A monoclonal antibody against a Na\(^+\)-L-Glutamate Cotransporter from Rat Brain*

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A monoclonal mouse IgM antibody (Z8E9) was raised against the Na\(^+\)-L-glutamate cotransporter from rat brain. In a preparation of brain plasma membrane vesicles, Z8E9 binds specifically to a polypeptide with an apparent molecular weight of 70,000 and inhibits Na\(^+\) gradient-dependent L-glutamate cotransport (up to 50\%) in brain membrane vesicles. In the membrane vesicles, the antibody does not alter the membrane permeability for Na\(^+\) and K\(^+\) nor the Na\(^+\) gradient-dependent uptake of \(\gamma\)-aminobutyric acid. Kinetic experiments showed that Z8E9 does not alter the \(K_{m}\) values for L-glutamate and Na\(^+\) activation of L-glutamate transport. However, an apparent cooperativity observed for L-glutamate activation was increased, and the \(V_{max}\) of L-glutamate transport was decreased. Immunostaining of rat cerebral identified antigenic sites of Z8E9 in Golgi epithelial cells and astrocytes (by light and electron microscopy), whereas no labeling at nerve terminals was detected. The data suggest that a component of a Na\(^+\)-L-glutamate cotransporter subtype has been identified that is specific for glia cells in brain.

Glutamate is thought to be the major excitatory transmitter in brain (1–3) and has an important role in neuronal plasticity and neurotoxicity (1, 4–9). Whereas several different types of glutamate-sensitive receptors have been characterized and cloned (2, 10–15) little is known about the molecular organization of the transporters which remove L-glutamate out of the synaptic cleft and thereby terminate the excitation by L-glutamate and keep the extracellular L-glutamate concentration below toxic levels (2, 16, 17). Denervation experiments, autoradiographic studies, and uptake measurements in cultured neuronal and glial cells show that glutamate transporters occur in glutaminergic neurons as well as in glia cells (18–26). Sodium-dependent and chloride-dependent glutamate uptake systems can be distinguished. Cotransport of Na\(^+\) and L-glutamate has been demonstrated in partially purified synaptosomal membranes (27), in membrane vesicles enriched in synaptosomal membranes (28), and in cultured neuronal and glial cells (29–31), and pharmacological studies suggest multiple types of Na\(^+\)-dependent L-glutamate transporters (16, 32, 33). Chloride-dependent high affinity glutamate uptake has been measured in preparations of rat brain synaptosomes and in glioma cells (21, 22).

In trying to identify and localize Na\(^+\)-L-glutamate cotransporters in brain, a protein preparation from plasma membranes highly enriched in Na\(^+\)-L-glutamate cotransporters (34) was employed to generate monoclonal antibodies. One antibody was obtained which binds to a M, 70,000 polypeptide, inhibits Na\(^+\)-L-glutamate cotransport in brain membranes, and shows selective binding to glial cells in cerebellum.

**EXPERIMENTAL PROCEDURES**

**Methods**

**Preparation of Renal Brush-border Membrane Vesicles and Brain Plasma Membrane Vesicles**—Brush-border membrane vesicles from rat renal cortex were isolated and frozen in liquid nitrogen exactly as previously described (35). To prepare plasma membrane vesicles from rat brain or rat retina for transport measurements and Western blots, a synaptosome-enriched subcellular fraction was prepared by differential centrifugation and density gradient centrifugation (36), and plasma membrane vesicles were generated after hypotonic lysis (28).

Briefly, 2 g of brain tissue or 10 retinas were homogenized in 50 or 20 ml, respectively, of 320 mM sucrose, 1 mM EDTA dipotassium salt, 10 mM Tris-HCl, pH 7.4 (SET buffer). After 3-min centrifugation (4 °C) at 1,300 \(\times\) g the supernatant was centrifuged 10 min (4 °C) at 17,000 \(\times\) g. The pellet of this centrifugation was suspended in 20 ml of SET buffer containing 12% (w/v) Ficoll 400 (Pharmacia LKB Biotechnology Inc.). Five-ml portions of this suspension were overlaid with 2.5 ml of SET buffer containing 7.5% (w/v) Ficoll 400 and with 2.5 ml of SET buffer. After 30 min (4 °C) centrifugation at 99,000 \(\times\) g in a swing-out rotor, the synaptosome-enriched fraction from the interphase between 7.5 and 12% (w/v) Ficoll 400 was collected, suspended in 30 ml of SET buffer, and spun down (10-min centrifugation at 5,500 \(\times\) g). The combined pellets were suspended in 40 ml of 5 mM Tris-HCl, pH 7.4, 1 mM EDTA dipotassium salt and incubated (30 min, 8 °C) for lysis. Then the suspension was centrifuged (20 min, 4 °C) at 27,000 \(\times\) g, and the pellet was suspended in 2 ml of 5 mM Tris sulfate, pH 7.4, 0.5 mM EDTA dipotassium salt, 1 mM MgSO\(_4\), 320 mM sucrose. Aliquots (200 \(\mu\)l) of this suspension were frozen in liquid nitrogen.

**Preparation of Protein Fractions from Brain Plasma Membrane Vesicles**—The Na\(^+\)-L-glutamate cotransporter was solubilized and partially purified exactly as previously described (34). The purification was started from a more crude preparation of brain plasma membranes which yielded a sufficient amount of starting material. These plasma membranes were prepared by lysing particles which were sedimented (20 min, 27,000 \(\times\) g) from rat brain tissue homogenized in isotonic mannitol/EDTA (0.52 M/1 mM). They were solubi-

1 The particles contain two populations of different density. In heavy and light subfractions, pinched-off nerve terminals and membrane vesicles from glia cells were identified by electron microscopy (unpublished data).
lized with CHAPS (20 mM), and the Na+-γ-glutamate cotransporter was enriched by successive chromatographic steps on wheat germ agglutinin-Sepharose, hydroxylapitate, and DEAE-cellulose. While in the last purification step on DEAE-cellulose some transporter did not bind to the column (DEAE flow-through, containing the γ-aminobutyric acid (GABA) transporter), the majority of the transport activity was eluted by 60 mM NaCl (DEAE peak). The specific activity of Na+/K+ gradient-dependent L-glutamate transport measured after reconstitution of the DEAE flow-through and DEAE peak was about 15 and 30 times higher than that measured after reconstitution of solubilized synaptic plasma membranes, respectively. After separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the DEAE peak and the DEAE flow-through contained a predominant polypeptide band with an apparent molecular weight between 65,000 and 80,000. This band was electroeluted from SDS-PAGE gels of the DEAE peak and the DEAE flow-through (DFE) as described before (37) and was dialyzed against phosphate-buffered saline (PBS).

Generation and Characterization of Hybridoma Cell Lines—Bath/c mice were immunized with the electroeluted polypeptides from the DEAE peak and the DEAE flow-through. The immunizations were performed with combined intraperitoneal and subcutaneous injections of 10 μg of protein on days 1 (with complete Freund’s adjuvant), 28, and 56 (with incomplete Freund’s adjuvant). On testing the antisera on day 66 it was found that the serum of one mouse (immunized with DFE) reacted with the DEAE peak in the ELISA and in Western blots. This mouse received a third intraperitoneal injection of 50 μg of DFE (without adjuvant) on day 94. Spleen cells were fused with myeloma cells of the nonsecreting mouse myeloma cell line X63-Ag 8.6.5.3 (38). The hybridoma supernatants were screened for their binding to the DEAE peak in the ELISA. Positive supernatants were tested for specific reaction with a M, 65,000–80,000 polypeptide in Western blots. Selected hybridomas were single cell-cloned by limiting dilution and grown in mass culture in Dulbecco’s modified Eagle’s medium containing 10% (v/v) fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin.

Measurements of Antibody Effects on Transport—To measure the effects of monoclonal antibodies on Na+/K+ gradient-dependent t-glutamate uptake into brain membrane vesicles or brush-border membrane vesicles from kidney, 150 μl of frozen vesicles containing 5–10 mg/ml protein were thawed (water bath, 37°C), diluted with 8 ml of

The abbreviations used are: CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; GABA, γ-aminobutyric acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; buffer mixtures: (i) 60 mM sodium chloride; (ii) 0.1 M potassium chloride; (iii) mixtures of 100 mM sodium chloride, pH 6.8, 100 mM tetramethylammonium chloride, pH 6.8; (iv) 100 mM sodium chloride, pH 6.8, containing 1 mM MgSO4, 100 mM potassium chloride, pH 6.8; (v) 100 mM sodium chloride, pH 6.8, containing 10 mM MgSO4, 100 mM potassium chloride, pH 6.8; (vi) 10 mM magnesium chloride; (vii) 100 mM potassium chloride; (viii) mixtures of 100 mM sodium chloride, pH 6.8, 100 mM potassium chloride, pH 6.8, and 100 mM magnesium chloride, pH 6.8; (ix) 100 mM potassium chloride; (x) phosphate-buffered saline containing 1 mg/ml of p-nitrophenylphosphate, and the absorption difference between 405 and 450 nm was measured (Titertec Multiscan 23276).
plastic slides were fixed with diaminobenzidene as substrate. The sections were investigated with thick sections which had been fixed with 2.5% (v/v) glutaraldehyde, postfixed with 1% (v/v) OsO₄, dehydrated with alcohol, and embedded in Spurr’s resin (45). Pre-embedding immunoperoxidase labeling for electron microscopic investigations was also performed with 100-μm thick sections which had been fixed with 2.5% (v/v) glutaraldehyde beforehand (see Fig. 7, d and e). These sections were incubated with Z8E9 antibody (12 h), treated with peroxidase-coupled anti-mouse IgM antibodies and with diaminobenzidene, postfixed, and embedded as described above. From plastic-embedded tissues ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and viewed in a Philips EM 300 electron microscope. In control experiments the primary antibody Z8E9 was replaced by a corresponding concentration of a nonspecific myeloma IgM antibody.

Materials

L-[14C]Glutamate (1.81 TBq/mmol), 42Rb (60 GBq/mmol), and 32PNa⁺ (330 GBq/mmol) were obtained from Du Pont de Nemours (Deinze) and 32Pi (75 TGBq/mmol), [γ-3H]Aminobutyric acid (1.48 TBq/mmol), and goat anti-mouse IgM antiserum from Amersham Buchler (Braunschweig). Ficoll 400 was delivered by Pharmacia (Freiburg), peroxidase-labeled anti-mouse IgM serum from goat and subclass-specific anti-mouse rabbit sera from rabbit by Renner (Dannstadt). The molecular mass marker proteins, mouse myeloma IgM, and all other chemicals were obtained as described earlier (39).

RESULTS

Antibody Production—Eight mice were immunized with polypeptides with apparent molecular weights between 65,000 and 80,000 which were eluted from SDS polyacrylamide gels of protein fractions containing the partially purified Na⁺-L-glutamate cotransporter from rat brain plasma membranes (see DEAE peak and DEAE flow-through in Ref. 34). Only one mouse produced an antiserum which reacted significantly by ELISA and Western blotting with the most purified protein fraction (DEAE peak) in which after reconstitution a 30-fold higher sodium- and potassium-coupled L-glutamate transport rate was measured compared with reconstitution of nonfractionated solubilized membrane vesicles from brain. This mouse was used for a fusion in which 550 hybridoma supernatants were screened by ELISA for their reaction with the DEAE peak. Eleven supernatants were positive in the ELISA, and four of these reacted in Western blots with three different polypeptides with apparent molecular weights in the range between 60,000 and 80,000. One antibody (monoclonal antibody Z8E9) inhibited Na⁺ gradient-dependent L-glutamate uptake into synaptosomal vesicles. Z8E9 was single cell-cloned, the antibody class (IgM) and the light chain (κ) were determined, and larger amounts of antibody were produced in mass culture.

Antigenic Polypeptide of Z8E9—Fig. 1 shows the reaction of Z8E9 antibody with brain plasma membranes and the DEAE peak in Western blots of SDS-polyacrylamide gels. The antibody reacts with a polypeptide with an apparent molecular weight of 70,000. The antigenic polypeptide forms a sharp band which appears to be a polypeptide subfraction of the DEAE peak. In Western blots Z8E9 did not react with synaptosomal membrane vesicles from rat retina nor with brush-border membrane vesicles from rat kidney cortex, although in both preparations significant rates of Na⁺,K⁺ gradient-dependent L-glutamate transport were detected (data not shown).

Specific Interaction of Z8E9 with the Na⁺-L-glutamate Cotransporter from Brain—To measure the effect of Z8E9 on glutamate transport, plasma membrane vesicles from rat brain and brush-border membranes from rat kidney were incubated 60 min (22 °C) with different concentrations of Z8E9 or of control IgM antibodies, and the initial rates of Na⁺,K⁺ gradient-dependent L-glutamate uptake were measured (see “Experimental Procedures”). In nine separate experiments with five different preparations of plasma membrane vesicles from rat brain1 uptake of 0.2 μM glutamate was inhibited by between 26 ± 10% (S.D.) and 55 ± 8% (S.D.) when vesicles containing 70 μg/ml of protein were incubated with 65 μg/ml of Z8E9. The antibody inhibition was identical whether the anion in the transport assay was chloride or phosphate (data not shown). In control experiments in which the vesicles were incubated with 65 to 200 μg/ml of seven different monoclonal IgM antibodies, no significant inhibition of glutamate uptake (0 ± 9% to 5 ± 9%) was detected. Fig. 2 shows that the inhibition of Na⁺,K⁺ gradient-dependent L-

4 In the different preparations of synaptosomal vesicles, the initial rates of Na⁺,K⁺ gradient-dependent uptake of 0.2 μM L-glutamate varied between 115 ± 16 and 396 ± 30 pmol/mg of protein/s (mean values and standard deviations of three measurements).
After different incubation times the radioactivity in the vesicles was analyzed as described under "Experimental Procedures." Mean values and standard deviations of three parallel experiments are presented. Only in membrane vesicles from brain glutamate uptake was significantly (*p < 0.01; **p < 0.001) inhibited by Z8E9.

<table>
<thead>
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<th>Sample</th>
<th>Antibody</th>
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<th>GABA uptake</th>
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<td>0.4 μM</td>
<td>0.2 μM</td>
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<tr>
<td>MV from rat</td>
<td>IgM</td>
<td>168 ± 14</td>
<td>424 ± 33</td>
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<td>124 ± 10a</td>
<td>237 ± 25b</td>
<td>31 ± 3</td>
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<tr>
<td>kidney Z8E9</td>
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FIG. 4. Effect of Z8E9 on the L-glutamate dependence of Na+-L-glutamate cotransport (b against control a). Synaptosomal vesicles from rat brain (67 μg of protein per ml) containing 100 mM potassium phosphate were incubated with 60 μg/ml of myeloma IgM (a) or of Z8E9 (b) as in Fig. 2. Then the initial Na⁺,K⁺ gradient-dependent glutamate uptake rates in the presence of different L-glutamate concentrations were measured as in Fig. 2. Mean values and standard deviations of triplicate measurements are presented. The curves represent fits of the Hill equation to the data (see "Experimental Procedures").

We tried to determine whether the inhibition of L-glutamate uptake by Z8E9 is due to a specific antibody interaction with the Na⁺,K⁺-dependent L-glutamate transporter rather than to the dissipation of the inwardly directed sodium gradient and/or to the outwardly directed potassium gradient. Therefore, we investigated whether 65 μg/ml Z8E9 alters the influx of ²²Na or the efflux of ⁸⁶Rb. In our transport assay (one second incubation of the vesicles) the intravesicular Na⁺ concentration increased from 0 to about 10 mM. After incubation of the vesicles with 60 μg/ml of Z8E9 the rate of Na⁺ influx was not altered significantly (Fig. 3a). The Rb⁺-efflux experiment in Fig. 3b shows that during the transport measurements (one second incubation) the K⁺ concentration in the vesicles decreased from 100 to about 90 mM and that Z8E9 did not increase the K⁺ efflux. Thus the inhibitory effect of Z8E9 on Na⁺,K⁺ gradient-dependent L-glutamate uptake is supposed to be due to direct antibody interaction with the glutamate transporter. To investigate the specificity of Z8E9 the effect of the antibody on Na⁺-GABA cotransport in brain plasma membrane vesicles (46, 47) and on Na⁺-L-glutamate cotransport in renal brush-border membrane vesicles (48) was tested. After incubation of brain plasma membrane vesicles with 60 μg/ml of Z8E9 Na⁺ gradient-dependent...
GABA transport was not inhibited, whereas Na\(^+\) gradient-dependent L-glutamate transport was inhibited in the same vesicle preparation (Table I). In brush-border membrane vesicles from rat kidney no inhibition of Na\(^+\) gradient-dependent L-glutamate uptake was observed by 60 \(\mu\)g/ml of Z8E9.

**Kinetics of the Inhibited Na\(^+\)-L-glutamate Cotransporter**—To characterize the inhibition of L-glutamate transport by Z8E9 the Na\(^+\)-glutamate dependence and the Na\(^+\) dependence of Na\(^+\),K\(^+\) gradient-dependent L-glutamate uptake was measured after incubation of synaptosomal vesicles with 60 \(\mu\)g/ml of IgM (control) or Z8E9. As has been described recently for glutamate dependence of high affinity Na\(^+\)-L-glutamate cotransport in oocytes of *Xenopus laevis* (49), also, in brain the glutamate dependence measured in the presence of an inwardly directed Na\(^+\) gradient (initially 90 mM) were calculated by subtracting the uptake in the presence of 100 mM NaCl and 100 mM tetramethylammonium chloride. Initial uptake rates were measured as in Fig. 2. The Na\(^+\)-dependent uptake rates (in the presence of an outwardly directed Na\(^+\) gradient of 90 mM) and the Hill coefficient was increased (control, 1.20 \pm 0.08; Z8E9, 1.37 \pm 0.08), whereas the \(K_{0.5}\) value was not significantly altered (control, 1.68 \pm 0.12 \(\mu\)M; Z8E9, 1.75 \pm 0.21 \(\mu\)M). The data indicate that binding of Z8E9 reduces the turnover in the presence of an inwardly directed gradient of 90 mM Na\(^+\).

Fig. 5 shows the dependence of L-glutamate transport on the inwardly directed Na\(^+\) gradient measured in the presence of an outwardly directed K\(^+\) gradient (initially 90 mM). In the control experiment (incubation of the vesicles with nonreacting IgM) and after incubation of the vesicles with Z8E9 two Na\(^+\) activation sites could be distinguished. The affinity of the high-affinity Na\(^+\) activation site (\(K_{0.5(high)}\): IgM, 1.56 \pm 0.11 mM; Z8E9, 1.94 \pm 0.04 mM) is slightly reduced by Z8E9 and the glutamate uptake activated by this site (\(V_{max(high)}\): IgM, 39 \pm 1 pmol/mg of protein/s; Z8E9, 47 \pm 1 pmol/mg of protein/s) is slightly increased. Z8E9 does apparently also decrease the affinity of the low-affinity Na\(^+\) activation site (\(K_{0.5(low)}\): IgM, 254 \pm 36 mM; Z8E9, 693 \pm 258 mM). However, the largest and most clear effect of Z8E9 is a reduction of the maximal transport rate at this transport site (\(V_{max(low)}\): IgM, 269 \pm 33 pmol/mg of protein/s; Z8E9, 90 \pm 31 pmol/mg of protein/s). The data show that Z8E9 mainly effects L-glutamate transport which is stimulated by a high concentration of Na\(^+\).

**Immunohistochemical Localization of the Na\(^+\)-L-glutamate Cotransporter in Rat Cerebellum**—To test whether the monoclonal antibody Z8E9 can be used to determine the distribution of the Na\(^+\)-L-glutamate cotransporter in brain, immunohistochemistry was performed in rat cerebellum. Light microscopic investigations were performed on frozen sections employing peroxidase-labeled secondary antibodies. Z8E9

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**Fig. 5.** Effect of Z8E9 on the Na\(^+\) dependence of Na\(^+\),K\(^+\) gradient-dependent L-glutamate uptake (● against control ○). Synaptosomal vesicles from rat brain containing 100 mM potassium phosphate, pH 6.8, were incubated with 60 \(\mu\)g/ml of IgM (○) or of Z8E9 (●) as in Fig. 2. Then 10 \(\mu\)l of vesicles were mixed with 90 \(\mu\)l of incubation medium containing 0.2 \(\mu\)M L-[^3H]glutamate plus either 100 mM tetramethylammonium chloride or 100 mM NaCl or mixtures of 100 mM NaCl and 100 mM tetramethylammonium chloride. Initial uptake rates were measured as in Fig. 2. The Na\(^+\)-dependent uptake rates (in the presence of an outwardly directed initial K\(^+\) gradient of 90 mM) were calculated by subtracting the uptake in the presence of different Na\(^+\) concentrations from that measured in the absence of Na\(^+\). Mean values and standard deviations of triple measurements are presented according to Hofstee. The curves were calculated as described under "Experimental Procedures."

**Fig. 6.** Light microscopic pictures of Golgi epithelial cells (arrow in c and d) and astrocytes (arrow in b) in rat cerebellum which are immunostained with monoclonal antibody Z8E9. The sections were prepared and stained with the help of a peroxidase-coupled secondary antibody as described under "Experimental Procedures." In b, c, and d 50 \(\mu\)g/ml of Z8E9 was employed as primary antibody, a, a control experiment in which Z8E9 was replaced by 50 \(\mu\)g/ml of myeloma IgM. Z8E9 binds to Golgi epithelial cells and astrocytes. The Golgi epithelial cells have their cell body next to the Purkinje cells (P) and send fibers (see parallel fibers in b and c) into the molecular layer (ML). The arrow in b points to an astrocyte in the granular layer; the arrow in c points to a subapical terminal of a Golgi epithelial cell fiber; and the arrowhead in d points to a Golgi epithelial cell which envelopes the soma and the dendritic trunk of a Purkinje cell (P).
dissection of the intracellular binding sites of Z8E9 was not performed.

The light microscopic labeling pattern indicated that Z8E9 does not react with parallel and mossy fibers which are thought to be glutamergic (50-53). Electron microscopic inspection revealed no immune reaction of Z8E9 with nerve terminals of these fibers (data not shown).

**Discussion**

In this paper we described a monoclonal antibody (Z8E9) which inhibits Na⁺-dependent L-glutamate cotransport into membrane vesicles isolated from rat brain. The vesicle preparation contains plasma membrane vesicles from synaptosomes and from glia cells (52), and the antibody binds to a polypeptide which has an apparent molecular weight of 70,000. Immunohistochemical investigations showed that the antibody reacts exclusively with glia cells in cerebellum, and preliminary investigations showed also a selective reaction with glia cells in hippocampus. In cerebellum Z8E9 binds to the plasma membrane of Golgi epithelial cells (Bergmann glia) and astrocytes and does not react with synapses of mossy fibers and parallel fibers which are supposed to be excitatory (50-53). Interestingly the pattern of immunostaining with Z8E9 observed in cerebellum is very similar to the pattern of D-[³H]aspartate uptake in cerebellar tissue sections which were analyzed by autoradiography (see Fig. 1 in Ref. 54 and Ref. 23). Thus in cerebellum the predominant L-glutamate uptake from the extracellular space may occur via Na⁺-L-glutamate cotransporters in glia cells. In other brain areas, e.g. hippocampus, where L-glutamate uptake into presynaptic excitatory nerve terminal has been demonstrated (18, 20) neuronal L-glutamate transporters may play a more significant role.

The antigenic M, 70,000 polypeptide of Z8E9 is supposed to be a component of a Na⁺-L-glutamate cotransporter which is exclusively localized in glia cells. This transporter is supposed to be a transporter subtype which may be completely or slightly different from Na⁺-L-glutamate cotransporters in presynaptic nerve terminals (16, 32, 33). That glia and neuronal cells contain pharmacological different Na⁺-L-glutamate cotransporters has been also demonstrated by a recent investigation in mouse brain cortex (55). In that study it was shown that the inhibition of sodium-coupled glutamate transport by glutamate analogs was different in membrane vesicles prepared from highly enriched synaptosomes and from primary cultures of astrocytes. At this time we cannot decide whether the identified M, 70,000 polypeptide is sufficient to build up the complete transporter or whether it represents a transporter subunit. The second possibility is not improbable for several reasons. First, the attempts on the purification of the transporter cannot exclude the existence of subunits with similar apparent molecular weights (34). Second, it has been shown that another Na⁺-cotransport system, the Na⁺-D-glucose transporter, contains two subunits with similar molecular weights: an integral membrane protein with 12 membrane-spanning domains (56) and a relatively hydrophilic polypeptide which contains one membrane anchor (57). Third, it is more probable to obtain antibodies against a hydrophilic transporter subunit than against a hydrophobic membrane-spanning component of a transporter.

In neuronal and glial cells chloride-dependent L-glutamate transporters and Na⁺-L-glutamate cotransporters have been detected (21, 29, 30), and it has been shown that functionally different Na⁺-L-glutamate cotransporters prevail in different brain areas (16, 32, 33). The antibody Z8E9 appears to be specific for a Na⁺-L-glutamate cotransporter subtype which is present in glia and apparently does not cross-react with the

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**Fig. 7.** Electron microscopic pictures of processes of cerebellar Golgi epithelial cells (arrows in a–c) and astrocytes (arrows in d, e) which are immunostained with the monoclonal antibody Z8E9. Unfixed cryosections (a–c) and glutaraldehyde-fixed sections (d, e) were immunostained, plastic-embedded, and investigated by electron microscopy as described under "Experimental Procedures." In the molecular layer labeling of Golgi epithelial cells (a–c) and in the granular layer labeling of astrocytes (d, e) was observed. Immunolabeling of Golgi epithelial cells is demonstrated in subapical terminals below the pia (P) (a), in longitudinal-sectioned processes (b), and in cross-sectioned processes (c). Labeling of astrocytes is shown (d) in cross-sectioned processes between granular cells (GC) or (e) in a process near a mossy fiber (MF). Specific binding of the monoclonal antibody Z8E9 to the plasma membranes is indicated by arrows. The dark appearance of the nucleus in d is due to the normal electron microscopic contrast of the uranyl acetate and lead citrate-stained sections.
Na⁺-L-glutamate cotransporter from kidney and retina, since no antibody reaction in Western blots and no transport inhibition was observed in these tissues (see Table I). Z8E9 is assumed to be directed against a conformational epitope of the transporter in glia cells which is assumed to be transport-relevant. The epitope of Z8E9 is denatured during plastic embedding of the tissue but remains intact after solubilization with SDS and blotting. Therefore Z8E9 may not be appropriate for detailed ultrastructural investigations but may be useful in the identification of the Na⁺-L-glutamate cotransporter (or parts of the transporter) during purification. Thus Z8E9 may enable the cloning of the Na⁺-L-glutamate cotransporter and may help to produce additional antibodies which are appropriate for a detailed investigation of the distribution and ultrastructural localization of Na⁺-L-glutamate cotransporters in brain.

REFERENCES

8. Monoclonal Antibody against Na⁺-L-glutamate Cotransporter from kidney and retina, since no antibody reaction in Western blots and no transport inhibition was observed in these tissues (see Table I). Z8E9 is assumed to be directed against a conformational epitope of the transporter in glia cells which is assumed to be transport-relevant. The epitope of Z8E9 is denatured during plastic embedding of the tissue but remains intact after solubilization with SDS and blotting. Therefore Z8E9 may not be appropriate for detailed ultrastructural investigations but may be useful in the identification of the Na⁺-L-glutamate cotransporter (or parts of the transporter) during purification. Thus Z8E9 may enable the cloning of the Na⁺-L-glutamate cotransporter and may help to produce additional antibodies which are appropriate for a detailed investigation of the distribution and ultrastructural localization of Na⁺-L-glutamate cotransporters in brain.