Original Paper

Interaction of Excitatory Amino Acid Transporters 1 – 3 (EAAT1, EAAT2, EAAT3) with *N*-Carbamoylglutamate and *N*-Acetylglutamate

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Key Words

Excitatory amino acid transporters • Hyperammonemia • *N*-carbamoylglutamate • *N*-acetylglutamate • Oxaloacetate • Sodium-dependent dicarboxylate transport • Urea cycle disorders

Abstract

Background/Aims: Inborn deficiency of the *N*-acetylglutamate synthase (NAGS) impairs the urea cycle and causes neurotoxic hyperammonemia. Oral administration of *N*-carbamoylglutamate (NCG), a synthetic analog of *N*-acetylglutamate (NAG), successfully decreases plasma ammonia levels in the affected children. Due to structural similarities to glutamate, NCG may be absorbed in the intestine and taken up into the liver by excitatory amino acid transporters (EAATs). **Methods:** Using *Xenopus laevis* oocytes expressing either human EAAT1, 2, or 3, or human sodium-dependent dicarboxylate transporter 3 (NaDC3), transport-associated currents of NAG, NCG, and related dicarboxylates were assayed. **Results:** L-aspartate and L-glutamate produced saturable inward currents with K_m values below 30 μ M. Whereas NCG induced a small inward current only in EAAT3 expressing oocytes, NAG was accepted by all EAATs. With EAAT3, the NAG-induced current was sodium-dependent and saturable (K_m 409 μ M). Oxaloacetate was found as an additional substrate of EAAT3. In NaDC3-expressing oocytes, all dicarboxylates induced much larger inward currents than did L-aspartate and L-glutamate. **Conclusion:** EAAT3 may contribute to intestinal absorption and hepatic uptake of NCG. With respect to transport of amino acids and dicarboxylates, EAAT3 and NaDC3 can complement each other.

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Published by S. Karger AG, Basel

Introduction

Glutamate is the predominant excitatory neurotransmitter in the central nervous system and is important in development, learning, memory, and higher cognitive functions (reviewed in [1, 2]). Besides its role in signal transduction, glutamate is needed for the synthesis of GABA and glutathione, and is, as a precursor of *N*-acetylglutamate, indirectly

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involved in the detoxification of ammonia by the urea cycle (reviewed in [1, 2]). Five glutamate transporters mediating glutamate uptake have been identified in the central nervous system and subsequently cloned from a human cDNA library. These transporters are members of the excitatory amino acid transporters (EAATs). The EAATs belong to the solute carrier 1 (SLC1) transporter family, including EAAT1, EAAT2, EAAT3, EAAT4, EAAT5, and the alanine serine cysteine transporters 1 and 2 (ASCT1, ASCT2) (reviewed in [3, 4]).

Glutamate transporters are specific for the acidic amino acids glutamate and aspartate as their natural substrates. Whereas aspartate is transported in its L- and the D-form, glutamate is transported only in the L-form. Both compounds are dicarboxylic amino acids with a four- or five-carbon backbone, possessing in addition an α -amino group. Whereas all EAATs accept glutamate and aspartate over the whole pH range, ASCT1 and 2 interact with glutamate only at low pH. Detailed studies on the coupling stoichiometry of EAAT1, EAAT2, and EAAT3 revealed that one glutamate is co-transported with three sodium ions and one proton in exchange for one potassium ion, resulting in a surplus of two positive charges entering the cell for each glutamate [5]. EAAT1 and 3 possess a substrate-activated chloride conductance, which has been proposed to function as a feedback sensor to reduce cell excitability by further glutamate release [6]. Whereas EAAT1 expression is largely restricted to brain and muscles [7], EAAT2 and EAAT3 mRNAs are present in various non-nervous tissues, including the intestine, the liver, and the kidneys [7, 8].

The urea cycle is the main pathway for disposal of excess nitrogen generated from the breakdown of protein and other nitrogen-containing compounds. Urea cycle disorders (UCD) are characterized by hyperammonemia and distorted amino acid metabolism. UCDs are due to an inborn deficiency of one of the enzymes in the urea cycle: the carbamoylphosphate synthetase 1 (CPS1), the ornithine transcarbamylase (OTC), the argininosuccinic acid synthetase (ASS1), the argininosuccinic acid lyase (ASL), and the arginase (ARG), or the *N*-acetylglutamate synthase (NAGS) which produces the co-factor *N*-acetylglutamate (NAG). NAG is synthesized in liver mitochondria from glutamate and acetyl CoA and is essential for CPS1 activation. Infants suffering from a severe NAGS deficiency rapidly develop lethargy, anorexia, hyper- or hypoventilation, hypothermia, seizures, and other neurological symptoms as well as cerebral edema, and coma (reviewed in [9]).

N-carbamoylglutamate (NCG, carglumatic acid) is the only known drug serving as a surrogate for the essential co-factor *N*-acetylglutamate, and application of *N*-carbamoylglutamate can completely restore the disease [10-13]. Using *Xenopus laevis* oocytes expressing the human sodium-dependent dicarboxylate transporter 3 (NaDC3), we recently demonstrated transport of *N*-acetylglutamate and *N*-carbamoylglutamate by NaDC3 [14]. In the rat, in-situ hybridization localized NaDC3 to perivenous hepatocytes [15], suggesting NaDC3 as a possible entry pathway for *N*-carbamoylglutamate at least in a subset of hepatocytes. Despite the fact that *N*-carbamoylglutamate is active after oral application, the transporter(s) mediating intestinal absorption are unknown. Since *N*-carbamoylglutamate is related structurally to glutamate (Fig. 1), we hypothesized that intestinal absorption and hepatic uptake may occur by members of the excitatory amino acid transporters (EAATs). To this end, currents evoked by the potential uptake of *N*-carbamoylglutamate and *N*-acetylglutamate in *Xenopus laevis* oocytes expressing human EAAT1, EAAT2, or EAAT3 were examined. The study also includes other C4- and C5-dicarboxylates to establish a more detailed picture on which dicarboxylates interact with the EAATs.

Materials and Methods

Reagents and Chemicals.

Chemicals used in this study, including the constituents of the oocyte Ringer (ORi), the test compounds α -ketoglutarate (α KG), L-aspartate, L-glutarate, L-glutamate, *N*-acetylglutamate (NAG), *N*-carbamoylglutamate (NCG), oxaloacetate (OAA), succinate (Fig. 1), and the antibiotic gentamycin were purchased from Merck (Darmstadt, Germany) or AppliChem (Darmstadt, Germany). For all experiments, an oocyte Ringer

(ORi) was used which contained (in mM): 110 NaCl, 3 KCl, 2 CaCl₂, 5 HEPES/Tris. Sodium-free conditions were achieved by replacing sodium by equimolar concentrations of *N*-methyl-D-glucamine (NMDG). α KG, L-aspartate, L-glutamate, glutarate, NAG, NCG, OAA, and succinate were added, if available, as sodium salts, otherwise as the free acid, to ORi in the concentrations indicated in the Figure legends, and the pH was always adjusted to pH 7.5.

In vitro transcription of human EAAT1-, EAAT2-, EAAT3-, and human NaDC3-cRNA

Plasmids from the human EAATs (Sequence accession IDs: NM_004170 (SLC1A1); NM_004171 (SLC1A2); NM_004172 (SLC1A3)) and the human NaDC3 (Sequence accession ID: AF154121 (SLC16A3)) were linearized with Not I and *in vitro* cRNA transcription was performed using the T7 mMessage mMachine kit (Ambion, Austin, TX) according to the manufacturer's instructions. The resulting cRNA was suspended in purified, RNase-free water to a final concentration of 1 μ g/ μ L.

Oocyte preparation, storage, and electrophysiological analysis

The isolation of oocytes from *Xenopus laevis* was approved by the government of Lower Saxony. Stage V and VI oocytes from ovary loops of *Xenopus laevis* (Nasco, Fort Atkinson, WI) were separated by an overnight treatment with collagenase (Typ CLS II; Biochrom, Berlin, Germany), a 10-min incubation in calcium-free ORi, subsequent washings in ORi, and maintaining again in ORi with 2 mM calcium. One day after removal from the frog, oocytes were injected with 23 nL cRNA coding for EAAT1-3, NaDC3 or an equivalent amount of RNase-free water. After one to three days of incubation at 16-18°C in ORi supplemented with 50 μ M gentamycin and 2.5 mM sodium pyruvate (Merck) with daily medium changes, oocytes were used for two-electrode voltage clamp (TEVC) studies. Oocytes were placed into a 0.5 mL-chamber on the stage of an inverted microscope and impaled under direct view with filament-containing borosilicate glass microelectrodes (BioMedical Instruments, Zöllnitz, Germany) filled with 3 M KCl. Using a TEVC device (OC725A; Warner, Hamden, CT) in the voltage clamp mode, substrate-mediated currents were detected at a clamp potential of -60 mV at room-temperature.

Fig. 1. Structures of the amino acids aspartate, glutamate, and of the tested C4- and C5-dicarboxylates. Structures are obtained from the manufacturer's data sheets.

Statistics and calculations.

Data are provided as means $\pm$ SEM. Data were tested for significance using OneWay Anova or paired Student's *t*-test, as appropriate. Results were considered statistically significantly different at $p < 0.05$. The Michaelis-Menten constants (K_m) were calculated and the respective graphs were designed using SigmaPlot software (Systat Software, San Jose, CA).

Results

When clamped at a potential of -60 mV, *Xenopus laevis* oocytes expressing the excitatory amino acid transporters 1, 2, or 3 (EAAT1, EAAT2, EAAT3), respectively, responded

with inward currents upon application of 1 mM L-aspartate or L-glutamate. The amplitudes of the inward currents depended on the EAAT subtype expressed and on the day post-injection. In EAAT1-expressing oocytes, one day post injection, L-aspartate and L-glutamate induced currents of -38 ± 2 and -46 ± 4 nA (3 oocytes, 2 donors), respectively. These currents rose to -78 ± 17 and -84 ± 21 nA (3 oocytes, 3 donors) at day 2 post injection and, at day 3 post injection, currents of -223 ± 68 for aspartate and -256 ± 93 nA for glutamate (3 oocytes, 3 donors) were detected. EAAT2- and EAAT3-cRNA-injected oocytes also revealed a time-dependent increase in currents, albeit the overall magnitude

Fig. 2. Currents mediated by the amino acids aspartate and glutamate and by several dicarboxylates in oocytes expressing EAAT1 (A), EAAT2 (B), and EAAT3 (C). Three days post injection of cRNA coding for the respective EAATs, oocytes were clamped to -60 mV. Upon superfusion with oocyte Ringer (ORi) containing in addition 1 mM of the indicated compound, the induced inward currents were registered. Intensive washings were performed and a recovery period of at least 10 min was allowed until the next compound was tested. Data present measurements with five oocytes from three frogs for EAAT1, four oocytes from four frogs for EAAT2, six oocytes from five frogs for EAAT3, and eight oocytes from six frogs for mock: n.s.: not significant; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; α KG, α -ketoglutarate; NAG, *N*-acetylglutamate; NCG, *N*-carbamoylglutamate; OAA, oxaloacetate.

Table 1. K_m values for aspartate, glutamate and several C4- and C5-dicarboxylates on EAAT1 – 3. K_m values were determined after measuring inward currents at -60 mV using a range of concentrations from 2–1000 μ M for aspartate and glutamate and from 100–5000 μ M for *N*-acetylglutamate (NAG), *N*-carbamoylglutamate (NCG), and oxaloacetate (OAA). *Due to large variations in currents at low concentrations, no exact K_m was calculated and the presented values are only an estimate. n.d., not determined; n, number of oocytes; m, number of frogs

	EAAT1		EAAT2		EAAT3	
Substrate	K_m , μ M	n/m	K_m , μ M	n/m	K_m , μ M	n/m
L-Aspartate	11.5 ± 4.1	5/3	18.6 ± 10.9	6/3	12.8 ± 2.8	3/3
L-Glutamate	17.9 ± 3.9	5/3	25.5 ± 6.5	3/3	16.1 ± 2.2	5/4
NAG	n.d.	-	>5000*	4/4	409 ± 67	4/4
NCG	n.d.	-	n.d.	-	>5000*	6/6
OAA	n.d.	-	n.d.	-	>5000*	3/2

of the currents was smaller ranging from -40 to -60 nA (c.f. Fig. 2). To obtain comparable results, all further experiments were performed at day 3 post cRNA injection. L-Aspartate and L-glutamate induced inward currents were abolished when sodium was replaced by *N*-methyl-D-glucamine, and decreased in magnitude when the oocytes were clamped to -30 mV instead to -60 mV (data not shown), indicating that the oocytes successfully expressed the respective EAATs. In water-injected oocytes (mocks), no substantial sodium- or potential-dependent L-aspartate- or L-glutamate-mediated inward currents were observed, indicating a low expression of endogenous electrogenic transporters for acidic amino acids.

Currents induced by L-aspartate and L-glutamate followed Michaelis-Menten kinetics. At -60 mV, K_m values for L-aspartate were 11.5, 18.6, and 12.8 μ M for EAAT1, EAAT2, and EAAT3, respectively (Table 1). For L-glutamate, K_m values of 17.9, 25.5, and 16.1 μ M were found. These values are similar to those previously published [7, 8, 16], again demonstrating successful expression of the EAATs.

The main aim of this study was to establish the transport of *N*-carbamoylglutamate (NCG) by one or more EAATs. For comparison, we included other C4- and C5-dicarboxylates containing amino- or keto-groups, i.e., *N*-acetylglutamate (NAG), α -ketoglutarate (α KG), glutarate, oxaloacetate (OAA), and succinate. All compounds were applied at 1 mM to the same individual oocyte at a clamp potential of -60 mV in random order, and their inward currents were compared to those evoked by L-aspartate and L-glutamate, which served as controls. EAAT1-expressing oocytes showed large inward currents with L-aspartate (-288 ± 41 nA) and L-glutamate (-342 ± 52 nA) and very small currents with the other compounds (5 oocytes, 3 donors, Fig. 2A). The currents induced by α KG, glutarate, and *N*-carbamoylglutamate (NCG) did not reach statistical significance as compared to water-injected oocytes (mock). The currents observed after addition of *N*-acetylglutamate (NAG), oxaloacetate (OAA), and succinate were below -10 nA, but significantly different from mock (Fig. 2A). In EAAT2-expressing oocytes, L-aspartate-, L-glutamate-, and NAG-sensitive currents were detected (Fig. 2B). The addition of all other compounds led to currents not different in amplitude from those observed in mock. As opposed, in EAAT3-expressing oocytes, all tested dicarboxylates, including *N*-carbamoylglutamate (NCG), resulted in inward currents that were significantly different from mock (Fig. 2C). These results reveal subtle differences between EAATs with respect to their interaction with dicarboxylates. Having an apparently wider substrate specificity than the other excitatory amino acid transporters, EAAT3 translocates also *N*-carbamoylglutamate, albeit at low rates.

N-acetylglutamate produced currents of different magnitudes in all EAAT-expressing oocytes (c.f. Fig. 2). In EAAT3-expressing oocytes, NAG-mediated currents were sodium-dependent (Fig. 3A, upper trace). In four oocytes from three frogs, upon application of 5 mM *N*-acetylglutamate, inward currents of -50.0 ± 11.6 nA were observed which dropped to 0.5 ± 1.8 nA in the absence of sodium. The mock responded only with a small outward current upon sodium-removal, possibly due to an impairment of the Na^+/K^+ -ATPase (Fig. 3A, lower trace). For *N*-acetylglutamate, a K_m value of 409 ± 67 μ M was evaluated (Fig. 3B). As compared to L-aspartate and L-glutamate, the affinity of *N*-acetylglutamate

Fig. 3. Impact of *N*-acetylglutamate (NAG) on EAAT3. (A) Sodium-dependence of NAG-evoked currents. Representative trace records of an EAAT3-expressing oocyte (upper trace) and a mock (water-injected oocyte, lower trace) obtained at a clamp potential of -60 mV are shown. Sodium replacement by *N*-methyl-D-glucamin resulted in the EAAT3- and in the water-injected oocyte in outward currents possibly due to the inhibition of the Na^+/K^+ -ATPase. In the EAAT3-expressing oocyte, the NAG-mediated current vanished in the absence of sodium. NAG evoked no current in the mock. (B) Concentration-dependent increase of currents mediated by L-aspartate (○), L-glutamate (●), and NAG (▼) in EAAT3-expressing oocytes. At a clamp potential of -60 mV, oocytes were superfused with ORi containing in addition L-aspartate, L-glutamate, or *N*-acetylglutamate (NAG) in concentrations up to 1 mM (aspartate, glutamate) or 5 mM (NAG). K_m values were calculated from the peak (maximal) inward currents obtained from three to four oocytes from three donors.

towards EAAT3 is approximately 30 times less (Table 1). For technical reasons, affinities for *N*-carbamoylglutamate and oxaloacetate could not be determined because, even at concentrations >5 mM, current amplitudes did not exceed -20 nA.

Finally, we investigated the impact of the dicarboxylates tested on the EAATs on human sodium-dependent dicarboxylate transporter 3 (NaDC3) expressed in *Xenopus laevis* oocytes. This transporter showed *N*-acetylglutamate (NAG)-, *N*-carbamoylglutamate (NCG)-, and oxaloacetate (OAA)-mediated inward currents which were only marginally smaller in magnitude than the currents obtained with the NaDC3 reference substrates α KG, glutarate, and succinate (Fig. 4A). For NAG and NCG, we recently determined in NaDC3-expressing oocytes K_m values of 125 ± 10 and 25.7 ± 2.0 μM , respectively [14]. Using similar experimental conditions, a K_m for OAA of 465 ± 59 μM was calculated (Fig. 4B).

Discussion

As a neurotoxic end product of amino acid metabolism, ammonia is detoxified by hepatocytes within the urea cycle. Urea is subsequently removed from the body by the kidneys. Urea cycle disorders (UCDs) are a group of inborn errors of nitrogen metabolism, leading to severe life-threatening hyperammonemia.

Carbamoylphosphate synthase 1 (CPS1) is the first enzyme in the urea cycle and is located in the mitochondria of hepatocytes and enterocytes. For the synthesis of carbamoylphosphate, CPS1 needs the allosteric co-factor *N*-acetylglutamate synthesized by the *N*-acetylglutamate synthase (NAGS). Failure of CPS1 either by mutations in the enzyme itself or by the inability of the NAGS to synthesize sufficient amounts of *N*-acetylglutamate (NAG), will compromise the entire urea cycle, resulting in an increase in blood ammonia with the above listed neurological defects. Allosteric stimulation of the CPS1 by the stable

Fig. 4. Currents mediated by the amino acids aspartate and glutamate and several dicarboxylates in oocytes expressing NaDC3: (A) Inward currents measured at -60 mV. Three days post injection of cRNA coding for human NaDC3, the indicated compounds were applied at a concentration of 1 mM and the maximal induced inward currents were registered. Intensive washings and a recovery period of at least 10 min was allowed until the next compound was tested. Data on NaDC3 were collected from five oocytes from four frogs, those for mock from eight oocytes from six frogs. Currents evoked by *N*-carbamoylglutamate (NCG), *N*-acetylglutamate (NAG), glutarate, α -ketoglutarate (α KG), oxaloacetate (OAA), and succinate were similar in magnitude, those evoked by L-aspartate and L-glutamate were much smaller and significantly different from those induced by the other compounds. For all tested compounds, currents in NaDC3 and mock were significantly different at $p < 0.05$ and higher. (B) Determination of the concentration dependence of OAA-induced currents. At a clamp potential of -60 mV, oocytes were superfused with ORi containing increasing concentrations of OAA. K_m was calculated from peak inward currents obtained from three oocytes from three donors.

and orally active NAG analog *N*-carbamoylglutamate accelerates the urea cycle, thereby reducing the ammonia load. To reach its site of action, orally applied *N*-carbamoylglutamate must be absorbed in the intestine and subsequently taken up into hepatocytes by hitherto ill-defined transporters.

In a recent paper [14], we demonstrated transport of *N*-carbamoylglutamate and *N*-acetylglutamate by the sodium-dependent dicarboxylate transporter 3 (NaDC3) with K_m values of 25.7 ± 2.0 and $125 \pm 10 \mu\text{M}$ (c.f. Table 2), respectively. The efficiency of transport was 2.38 and 0.53 nA/ μM , indicating that the turnover rates for NCG and α KG, one of the reference substrates of NaDC3 (2.88 nA/ μM ; Table 2, [14]), are similar. At least in the rat, NaDC3 mRNA appears to be restricted to perivenous hepatocytes that are not primarily involved in urea synthesis, but utilize ammonia mainly for glutamine synthesis [15]. Moreover, NaDC3 is not present in the intestine. Therefore, we hypothesized that uptake of *N*-carbamoylglutamate may also occur by one of the EAATs, especially EAAT3 whose RNA was detected in liver and intestine [8].

Since *N*-carbamoylglutamate is not available in radio-labeled form, we expressed human EAAT1, EAAT2, or EAAT3, respectively, in *Xenopus laevis* oocytes and tested for inward currents occurring during electrogenic substrate translocation. In case of L-aspartate and

Table 2. Comparison of the kinetic parameters for selected dicarboxylates on NaDC3 and EAAT3. K_m , I_{max}/K_m for *N*-acetylglutamate (NCG), *N*-carbamoylglutamate (NCG), and L-glutamate on NaDC3 were obtained from *[14]. The plasma concentrations (c_{plasma}) are from the respective references: L-glutamate, **[23, 24]; NAG, ***[25]; NCG, ****[26]; OAA, *****[27, 28]. Plasma concentrations of the respective compound were measured in healthy volunteers [23-25, 27, 28] or from patients [26], K_m and I_{max}/K_m for OAA on NaDC3 and for L-glutamate, NAG, NCG, and OAA were calculated from the data of the present study

Compound	NaDC3				EAAT3		
	c_{plasma}	K_m , μM	I_{max} , nA	I_{max}/K_m , nA/ μM	K_m , μM	I_{max} , nA	I_{max}/K_m , nA/ μM
L-glutamate	61 – 132**	>2000*	>-70*	<0.03*	10.6 $\pm$ 1.9	-52 $\pm$ 13	4.9
NAG	5.17 $\pm$ 0.22***	125 $\pm$ 10*	-67.4 $\pm$ 1.2*	0.53*	409 $\pm$ 67	-38 $\pm$ 2	0.09
NCG	17.25 $\pm$ 7.75****	25.7 $\pm$ 2.0*	-61.2 $\pm$ 0.9*	2.38*	>1000	<-20	<0.02
OAA	<10-551*****	465 $\pm$ 59	-70.9 $\pm$ 2.7	0.15	>1000	<-20	<0.02

L-glutamate possessing two negatively charged carboxyl groups and one positively charged amino group (one net negative charge), two positive charges are taken up during co-transport with three sodium ions and one proton and antiport of one potassium. Indeed, the physiological substrates, L-aspartate and L-glutamate, produced clearly detectable inward currents that were largest with EAAT1, and smaller with EAAT2 and EAAT3. The currents were sodium-dependent and saturable, exhibiting K_m and I_{max} values in good agreement with published data [K_m , 7, 8, 16; I_{max} , 17, 18].

N-carbamoylglutamate (Fig. 1) has two negatively charged carboxyl groups and no positive charge. The symport of *N*-carbamoylglutamate with three sodium ions and one proton in exchange with one potassium ion should result in an uptake of one surplus positive charge and, hence, a measurable inward current. However, in EAAT1 and EAAT2 expressing oocytes, *N*-carbamoylglutamate did not produce a measurable inward current, suggesting that it is not translocated by these transporters. With EAAT3, a small, but significant inward current was observed after application of *N*-carbamoylglutamate. Therefore, EAAT3 is able to transport *N*-carbamoylglutamate, albeit at low rates and could contribute to the absorption of *N*-carbamoylglutamate in the small intestine and to the uptake in the liver.

The allosteric activator of CPS1, *N*-acetylglutamate, produced small, but significant inward currents with EAAT1, EAAT2, and EAAT3. The NAG-induced current in EAAT3-expressing oocytes was blocked by sodium removal. *N*-acetylglutamate (Fig. 1) has two negatively charged carboxyl groups and no positive charge. Given the above-mentioned transport stoichiometry, one net positive charge is taken up with each *N*-acetylglutamate molecule. Thus, our data indicate that *N*-acetylglutamate, a substrate of NaDC3 [14], is also a substrate of EAATs. In particular, EAAT3 should contribute to intestinal absorption and hepatic uptake of *N*-acetylglutamate. Unfortunately, this compound is rapidly degraded in the blood and is thus useless in therapy of urea cycle disorders [9, 11].

Oxaloacetate was recently introduced as a neuroprotective drug [19-22] due to ability to decrease blood glutamate levels by the activation of the blood-resident enzyme glutamate-oxaloacetate transaminase (GOT). This enzyme catalyzes, by transfer of an amino group, the transformation of glutamate into α -ketoglutarate and of oxaloacetate into L-aspartate. Artificially rising oxaloacetate in the blood shifts the equilibrium of the reaction to the right, thereby lowering blood L-glutamate levels. Oxaloacetate produced considerable inward currents in EAAT3-expressing oocytes, suggesting that it is a hitherto unknown substrate of this transporter.

EAAT3 and NaDC3 are expressed in the liver. It appears that both sodium-dependent transporters complement each other. EAAT3 transports L-alanine and L-glutamate with high, and NaDC3 with low efficiency, as determined from our expression studies in oocytes. As opposed, EAAT3 is weak transporter of dicarboxylates that are all translocated by NaDC3.

Obviously, EAATs require a positively charged α -amino group for full transport activity and interact only slowly, or not at all, with compounds in which the α -amino group is modified by an acetyl- or carbamoyl residue. In contrast, NaDC3 nearly excludes compounds with a positively charged α -amino group and accepts dicarboxylates with substitutions at the α -C-atom. It remains to be determined, whether EAAT3 and NaDC3 are co-expressed, at least in a subset of hepatocytes in the human liver involved in urea synthesis.

Acknowledgements

The authors wish to thank Prof. Florian Lang, Physiology I, University of Tübingen, for the generous gift of the EAAT1, EAAT2, and EAAT3 cRNA. The skillful technical assistance of Andrea Paluschkiwitz and Sören Petzke is gratefully acknowledged. The study was founded by the Deutsche Forschungsgemeinschaft (BU998/9-1 to B.C.B.) and by a grant in aid from Orphan Europe.

Disclosure Statement

The authors have nothing to declare.

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