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Jeffrey L. Arriza

Scott Eliasof

Michael Kavanaugh
University of Montana - Missoula

Susan G. Amara

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Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance

(uptake/chloride ion/PDZ domain)

JEFFREY L. ARRIZA*, SCOTT ELIASOF*, MICHAEL P. KAVANAUGH*, AND SUSAN G. AMARA*†‡

*Vollum Institute for Advanced Biomedical Research and †Howard Hughes Medical Institute, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97210

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ABSTRACT **Although a glutamate-gated chloride conductance with the properties of a sodium-dependent glutamate transporter has been described in vertebrate retinal photoreceptors and bipolar cells, the molecular species underlying this conductance has not yet been identified. We now report the cloning and functional characterization of a human excitatory amino acid transporter, EAAT5, expressed primarily in retina. Although EAAT5 shares the structural homologies of the EAAT gene family, one novel feature of the EAAT5 sequence is a carboxy-terminal motif identified previously in *N*-methyl-D-aspartate receptors and potassium channels and shown to confer interactions with a family of synaptic proteins that promote ion channel clustering. Functional properties of EAAT5 were examined in the *Xenopus* oocyte expression system by measuring radiolabeled glutamate flux and two-electrode voltage clamp recording. EAAT5-mediated L-glutamate uptake is sodium- and voltage-dependent and chloride-independent. Transporter currents elicited by glutamate are also sodium- and voltage-dependent, but ion substitution experiments suggest that this current is largely carried by chloride ions. These properties of EAAT5 are similar to the glutamate-elicited chloride conductances previously described in retinal neurons, suggesting that the EAAT5-associated chloride conductance may participate in visual processing.**

The uptake of glutamate and other excitatory amino acids is mediated by a gene family of high affinity sodium-dependent transporters that includes four known mammalian subtypes; in humans, we have termed these glutamate transporters excitatory amino acid transporter (EAAT) 1 through 4 (1, 2). The transport of glutamate is driven by the cotransport of sodium ions and the countertransport of potassium ions down their electrochemical gradients, and recent studies suggest that this complex process involves the cotransport of protons as well (3–6). Because there is net inward movement of positive charge with the transport of each molecule of glutamate, the transport process is readily studied in the *Xenopus* oocyte expression system by observing the associated current. In addition to these transport currents, however, we have found that application of substrate to the transporter also gates an uncoupled, passive flux of chloride ions (2, 7). The relative magnitude of this associated chloride conductance varies with each cloned EAAT subtype; for EAAT1–EAAT3, the magnitude of the chloride current at physiological membrane potentials is similar to that of the electrogenic cotransport current, but the currents generated by EAAT4 are almost entirely due to the flux of chloride ions. *In vivo*, a glutamate-dependent current that has a transporter-like pharmacology is carried largely by chloride ions in retinal cone (8) and rod (9) photoreceptors and bipolar cells (10). In bipolar cells, this chloride current has been proposed to mediate the cone component of the ON bipolar cell light response (10). Although the properties of EAAT4 are similar to the glutamate-elicited current in the retinal neurons, EAAT4 is almost exclusively expressed in cerebellum (2) and therefore was unlikely to account for the retinal activity.

To gain insight into the molecular basis of glutamate transporter physiology in the retina, we are currently characterizing EAAT subtypes expressed in a well described model system, the salamander retina. One of the glutamate transporters isolated from this tissue is associated with a large chloride conductance when expressed in *Xenopus* oocytes and exhibits a pharmacology similar to that previously reported in the retinal cells (unpublished work). We have isolated the human homolog of this gene by screening a human retinal cDNA library with the salamander cDNA. Here, we report the cloning and characterization of a human retinal glutamate transporter subtype, EAAT5.

EXPERIMENTAL PROCEDURES

Molecular Cloning and Sequencing. A glutamate transporter cDNA isolated from salamander retina (unpublished work) was used to screen a human retinal λgt10 cDNA library [provided by J. Nathans (11)] under conditions of reduced stringency. Plaque filter lifts prepared as per the manufacturer's instructions (GeneScreen; New England Nuclear) were hybridized overnight at 55°C in 0.5 M Na_2HPO_4, pH 7.15/7% SDS/1 mM EDTA with the salamander cDNA probe coding sequence ^{32}P-radiolabeled by random priming at 1×10^6 cpm/ml (Boehringer Mannheim). Filters were washed at 55°C in 2× standard saline phosphate/EDTA (SSPE) (20× SSPE = 3 M NaCl/0.2 M NaH_2PO_4/0.02M Na_2 EDTA, pH 7.4) and 1% SDS. The *Eco*RI inserts from purified phage clones were subcloned into pBluescript II SK (Stratagene) and characterized. The complete coding sequences of two clones were subsequently sequenced on both strands using double-stranded template, synthetic oligonucleotide primers, PRISM PCR-based sequencing reactions (Applied Biosystems), and an Applied Biosystems 373 Stretch DNA Sequencer. Sequence data analyses and comparisons were performed using MacVector (Kodak). The 2.9-kbp insert of one clone, termed "pEAAT5," has ≈180 bp of 5′ untranslated region containing two in-frame stop codons upstream of the Kozak consensus

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Abbreviations: EAAT, excitatory amino acid transporter; SSPE, standard saline phosphate/EDTA; NMDG, *N*-methly D-glucamine; PSD-95, postsynaptic density-95 protein; PDZ, a modular protein-binding motif; THA, *threo* β-hydroxyaspartate. tPDC, L-*trans*-pyrollidine-2,4-dicarboxylic acid.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. U76362).

‡To whom reprint requests should be addressed.

(ACCATGG) initiator methionine, 1683 bp of coding sequence, and ≈1.1 kbp of 3′ untranslated region. Polyadenylylation in the other EAAT5 isolate suggested a total message size of ≈3.1 kb, consistent with retinal Northern blot analyses.

Northern Blot Analyses. Human retinal poly(A)$^+$ RNA (2 μg) was size-fractionated on a denaturing formaldehyde gel and transferred to nylon membrane. The human retina RNA and Multiple Tissue Northern blot (model 7760–1) were obtained from CLONTECH. The coding sequence of EAAT5 was excised and radiolabeled with α[^{32}P]dCTP (New England Nuclear) by the random priming method (Boehringer Mannheim). Filters were hybridized overnight at 42°C with cDNA probe (10^6 cpm/ml) in 5X SSPE, 50% formamide, 7.5X Denhardt's solution, 2% SDS, and 100 μg/ml of denatured salmon sperm DNA. Autoradiography was performed after two 30-min room temperature washes in 2X SSPE/0.1% SDS and two 20-min washes at 50°C in 0.1X SSPE/0.1% SDS. Membranes were subsequently stripped and rehybridized with a β-actin probe according to the manufacturer's protocol.

Expression and Electrophysiology. The coding sequence of EAAT5 was isolated by PCR for subcloning. Unique flanking restriction sites (Asp718 and *Xba*I) were incorporated via the oligonucleotide primers 5′-CGCG GGTAC CTC ACC *ATG* GTG CCG CAT-3′ and 5′-CGCG TCTAGA GGC *TCA* GAC ATT GGT CTC-3′. Twenty five cycles of denaturation (30 s, 94°C), annealing (30 s, 55°C), and extension (2 min, 72°C) were performed in 100-μl reactions that contained oligonucleotide primers at 1 μM each, 10 ng of plasmid cDNA template, and 300 μM each of deoxynucleotide, Vent polymerase, and reaction buffer (New England Biolabs). Digestion of the reaction products with Asp718 and *Xba*I allowed each coding sequence to be subcloned into plasmid pOTV for expression in *Xenopus laevis* oocytes as described (1). The resulting plasmid, termed "pOTV-EAAT5," was linearized with *Spe*I and synthetic RNA transcribed using T7 RNA polymerase and an mMessage mMachine RNA capping kit (Ambion, Austin, TX). EAAT5 RNA was microinjected into defolliculated stage V-VI *Xenopus* oocytes and used for experiments 2–5 days later.

For uptake and electrophysiological experiments, the extracellular Ringer's solution consisted of (in millimolars): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, and 5 Hepes. The pH, adjusted with NaOH, was 7.5. For sodium-free solutions, 96 mM NaCl was replaced with 96 mM *N*-methyl D-glucamine (NMDG)-chloride, and the pH was adjusted with NMDG. For chloride-free solutions, all chloride salts were replaced with gluconate salts. Nitrate extracellular solution contained 96 mM $NaNO_3$ instead of NaCl.

Radiolabeled glutamate uptake was performed under voltage clamp at −60 mV (except, as noted, +10 mV). Currents were recorded during bath application of 100 μM [^{3}H]-L-

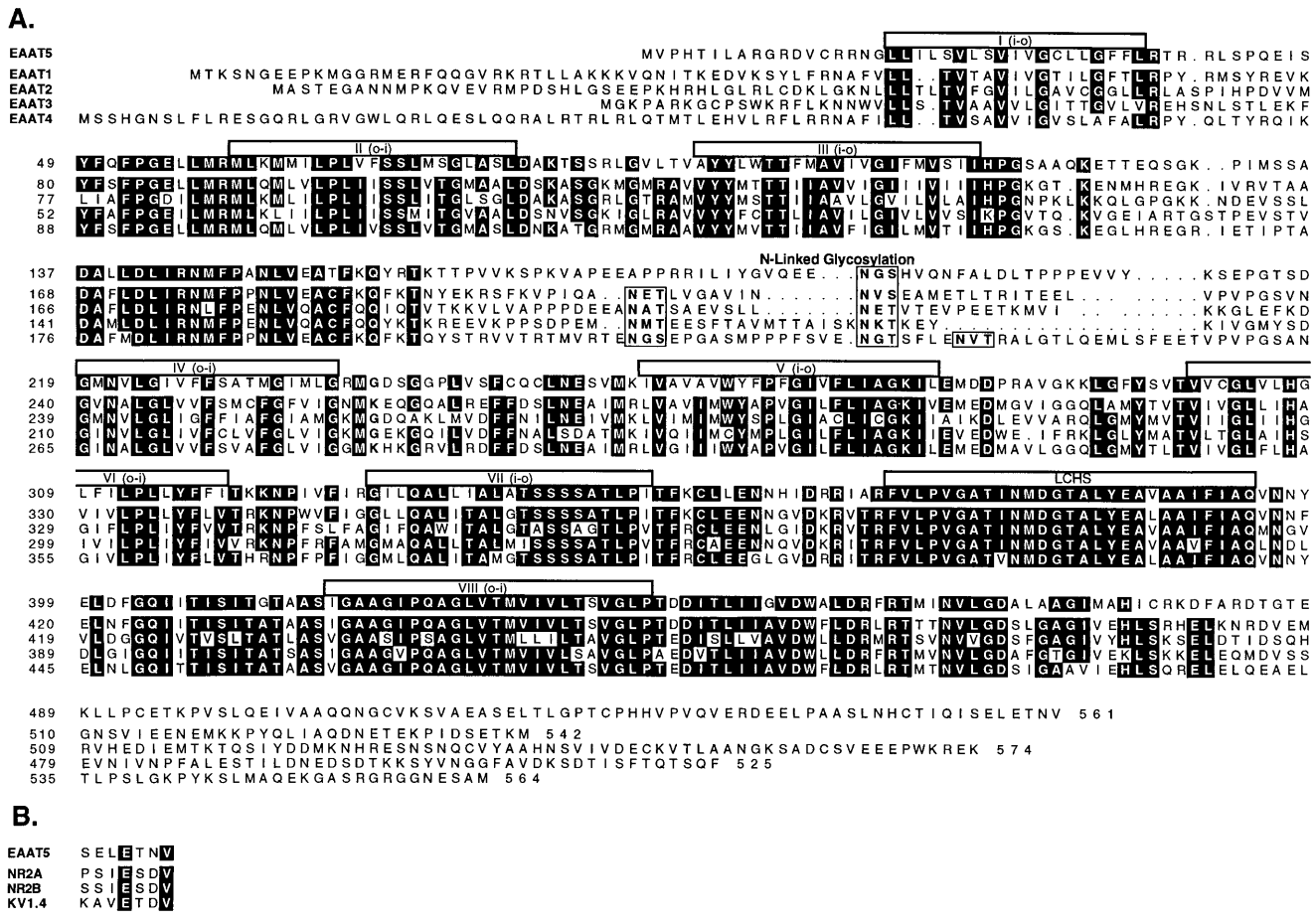

FIG. 1. EAAT5 is a member of the human glutamate transporter gene family. (*A*) The predicted amino acid sequences of human EAAT5 is shown in alignment with the other known human EAAT subtypes (1, 2). In this alignment, amino acid residues identical in four of the five sequences are shown in white on black lettering to illustrate the extensive amino acid sequence conservation in this gene family. Although the transmembrane topology of these transporters is not well defined, one possible model is indicated here. The poorly conserved amino- and carboxy-terminal sequences are likely to be intracellular, and eight regions with strong transmembrane (TM) scores are indicated by bars and suggested orientation (i, inside; o, outside). The large conserved hydrophobic sequence (LCHS) indicated between TMs VII and VIII may be membrane-associated. Possible N-linked glycosylation sites (N-X-S or T) in a large extracellular loop are boxed. (*B*) The carboxyl terminus of EAAT5 conforms to a sequence motif (E-S or T-X-V) involved in subcellular targeting. NMDA receptor subunits NR2A and NR2B (12) and potassium channel Kv1.4 (13) interact with PSD-95 via their C termini.

glutamate for 100 s. The specific activity of the [^{3}H]-L-glutamate (Amersham) in this solution was 20 Ci/mol (1 Ci = 37 GBq). Oocytes were washed in the bath for 3 min to minimize background radioactivity and then individually lysed in a scintillation vial containing 1% SDS for >15 minutes before adding scintillation fluid and counting.

Two microelectrode voltage-clamp recordings were performed using a GeneClamp 500 amplifier interfaced to a Digidata 1200 A/D and controlled using pClamp6 (Axon Instruments, Foster City, CA). Recording electrodes contained 3 M KCl with resistances <1 MOhm. Currents were filtered at 2 kHz and digitized at 5 kHz. For current–voltage curves, the oocyte membrane potential was held to −30 mV and stepped through a range of potentials from +50 to −100 in 100-ms steps. Steady state currents were measured during the final 20 ms of the command step.

RESULTS

Cloning of EAAT5. The isolation of an EAAT subtype from salamander retina whose sequence differed substantially from previously reported mammalian sequences lead us to isolate the human homolog EAAT5. The predicted human EAAT5 gene product is 560-amino acid residues in length, as depicted in the sequence comparison with other EAAT subtypes in Fig. 1*A*, and has a theoretical molecular mass of 61 kDa. Like other members of this gene family, EAAT5 is likely to be a glycoprotein, but EAAT5 differs in that there is only a single N-linked glycosylation site (N-X-S or T) in the putative large extracellular loop. With an optimal sequence alignment, EAAT5 has 46% identity with EAAT1, 43% identity with EAAT4, 37% with EAAT3, and 36% identity with EAAT2. For comparison, EAAT1 has 52% identity with EAAT4 and 49% with EAAT3. The most striking sequence conservation is found in a large conserved hydrophobic sequence that includes the sequence AAIFIAQ (residues 388–394 in EAAT5; Fig. 1*A*). However, the amino- and carboxy-terminal sequences of this gene family, thought to be intracellular, are poorly conserved. Of interest, the amino acid residues at the EAAT5 C terminus conform to a sequence motif found in synaptic membrane proteins: E-S or T-X-V-COOH [see review by Sheng (14)]. A comparison of the EAAT5 C-terminal sequence with those of the NMDA receptor subunits NR2A and NR2B and the Shaker-type potassium channel Kv1.4 ion channels, whose interactions with postsynaptic density-95 protein (PSD-95) have been studied extensively, is shown in Fig. 1*B*. The C termini interact with several PDZ (a modular protein-binding motif) domains in PSD-95 (14), and preliminary results using the yeast two hybrid assay for protein–protein interactions indicate an ability of EAAT5 to interact with these PDZ domains (J.A., unpublished observations).

Distribution by Northern Blotting. Northern Blot analyses indicate that a 3.1-kb EAAT5 RNA species is abundantly expressed in human retina (Fig. 2). A band of approximately the same size also was detectable in liver, but at a level at least 20-fold lower. Weak, ≈2.0-kb bands also were seen in heart and muscle, and a very light band at ≈4.5 kb was found in brain. It is not known if these differently sized bands reflect differential processing of the same gene or if there is cross-hybridization with a very closely related gene, but the sizes do not correspond to those reported for other human EAATs (1, 2). The weak hybridization with total brain RNA might reflect a restricted brain distribution; to address this issue, 20 μg of total RNA isolated from six human brain regions was analyzed by Northern blotting (frontal and motor cortex, hippocampus, thalamus, basal ganglia, and cerebellum), and no signal was detected (data not shown). Although these distribution studies were not exhaustive, the data suggest that expression of EAAT5 is relatively retina-specific.

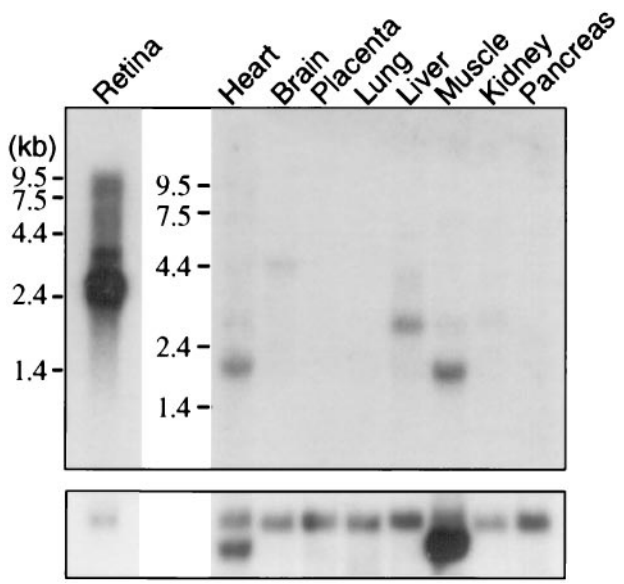

FIG. 2. EAAT5 mRNA is most abundantly expressed in the retina. The EAAT5 cDNA was used for hybridization of Northern-blotted poly(A)$^+$ RNAs (2 μg) from retina and other human tissues. The retinal signal, a single 3.1-kb band, was overexposed here so that additional EAAT5-hybridizing bands in other tissue RNAs could be seen. *Lower*, the same blots were hybridized with a β-actin probe to control for RNA loading in each lane.

Functional Properties. *Xenopus* oocytes injected with RNA transcribed from the EAAT5 cDNA were tested for their ability to take up exogenously applied glutamate. Uptake of radiolabeled glutamate was significantly increased over uninjected control oocytes (typically 2- to 10-fold) but was less than observed for EAAT1-, EAAT2-, or EAAT3-expressing oocytes (typically >50-fold). It is not clear whether this difference reflects a lower turnover rate for EAAT5 or reduced expression, but this is a characteristic shared with EAAT4. Radiolabeled L-glutamate (100 μM) was applied for 100 s while the oocyte membrane potential was held at −60 mV using a two-electrode voltage clamp (Fig. 3*A*). The [^{3}H]-L-glutamate content of EAAT5 oocytes was 2.3-fold greater than in uninjected oocytes not expressing EAAT5 ($P = 0.013$, unpaired two-tailed t test). EAAT5 uptake was sodium-dependent and voltage-dependent, with no significant difference from controls observed when external sodium was replaced by NMDG or when the membrane potential was clamped at +10 mV ($P > 0.29$). Similar to EAAT1–4 (2, 7), uptake was not significantly affected by replacing external chloride with the larger anion gluconate ($P = 0.55$).

Application of glutamate to oocytes expressing EAAT5 generated a current that was both voltage- and concentration-dependent (Fig. 3*B*). The current reverses at −20 ± 1 mV, and this reversal potential was not affected by the glutamate concentration. An outward current is not predicted for an electrogenic transporter mediating coupled flux but is similar to the currents present in other glutamate transporters, particularly the EAAT4 subtype (2). The ionic basis for this outward current is explored below. Currents also were elicited by both L- and D-aspartate and, much less potently, by D-glutamate. The apparent affinity (EC_{50}) and relative maximal current (I_{max}) for these compounds with the membrane potential held at −60 mV are shown in Table 1. EAAT5 exhibits considerable stereospecificity for L-glutamate vs. D-glutamate and a slight preference for L-aspartate over D-aspartate. The affinity for L-glutamate is modestly voltage-dependent; the EC_{50} increases e-fold per 86 mV.

L-*trans*-pyrollidine-2,4- dicarboxylic acid (tPDC) and *threo* β-hydroxyaspartate (THA) are potent blockers of both glu-

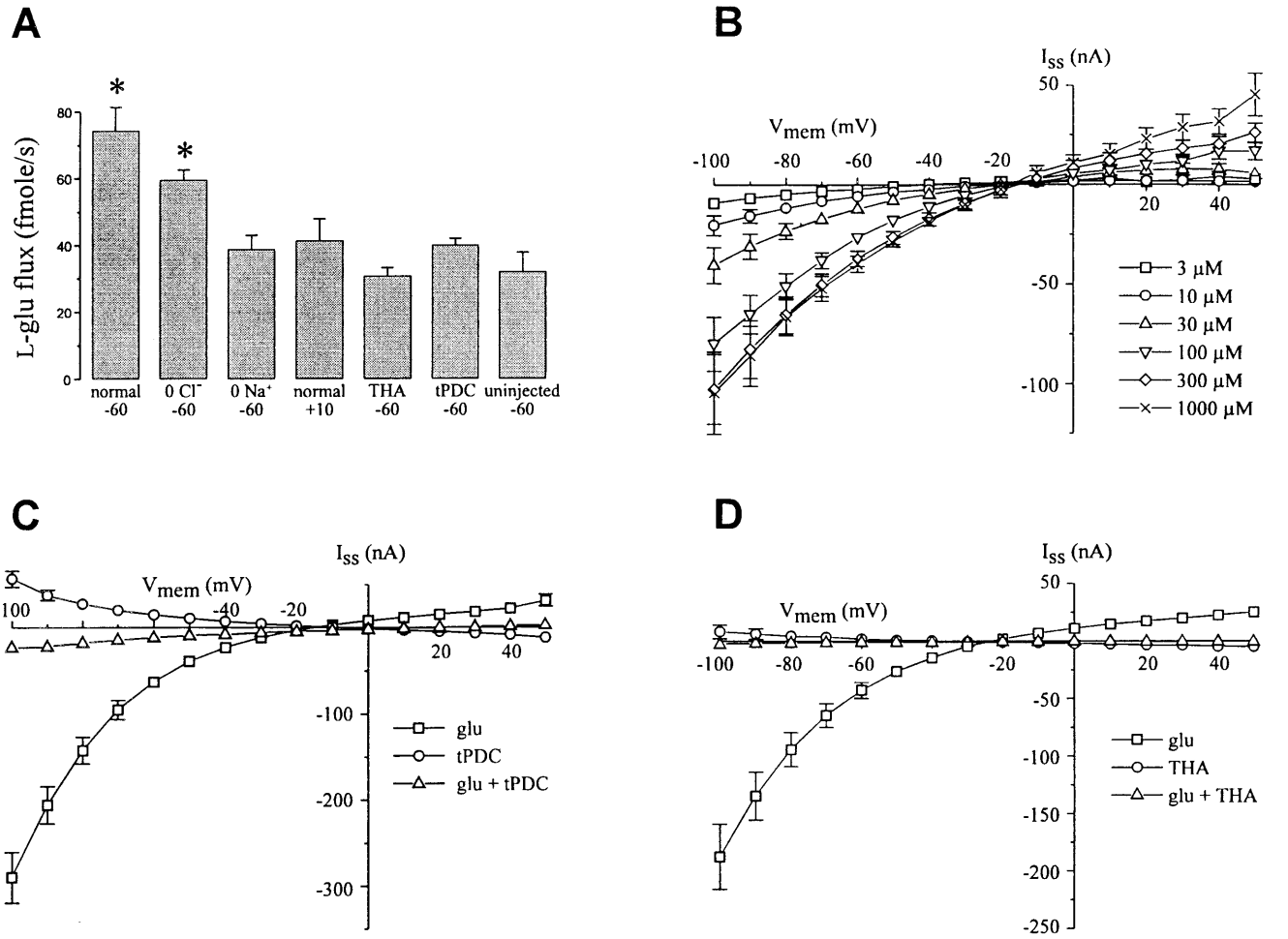

FIG. 3. Pharmacology of EAAT5-mediated uptake and currents. (*A*) Uptake of [^{3}H]-L-glutamate (100 μM) in oocytes voltage-clamped at −60 mV in normal Ringers solution (96 mM NaCl) (normal) or sodium-free (0 Na^+) or chloride-free Ringers (0 Cl^-) or voltage-clamped at +10 mV in normal Ringers. Uptake was sodium- and voltage-dependent. Additionally, uptake (−60 mV, normal Ringers) was blocked by coapplication of 100 μM THA or tPDC. *, Differs significantly from uptake measured in control uninjected oocytes. Data are the average of five cells each. (*B*) Dose- and voltage-dependent steady-state currents due to the application of L-glutamate to EAAT5-expressing oocytes. Concentration of L-glutamate indicated in the legend. Data are the average of seven cells. (*C*) Steady-state current elicited by 100 μM L-glutamate (squares) is blocked by coapplication of 100 μM tPDC (triangles); 100 μM tPDC alone (circles) elicits a small outward current at hyperpolarized potentials. Data are the average of four cells. (*D*) Steady-state current elicited by 100 μM L-glutamate (squares) is blocked by coapplication of 100 μM THA (triangles); 100 μM THA alone (circles) elicits a small outward current at hyperpolarized potentials. Data are the average of six cells. All error bars represent SEM. Error bars smaller than the symbols are not shown.

tamate uptake (Fig. 3*A*) and the glutamate-elicited current in EAAT5-expressing oocytes (Fig. 3, *C* and *D*). Currents elicited by 100 μM glutamate were almost entirely blocked by coapplication of 100 μM tPDC or 100 μM THA. From Schild analysis, EAAT5 has an apparent affinity (K_i) for tPDC and THA of 6.2 ± 1.7 μM and 1.0 ± 0.1 μM, respectively. Although both compounds are competitive substrates of other EAAT subtypes (1), neither blocker generated a current with a voltage dependence similar to that of glutamate. On the contrary, either tPDC or THA alone elicited currents that were outward at negative potentials and became small and inward at positive potentials (Fig. 3, *C* and *D*); this current, which is not seen in uninjected controls, may reflect the block of an EAAT5-mediated current that is independent of external amino acid. In contrast to the effects of tPDC and THA, the high affinity EAAT2 subtype blocker kainate had minimal effects on EAAT5 function. In five cells, 1 mM kainate reduced the response to 100 μM glutamate to 84 ± 11% of control over the range of −100 to −40 mV (data not shown).

Table 1. EAAT5 pharmacology

Compound	*n*	EC_{50}, μM	I_{max}
L-glutamate	5	64 ± 6	(1)
D-glutamate	4	>10,000	(0.21 ± 0.06)*
L-aspartate	5	13 ± 5	0.67 ± 0.20
D-aspartate	4	64 ± 10	0.72 ± 0.03
THA	6	1.0 ± 0.1	(0)†
tPDC	4	6.2 ± 1.7	(0)†

I_{max} is normalized to L-glutamate I_{max} in the same oocyte.
*Relative current elicited by 10 mM D-glutamate.
†Determined by Schild analysis; THA and PDC did not induce currents.

The dependence of EAAT5 glutamate-elicited currents on sodium and chloride ions was examined. As with uptake, glutamate-elicited currents in EAAT5 expressing oocytes require extracellular sodium; no currents were observed when sodium was replaced with NMDG (Fig. 4*A*). Replacing extracellular chloride with the larger anion gluconate eliminated the outward current elicited by 100 μM glutamate but had only a negligible effect on the inward current (Fig. 4*B*). This suggests that at least a portion of the glutamate-elicited outward current is due to the passive flux of chloride ions, as has been reported for the other human glutamate transporters (2, 7). To determine the relative contribution of chloride flux

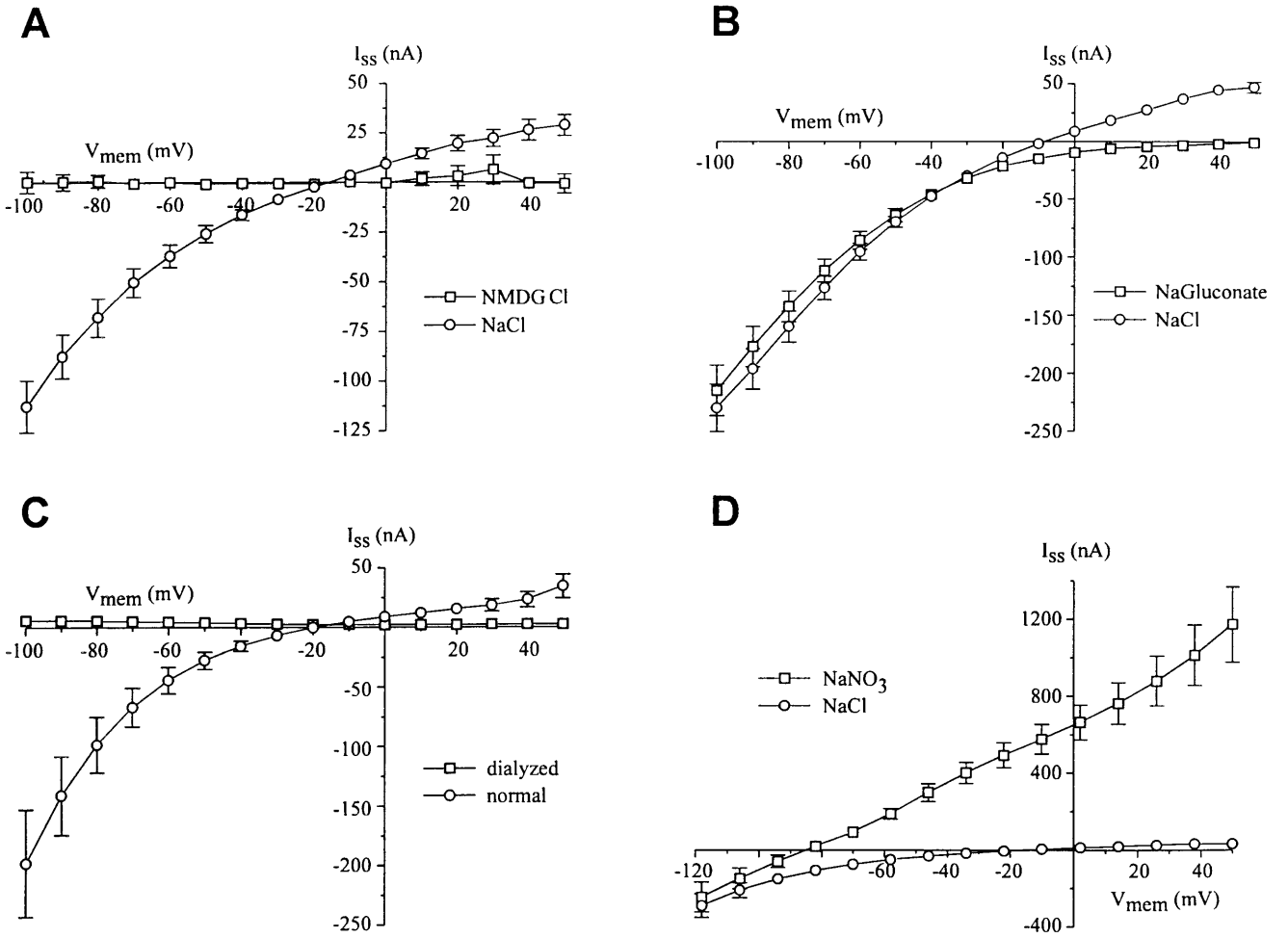

FIG. 4. Ionic dependence of EAAT5-mediated currents. (*A*) Steady-state current elicited by 100 μM L-glutamate (circles) is abolished when external sodium is replaced with NMDG (squares). Data are the average of five cells. (*B*) Steady-state current elicited by 100 μM L-glutamate in normal chloride (circles) or when external chloride was replaced with gluconate (squares). Removal of external chloride ions blocks outward current at positive potentials and has no effect at negative potentials. Data are the average of five cells. (*C*) Steady-state current elicited by 100 μM L-glutamate in normal oocytes (circles) or in oocytes dialyzed in chloride-free solution for >48 h (squares). No currents are seen in oocytes dialyzed to remove chloride ions. Data are the average of 10 cells for each condition. (*D*) Steady-state current elicited by 100 μM L-glutamate in normal chloride (circles) or when most external chloride was replaced with nitrate (squares). L-glutamate elicited current in nitrate produces a large outward current. Data are the average of six cells. All error bars represent SEM. Error bars smaller than the symbols are not shown.

to the inward current, internal chloride was reduced by dialyzing the oocytes in a chloride-free medium for at least 48 h. Under these conditions, application of 100 μM glutamate in the absence of external chloride produced no measurable current in 10 cells (Fig. 4*C*; dialyzed). In contrast, 10 cells from the same batch of oocytes were kept in a normal chloride medium and exhibited normal glutamate-elicited currents (Fig. 4*C*; control). Thus, it appears that both the inward and outward currents elicited by glutamate in EAAT5 are due primarily to the passive flux of chloride ions, similar to what has been reported for EAAT4. Finally, we investigated the effect of replacing extracellular chloride with the anion nitrate (Fig. 4*D*). As with other glutamate transporters (7, 15), nitrate substantially increased the glutamate-elicited outward current due to the influx of a more permeant external anion.

DISCUSSION

We have identified a novel mammalian glutamate transporter, EAAT5, whose mRNA is abundantly expressed in the retina and whose properties are consistent with a glutamate-gated chloride conductance associated with both presynaptic (16) and postsynaptic (10) aspects of the retinal light response. Electrophysiological studies in retinal photoreceptors (9, 17) and bipolar cells (10) have reported glutamate-gated currents that reverse at potentials close to the equilibrium potential for chloride and are diminished by removal of extracellular or intracellular chloride, suggesting that the currents are carried largely by chloride ions. These currents are not associated with known types of glutamate receptors because they are neither activated by receptor agonists nor inhibited by receptor blockers (8–10). However, the currents share several properties with glutamate transporters: They are sodium-dependent, activated by L-glutamate and L- and D-aspartate but not D-glutamate, and reduced by the coapplication of transport inhibitors such as THA and tPDC.

We find that human EAAT5 exhibits the ion-dependence and most of the pharmacological properties of the retinal activities described previously, yet there are a few differences. Unlike salamander rods (9) or fish bipolar cells (10), in which THA and tPDC alone produce small but measurable inward currents, these compounds do not induce EAAT5-mediated inward currents. Rather, the application of these compounds alone appears to reduce an EAAT5 leak current. This difference may be the result of differences between the human and lower vertebrate homologs of EAAT5 because THA and tPDC produce inward currents in the salamander homolog of EAAT5 (unpublished work). Another pharmacological differ-

ence between EAAT5 and the photoreceptor currents is that the currents in both rods (9) and cones (8) are somewhat sensitive to dihydrokainate, a transporter blocker similar to kainate. This may be caused by expression of multiple subtypes of glutamate transporters in a single cell. Such colocalization of glutamate transporter subtypes has been observed in the brain (18–20), and we have immunocytochemical evidence to suggest that this occurs extensively in the salamander retina as well (unpublished work).

There is accumulating evidence that the functional activities of glutamate transporters may extend beyond a conventional role in neurotransmitter clearance. Recent antisense knockout experiments suggest that the rat homologs of EAAT1, EAAT2, and EAAT3 [GLAST (21), GLT1 (22), and EAAC1 (23)] mediate the bulk of glutamate and aspartate uptake in the brain (24). This activity is essential in the nervous system, where glutamate serves as the major excitatory neurotransmitter yet is also a potent excitotoxin. Electrophysiological studies of EAAT4, and now EAAT5, demonstrate a relatively large chloride conductance associated with transport activity. The chloride conductances of EAAT4 and EAAT5 are of great interest because they suggest a function more akin to ligand-gated chloride channels than classic transporters. Both pre- and postsynaptic glutamate-gated chloride conductances have been examined in the vertebrate retina. In perch retina, the cone-mediated light response in depolarizing bipolar cells is due to the closing of a postsynaptic chloride conductance that has the pharmacology and ionic dependence of a glutamate transporter (10). Presynaptically, a glutamate-elicited chloride conductance with transporter-like properties in salamander cone photoreceptors recently has been demonstrated to respond to the release of glutamate from the same cell (16). The chloride equilibrium potential is more negative than the voltage-operating range of cones, so this EAAT5-like molecule may act as an inhibitory autoreceptor.

The protein-binding structure at the C terminus of EAAT5 has intriguing functional implications. We have found that the EAAT5 coding sequence contains a protein-binding motif recently identified as a mechanism for receptor and ion channel clustering at the synapse (14). It has been demonstrated that the cytoplasmic C terminus of NMDA receptor subunits (12, 25) and of Shaker-type potassium channels (13) physically bind PSD-95, an abundant synaptic protein associated with the membrane cytoskeleton (26). These C termini bind to multiple PDZ domains present in PSD-95, and the crystal structure of PDZ domains recently was examined to determine how a relatively short C-terminal peptide sequence is specifically bound (27, 28). The critical C-terminal determinants for binding to PDZ domains are present in the EAAT5 C terminus (Fig. 1*B*), and we have preliminary data from the yeast two-hybrid system that support a EAAT5–PDZ domain interaction (J.A., unpublished observations). PSD-95 is itself a member of a family of synaptic proteins containing PDZ domains (14, 25, 29–31), so it is not readily apparent which protein or proteins might serve as functional partners of EAAT5 *in vivo*. The discovery of this binding motif in EAAT5 suggests that EAAT5 resides in the vicinity of components of the signal transduction pathway where its channel-like properties may indicate a role in retinal physiology distinct from neurotransmitter clearance.

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