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Research report

Expression and functional analysis of Na^+-dependent glutamate transporters from zebrafish brain

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ABSTRACT

High-affinity excitatory amino acid transporters (EAATs) regulate extracellular glutamate levels. Zebrafish (*Danio rerio*) provides an excellent model to study the function of different neurotransmitter systems. Although the identification of the EAAT family is well established in the mammalian central nervous system (CNS), EAAT-related genes and their expression profile in zebrafish have not yet been reported. Here we identify and describe the expression profile of EAATs-related genes and functional properties of glutamate uptake in three major brain structures from zebrafish (telencephalon, optic tectum and cerebellum). Searches on zebrafish genome databases and a phylogenetic analysis confirmed the presence of several EAAT-related genes (EAAT2, EAAT3, three EAAT1 paralogs and two EAAT5 sequences). All sequences identified were expressed in the structures analyzed. EAAT2 and EAAT3 were the most prominent glutamate transporters expressed in all brain areas. A uniform expression was observed for EAAT1A, whereas higher EAAT1B transcript levels were detected in telencephalon. Lower amounts of EAAT1C transcripts were observed in cerebellum when compared to other structures. No EAAT4-related sequence was found in the zebrafish genome. The EAAT5A expression was similar to EAAT5B in the telencephalon, while EAAT5B was less expressed than EAAT5A in optic tectum and cerebellum. Moreover, the glutamate uptake was significantly higher in optic tectum, which indicates functional differences within zebrafish brain structures. Altogether, the study of glutamate uptake in zebrafish could be important to evaluate the modulation of glutamatergic signaling through pharmacological and toxicological studies.

1. Introduction

Glutamate is the most widespread excitatory neurotransmitter in the mammalian CNS, being involved in many aspects of brain function such as learning and memory [48,26], development and ageing [50,7], and environmental adaptation [11]. However, besides its essential roles in brain activity, the neurotransmitter glutamate may be potently toxic (excitotoxicity), when present in high concentrations in the synaptic cleft [11,64,29], and it has been shown that this excitotoxic effect is involved in various acute and chronic neurological disorders [32,33,53,58]. Thus, the clearance of extracellular glutamate, mainly mediated by sodium-dependent transport into astrocytes [1,52,64], is an essential parameter involved in the physiological/excitotoxic tonus of the glutamatergic system.

The EAATs represent a protein family that displays considerable homology (50–60% at the amino acid level) [6]. To date, five structurally distinct subtypes of excitatory amino acid transporters have been identified and characterized in the mammalian brain: EAAT1 [56], EAAT2 [40], EAAT3 [27], EAAT4 [18] and EAAT5 [4]. EAAT1 is primarily an astroglial transporter and the main transporter pro-

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Table 1
PCR primer design.

EAAT member	Primers (5′–3′)	Tm (°C)	Cycles	PCR product (bp)	GenBank accession numbers	ZFIN ID (ZDB-GENE)
EAAT1A	TGTCACGTCACGAGCTGCGCTC (F) ACAAGAAAACAGTGGACCGCTCG (R)	57	30	321	mRNA BC063233 Protein AAH63233	030131-2159
EAAT1B	AGAAACCGCGGTCGCGCAGC (F) TGCCAATGAACACTGCGATGAAGG (R)	57	30	396	mRNA XM_679025 Protein XP_684117	—
EAAT1C	CGTGATCTTCACCGTGGCTGCTG (F) AGTTGACGTTCTCTGTCGCATTGACC (R)	57	35	497	mRNA NM_001109703 Protein NP_001103173	071004-45
EAAT2	GCTGTCTGGAGGAGAACCTGGGCATTG (F) TCATCTCGATGTCGTCAGTCTTCCCGTG (R)	61	30	432	mRNA BC056751 Protein AAH56751	030131-7779
EAAT3	GGATGGAACTGCACTGTATGAAGCGGTG (F) GGACGATTCCAGCACCGTAGGCGTC (R)	61	30	287	mRNA NM_001002666 Protein NP_001002666	040718-414
EAAT5A	CGTTTGGCATTGTGTTTCTGGTGGCTG (F) TGAGCGATAAATATGGCCGCCACAG (R)	61	35	376	mRNA XM_678579 Protein XP_683671	—
EAAT5B	ATCGTCCTCACTTCAGTGGGTTTGC (F) GCAAAGGTTGTAACAGGCGGTGG (R)	57	35	330	mRNA XM_687808 Protein XP_692900	—
β-actin	GTCCCTGTACGCCTCTGGTCG (F) GCCGGACTCATCGTACTCCTG (R)	54	35	678	mRNA AAC13314 Protein AF057040	000329-1

tein present during CNS development [20]. EAAT2 is one of the two most abundant glutamate transporters in the adult CNS [30], and is an astroglial transporter expressed postnatally and responsible for up to 90% of all glutamate transport in adult tissue [45,61,63,31]. EAAT3, a neuronal glutamate transporter found at high densities on postsynaptic membranes, is present most notably in the hippocampus, cerebellum, and basal ganglia [21]. EAAT4 is a glutamate transporter largely limited to the Purkinje cells of the cerebellum [18,21], whereas EAAT5 is found primarily in the retina on photoreceptors and bipolar cells [4,43].

The zebrafish is a small freshwater teleost that has recently attained a pre-eminent position in biomedical research, being considered an important and emerging vertebrate model in many fields of biology including neuroscience [22]. This species exhibits genetic and anatomic conservation in relation to both mice and humans and a high degree of genetic homology [5,13]. Moreover, the zebrafish appears to be an attractive organism for high throughput screening applications, e.g., mutagenesis screening, forward genetics or drug discovery efforts applied to neurotoxicity tests [68,39].

Recently, the choice of this animal model to investigate some aspects of brain neurotransmission, including the glutamatergic system, has become more common. Studies have demonstrated the distribution and function of ionotropic glutamate receptors in the olfactory bulb [15,59], as well as embryonic expression of NMDA receptor subunit genes [10]. The glutamatergic modulator MK-801 was employed to examine behavioral parameters [57] as well as the role of glutamatergic receptors in learning and memory processes [36]. Furthermore, vesicular glutamate transport has been described in mutant zebrafish larvae, playing a key role in visual perception and behavior [54,37].

Although some parameters of the glutamatergic signaling in zebrafish have already been characterized, the expression and functional profile of glutamate transporters have not yet been reported. Theories about vertebrate neural and behavioral bases propose that brain evolution occurred in successive stages and that these bases have been conserved through phylogenesis. However, recent developmental, neuroanatomical and functional data indicate that the brain and behavioral evolution may have been more conservative than previously thought [47].

To provide new insights into the primary characteristics of the glutamatergic system in zebrafish, the aims of the present study were to identify the relative gene expression profiles of distinct members of the EAAT family and to carry out a preliminary investigation of some parameters of glutamate uptake in three brain structures in this species: the telencephalon, optic tectum, and cerebellum.

2. Experimental procedures

2.1. Materials

Reagents were purchased from Sigma Chemical CO (St. Louis, MO, USA). L-[^{3}H]glutamate (specific activity 30 Ci mmol^{-1}) was purchased from Amersham International, UK. Platinum Taq DNA polymerase, TRIzol reagent, and SuperScript™ First-Strand III (Synthesis System for RT-PCR) were purchased from Invitrogen (Carlsbad, CA, USA).

2.2. Animals

Adult wild-type zebrafish (*Danio rerio*) of both sexes (3–6 months-old) were obtained from a commercial supplier (Delphis, RS, Brazil). All fish were acclimated to their new environment for at least 2 weeks in a 50-l thermostated aquarium. The water was kept at 26 ± 2 °C under a 12-h light–dark controlled photoperiod and the animals were fed with commercial flake fish food twice a day. They were used according to the National Institutes of Health Guide for Care and Use of Laboratory Animals, being healthy and free of any signs of disease. All procedures in the present study were approved by the Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS), protocol number 477/05-CEP.

2.3. Phylogenetic analysis and primers design

EAAT members were identified by NCBI Blast searches of GenBank, using the well-known *Homo sapiens* and *Rattus norvegicus* proteins as queries. The obtained sequences (supported by mRNA or EST data) were compared with the zebrafish protein database of the Zebrafish Information Network (ZFIN) (University of Oregon, Eugene, OR 97403-5274; World Wide Web URL: http://zfin.org) and the sequences were aligned using the ClustalX program [62]. A phylogenetic tree was constructed according to the Neighbor-Joining method [49] using proportional (p) distance with the MEGA 2.1 program [46]. In order to compare the zebrafish deduced amino acid sequences, an alignment was performed using ClustalX. Regions with low scores for similarity among the sequences were used to search for specific primers, which were designed using the program Oligos 9.6. The primer specificities were checked by comparing each primer with the zebrafish genome to confirm that it would recognize only its specific target sequence. Thus, the strategy adopted for the design of the primers avoided cross-amplification. The optimal conditions for primer annealing were determined (Table 1) and the β-actin primers were designed as described previously [9].

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

In order to obtain distinct brain structures from zebrafish, the animals were cryoanaesthetized and further euthanized by decapitation. Total RNA was isolated from telencephalon, optic tectum, and cerebellum using the TRIzol® reagent in accordance with the manufacturer's instructions. The purity of the RNA was spectrophotometrically determined by calculating the ratio between absorbance values at 260 and 280 nm and its integrity was confirmed by electrophoresis through a 1.0% agarose gel. Afterwards, all samples were adjusted to 160 ng/μl and cDNA species were synthesized using SuperScript™ III First-Strand Synthesis SuperMix (Synthe-

sis System for RT-PCR) following the suppliers instructions. Each RNA sample was mixed with 1 μl of 50 μM Oligo (dt) and 1 μl annealing buffer (final volume of 8 μl) and then incubated in a thermal cycler at 65 °C for 5 min. The samples were immediately placed on ice for 1 min and 10 μl 2× First-Strand Reaction Mix and 2 μl SuperScript™ III/RNaseOUT™ Enzyme Mix were added. The products were mixed, incubated for 50 min at 50 °C and the reaction was terminated at 85 °C for 5 min. PCR reactions for different EAATs and β-actin genes were performed in a total volume of 20 μl, with 0.1 mM primers (Table 1), 0.2 mM dNTP, 2 mM $MgCl_2$ and 0.5 U Platinum Taq DNA polymerase. The following conditions were used for the PCR reactions: 1 min at 94 °C, 1 min at the annealing temperature (see Table 1), and 1 min at 72 °C for a number of cycles to ensure the linearity of transcript amplification. Post-extension at 72 °C was performed for 10 min. A negative control was included for each set of PCR reactions. PCR products were separated on a 1.0% agarose gel with GelRed 10× and visualized with ultraviolet light. The fragment lengths expected for the PCR reactions were confirmed using Low DNA Mass Ladder and β-actin was determined as an internal standard. Band intensities were analyzed by optical densitometry using the Kodak 1D Image Analysis Software after running all PCR products in a single gel.

2.5. Glutamate uptake

2.5.1. Tissue preparation

The animals were cryoanaesthetized and further euthanized for total brain excision. Telencephalon, optic tectum and cerebellum were dissected into Petri dishes humidified with Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 1.11 glucose, pH 7.2. Each structure was separated with the help of a magnifying glass and entirely transferred to paired 24-well culture plates containing 0.5 ml of HBSS. One plate from each pair was maintained at 37 °C and the other at 4 °C. The structures from the first plate were washed once with l ml of 37 °C HBSS and those of the second were washed with 1 ml of ice-cold HBSS containing N-methyl-D-glucamine (4 °C) instead of sodium chloride.

2.5.2. Uptake assay

Brain structures were preincubated at 37 °C for 15 min in 0.28 ml of HBSS. The uptake assay was carried out by adding 20 μl of a solution containing 0.33 μCi/ml L-[2,3–3 H]glutamate with unlabeled glutamate (to appropriate concentrations) at 37 °C. Incubations were stopped after 5 min (for telencephalon) and 7 min (for optic tectum and cerebellum) by washing out the glutamate remaining in the incubating medium followed by two washes with l ml ice-cold HBSS. The brain structures were immediately transferred to 0.5N NaOH and incubated overnight, resulting in a homogenate. Protein content was measured using aliquots of homogenate (10 μl) following the method described by Peterson [38]. Samples were taken for determination of the intracellular content of L-[2,3–3 H]glutamate by scintillation counting. Sodium-independent uptake was determined by using ice-cold (4 °C) HBSS containing N-methyl-D-glucamine instead of sodium chloride. The results were subtracted from the total uptake to obtain the sodium-dependent uptake.

2.6. Statistical analysis

EAATs expression data were analyzed using one-way ANOVA for multiple group comparison followed by post hoc analysis carried out by Duncan's multiple range tests. The glutamate uptake was expressed as nmol glutamate min^{-1} mg $protein^{-1}$. For all parameters analyzed, $p \leq 0.05$ was considered significant.

3. Results

3.1. Identification of zebrafish EAATs ortholog gene sequences and phylogenetic analysis

Proteins of *H. sapiens* (EAAT1: AAH37310; EAAT2: P43004; EAAT3: NP_004161; EAAT4: NP_005062; EAAT5: NP_006662) and *R. norvegicus* (EAAT1: NP062098; EAAT2: NP_058911; EAAT3: NP_037164; EAAT4: AAB72086; EAAT5: NP_001102443) were retrieved from GenBank and used as queries for the identification of EAAT-related ortholog sequences in zebrafish. Another non-mammalian organism, *Ambystoma tigrinum*, was added in order to compare the grouping of EAAT sequences since previous studies demonstrated the existence of different EAAT members in salamander [16,17].

The phylogenetic tree was constructed using the Neighbor-Joining method [49] and proportional (p) distance (Fig. 1). Five well-resolved terminal clades supported by high bootstrap values were identified. When both human and rat EAAT genes were used as queries, NCBI Blast searches of GenBank yielded seven complete EAAT-similar deduced amino acid sequences in zebrafish (Fig. 1). In order to confirm the sequence identities and to obtain the information about the current data annotation, these sequences were compared with the zebrafish protein database at ZFIN. The AAH56751 and NP_001002666 sequences grouped consistently with both *H. sapiens* and *R. norvegicus* EAAT2 and EAAT3 clades, respectively, suggesting homologous function in zebrafish. The AAH63233, XP_684117 and NP_001103173 sequences (named EAAT1A, EAAT1B, and EAAT1C, respectively) must be paralogs since they were grouped together and formed the EAAT1 clade with *H. sapiens* and *R. norvegicus* sequences. The phylogenetic analysis did not enable any zebrafish sequence retrieved from the GenBank database to be grouped consistently with the EAAT4. Two other sequences, XP_683671 and XP_692900, also formed a clade with EAAT5 *H. sapiens* and *R. norvegicus* sequences. These sequences were named EAAT5A and EAAT5B, respectively. The sequences Dr XP_684117 (EAAT1B), Dr XP_683671 (EAAT5A) and Dr XP_692900 (EAAT5B) were classified by the GenBank database as predicted. Due to the high degree of similarity with the respective *H. sapiens* and *R. norvegicus* EAAT sequences and their consistent position on the phylogenetic tree, these paralog members were also analyzed.

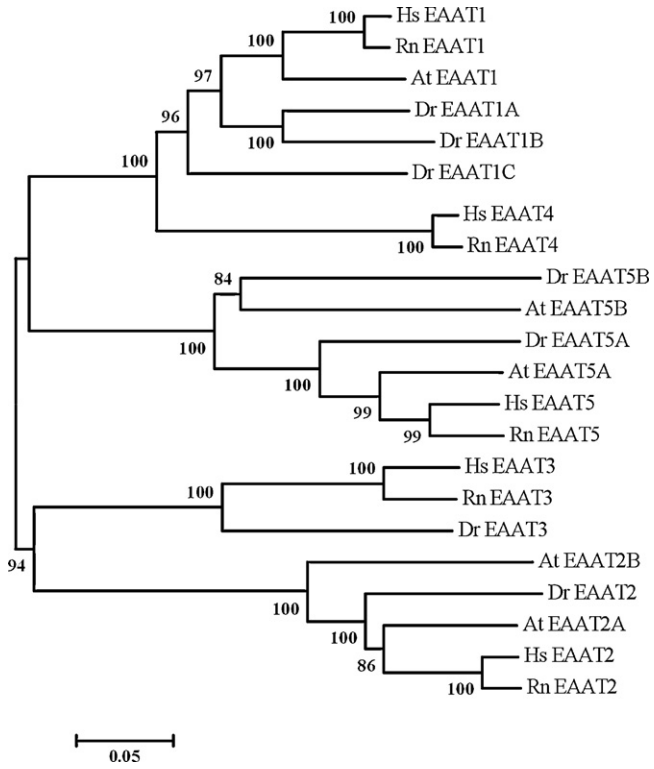

Fig. 1. Phylogenetic analysis of EAAT-related genes. The deduced amino acid sequences were aligned with the ClustalX program and the phylogenetic tree was constructed using the Neighbor-Joining method, proportional (p) distance with the MEGA 2.1 program. The phylogenetic tree grouped consistently *Danio rerio* (Dr), *Ambystoma tigrinum* (At), *Rattus norvegicus* (Rn) and *Homo sapiens* (Hs) EAATs ortholog sequences.

3.2. Gene expression of EAAT members in brain structures

The relative expression profiles of EAAT-related genes were evaluated in different brain structures of zebrafish. RT-PCR experiments showed that transcripts for the seven glutamate transporters identified by phylogenetic analysis were present in all analyzed structures (Fig. 2). EAAT2 and EAAT3 mRNAs were detected at higher levels in all structures, compared with all the other mRNAs. Gene expression of the paralogs EAAT1A and EAAT1B was similar in telencephalon, whereas significantly higher levels of EAAT1A transcripts were detected in the optic tectum and cerebellum when

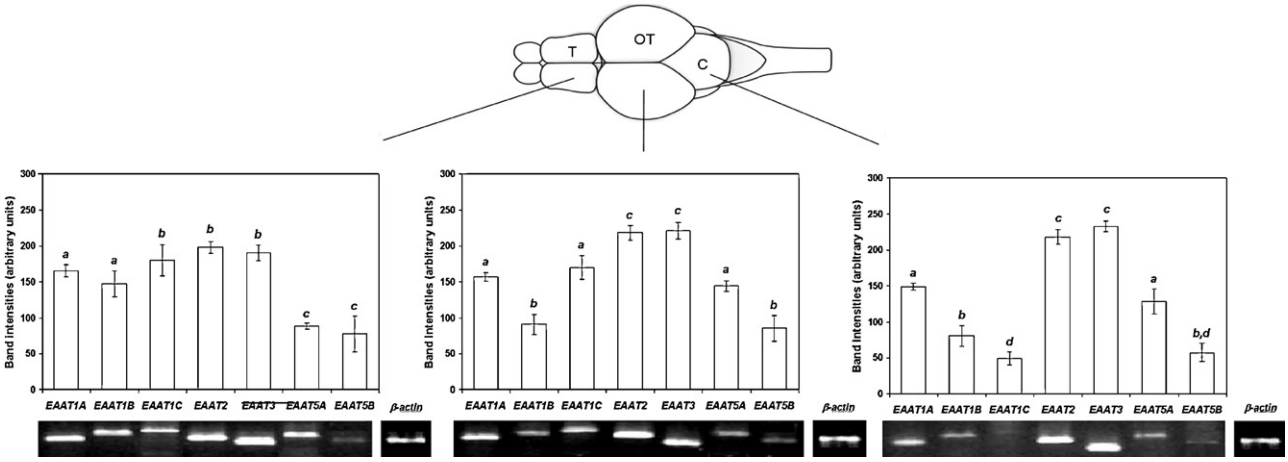

Fig. 2. Relative expression profile of EAAT genes in zebrafish brain. The amplifications resulted in a single product. The band intensities were measured by optic densitometry analysis for telencephalon (T), optic tectum (OT) and cerebellum (C) using the Kodak 1D Image Analysis Software. The results were expressed as mean ± S.E. of optical densitometry arbitrary units of four independent replicate RT-PCR experiments. Distinct letters mean statistically significant differences for EAAT genes expression in each structure ($p \leq 0.05$; one-way ANOVA followed by the Duncan post hoc test).

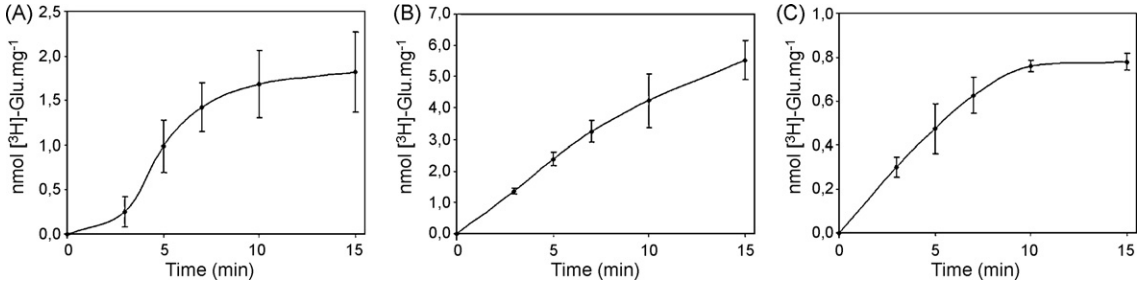

Fig. 3. Time course of Na^+-glutamate uptake in zebrafish telencephalon (A), optic tectum (B) and cerebellum (C). Brain structures were transferred to 24-well culture plates and preincubated at 37 °C for 15 min in 0.28 ml of HBSS. Uptake was assessed by adding 20 μl of a solution containing 0.33 μCi/ml L-[2,3–3 H]glutamate with 100 μM of unlabeled glutamate at 37 °C. The sodium-independent uptake was subtracted from the total uptake to obtain the sodium-dependent uptake. The data were expressed as mean ± S.E. of four different experiments, each in duplicate.

compared to EAAT1B. Concerning the EAAT1C, the results show the presence of similar mRNA levels in telencephalon and optic tectum and a lower amount in the cerebellum. The relative gene expression of EAAT5A was similar to that of EAAT5B in the telencephalon, while EAAT5B was significantly less expressed when compared to EAAT5A in optic tectum and cerebellum.

3.3. Glutamate uptake

Considering the identification of distinct EAAT mRNAs and their expression profiles in zebrafish brain, some preliminary parameters of glutamate transporter activity were investigated. In order to verify the influence of temperature on L-[^{3}H]glutamate uptake, the assay was carried out in over a range from 21 to 45 °C (7 min of incubation, 100 μM of glutamate). Since the temperatures tested did not alter the glutamate uptake in all brain structures analyzed (data not shown), subsequent experiments were performed at 37 °C. Time courses for glutamate uptake in telencephalon, optic tectum and cerebellum were obtained over 3–15 min and these are represented in Fig. 3. A distinct glutamate uptake profile was observed in zebrafish brain structures. The uptake in telencephalon and cerebellum remained constant after 10 min incubation, whereas the glutamate uptake activity in optic tectum increased over the time period analyzed.

4. Discussion

In mammalian brain, it has been clearly demonstrated that glutamate transporter activity modulates the excitotoxic/physiological tonus of the glutamatergic system, presenting a central role in brain function [11,33,64]. By way of comparison, therefore, here we investigated some EAAT parameters in zebrafish brain. In addition to the confirmation of EAAT-related sequences by a phylogenetic analysis, this is the first study to demonstrate the mRNA expression profile in zebrafish CNS of EAAT1, EAAT2, EAAT3 and EAAT5 members of the glutamate transporter family. Surprisingly, the EAAT4 glutamate transporter was not identified in zebrafish in this work. Despite this, it is clear that structures from zebrafish brain have the ability to transport glutamate in a Na^+-dependent fashion.

Searches in the GenBank database yielded seven EAAT-similar deduced amino acid sequences in zebrafish, which were consistently grouped to human, rat, and salamander EAAT orthologs, supporting the evolutionary conservation of each protein. For EAAT2 and EAAT3 only one related gene was found in the zebrafish genome. However, for EAAT1 three paralog members were found which were named EAAT1A, EAAT1B, and EAAT1C. For EAAT5 two paralog members were found which were named EAAT5A and EAAT5B. The presence of these paralog genes may be due to evolutionary genome duplication in the Teleostei infraclass [2,42], since previous studies have shown that teleosts tend to have expanded gene families as compared with mammals [19,41]. This gene family expansion could be caused by extra tandem duplication in the fish lineage, extensive loss of preexisting duplicates in the mammalian lineage or extra duplication of chromosomal segments, chromosomes, or the entire genome in the fish lineage [66]. Previous phylogenetic studies have argued against the idea that expanded families result from retention in the fish lin-

eage of a large number of duplicates that were present in the last common ancestor of zebrafish and humans [66]. Moreover, EAAT paralog genes have been found in organisms other than teleosts. Thus, five distinct glutamate transporter genes were identified expressed in the salamander retina: one EAAT1 subtype, two distinct EAAT2 subtypes (EAAT2A and EAAT2B), and two distinct members of the retina-specific subtype, EAAT5A and EAAT5B [16].

In this study, semiquantitative RT-PCR assays were performed to verify the mRNA expression of EAAT members of the glutamate transporter family in zebrafish brain structures. In mature mammalian brain, EAAT1 (GLAST) and EAAT2 (GLT-1) are expressed primarily in astrocytes and are responsible for up to 80% of glutamate removal from the synaptic cleft [45,11,24]. Here, we show that the orthologs EAAT1A, EAAT1B, EAAT1C, and EAAT2 genes are expressed and have a wide distribution in the zebrafish brain, as reflected in considerable levels of transcripts for these genes.

EAAT3 is a mammalian neuronal EAAT. Unlike the astrocytic glutamate transporters, it does not play a major role in clearing glutamate from the extracellular space [45,23,28], in spite of being an essential brain transporter. In the rat brain, the highest concentrations of EAAT3 are found in the hippocampus, cerebellum, and basal ganglia [55,11]. Here we show that there were high expression levels in several brain structures, as well as a wide distribution of transcripts for the EAAT3 gene. There is evidence pointing to the involvement of this transporter in the re-synthesis of GABA in presynaptic GABAergic neuronal terminals [51]. Moreover, it is far more effective at transporting cysteine than the astrocyte glutamate transporters [67,3] and cysteine is a substrate for the synthesis of glutathione, the main thiol antioxidant [14].

Electrophysiological studies of EAAT4 and EAAT5 have demonstrated a relatively large chloride conductance associated with transport activity. Here, we show that both EAAT5A and EAAT5B are expressed in zebrafish brain and their mRNAs were detectable in all the investigated structures, with the expression of EAAT5B being lower than that of EAAT5A. This result differs from data obtained in mammalian neuronal tissue [4], for which Northern blot assays showed a strong signal only in the retina. Furthermore, two clones of EAAT5 (sEAAT5A and sEAAT5B) have been isolated from salamander [16] and appear to be expressed both in Müller cells and in most neurons [16]. The significance of these findings still remains unclear, but it is possible to speculate that the functions of EAAT5 in fish and other vertebrates might be distinct.

Based upon the observed data for EAAT gene expression in zebrafish, we performed some preliminary experiments on the [^{3}H] glutamate uptake by the three brain structures and observed that all the structures presented glutamate uptake activity. It has been clearly demonstrated [8] that the equilibrium between the physiological/excitotoxic glutamatergic tonus is dependent on adequate activity in glutamate transporters. As there is no extracellular metabolism of glutamate, uptake of this amino acid is the unique mechanism responsible for maintaining extracellular concentrations below toxic levels in the long term, as well as for the control of glutamatergic function. Consequently, brain tissue needs a very efficient glutamate uptake system to protect itself against glutamate toxicity.

The presence of all the observed EAATs in all the structures investigated is intriguing, as compared with mammalian brain distribution. The area responsible for learning in zebrafish is the telencephalon, which is analogous to the hippocampus and amygdala in mammalian brain [44]. After induction of long-term potentiation (LTP), the activation of NMDA receptors and GLT-1 function play important roles through the regulation of extracellular levels of glutamate [35,60]. Electrophysiological evidence of NMDA receptor-mediated activity and synaptic plasticity, such as LTP, has already been reported in zebrafish brain [36]. Accordingly, our results demonstrate the expression pattern of GLT-1 (EAAT2) in the telencephalon. Four EAAT subtypes are expressed in zebrafish brain and they may contribute to glutamate uptake in the optic tectum of zebrafish, which has been considered a useful vertebrate model in visual neuroscience. Given that the optic tectum is the area responsible for visual processing in the zebrafish brain [34], an interesting possibility is that the glutamate transporters may participate in visual functions in this species. Furthermore, the influence of central glutamatergic synapses on the acuity of visual perception in zebrafish has been demonstrated [54].

Cerebellar compartments in teleosts correspond to the mammalian vestibulocerebellar and non-vestibulocerebellar systems, participating in the control of balance and locomotion, respectively [65]. The Purkinje cells of the cerebellar molecular layer express EAAT4 in the CNS of adult rat [12] and human [25]. Considering that the EAAT4 gene has not been found in the zebrafish genome so far, other members of the EAAT family could play a role in the glutamatergic transmission involved in cerebellar function.

The different gene expression patterns of EAATs in zebrafish in comparison to those observed in human and rat reinforces the idea that the vertebrate neural and behavioral changes that support brain evolution occurred in successive stages and have been conserved through phylogenesis. In addition, knowledge of EAATs in this species sheds light upon their localization as well as functional differences in vertebrate glutamatergic signaling.

In summary, this report presents a phylogenetic analysis and the relative gene expression profiles of glutamate transporters in zebrafish, as well as preliminary data on glutamate uptake. The identification of EAAT-related genes involved in glutamate transport and the demonstration of uptake activity, together with the powerful genetic approaches that this organism offers could be important in determining neurotoxicity parameters and in providing a better understanding of the role of glutamatergic signaling in the neurobiology of this model species.

Conflict of interest

None.

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