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Functional Properties and Substrate Specificity of the Cloned L-Glutamate/L-Aspartate Transporter GLAST-1 from Rat Brain Expressed in *Xenopus* Oocytes

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The rat brain L-glutamate/L-aspartate transporter GLAST-1 is a member of a family of Na^+-dependent high-affinity L-glutamate transporters proposed to be involved in the termination and modulation of excitatory neurotransmitter signals. Application of electrophysiological and radiotracer techniques on *Xenopus* oocytes expressing cloned GLAST-1 revealed that the apparent K_m value of the transporter for L-glutamate and Na^+ ions did not depend on voltage while the maximal transport rate increased with more negative potentials, indicative of a low-field access channel. The apparent K_m value of the transporter for L-glutamate depends on the Na^+ concentration, suggesting that substrate and ions are transported by GLAST-1 in a simultaneous manner. All of the L-glutamate uptake blockers tested either were substrates or did not affect the current induced by L-glutamate. The changes in the amplitude of the current induced by simultaneous application of two substrates can be interpreted by a competition for one binding site.

[Key words: GLAST-1, glutamate, transport, functional properties, electrophysiology,* Xenopus *oocytes]

The amino acid L-glutamate is the major fast acting neurotransmitter in the mammalian CNS (Collingridge and Lester, 1989). Activating iono- and metabotropic receptors, L-glutamate plays an important role in the complex process of learning and the establishment of memory. However, at increased concentrations L-glutamate leads to neuronal degeneration (excitotoxicity of L-glutamate; see Choi, 1988). In order to maintain synaptic signaling and to prevent the excitotoxic action of L-glutamate, the amino acid has to be rapidly removed from the extracellular space, a process thought to be mediated by Na^+-dependent high-affinity glutamate transport systems (Flott and Seifert, 1991). The existence of multiple Na^+-dependent glutamate transport systems was initially inferred from the regional heterogeneity of the uptake process (Robinson et al., 1993) and proven by the recent cloning of three cDNAs coding for rodent L-glutamate transporters (Kanai and Hediger, 1992; Pines et al., 1992; Storck et al., 1992). The encoded membrane proteins belong to a new family of Na^+-dependent high-affinity glutamate transporters deviating from the topology of previously discovered neurotransmitter transporters (Storck et al., 1992; for review, see Kanai et al., 1993). Single members of this family can now be studied for the first time independently upon expression of the cloned transporters in heterologous systems. Besides catalyzing the uptake of L-glutamate and L-aspartate, the transport systems are proposed to be involved in the uptake of L-cysteate (L- CA), L-cysteinesulfinate (L-CSA), L-homocysteate (L-HCA), and L-homocysteinesulfinate (L-HCSA), sulfur-containing amino acids, which are discussed as putative neurotransmitters (Griffiths, 1990; Cuenod et al., 1991). Other substances like D,L-threo-3-hydroxyaspartate (THA) and L-α-aminoadipate have been described as L-glutamate uptake blockers.

In the present study, we further characterize the functional properties and the substrate specificity of GLAST-1, which has been demonstrated to catalyze the electrogenic uptake of L-glutamate in the *Xenopus* expression system (Storck et al., 1992; Klöckner et al., 1993). The results demonstrate that L-glutamate and Na^+ ions bind in a simultaneous manner to their recognition sites, which are located outside the electrical field of the membrane. The L-glutamate uptake blockers tested either were substrates carrying a current by themselves or did not affect the L-glutamate–induced current. Simultaneous application of two substrates induced a current, which can be described by simple competition for one binding site.

Materials and Methods

cRNA preparation and oocyte injection. Details of cRNA preparation and injection of the oocytes were published elsewhere (Storck et al., 1992). Briefly, cRNA was synthesized from the template pSP-GLAST using SP6 RNA polymerase (Bethesda Research Labs). Collagenase-treated stage V–VI oocytes were injected with 40 nl of GLAST-cRNA (0.4 $\mu g/\mu l$). Oocytes were maintained in Barth's modified saline at room temperature.

Electrophysiological recordings. For the electrophysiological experiments the oocytes were superfused with a solution composed of (mM) NaCl (120), $CaCl_2$ (1.8), KCl (2), and HEPES-Tris (10) pH 7.4. Oocytes were voltage clamped using a two-electrode voltage-clamp amplifier (Warner Instruments Corp., Hamden, CT). Microelectrodes filled with 3 M KCl had resistances ranging from 1 to 2 MΩ. In order to generate the voltage commands as well as to acquire and evaluate the data, the software and hardware package ISO2 (MFK, Frankfurt, Germany) was used. Data were filtered at 10 Hz (−3 dB) and digitized at 100 Hz. Provided the different substrates had no other electrical effects apart from activating the transporter, I_{GLAST} expresses the difference of the membrane current before and during the application of the substrates. In some experiments Na^+ was replaced by equimolar amounts of choline. When 105 mM choline was added under control conditions, no

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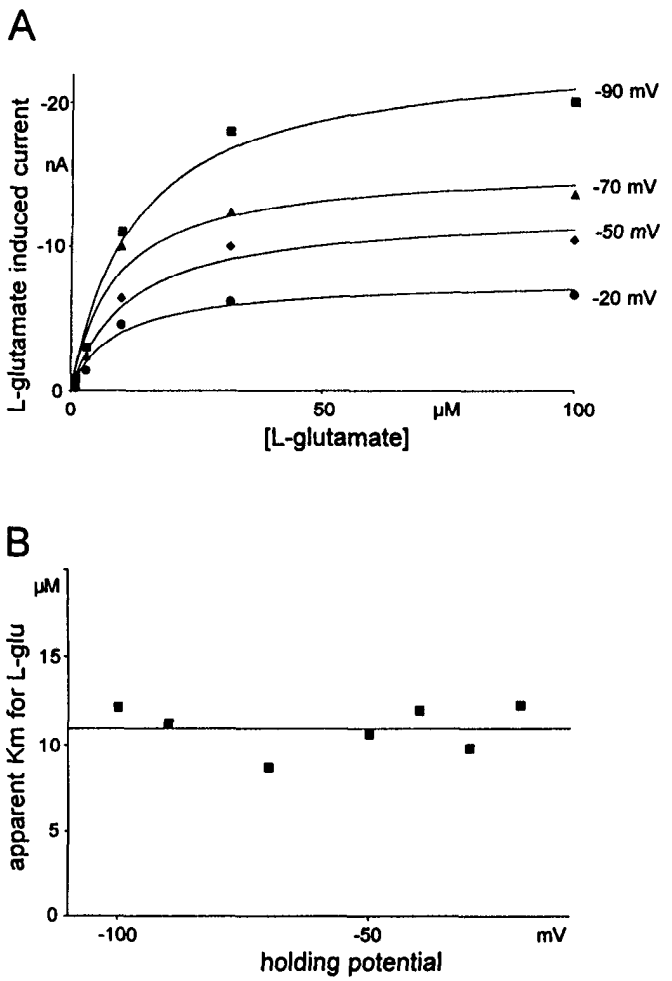

Figure 1. Voltage dependence of the concentration-response curves for L-glutamate. *A*, Superimposed concentration–response curves recorded at the indicated holding potential. The *solid line* was fitted to the data according to a Hill equation to minimize the squared errors. *B*, K_m values of GLAST-1 for L-glutamate recorded at −110, −90, −70, −50, −40, and −20 mV were plotted against the holding potential.

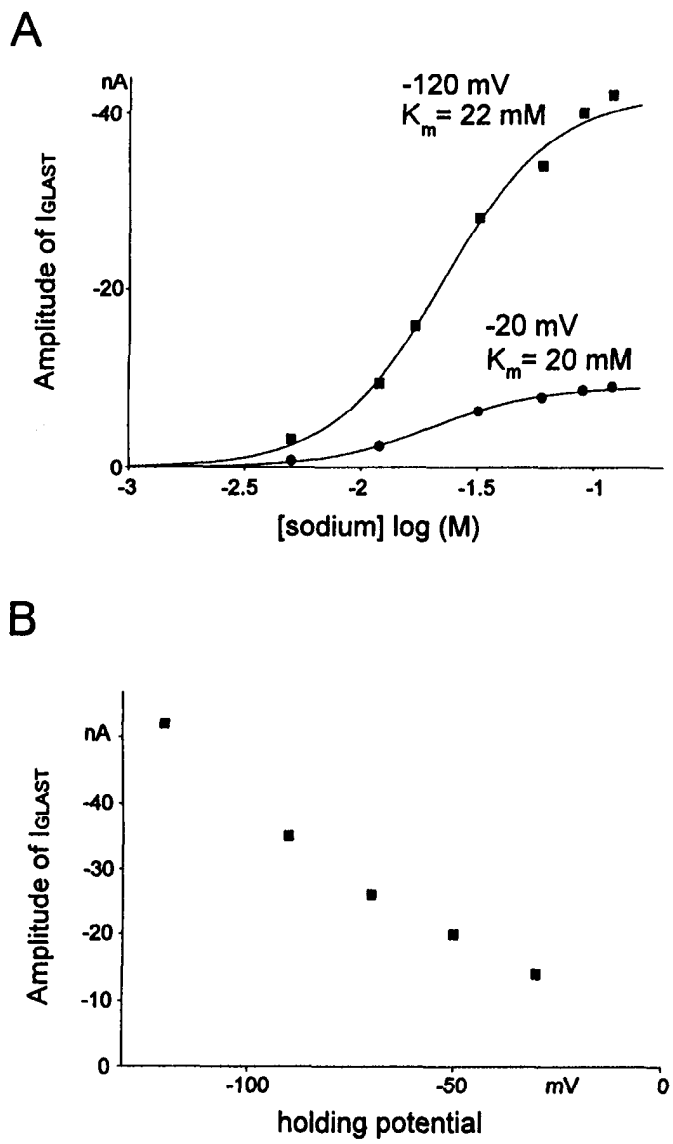

Figure 2. Voltage dependence of I_{max} and apparent K_m as a function of $[Na^+]_o$. *A*, Voltage dependence of the apparent K_m value of GLAST-1 for Na^+ ions. The curves were fitted to the data according to a Hill equation with the indicated K_m values. *B*, I_{max} of the inward current plotted as a function of voltage. The currents were recorded at 120 mM $[Na^+]_o$ and 100 μM L-glutamate.

decrease of I_{GLAST} was detected, demonstrating that choline has no inhibitory effect on the transport rate itself.

The data of the concentration–response curves were fitted by nonlinear regression to minimize the squared errors to the Hill equation $I = I_{max} \cdot [S]^n/([S]^n + K^n_{mS})$, where I_{max} is the normalized maximum current amplitude; $[S]$, the concentration of the substrate in the bath; K^n_{mS}, the apparent affinity constant; and n the cooperativity parameter. All measurements were done at room temperature (21–23°C). The data are presented as mean ± SD.

Flux measurements. Twenty-four to 72 hr after injection of GLAST-cRNA, single oocytes were placed in 1.5 ml reaction tubes and incubated for 15 min at 21–23°C in Barth's modified saline (BS) supplemented with 100 μM L-^{14}C-glutamate at a specific activity of 45 mCi/mmol. The oocytes were washed three times with 1 ml of BS, homogenized in 0.1 ml of 1% SDS, and transferred into 10 ml of Bray's solution. The radioactivity taken up by the oocytes was determined in a Beckman liquid scintillation counter.

Results

Voltage dependence of the affinity of GLAST-1 for L-glutamate and Na^+ ions

There are two structural limits for the access channel, which ions and substrates have to pass on their way to the binding sites of the transporter (Läuger and Jauch, 1986). The access channel may be a wide, water-filled pore with low electric field strength so that a large fraction of the voltage drops across the narrow part of the transporter (low-field-access channel). As a consequence, the rate constants for binding and release (i.e., the apparent K_m values) of ions and substrates are considered to be independent of the membrane potential. In the other case, the access channel is narrow and part of the transmembrane voltage drops between binding site and the external medium leading to an "ion-well behavior" of the access channel (high-field-access channel). Since ions and substrates have to pass part of the transmembrane field on their way to the binding site, the rate constants for binding and release should exhibit voltage dependence.

In order to discriminate between these two models, we examined at first the influence of voltage on the transport rate of L-glutamate (Fig. 1*A*). To determine the voltage dependence of

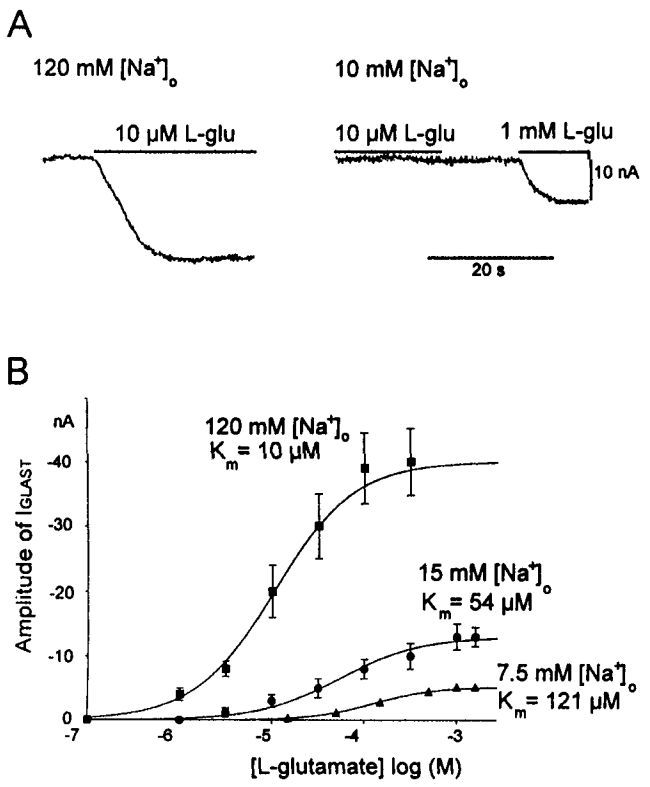

Figure 3. $[Na^+]_o$ dependence of the affinity of GLAST-1 for L-glutamate. *A*, Original traces recorded at the indicated $[Na^+]_o$. Changing the extracellular sodium concentration ($[Na^+]_o$) reduced the affinity of GLAST-1 for L-glutamate. In addition, I_{max} was diminished. *B*, Concentration–response curves. At 120 mM $[Na^+]_o$, the apparent K_m value for L-glutamate was 10 µM and I_{max} −39 nA. Reducing $[Na^+]_o$ to 15 mM increased the K_m value to 54 µM and decreased I_{max} to −12 nA. At 7.5 mM, $[Na^+]_o$ I_{max} amounted to −7 nA and the apparent K_m was 121 µM.

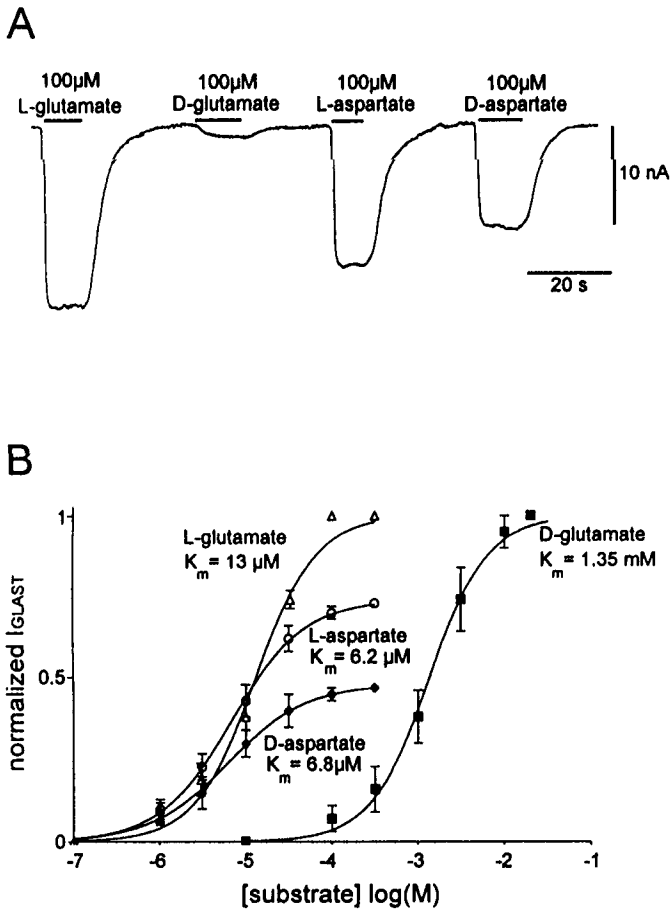

Figure 4. Stereoselective transport of glutamate but not aspartate by GLAST-1. *A*, Inward currents elicited by the indicated substrates (holding potential = −90 mV). *B*, Concentration–response curves for the stereoisomers of glutamate and aspartate. The amplitudes of the currents were normalized to the maximal current evoked by 100 µM L-glutamate in each oocyte and plotted versus the substrate concentration. The data were least square fitted according to a Hill equation with cooperativity coefficients between 0.9 and 1.1. The apparent K_m values are indicated.

the apparent K_m, we recorded concentration–response curves at different potentials. Changing the holding potential from −90 to −70, −50, and −20 mV resulted in apparent K_m values of 11.2, 8.7, 10.6, and 12.2 µM, respectively. There was no systematic voltage dependence of the transport rate of GLAST-1 for L-glutamate between −20 and −100 mV (Fig. 1*B*). The straight line fitted to the K_m values at different holding potentials represents a mean value of 10.9 µM. These findings imply that the voltage dependence of I_{GLAST} is the same at all L-glutamate concentrations. Similar results were obtained in three other oocytes.

Next we studied the influence of voltage on the transport rate of GLAST-1 for Na^+ ions (Fig. 2). The apparent K_m value for Na^+ was determined at a constant L-glutamate concentration of 2 mM and holding potentials of −120 and −20 mV. Fitting the data recorded at a potential of −120 mV to a Hill equation yielded a K_m value of 22 mM (cooperativity coefficient = 1.8). Changing the membrane potential to −20 mV decreased the amplitude of I_{max} to 20% (120 mM $[Na^+]_o$; Fig. 2*A*, circles), but there was no significant difference in the observed K_m value (cooperativity coefficient = 1.9). In six other oocytes K_m values of 27 ± 6 mM and 26 ± 4 mM were determined at −20 mV and −140 mV, respectively. In Figure 2*B*, I_{max} of the inward current is plotted as a function of voltage (2 mM L-glutamate, 120 mM $[Na^+]_o$). A similar voltage dependence of I_{max} was obtained in repeated experiments. The observation that voltage did not influence the apparent K_m values for L-glutamate or Na^+ ions suggest, that the major kinetic terms involved in determining these K_m values are voltage independent.

Sodium dependence of the affinity of GLAST-1 for L-glutamate

Two alternative mechanisms for the transport of ions and substrates by cotransporters are discussed: the transport can be accomplished consecutively or in a simultaneous manner (for references, see Jauch and Läuger, 1986). During "simultaneous transport" ions and substrates bind to the transporter, which then switches between states with inward-facing and outward-facing binding sites, while in the consecutive mechanism the conformation transitions occur when either ion or substrate is bound. In order to determine experimentally which model describes the transport mechanism of GLAST-1, we examined the influence of Na^+ ions on the transport rate of the transporter for L-glutamate. Figure 3 shows that reducing $[Na^+]_o$ not only diminished I_{max} but also decreased the apparent K_m value of GLAST-1 for L-glutamate. At 120 mM $[Na^+]_o$ 10 µM L-glutamate induced an inward current with an amplitude of −20 nA (Fig.

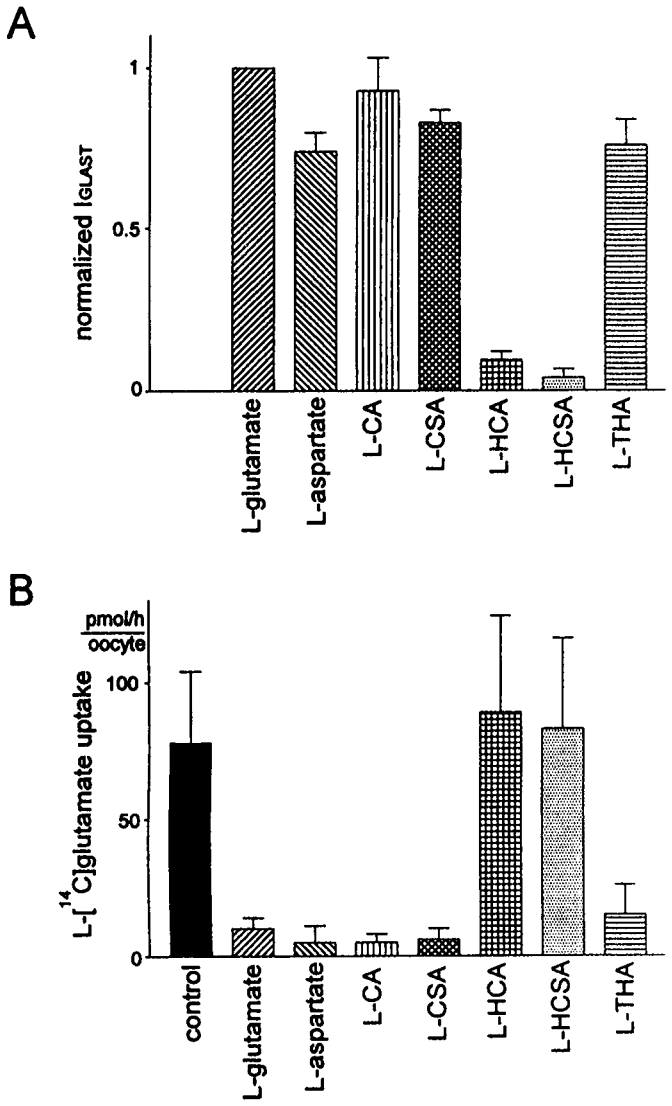

Figure 5. Potency of different substrates to induce I_{GLAST} and to inhibit the L-^{14}C-glutamate uptake into oocytes. Each column represents the mean ± SD (n = 5–8). *A*, Inward currents evoked by the indicated substrates were normalized to the mean amplitude of I_{GLAST} induced by 100 μM L-glutamate (holding potential = −90 mV). *B*, Uptake of L-^{14}C-glutamate (100 μM) under control conditions and in the presence of 1 mM of the indicated substrates.

3*A*). When $[Na^+]_o$ was reduced to 10 mM, the same concentration of L-glutamate was not able to evoke an inward current in contrast to 1 mM L-glutamate, which induced an inward current with an amplitude of −10 nA, indicating that the apparent K_m value of the transporter for L-glutamate had changed. The concentration–response curves for M-glutamate at different $[Na^+]_o$ are shown in Figure 3*B*. Lowering $[Na^+]_o$ from 120 to 15 mM decreased I_{max} from −38 to −15 nA and increased the apparent K_m value of GLAST-1 for L-glutamate from 10 to 54 μM (n = 4). At 7.5 mM, $[Na^+]_o$ I_{max} decreased to −7 nA and K_m increased to 121 μM (n = 5). These results support the hypothesis that a simultaneous mechanism underlies the transport mediated by GLAST-1.

Stereoselectivity of GLAST-1

We examined the capability of GLAST-1 to discriminate between the stereoisomers of glutamate and aspartate (Fig. 4*A*).

Table 1. I_{GLAST} induced by different substrates

Substrate	Apparent K_m (μM)	Normalized I_{max} (mean ± SD)	n
L-Glutamate	12 ± 4	1	43
D-Glutamate	1500 ± 300	0.90 ± 0.20	5
L-Aspartate	6.5 ± 3*	0.76 ± 0.1*	11
D-Aspartate	6.1 ± 3*	0.50 ± 0.1*	5
L-Cysteinesulfinate	8 ± 4	0.86 ± 0.0*	11
L-Cysteate	6.3 ± 3*	0.93 ± 0.11	10
L-Homocysteinesulfinate	50 ± 100	0.92 ± 0.10	3
L-Homocysteate	1500 ± 250	0.91 ± 0.08	4
D,L-Threo-3-hydroxyaspartate	16 ± 5	0.75 ± 0.1*	8
L-α-Aminoadipate	1100 ± 400	0.93 ± 0.13	7
β-Aminoadipate (1 mM)	—	0	3
D-α-Aminoadipate (1 mM)	—	0	3
Quisqualate (1 mM)	—	0	3
Dihydrokainate (1 mM)	—	0	3
N-*p*-aminobenzoyl-L-glutamate (1 mM)	—	0	3

* Significantly smaller (paired t test, $p < 0.05$) than the value for L-glutamate.

Applied at the same concentration (100 μM), D-glutamate evoked a 15 ± 3-fold smaller I_{GLAST} than L-glutamate. The concentration–response curves for the stereoisomers are shown in Figure 4*B*. To facilitate a direct comparison, the amplitudes of currents induced by the different substrates were normalized to the amplitude of the current induced by 100 μM L-glutamate for each oocyte. The values obtained with L-glutamate can be fitted by the Hill equation with a relative I_{max} of 1 and an apparent K_m of 13 μM. The data for D-glutamate yielded a similar relative I_{max} of 1 but an apparent K_m of 1.35 mM.

In contrast, the cloned transporter did not discriminate between the stereoisomers of aspartate. Applied at a concentration of 100 μM, L- and D-aspartate induced a current with an amplitude of −14 nA and −10 nA, respectively (Fig. 4*A*, right). The concentration–response curves revealed an about twofold higher affinity of GLAST-1 for the stereoisomers of aspartate than for L-glutamate (Fig. 4*B*). However, the maximal current induced by D- and L-aspartate is only 50 ± 14% and 76 ± 16% of that induced by M-glutamate (n = 5).

Sulfur-containing amino acids

From the amino acid uptake studies on synaptosomes there is evidence that at least some of the sulfur-containing excitatory amino acids may be taken up by the glutamate transport system (Griffiths, 1990). Measuring the transport of these substances in oocytes expressing GLAST-1 offers the advantage of excluding the presence of other transport systems, which might also be engaged in the uptake of sulfur-containing excitatory amino acids. L-CA induced at a concentration of 100 μM a current with a similar amplitude as 100 μM L-glutamate (Fig. 5*A*). In addition, the potency to compete with L-^{14}C-glutamate for the uptake was not distinguishable from that of L-glutamate (Fig. 5*B*). There was no significant difference between the apparent K_m values of L-CA and L-glutamate (Table 1). Similar results were obtained with L-cysteinesulfinate (L-CSA). In contrast, the L-glutamate analog sulfur-containing amino acids displayed a

much lower affinity for GLAST-1. In comparison to L-glutamate, the amplitude of the current induced by application of 100 μM L-HCA and L-HCSA was only 9.4 ± 2.6% and 4 ± 2.6% (Fig. 5*A*). Accordingly, the addition of 1 mM L-HCA or L-HCSA did not significantly reduce the L-^{14}C-glutamate uptake (Fig. 5*B*). Despite the low affinity of GLAST-1 for L-HCA and L-HCSA, the recorded I_{max} values are comparable to that for L-glutamate (Table 1).

L-Glutamate uptake blockers

D,L-Threo-3-hydroxyaspartate (THA) is a well-known inhibitor of the L-glutamate transport (Balcar and Johnston, 1972). Electrophysiological measurements have shown that, although THA carries a current itself, it also reduces the current induced by L-glutamate in retina glia cells and retinal cones of the salamander (Barbour et al., 1991; Eliasof and Werblin, 1993), as well as the current directed by the expression of a high-affinity glutamate transporter from rabbit small intestine (EAAC1) in oocytes (Kanai and Hediger, 1992). Figure 6*A* shows that 1 mM THA generated an inward current with an amplitude of −24 nA in oocytes expressing GLAST-1. This amplitude is significantly smaller than the current induced by 100 μM L-glutamate (−34 nA). Simultaneous application of 1 mM THA and 100 μM L-glutamate evoked a current with an amplitude of −26 nA; that is, the high dose of THA reduced the L-glutamate–induced current by 24%. However, no appreciable inhibition was seen when equimolar concentrations of L-glutamate and THA were applied (data not shown). The apparent K_m value of GLAST-1 for THA amounted to 16 ± 5 μM (n = 5), which was not statistically different from that determined for L-glutamate. Evaluated in the same oocytes, the maximal inward currents for L-glutamate and THA were −22 ± 4 nA and −15 ± 2 nA, respectively; that is, the I_{max} of THA was 75% of that of L-glutamate.

We further studied the influence of dihydrokainate (DHK), *p*-chloromercuriphenylsulfonate (p-CMPS), and L-α-aminoadipate, compounds that have been shown to inhibit the L-glutamate–induced current in some preparations (Brew and Attwell, 1987; Kanai and Hediger, 1992). Up to a concentration of 1 mM, none of them had any influence on the amplitude of I_{GLAST} induced by 100 μM L-glutamate. Only L-α-aminoadipate was a poor substrate for GLAST-1. Applied at a concentration of 1 mM, L-α-aminoadipate induced a current with an amplitude of −10 nA, while 100 μM L-glutamate evoked an current with an amplitude of −22 nA (Fig. 6*B*). From dose–response curves for L-α-aminoadipate, an apparent K_m value of 1.2 mM was evaluated (Table 1). Applied simultaneously with L-glutamate, even at high concentrations, L-α-aminoadipate did not reduce the L-glutamate–induced current.

Competition of L-glutamate and other substrates for GLAST-1

There is evidence that the transport of more than one substrate by glutamate transporters can be accomplished either in a competitive or in a noncompetitive way (Barbour et al., 1991). Application of 7.5 μM L-glutamate induced an inward current with an amplitude of −13 nA (Fig. 7*A*). Addition of 7.5 μM L-aspartate increased the amplitude to −19 nA. Upon washout of L-aspartate, I_{GLAST} returned to the control level. The amplitude of the current induced by 7.5 μM L-glutamate and 7.5 μM L-aspartate was larger than the current induced by the application of 15 μM L-glutamate alone (−16 nA). Similar data were obtained in four oocytes. These results are compatible with the hypothesis that L-glutamate and L-aspartate simply compete for the same binding site.

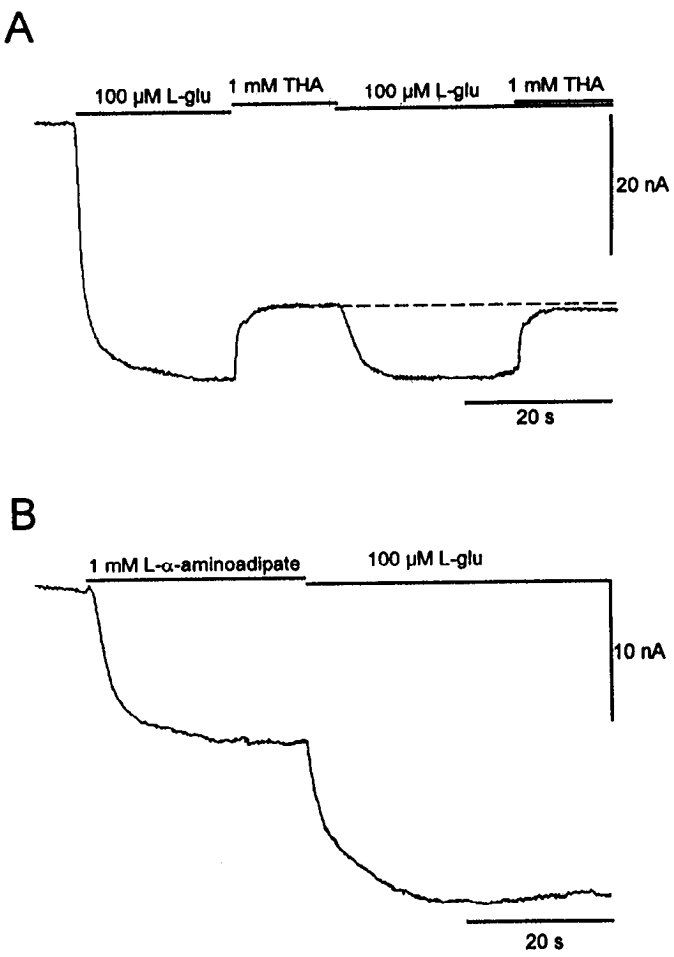

Figure 6. Effect of L-glutamate uptake blockers on I_{GLAST}. *A*, Simultaneous application of 1 mM THA and 100 μM L-glutamate induced a current with smaller amplitude than I_{GLAST} induced by 100 μM L-glutamate alone. The *dashed line* was drawn by eye to facilitate the comparison of the amplitudes of I_{GLAST}. *B*, Application of 1 mM L-α-aminoadipate induced a current with less than half the amplitude as the current induced by 100 μM L-glutamate. Holding potential = −90 mV.

To test this hypothesis further, 1 mM D-aspartate was applied simultaneously with 100 μM L-glutamate. Now, I_{GLAST} should decline because the maximal transport rate of GLAST-1 for D-aspartate is only about 50% of that for L-glutamate and most binding sites should be saturated with D-aspartate. Figure 7*B* demonstrates that administration of 1 mM D-aspartate indeed reversibly reduced I_{GLAST} from −14 to −7.5 nA. On average, the reduction amounted to 55 ± 4% (n = 4). This value is only slightly larger than the I_{max} for L-aspartate. At saturating concentrations, D-aspartate is able to reduce I_{GLAST} if the amplitude of the current evoked by L-glutamate is larger than the I_{max} for D-aspartate (Fig. 7*C*, squares). This finding is in line with the hypothesis that the two substances compete for one binding site.

Discussion

Kinetic properties of GLAST-1

One important finding of our study is that the apparent K_m values of GLAST-1 for L-glutamate and Na^+ ions do not depend on membrane voltage (Figs. 1,2). Similar results were reported

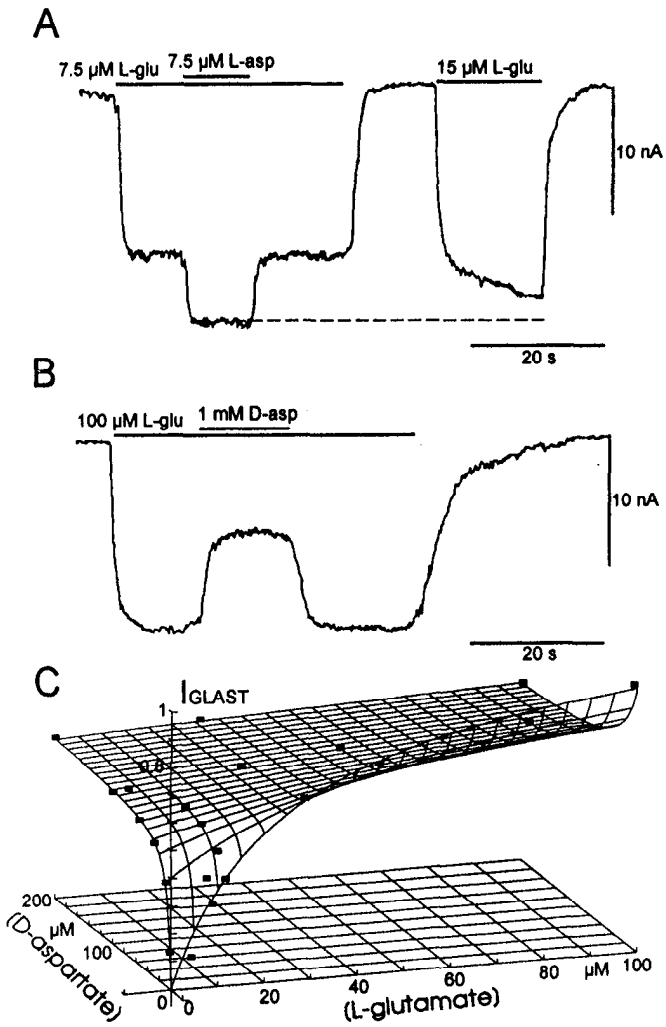

Figure 7. Competition of L-glutamate and L- or D-aspartate for GLAST-1. *A*, Inward currents induced by the application of 7.5 μM L-glutamate, 7.5 μM L-glutamate together with 7.5 μM L-aspartate, and 15 μM L-glutamate (*dashed line* drawn by eye). *B*, Addition of 1 mM D-aspartate reduces the current induced by 100 μM L-glutamate to 50%. *C*, I_{GLAST} induced by simultaneous application of different concentrations of L-glutamate and D-aspartate (*squares*). The data are displayed in a three-dimensional plot of I_{GLAST} as a function of [D-aspartate] and [L-glutamate]. The lines of the plot were calculated by solving the following equations: $K_m^{glu} = [Tr \cdot Glu]/[Tr] \cdot [Glu]$, $K_m^{asp} = [Tr \cdot Asp]/[Tr] \cdot [Asp]$, and $[T] + [Tr \cdot Glu] + [Tr \cdot Asp] = 1$, where [Tr] is the concentration of the transporter, [Tr•Glu] the concentration of L-glutamate bound by the transporter, and [Tr•Asp] is the concentration of the D-aspartate/transporter complex. The amplitude of I_{GLAST} was normalized to the maximal amplitude induced by application of 100 μM L-glutamate. The holding potential was throughout −90 mV. The squares represent the mean values obtained from three oocytes.

by Schwartz and Tachibana (1990) for the aspartate transport in glial cells from the salamander retina. Also, Barbour et al., (1991) reported that the voltage dependence of uptake is the same at all glutamate concentrations in the same preparation. Basically, these data indicate, that the major kinetic term involved in determining the apparent K_m is voltage insensitive. Since I_{max} is voltage dependent (Fig. 2*B*), while the apparent K_m values for glutamate and Na^+ ions are not, it can be assumed that the conformational transition switching the binding sites from the outward-facing to the inward-facing configuration is the voltage-dependent rate-limiting step in the transport process. Therefore, the transporter can be regarded to be in a "quasi equilibrium"; that is, the occupation of the binding sites is in equilibrium with the concentration of the substrates in the medium. According to Läuger and Jauch (1986), this behavior is compatible with a model in which GLAST-1 possesses a low field access channel with the binding sites for ions and substrate located outside the electrical field of the membrane.

The strict dependence of the affinity of the transporter for L-glutamate on $[Na^+]_o$ has significant impact on the mechanism of transport accomplished by GLAST-1. There are two distinct mechanisms by which cotransporters translocate their substrates (for references, see Jauch and Läuger, 1986). In the simultaneous mechanism, the ion(s) and the substrate bind to the transporter to form a ternary complex with inward- or outward-facing binding sites. In the consecutive mechanism, the ion(s) and the substrate are transported separately. A consecutive model predicts that at low $[Na^+]_o$ the apparent K_m for L-glutamate should become smaller and vanish at $[Na^+]_o = 0$. Since in our experiments intracellular $[Na^+]$ and [L-glutamate] are not negligible (the predictions made above are only strictly valid under "zero-trans conditions"), we may interpret our results only qualitatively. However, from the facts that the apparent K_m of GLAST-1 for L-glutamate increases while lowering $[Na^+]_o$ and the apparent K_m for Na^+ decreases while elevating [L-glutamate], it may be concluded that GLAST-1 transports Na^+ ions and L-glutamate simultaneously, although an allosteric regulation exerted via an external Na^+ binding site cannot be excluded.

Substrates of GLAST-1

There is strong evidence for a family of transporters each with a distinct and characterizing substrate specificity according to their location (for references, see Robinson et al., 1993). Studying the affinity of GLAST-1 for D- and L-aspartate, and D- and L-glutamate we determined apparent K_m values of 6.5, 6.1, 1500, and 12 μM, respectively. Although there are slight quantitative differences, these results are in line with the specificity of the glutamate transporters described in Müller retinal glia cells, photoreceptors of the turtle, retinal cones brain synaptosomes, and other tissue (Brew and Attwell, 1987; Tachibana and Kaneko, 1988; Eliasof and Werblin, 1993; Kanai et al., 1993).

Sulfur-containing excitatory amino acids were transported by GLAST-1 with similar characteristics as has been reported for retinal glial cells (Bouvier et al., 1991). L-CA and L-CSA were taken up with high affinities (apparent K_m values = 6 and 8 μM, respectively). Therefore, the affinity of GLAST-1 for these substrates is sufficient to reduce their concentrations under the activation threshold of the responsible receptors. In contrast, L-HCA and L-HCSA are transported with a very low efficiency according to their apparent K_m values of 1500 and 450 μM, respectively. These results implicate that at least the L-aspartate homologous excitatory sulfur-containing amino acids share a common transport system with L-glutamate and L-aspartate.

Comparison with previous studies

Glutamate uptake blockers. D,L-Threo-3-hydroxyaspartate (THA) is a well-known and potent inhibitor of the L-glutamate uptake into synaptosomes (Balcar and Johnston, 1972). When we applied THA alone to oocytes expressing GLAST-1, THA generated a current with an I_{max} of 75% in comparison to that induced by L-glutamate and an apparent K_m value of 16 ± 5 μM. Like aspartate, THA reduced the L-glutamate–induced cur-

rent only at high doses (Fig. 4). In retinal glia cells, THA also carries a current itself and reduces at slightly lower concentrations the current induced by L-glutamate (Barbour et al., 1991). However, GLAST-1 behaves completely different from the L-glutamate transporter described in retinal cones (Eliasof and Werblin, 1993), as well as from EAAC1 examined in the *Xenopus* expression system (Kanai and Hediger, 1992). In retinal cones, THA did not induce a current at all, but reduced the L-glutamate–induced current by 57–68%, while in EAAC1-expressing oocytes the current induced by 20 μM L-glutamate was inhibited by THA in a dose-dependent manner with 50% inhibition at 7 μM. Both effects of THA cannot be explained by a competition for one binding site. There must be some additional functional difference between these transporters and GLAST-1. At least two pharmacologically distinct Na^+-dependent high-affinity L-glutamate transport processes have been observed in rat brain (Robinson et al., 1991). This favors the idea that the differences are inherent to different transporters. In cerebellum, the transport is insensitive toward dihydrokainate, while the competitive inhibitor L-α-aminoadipate reduces the transport activity with an IC_{50} of 40 μM. In the forebrain, dihydrokainate inhibits the transport with an IC_{50} of 100 μM, while L-α-aminoadipate inhibits the transport with an IC_{50} of 700 μM. Obviously, GLAST-1 does not belong to one of these transporters since DHK has no effect on I_{GLAST} and L-α-aminoadipate was a substrate with a low affinity to GLAST-1 (Table 1). However, in contrast to other glutamate transporters studied, the GLAST-1–mediated transport was not affected by *p*-chloromercuriphenylsulfonate (p-CMPS), a compound that is able to reduce the current associated with the uptake of glutamate by Müller cells and EAAC1-expressing *Xenopus* oocytes (Brew and Attwell, 1987; Kanai and Hediger, 1992).

Competition of substrates for GLAST-1. When low doses of L-glutamate and D- or L-aspartate were applied simultaneously, no inhibition of I_{GLAST} evoked by L-glutamate alone was observed (Fig. 7). Only at higher concentrations D- and L-aspartate are able to reduce the L-glutamate–induced current, which can be explained by their lower I_{max} values. These results suggest that the substrates compete for one common binding site (Fig. 7*C*). The observations are in line with the results reported by Barbour et al. (1991), observing a similar phenomenon by simultaneous application of equimolar doses of L-glutamate and L-aspartate. Very recently an analogous competitive model was proposed for the inhibition of the L-glutamate uptake into glial cells from the tiger salamander retina by L-*trans*-pyrrolidine-2,4-dicarboxylate (PDC) (Sarantis et al., 1993)

In this article we present data characterizing the functional properties and the substrate specificity of GLAST-1. Comparable studies on the two other cloned glutamate transporters are required. Together with these results the findings reported here will contribute to our understanding of the role individual members of the Na^+-dependent glutamate transporter family localized partially in different areas of the brain may play in the regulation of the excitatory neurotransmitter concentration.

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