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GLIAL AND NEURONAL GLUTAMATE TRANSPORT FOLLOWING GLUTAMINE SYNTHETASE INHIBITION

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Abstract—Glutamate transport into striatal tissue preparations was studied following inhibition of glutamine synthetase with methionine sulfoximine (MSO). Glutamate uptake in striatal tissue prisms was elevated for up to 7 days following an intraventricular (i.c.v.) injection of MSO. Kinetic analysis of glutamate uptake revealed that a high- and a low-affinity carrier system mediated the transport of glutamate into tissue slices. MSO altered the transport of glutamate via the high-affinity carrier without changing the characteristics of low-affinity glutamate transport. MSO increased the K_m for glutamate and the V_{max} at the high-affinity uptake site. The changes in the K_m and the V_{max} for glutamate uptake were maximal 24 hr after administration of MSO, but the transport system returned to normal by 14 days after injection. In addition, MSO increased high-affinity aspartate uptake into tissue slices, but it was without effect on leucine uptake. Glutamate uptake into striatal synaptosomes and bulk-isolated glial cells or neurons was, in all cases, mediated by a low- and high-affinity carrier. The K_m and V_{max} values for high-affinity glial-glutamate uptake were increased 24 hr after i.c.v. injection of MSO, while the low-affinity kinetic parameters for glial glutamate uptake were not altered by MSO. Neither high-affinity nor low-affinity glutamate uptake into bulk-isolated neurons or synaptosomes was altered by MSO 24 hr after injection. These results suggest that MSO induced alterations in glutamate transport within striatal slices may be due to changes in glial glutamate transport arising from the disruption of glutamate metabolism.

Early studies on glutamate uptake by brain tissue established the existence of transport carriers with a high and a low affinity for glutamate [1]. Subsequent studies demonstrated that both astroglia [2–7] and neurons [7–10] possessed an active high-affinity glutamate carrier. Radioautographic evidence, however, suggested that glial glutamate uptake was more active [11, 12] in brain tissue compared to neuronal glutamate uptake, and such contentions were supported by *in vitro* uptake studies which demonstrated a greater capacity for high-affinity glutamate uptake by glia [13, 14] compared to glutamate uptake by neuronal elements.

Astroglia are responsible for one of the major routes of glutamate metabolism in the CNS. Glutamate is metabolized to glutamine by the enzyme glutamine synthetase (GS§) (L-glutamate:ammonia ligase (ADP forming); EC 6.3.1.2), and this enzyme has been shown by immunohistochemical studies [15, 16] to be localized exclusively in astrocytes.

Although the capacity of glial cells for transporting glutamate has been well established, little is known of whether glial glutamate metabolism is, in any way, coupled to the transport of glutamate into these cells. Methionine sulfoximine (MSO) is an irreversible inhibitor of GS [17, 18], and the present study was designed to determine if inhibition of glial glutamate metabolism by MSO would alter glutamate uptake in slices of the rat striatum. Since glutamate is transported by both glial cells and neurons, but GS is located only in glia, we also examined the transport of glutamate into preparations of bulk-isolated neurons and glia following treatment of animals with MSO.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats, weighing 175–200 g, purchased from King Animal Laboratories, Oregon, WI, were housed four per cage in our laboratories (22°; 12-hr light cycle), with free access to Purina lab chow and water prior to being used as experimental subjects.

Surgery and sacrifice. Unilateral intraventricular (i.c.v.) injections were performed by the method of Noble *et al.* [19]. Animals were injected with 15 μl of solution containing 0.2 μmole of MSO dissolved in artificial cerebral spinal fluid (CFS) (pH 7.4). Control animals received i.c.v. injections of the artificial CSF.

Animals were killed for uptake studies at 4 hr, 1 day, 3 days, 7 days and 14 days after the i.c.v. injections. Injection sites were verified in a number of animals by examining 30 μm frozen sections of tissue stained with cresyl violet. The site of the injection in the other experimental animals was visually verified during dissection.

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§ Abbreviations: GS, glutamine synthetase; MSO, methionine sulfoximine; DA, dopamine; BSO, buthionine sulfoximine; KRB, Krebs–Ringer bicarbonate; i.c.v., intraventricular; i.p., intraperitoneal; PTZ, pentylenetetrazol; and T/M, tissue:medium.

Preparation of striatal prisms, synaptosomes and bulk-isolated neurons and glia. Animals were decapitated, and their brains were placed in the trough of an ice-cold aluminium cutting block, as described by Hartman and Halaris [20]. A 1.5 mm coronal slice of tissue, anterior to the caudal tip of the olfactory tubercle, was obtained. The striatum was removed from both hemispheres by cutting along the internal capsule and globus pallidus at the medical border, and along the lateral border of the external capsule. This dissection provided anatomically uniform tissue samples. Striata were placed on the platform of a McIlwain tissue chopper (Brinkmann Instruments, Westbury, NY) and were sliced into prisms 0.3 mm × 0.3 mm. Prisms were suspended in a Krebs–Ringer bicarbonate (KRB) buffer (pH 7.4; gassed with 95% O_2 and 5% CO_2). The KRB solution consisted of the following ingredients: NaCl, 119 mM; KCl, 4.8 mM; $MgCl_2$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 1.7 mM; $NaHCO_3$, 23.8 mM; and glucose, 10 mM.

Synaptosomes were isolated by the procedure of Cotman [21]. The recovered synaptosomes were washed with 5 vol. of KRB buffer and centrifuged at 34,000 *g* for 30 min. The synaptosomal pellet was resuspended in KRB buffer so that the protein concentration in the final suspension was approximately 1 mg/ml.

Glial and neuronal cell fractions were isolated as described by Henn [22]. Fractions were diluted with 5 vol. of KRB buffer and centrifuged at 1000 *g* for 20 min at 0–4°. The pellets of glial and neuronal fractions were resuspended in KRB buffer so that the protein concentration in the final suspensions was 5–10 and 1–5 mg/ml respectively.

Glutamate uptake. Prisms from individual striata were preincubated in KRB buffer at 37° under an atmosphere of 95% O_2–5% CO_2 for 30 min in a shaking water bath. In certain studies, MSO, at a final concentration of 1 mM, was also added to the KRB buffer. Glutamate at a final concentration of 10 μM (0.2 μCi) and 0.5 μCi/ml of [^{3}H-G]inulin were then added to the medium. The final assay volume was 6 ml. Uptake was terminated 2 min later by filtration over glass fiber filters (Whatman GF/B, Whatman, NJ) in a Millipore Sampling Manifold (Millipore Corp., Bedford, MA). An aliquot of the medium was saved for determination of radioactive glutamate present in the medium. Filters were placed in glass scintillation vials, and 1 ml of TS-1 tissue solubilizer (Research Products International (RPI), Mt. Prospect, IL) was added. Following solubilization, 10 ml of Neutralizer Scintillation Cocktail (RPI) was added to the scintillation vials.

Glutamate uptake was measured in synaptosomes by suspending tissue in KRB buffer in a final assay volume of 3 ml. The tissue suspension (200–300 μg protein) was preincubated at 37° for 5 min. Uptake was initiated by the addition of glutamate (0.2 μCi) and, after a 2-min incubation, uptake was terminated by filtration, and filters were dried and placed in 3a70b Scintillation Cocktail (RPI).

To measure glutamate uptake into bulk-isolated neurons or glia, the tissue was first preincubated at 37° for 5 min. Uptake was initiated by the addition of glial cells (100–500 μg protein) or neuronal cells (50–150 μg protein) to 1.5 ml polypropylene microfuge tubes containing KRB buffer and glutamate (0.2 μCi) in various concentrations. Uptake was terminated after 2 min by a 30-sec rapid centrifugation on a Beckman Microfuge B. The supernatant fraction was removed, the pellet was dissolved in 1 ml of TS-1 solution, and Neutralizer Scintillation Cocktail was added. Radioactivity was quantitated by liquid scintillation counting using a Beckman LS7500 equipped with a Data Reduction Accessory for dual- and single-labeled samples. Protein was measured according to the method of Lowry *et al.* [23] with bovine serum albumin as a standard.

For all preparations, uptake was linear with time for up to 5 min. Uptake experiments were also performed at 0° or in a sodium-free KRB buffer in which equimolar amounts of choline chloride and $KHCO_3$ were substituted for NaCl and $NaHCO_3$ respectively. Uptake values obtained under these conditions were less than 5% of those obtained at 37° in the presence of sodium. Active, sodium-dependent uptake was calculated after subtracting the values determined at 0° under sodium-free conditions. Active uptake was expressed as either tissue:medium ratios, or as intracellular accumulation of glutamate per wet weight of tissue, or per mg protein. Values were also corrected for the presence of radioactive glutamate in the extracellular space in tissue slices, as determined by inulin distribution between tissue and medium.

For determination of kinetic parameters of glutamate or aspartate uptake, tissue was incubated with glutamate or aspartate at concentrations between 1 and 600 μM. This range was chosen to include both high- and low-affinity uptake for glutamate and aspartate [7, 24]. For leucine uptake studies, slices were incubated with leucine at concentrations from 50 μM to 4 mM. The kinetic constants and their standard errors were calculated by non-linear, weighted, least-squares analysis of the non-linear rate equation:

$$V = \frac{V_{\text{maxH}} \cdot S}{K_{m\text{H}} + S} + \frac{V_{\text{maxL}} \cdot S}{K_{m\text{L}} + S}$$

for a two-component uptake system. Fortran programs (PENNZYME) written by Kohn *et al.* [25] were obtained from the Share Program Library Agency (No. 360D.13.2.004; Research Triangle Park, NC). PENNZYME was adapted in our laboratories for routine use on a PDP-11 computer.

Chemicals. L-[^{14}C-U]Glutamic acid (270–290 mCi/mmole), L-[^{14}C-U]aspartic acid (220 mCi/mmole), L-[^{14}C-U]leucine (353 mCi/mmole) and [^{3}H-G]inulin (443.8 mCi/g) were purchased from the New England Nuclear Corp. (Boston, MA). [^{3}H-G]Inulin was purified prior to use by passage through Bio-Gel P2 (Bio-Rad Laboratories, Richmond, CA). [^{14}C-U]Glutamate was purified using ion exchange chromatography (AG1x8, chloride form), as described by Pruisner and Milner [26]. DL-Buthionine-*S*-*R*-sulfoximine was obtained from the Chemical Dynamics Corp. (South Plainfield, NJ). Pentylenetetrazol was obtained from the City Chemical Corp. (New York, NY). All other reagents and biochemicals were purchased from the Sigma Chemical Co. (St. Louis, MO).

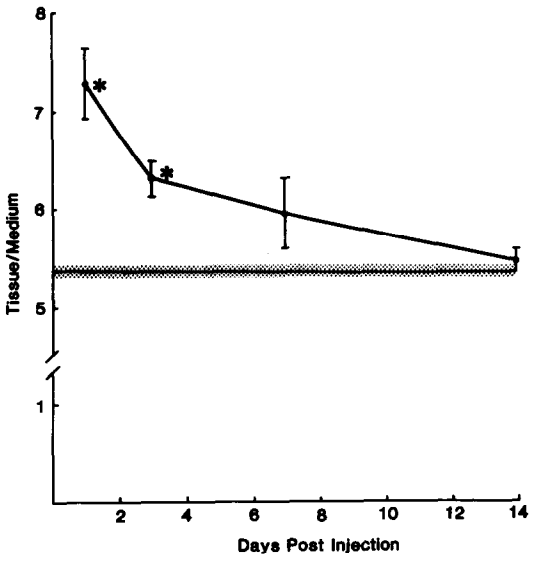

Fig. 1. Changes in high-affinity glutamate uptake in striatal tissue slices at various time points after animals received an i.c.v. injection of MSO or artificial CSF vehicle (control). Glutamate uptake was determined in striatal slices suspended in 6 ml of oxygenated KRB buffer and preincubated at 30° for 30 min. Assays were initiated by the addition of glutamate (0.2 μCi) at a final concentration of 10 μM. The uptake was terminated 2 min later by vacuum filtration over glass-fiber filters, and uptake was calculated and expressed as a tissue-to-medium (T/M) ratio as described in Materials and Methods. Results are the means ± S.E.M. of six experiments. Shaded areas represent control values ± S.E.M. Asterisks(*) denote statistically significant differences from control, $P < 0.01$.

Statistical analysis. For statistical analysis of the time course of changes in glutamate uptake, analysis of variance (ANOVA) was employed. Comparisons between groups within time course studies were performed using planned multiple comparison [27]. When appropriate, statistical analysis of data was performed using a two-tailed Student's *t*-test. For all statistics, P values <0.05 were taken to indicate significant differences in the values being compared.

RESULTS

Glutamate uptake into striatal slices. Glutamate uptake by slices of the striatum was altered significantly [ANOVA; $F(4,21) = 3.426$; $P < 0.05$] during the 2-week period following i.c.v. injection of MSO (Fig. 1). Glutamate uptake into striatal slices, measured 4 hr after i.c.v. injection of MSO, was unaltered compared to control glutamate uptake. However, by 24 hr after i.c.v. injection of MSO, glutamate uptake was increased significantly ($P < 0.01$), as reflected by the 40% increase in the T/M ratio for glutamate. By 3 days after injection of MSO, T/M ratios began to decrease, but they still remained significantly elevated ($P < 0.01$) above control T/M ratios. Fourteen days after injection of MSO, T/M ratios returned to control values. Glutamate uptake into striatal slices from control animals was not altered when concentrations of MSO up to 1 mM were added directly to the incubation medium.

To further characterize the changes in glutamate uptake produced by the administration of MSO, the kinetics of glutamate uptake into striatal slices were

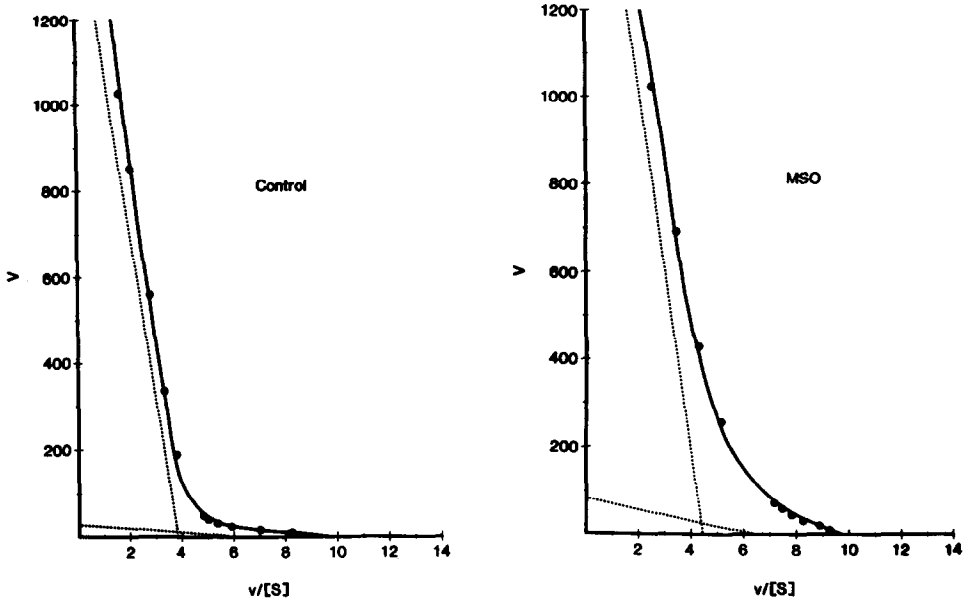

Fig. 2. Eadie–Hofstee plot of glutamate uptake intro striatal tissue slices from control animals and from MSO-pretreated animals. Glutamate uptake was determined in slices suspended in 6 ml of oxygenated KRB buffer and preincubated at 37° for 30 min. Assays were initiated by the addition of various concentrations of glutamate (0.2 μCi). The assays were terminated 2 min later by vacuum filtration over glass-fiber filters, and uptake was calculated as described in Materials and Methods. Each point represents the mean of four to six determinations. The coefficient of variation at each point was no greater than 10% of the mean. The units for velocity (v) are pmoles/min/mg wet weight and the units fo S (glutamate) are μM. Dotted lines represent "PENNZYME" generated kinetic estimates of the high- and low-affinity uptake systems (see Materials and Methods).

Table 1. Kinetic parameters for amino acid uptake into striatal tissue slices*

Glutamate				
	High-affinity uptake		Low-affinity uptake	
Group	K_m (μM)	V_{max} ($\times 10^{-12}$)	K_m (μM)	V_{max} ($\times 10^{-12}$)
Control (5)	2.18 ± 0.54	9.9 ± 1.1	393 ± 36	1524 ± 122
MSO, day 1 (5)	10.30 ± 0.69†	55.7 ± 6.1†	419 ± 18	1848 ± 212
Aspartate				
	High-affinity uptake		Low-affinity uptake	
Group	K_m (μM)	V_{max} ($\times 10^{-12}$)	K_m (μM)	V_{max} ($\times 10^{-12}$)
Control (3)	1.48 ± 0.18	10.83 ± 2.43	419 ± 29.9	1440 ± 74
MSO, day 1 (3)	7.10 ± 0.60†	69.10 ± 8.25†	468 ± 27.9	1950 ± 400
Leucine				
	Uptake			
Group	K_m	V_{max} ($\times 10^{-9}$)		
Control (3)	823.0 ± 73.4	110.0 ± 1.0		
MSO, day 1 (3)	921.3 ± 41.3	114.9 ± 10.3		

* Animals received a 15 μl i.c.v. injection of a solution containing 0.2 μmole of MSO or artificial CSF vehicle (control), and were killed 24 hr later. Amino acid uptake was determined in striatal tissue slices suspended in 6 ml of oxygenated KRB buffer and preincubated at 37° for 30 min. Assays were initiated by the addition of ^{14}C-labeled amino acids at various concentrations. The assay was terminated 2 min later by vacuum filtration over glass-fiber filters, and uptake was calculated as described in Materials and Methods. The data for glutamate and aspartate uptake in MSO and control groups best fit a transport model consisting of two independent carrier sites, while the data for leucine uptake best fit a one-carrier model. Kinetic parameters were generated by computer analysis (see text). The number of separate experiments is noted in parentheses, and results shown are the means ± S.E.M. of these experiments. V_{max} is expressed as moles/min/mg wet weight.

† Statistically significant difference from control values, $P < 0.01$.

examined. Eadie–Hofstee plots of the glutamate uptake data in control tissue, and in tissue at all time points following MSO injection, were non-linear (Fig. 2). The data from control or MSO-treated groups were analyzed using rate equations for one, two or three independent carrier systems. An analysis of the residual error indicated that a two or three independent carrier model was statistically acceptable ($P < 0.01$), while a one-carrier model was not. The three-carrier rate model was discarded, since it did not produce a significantly better fit of the data than did the two-carrier model. We refer to the two-carriers as the high- and low-affinity transport sites.

The kinetic parameters for low affinity glutamate uptake were unaltered 24 hr after MSO injection (Table 1). However, the K_m and V_{max} values for the high-affinity glutamate transport site in striatal slices were significantly altered 24 hr after animals were injected with MSO. Analysis of the changes in kinetic parameters for high-affinity glutamate uptake into striatal slices over time showed a significant effect of MSO on the K_m for glutamate [ANOVA; $F(4, 21 = 16.176$, $P < 0.01$] and the V_{max} for glutamate uptake [ANOVA; $F(4, 21) = 14.104$, $P < 0.01$]. The high-affinity K_m values for striatal glutamate uptake were elevated significantly for up to 7 days after MSO injections, but returned to control values by day 14 (Fig. 3A). Similarly, the V_{max} for high-affinity glutamate transport was elevated significantly ($P < 0.01$) on days 1 through 7 and returned to control values by day 14 (Fig. 3B). Calculations of the low-affinity transport site kinetic parameters revealed that at no time point following MSO administration did the K_m of V_{max} values for low-affinity glutamate uptake vary from control values.

Since aspartate may be transported by the same carrier as glutamate, the effects of MSO on aspartate uptake were examined. Aspartate uptake into striatal tissue was also altered 24 hr after an i.c.v. MSO injection (Table 1). The changes in kinetic parameters for aspartate uptake following MSO treatments were similar in magnitude to those determined for glutamate uptake after MSO treatments. As with glutamate uptake, low-affinity aspartate uptake kinetics were not altered following MSO pretreatment.

The effect of MSO pretreatment on leucine uptake into striatal prisms was also examined. Only one uptake carrier could be distinguished in the con-

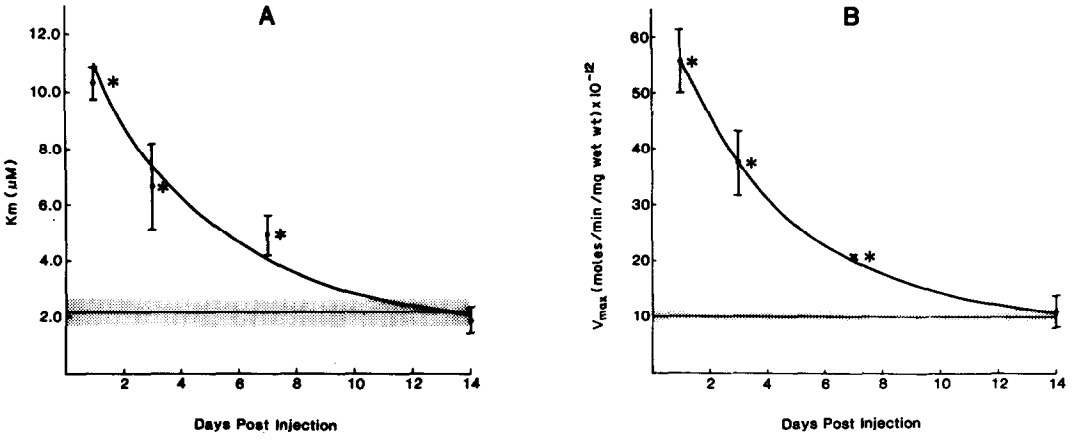

Fig. 3. Changes in the kinetic parameters for high-affinity glutamate uptake into striatal tissue slices at various time points after animals received an i.c.v. injection of MSO or artificial CSF vehicle (control). Glutamate uptake was determined in striatal slices suspended in 6 ml of oxygenated KRB buffer and preincubated at 37° for 30 min. Assays were initiated by the addition of various concentrations of glutamate (0.2 μCi). The assays were terminated 2 min later by vacuum filtration of glass-fiber filters, and uptake was calculated as described in Materials and Methods. Kinetic parameters were calculated by computer analysis (see Materials and Methods). Results are the means ± S.E.M. of five experiments. Panel A = K_m values; panel B = V_{max} values. Shaded areas represent control values ± S.E.M. Asterisks (*) denote statistically significant differences from control, $P < 0.01$.

centration range of leucine used in our assays (Table 1). Pretreatment with MSO did not alter the kinetics of leucine transport into striatal slices.

Behaviourally, animals exhibited tonic–clonic convulsions 4–6 hr after i.c.v. injections of MSO, but were calm and alert 24 hr after injection. To examine whether the changes in uptake were secondary to the convulsant episodes after injection of MSO, rats were injected with the convulsant pentylenetetrazol (PTZ) (45 mg/kg), and glutamate uptake into striatal slices was examined 24 hr later. At the dose of PTZ used, all animals exhibited convulsions, yet glutamate uptake, as measured by T/M ratios, was not significantly different between PTZ-pretreated and control groups (Table 2).

The effect of the MSO analog, buthionine sulfoximine (BSO), on glutamate uptake into striatal slices was also investigated. One day following i.c.v. injections of either 0.4 μmole BSO, or 4.0 μmoles BSO, glutamate uptake, as measured by T/M ratios, was not significantly different from control values (Table 2). At no time, up to 24 hr after BSO injection, did animals exhibit convulsions.

Glutamate uptake into bulk-isolated glia, neurons and synaptosomes. When glutamate uptake was examined using isolated neurons, glia or synaptosomes prepared from control or MSO-pretreated animals, the data were non-linear when graphed as Eadie–Hofstee plots.

As with striatal slices, PENNZYME analysis for each tissue preparation revealed that the data for glutamate uptake in both MSO-treated and control groups best fit ($P < 0.01$) a model of two independent carriers.

Glial glutamate uptake was altered in striatal tissue from animals treated with MSO. The apparent high-affinity K_m for glutamate was significantly ($P < 0.01$) elevated by 93% 24 hr after MSO treatment (Table 3). Similarly, the V_{max} for glutamate uptake into glial cell preparations was increased significantly by MSO pretreatment ($P < 0.01$). The kinetic parameters for low-affinity glial glutamate uptake were unchanged in glial cells obtained from MSO-treated animals compared to controls.

When kinetic parameters for glutamate transport into isolated neurons were examined 24 hr after MSO injection, neither the low-affinity nor the high-affinity transport systems were altered (Table 3). Notably, the kinetic parameters for high-affinity glutamate transport into glial cells were different from the kinetic parameters of glutamate transport into neurons. Bulk-isolated neurons displayed a 7-fold lower K_m for "high-affinity" glutamate uptake, while bulk-isolated glial cells possessed a greater high-affinity transport capacity as reflected by the 14-fold greater V_{max} for glutamate uptake.

Table 2. Effects of buthionine sulfoximine and pentylenetetrazol on glutamate uptake in striatal tissue slices*

Treatment	N†	T/M ratio
Pentylenetetrazol (PTZ)		
Control	6	4.07 ± 0.08
PTZ (45 mg/kg)	6	3.94 ± 0.19
Buthionine sulfoximine (BSO)		
Control	8	4.16 ± 0.21
BSO (0.4 μmole)	8	4.76 ± 0.33
BSO (4.0 μmole)	8	3.84 ± 0.22

* Animals were injected i.c.v. with BSO, or i.p. with PTZ 24 hr prior to being killed. Control animals received either i.c.v. injections or artificial CSF vehicle, or i.p. injections of saline. Glutamate uptake was measured in striatal tissue prisms suspended in 6 ml of oxygenated KRB and preincubated at 37° for 30 min. Assays were initiated by the addition of glutamate (0.2 μCi) at a final concentration of 10 μM. The assay was terminated 2 min later by vacuum filtration over glass-fiber filters, and uptake was calculated as T/M ratios described in Materials and Methods. Results shown are mean values ± S.E.M.

† Number of experiments.

Table 3. Kinetic parameters for the uptake of glutamate by synaptosomes, bulk-isolated glial cells and neurons*

Group	High-affinity uptake		Low-affinity uptake	
	K_m (μM)	V_{max}	K_m (μM)	V_{max}
Glia				
Control	8.05 ± 1.2	1.56 ± 0.39	204.44 ± 46.0	5.71 ± 1.14
MSO	15.57 ± 1.88†	3.99 ± 0.45†	281.79 ± 84.0	5.41 ± 0.72
Neurons				
Control	1.15 ± 0.10	0.108 ± 0.011	216.2 ± 0.7	2.29 ± 0.49
MSO	0.85 ± 0.20	0.103 ± 0.038	352.4 ± 0.8	2.63 ± 0.09
Synaptosomes				
Control	7.25 ± 1.02	2.02 ± 0.23	222.9 ± 58.2	9.45 ± 0.45
MSO	8.95 ± 0.9	2.61 ± 0.47	313.98 ± 40.0	10.75 ± 1.66

* Animals were injected i.c.v. with 15 μl of a solution containing 0.2 μmole MSO or artificial CSF vehicle (control), and were killed 24 hr later. Striata were dissected, pooled from ten to fifteen rats, and used for the isolation of either synaptosomes or neuron/glial fractions. Aliquots of either synaptosomes (200–300 μg protein), isolated glia (100–500 μg protein) or isolated neurons (50–150 μg protein) were preincubated for 5 min at 37° and then incubated for 2 min with various concentrations of glutamate (0.2 μCi). Tissue was rapidly pelleted and glutamate uptake was calculated as described in Materials and Methods. The data for all three tissue preparations, for both MSO and control groups, best fit ($P < 0.01$) a two independent carrier model for glutamate transport. Kinetic constants were generated by computer analysis. Results shown are the means ± S.E.M. of five experiments; V_{max} is expressed as nmoles/min/mg protein.

† Statistically significant difference from control, $P < 0.01$.

Glutamate uptake into synaptosomes isolated from striatal tissue was also examined 24 hr after MSO injections. Glutamate uptake into this tissue preparation was not altered noticeably by MSO pretreatment (Table 3). The K_m and V_{max} for both high- and low-affinity glutamate uptake were similar in synaptosomes from control and MSO-treated animals.

DISCUSSION

The results of the present experiments demonstrate that i.c.v. MSO administration can increase glutamate uptake into striatal tissue slices, and that the high-affinity uptake kinetic parameters are altered in conjunction with the altered transport. Furthermore, by analyzing cellular fractions, our data indicate that high-affinity glutamate transport into glial cells in striatal tissue was specifically altered by administration of MSO. The differences in the magnitude of effect noted in glial preparations as compared to striatal slices may be a consequence of the procedure for preparing the glial fractions. The glial fractions were noted, by microscopic examination, to contain a number of cells whose processes had been damaged.

The concomitant increase in high-affinity aspartate transport into striatal tissue slices would be expected, since aspartate has been demonstrated to be transported via the same carrier as glutamate in brain tissue [24, 28–30]. The changes in glutamate and aspartate transport produced by MSO could reflect a general alteration in the uptake of all amino acids by brain tissue. Leucine is transported into brain tissue by a carrier distinct from the glutamate/aspartate carrier [31, 32], but the lack of effect of MSO on leucine transport indicates a specific action of MSO on the glutamate/aspartate transport system.

MSO is a potent convulsant, and the changes in glutamate uptake could have been secondary to the convulsant episodes observed after MSO. However, administration of PTZ, another strong convulsant agent, did not alter glutamate uptake into brain tissue. It is, therefore, unlikely that the changes in glutamate uptake after MSO administration were simply the results of convulsions.

The effect of MSO on glutamate transport was, however, not a direct consequence of an interaction between MSO and this amino acid transport system. MSO did not alter uptake when it was added directly to assays containing striatal slices, or when measurements were performed 4 hr after i.c.v. injection of MSO. These data agree with previous reports concerning the lack of a direct effect of MSO on glutamate uptake [24, 33, 34].

MSO is a specific, irreversible inhibitor of GS [17, 18] and has little effect on other enzymes involved in glutamate metabolism, such as glutaminase, glutamate dehydrogenase, glutamate decarboxylase, glutamine transaminase, and aspartate aminotransferase [35–38]. MSO, however, has been reported to inhibit the enzyme γ-glutamyl cysteine synthetase [39], which forms the glutathione precursor γ-glutamyl-cysteine. Glutathione is believed to have many functions, including maintenance of -SH groups on proteins, scavenging of hydrogen peroxide and free radicals, and the transport of amino acids and peptides into cells [40]. BSO, a structural analog of MSO, is an inhibitor of γ-glutamyl cysteine synthetase, but does not significantly inhibit GS [41, 42]. Since BSO did not alter striatal glutamate uptake, it is unlikely that MSO effects on γ-glutamyl cysteine synthetase are responsible for our observed changes in glutamate uptake.

Previously, we had demonstrated that, 24 hr after an i.c.v. injection of MSO, GS was inhibited 50%,

and by 2 weeks after administration GS activity returned to control values [43]. These changes in GS activity significantly correlate with the changes in K_m or V_{max} values for high-affinity glutamate transport ($r = 0.9481$, $P < 0.05$ and $r = 0.9486$, $P < 0.05$ respectively) observed in our present study. It is possible, then, that the effect of MSO on glial glutamate uptake is due to actions of MSO on systems such as GS or other glial enzymes. We would propose that the altered metabolism of glutamate via the glutamate–glutamine cycle would produce changes in glial glutamate uptake as an adaptive response to inhibition of the enzymes which inactivate the accumulated glutamate.

It has been demonstrated that glutamate is transported into astroglia via a sodium-dependent, high-affinity, high-capacity carrier [2–5, 7]. Bulk-isolated neurons [7, 8], cultured neurons [9], and synaptosomes [7, 8, 10] also contain a high- and a low-affinity transport system for glutamate but, due to the relatively higher capacity of glial cells to accumulate glutamate, it has been suggested that glial cells may be the primary elements which accumulate glutamate in brain tissue [13]. Our data demonstrate that changes in transport of glutamate into striatal tissue are a result of changes in glial glutamate uptake.

Previously, we had demonstrated that MSO alters striatal dopamine (DA) release from nigrostriatal DA terminals [43]. A change in the rate of glutamate uptake from the synaptic cleft, or its release from glutamatergic corticostriatal terminals [44, 45], could modify DA release [46–48]. The increase in glutamate uptake in striatal tissue from animals treated with MSO could decrease the normal activation of glutamate receptors on striatal DA terminals. These experiments demonstrate that MSO can be a useful tool to examine neuron–glial interactions, as well as neuron–neuron interactions in the CNS.

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