723.
[10] Coady, M.J., Pajor, A.M., Toloza, E.M. and Wright, E.M. (1990) Arch. Biochem. Biophys. 283, 130–134.
[11] Tate, S.S., Urade, R., Getchell, T.V. and Udenfriend, S. (1989) Arch. Biochem. Biophys. 275, 591–596.
[12] Aoshima, H., Tomita, K. and Sugio, S. (1988) Arch. Biochem. Biophys. 265, 73–81.
[13] Su, T., Logsdon, C.D. and Oxender, D.L. (1992) Mol. Cell. Biol. 12, 5281–5287.
[14] McNamara, P.D., Rea, C.T. and Segal, S. (1991) J. Biol. Chem. 266, 986–989.
[15] Magagnin, S., Bertran, J., Werner, A., Markovich, D., Biber, J., Palcin, M. and Murer, H. (1992) J. Biol. Chem. 267, 15384–15390.
[16] Harvey, C., Muzyka, W.R., Yao, S.Y., Cheeseman, C.I. and Young, J.D. (1993) Am. J. Physiol. 265, G99–G106.
[17] Van Winkle, L.J., Campione, A.L. and Gorman, J.M. (1988) J. Biol. Chem. 263, 3150–3163.
[18] Bertran, J., Werner, A. Chillaron, J., Nunes, V., Biber, J. Testar, X., Zorzano, A., Estivill, X., Murer, H. and Palacin, M. (1993) J. Biol. Chem. 268, 14842–14849.
[19] Bertran, J., Werner, A., Moore, M.L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M. and Murer, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5601–5605.
[20] Wells, R.G. and Hediger, M.A. (1992) Proc. Natl. Acad Sci. USA 89, 5596–5600.
[21] Kong, C., Yet, S. and Lever, J. (1993) J. Biol. Chem. 263, 1509–1512.
[22] Arriza, J.L., Kavanaugh, M.P., Fairman, W.A., Wu, Y., Murdoch, G.H., North, R.A. and Amara, S.G. (1993) J. Biol. Chem. 268, 15329–15332.
[23] Shafquat, S., Tamarapoo, B.K., Kilberg, M.S., Puranam, R.S., McNamara, J.O., Guadano-Ferraz, A. and Fremeau, R.T. (1993) J. Biol. Chem. 268, 15351–15355.
[24] Kim, J.W., Closs,E.I., Albritton, L.M. and Cunningham, J.M. (1991) Nature 352, 725–728.
[25] Wang, H., Kavanaugh, M.P., North, R.A. and Kabat, D. (1991) Nature 352, 729–731.
[26] Bertran, J., Magagnin, S., Werner, A., Markovich, D., Biber, J., Testar, X., Zorzano, A., Kühn, L.C., Palacin, M. and Murer, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5606–5610.
[27] Tate, S.S., Yan, N. and Udenfriend, S. (1992) Proc. Natl. Acad. Sci. USA 89, 1–5.
[28] Yan, N., Mosckovitz, R., Udenfriend, S. and Tate, S.S. (1992) Proc. Natl. Acad. Sci. USA 89, 9982–9985.
[29] Clark, J.A. and Amara, S.G. (1993) Bioessays 15, 323–332.
[30] Hamprecht, B. and Löffler, F. (1985) Methods Enzymol. 109, 341–345.
[31] Amano, T., Hamprecht, B. and Kemper, W. (1974) Exp. Cell Res. 85, 399–408.
[32] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[33] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[34] Smith, L.D.,Xu, W. and Varnold, R.L. (1991) Methods Cell Biol. 36, 45–60.
[35] Shotwell, M.A., Jayme, D.W., Kilberg, M.S. and Oxender, D.L. (1981) J. Biol. Chem. 256, 5422–5427.
[36] Weissbach, L., Handlogten, M.E., Christensen, H.N. and Kilberg, M.S. (1982) J. Biol. Chem. 257, 12006–12011.
[37] Oja, S.S. and Korpi, E.R. (1983) in Handbook of Neurochemistry, Vol. 5 (Lajtha, A., ed.), pp. 311–337, Plenum Press, New York..
[38] Wiesinger, H. and Schmidlin, A. (1993) Biol. Chem. Hoppe-Seyler 374, 698.
[39] Blondeau, J., Beslin, A., Chantoux, F. and Francon, J. (1993) J. Neurochem. 60, 1407–1413.
[40] Steffgen, J., Scheyerl, F., Gründemann, D., Kienle, S., Franz, H.E. and Koepsell, H. (1993) Biochim. Biophys. Acta 1149, 145–150.
[41] Silbernagl, S. (1988) Physiol. Rev. 68, 911–1007.
[42] Van Winkle, L.J. (1993) Biochim. Biophys. Acta 1154, 157–172.