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# Glutamate Increases Glycogen Content and Reduces Glucose Utilization in Primary Astrocyte Culture

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**Abstract:** The glycogen content of primary cultured astrocytes was approximately doubled by incubation with 1 m*M* L-glutamate or L-aspartate. Other amino acids and excitatory neurotransmitters were without effect. The increase in glycogen level was not blocked by the glutamate receptor antagonist kynurenic acid but was completely blocked by the glutamate uptake inhibitor *threo*-3-hydroxy-D,L-aspartate and by removal of $Na^+$ from the medium. Incubation with radiolabeled glucose and glutamate revealed that the increased glycogen content was derived almost entirely from glucose. Glutamate at 1 m*M* was also found to cause a 53 ± 12% decrease in glucose utilization and a 112 ± 69% increase in glucose-6-phosphate levels. These results suggest that the glycogen content of astrocytes is linked to the rate of glucose utilization and that glucose utilization can, in turn, be affected by the availability of alternative metabolic substrates. These relationships suggest a mechanism by which brain glycogen accumulation occurs during decreased neuronal activity. **Key Words:** Aspartate—Glia—2-Deoxyglucose—Excitatory amino acid—Glucose-6-phosphate—Methionine sulfoximine. **Swanson R. A. et al.** Glutamate increases glycogen content and reduces glucose utilization in primary astrocyte culture. *J. Neurochem.* **54,** 490–496 (1990).

Brain glycogen is found primarily in astrocytes (Koizumi, 1974; Phelps, 1975; Cataldo and Broadwell, 1986). The physiologic function of this glycogen reserve has not been firmly established, but several lines of evidence support the notion that this energy reserve is mobilized in response to local increases in neuronal activity (Lipton, 1973; Quach et al., 1978; Swanson et al., 1987; Cambray-Deakin et al., 1988; Fox et al., 1988; Magistretti, 1988). Conversely, an increase in glycogen content is associated with reduced neuronal activity, such as results from anesthesia (Nelson et al., 1968; Brunner et al., 1971), hibernation (Wolff, 1970; Ibrahim, 1975), and after neuronal death (Guth and Watson, 1968; Pudenz et al., 1975). Although the reason for glycogen accumulation under these conditions is unknown, a common element may be a decreased metabolic demand on the glia secondary to decreased neuronal activity (Ibrahim, 1975).

A potent inducer of astrocyte glycogen content in vitro and in vivo is the glutamine synthetase inhibitor L-methionine-D,L-sulfoximine (MSO) (Rao and Meister, 1972; Folbergrová, 1973; Hevor et al., 1985; Swanson et al., 1989). Glutamine synthetase in brain is localized primarily to astrocytes (Martinez-Hernandez et al., 1977) and represents a major pathway of brain glutamate metabolism (Benjamin and Quastel, 1975; Yu et al., 1982; Rothstein and Tabakoff, 1986; Juurlink, 1987; Yudkoff et al., 1988). The colocalization of glutamine synthetase and the MSO-induced glycogen accumulation to astrocytes suggests a possible link between glutamate and glycogen metabolism. The present study examines the mechanisms by which glutamate metabolism, glucose utilization, and glycogen content are related in cultured astrocytes.

## MATERIALS AND METHODS

### Materials

Newborn Sprague–Dawley rats were obtained from Bantin and Kingman (Fremont, CA, U.S.A.). Horse and fetal calf sera were supplied by Hyclone (Logan, UT, U.S.A.). Falcon tissue culture dishes were obtained from Becton Dickinson (Oxnard, CA, U.S.A.), and Nitex sieves were from Tetko (Elmford, NY, U.S.A.). Radiochemicals were purchased from American Radiochemicals (St. Louis, MO, U.S.A.). All other

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*Abbreviations used:* 2DG, 2-deoxyglucose; G6P, glucose-6-phosphate; MEM, Eagle's minimal essential medium; MSO, L-methionine-D,L-sulfoximine; SDS, sodium dodecyl sulfate; THPA, *threo*-3-hydroxy-D,L-aspartate.

reagents and enzymes were obtained through Sigma Chemical Co. (St. Louis, MO, U.S.A.).

### Astrocyte culture

Primary astrocytes were prepared as described by Hertz et al. (1985) from the cerebral cortex of newborn Sprague–Dawley rats. The portions of cortex dorsal and lateral to the lateral ventricles were cut into small cubes (<1 $mm^3$) in a modified Eagle's minimal essential medium (MEM) (Hertz et al., 1985). The tissue was disrupted by vortex-mixing for 1 min, and the suspension was passed through two sterile nylon Nitex sieves with pore sizes of 80 (first sieving) and 10 μm (second sieving). The cell suspension was diluted into MEM containing 20% fetal calf serum to a final volume of 30 ml/dissected brain and placed into 60-mm-diameter tissue culture dishes in 3-ml aliquots. Cultures were incubated at 37°C in a 95/5% mixture of air and $CO_2$, with 95% humidity. The medium was changed 3 days after seeding and subsequently twice per week with MEM containing 5% horse serum and 5% fetal calf serum. After 2 weeks, the cultures reached confluence and were grown in the additional presence of 0.25 m*M* dibutyryl cyclic AMP to induce differentiation (Schousboe et al., 1980; Kimelberg, 1983; Hertz et al., 1985). The dibutyryl cyclic AMP was omitted, however, on the day preceding experiments so that the experiments were not confounded with the glycogenolytic effect of dibutyryl cyclic AMP (Magistretti et al., 1983). The cultures were used for studies when they were between 4 and 6 weeks old, with a cell density of ~0.6 mg of protein/dish. Cultures prepared in this way contained a monolayer of cells of which >95% stained for glial fibrillary acidic protein.

### Biochemical determinations

All experiments were begun 24 h after exchange of the culture medium, at which time the astrocyte glycogen content is stable (Swanson et al., 1989). Compounds were added from pH-neutralized stock solutions, and the cultures were returned to the 5% $CO_2$ incubator. Experiments were terminated by twice washing the cells for 1 s with ice-cold normal saline containing 0.2 m*M* phloretin [to prevent glucose egress (Kletzien et al., 1975)] and immediately freezing the cells with liquid nitrogen. The culture dishes were warmed to −10°C in an ethanol–ice bath, thawed, and lysed by addition of 1 ml of 0.03 *M* HCl with 0.25% sodium dodecyl sulfate (SDS). The dissolved cells were stored at −20°C.

Glucose and glucose-6-phosphate (G6P) were quantified by the fluorometric methods of Lowry and Passonneau (1972). Glycogen content was determined by the amylo-α-1,4-α-1,6-glucosidase (EC 3.2.1.3) procedure of Passonneau and Lauderdale (1973) and expressed as nanomole glucosyl equivalents. All values were normalized to protein content, as determined with the method of Lowry et al. (1951).

### Incorporation of glucose and glutamate into glycogen

Cultures received 60 μl of either 50 m*M* glutamate (for final media concentrations of 1 m*M*) or 60 μl of water. After 90 min, each culture also received 50 μCi of [3-$^3$H]glucose (15 Ci/mmol) and 10 μCi of [U-$^{14}$C]glutamate (250 mCi/mmol). After 4 h, the cells were harvested as above. Aliquots were removed for protein and glycogen content determinations, and the remainder was divided into two fractions. One fraction was digested with amyloglucosidase at pH 5 to hydrolyze glycogen, whereas the other fraction was placed in pH 5 acetate buffer without the enzyme. Both fractions were deproteinized by addition of perchloric acid to a final concentration of 0.25 *M*. The supernatants were removed and added to 3 volumes of ethanol to precipitate glycogen. The precipitates were washed twice with 75% ethanol. Preliminary experiments showed cpm in the last wash supernatant to be a negligible fraction of cpm in the precipitate. The precipitates from the last wash were dissolved in water, and aliquots were taken for glycogen content determinations (to establish the percentage recovery of glycogen) and for scintillation counting. Calculation of $^3$H and $^{14}$C dpm was performed with a [$^{14}$C]toluene and [$^3$H]toluene standardized quench curve and a software program supplied with the Beckman LS5801 scintillation counter. The differences in dpm between the untreated and amyloglucosidase-treated fractions from each sample represented label incorporated into glycogen.

### Glucose utilization

Ninety minutes after addition of 1 m*M* glutamate or MSO, 13.5 μCi of 2-deoxy[1,2-$^3$H]glucose ([$^3$H]2DG; 40 Ci/mmol) was added to each culture. After a 45-min incubation, the media were removed, and the cells were washed three times by 15-min incubations in fresh media (MEM plus serum) to remove unphosphorylated 2DG. The fresh media were preequilibrated with $CO_2$ in the incubator before use to prevent changes in the intracellular pH of the astrocytes, which could alter the rate of glycolysis (Trivedi and Danforth, 1966). After removal of the third wash, the cells were lysed in 0.03 *M* HCl with 0.25% SDS. Aliquots of the lysate were taken for determination of protein content and for scintillation counting. Aliquots of media were taken for determination of specific activity and to assess the adequacy of the washes.

## RESULTS

The mean glycogen content of the control cultures from 19 separate batches of astrocytes varied from 34.2 to 60.4 nmol/mg of protein. Therefore, pooled results were expressed as percentages of the control values for each experiment. As shown in Fig. 1, a 4-h incubation with 1 m*M* L-glutamate or L-aspartate caused an approximate doubling of the astrocyte glycogen content. MSO also induced a significant increase, although a longer incubation (24 h) was required. Other amino acids and metabolic intermediates had no significant effect (Fig. 2).

Figure 3 shows the effects of different initial media glutamate concentrations and incubation times on astrocyte glycogen content. With 1 m*M* glutamate, maximal glycogen accumulation required at least 4 h (Fig. 3A). With the incubation time fixed at 4 h, 1 m*M* glutamate was sufficient to produce a maximal accumulation (Fig. 3B). There was no discernible effect at initial glutamate concentrations below 0.1 m*M*.

Glutamate receptor agonists and antagonists were used to test whether the glutamate effect was receptor mediated. The agonists kainate and *N*-methyl-D-aspartate (Watkins and Evans, 1981) did not alter astrocyte glycogen content, and the antagonist kynurenate (Watkins and Evans, 1981) failed to block the effect of glutamate (Fig. 4). However, blockade of glutamate uptake with 1 m*M* *threo*-3-hydroxy-D,L-aspartate (THPA) (Cox and Bradford, 1978) or by replacing $Na^+$ with choline (Schousboe et al., 1977) completely pre-

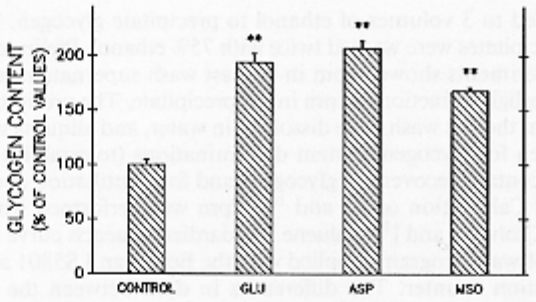


**FIG. 1.** Effects of 1 m*M* L-glutamate, L-aspartate, and MSO on astrocyte glycogen content. Incubations with glutamate and aspartate were for 4 h, and those with MSO were for 24 h. Data are mean ± SEM (bars) values from three experiments, each performed in quadruplicate. Significant differences by Student's *t* test from the control are indicated: $^{**}p < 0.001$.

vented the glutamate-induced increase in glycogen content (Fig. 4).

These experiments suggested that the glutamate-induced glycogen accumulation is a result of glutamate uptake or metabolism. Glutamate, once taken up, might become incorporated into glycogen through gluconeogenesis. Alternatively, glutamate or a metabolite might influence either the rate of glucose incorporation into glycogen or the rate of glycogenolysis. These possibilities were investigated using [$^3$H]glucose and [$^{14}$C]glutamate tracers (Table 1). Addition of 1 m*M* glutamate produced increases in glycogen content and in $^3$H incorporation into glycogen. In contrast, there was essentially no incorporation of $^{14}$C from glutamate into glycogen either with or without addition of 1 m*M* unlabeled glutamate.

A plausible mechanism for the glutamate-induced increase in glucose incorporation into glycogen is that the rapid metabolism of glutamate can reduce the astrocyte requirement for glucose. This was tested by measuring glucose utilization rates in the presence and absence of 1 m*M* added glutamate, using a modification of the 2DG method (Sokoloff et al., 1977). Glutamate was found to reduce the amount of retained [$^3$H]2DG by 53 ± 12% (Table 2). MSO at 1 m*M* caused a decrease of comparable magnitude: 42 ± 16%.

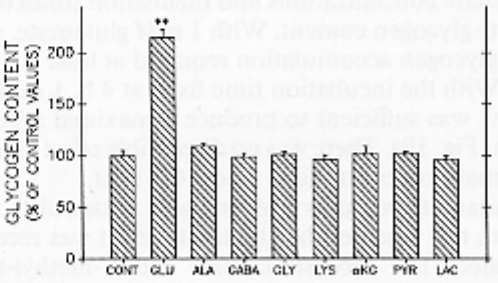


**FIG. 2.** Effects of other amino acids and metabolic intermediates on astrocyte glycogen content: L-glutamate, L-alanine, γ-aminobutyrate (GABA), glycine, L-lysine, α-ketoglutarate (αKG), pyruvate (PYR), and lactate (LAC). Incubations with each agent were at 1 m*M* concentration for 4 h. Data are mean ± SEM (bars) values from two experiments, each performed in quadruplicate. Significant difference by Student's *t* test from the control (CONT) is indicated: $^{**}p < 0.001$.

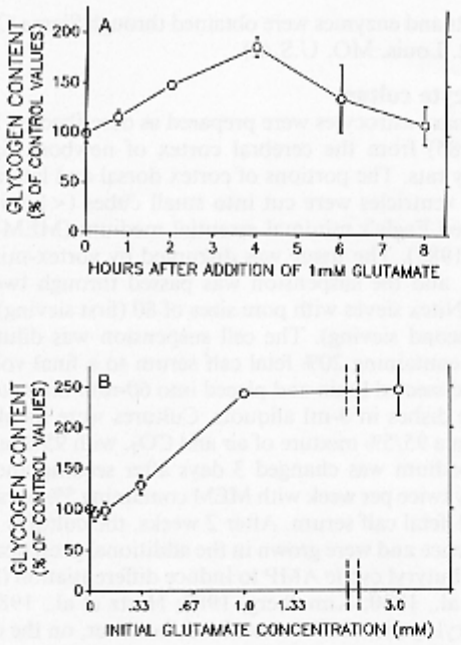


**FIG. 3.** Glutamate-induced increases in astrocyte glycogen content **(A)** after varying incubation times and 1 m*M* initial glutamate concentration and **(B)** with varying initial glutamate concentrations and 4-h incubations. Data are mean ± SEM (bars) values from three experiments, each performed in triplicate.

Glutamate is not known to regulate directly either glucose utilization or glycogen metabolism. Rapid glutamate metabolism could, however, indirectly increase G6P levels (see Discussion). G6P has allosteric effects on brain hexokinase, glycogen phosphorylase, and glycogen synthetase (McIlwain and Bachelard, 1985).

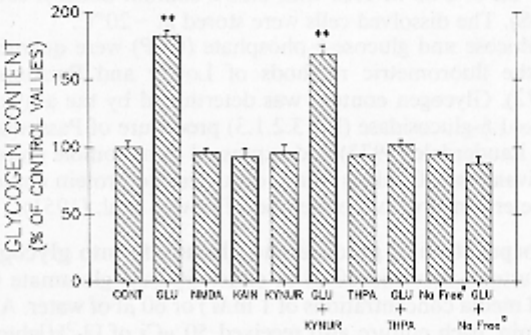


**FIG. 4.** Effects of glutamate receptor agonists and antagonists and glutamate uptake inhibition on glutamate-induced glycogen accumulation: L-glutamate, *N*-methyl-D-aspartate (NMDA), kainate (KAIN), kynurenate (KYNUR), and THPA. All agents were added at 1 m*M* for 4-h incubations. Data are mean ± SEM (bars) values from three experiments, each performed in triplicate. Na Free[a], $[Na^+] < 10$ m*M*; $Na^+$ in MEM was replaced by choline before addition of serum, and the cells were preincubated in this medium for 4 h before addition of GLU. Significant differences by Student's *t* test from the control (CONT) are indicated: $^{**}p < 0.001$.

**TABLE 1.** *Incorporation of [3-$^3$H]glucose and [U-$^{14}$C]glutamate into glycogen*

| | Control | Glutamate (1 m*M*) |
|---|---|---|
| Glycogen content (nmol/mg of protein) | 33.1 ± 2.7 | 55.9 ± 2.2[a] |
| Total label in glycogen[b] | | |
| $^3$H | 47,000 ± 7,400 | 144,000 ± 26,600[c] |
| $^{14}$C | 99 ± 8 | 112 ± 4 |
| Specific activity of glycogen[d] | | |
| $^3$H | 1,350 ± 230 | 2,580 ± 470[c] |
| $^{14}$C | 3 ± 2 | 2 ± 2 |
| Specific activity of medium | | |
| $^3$H (dpm/nmol glucose) | 11,200 ± 600 | 11,000 ± 550 |
| $^{14}$C (dpm/nmol glutamate) | —[e] | 12,000 ± 300 |

Data are mean ± SEM values from one of two experiments with similar results. Control cultures (n = 4) were incubated for 4 h in medium containing 6 m*M* glucose and tracer amounts of [3-$^3$H]glucose and [U-$^{14}$C]glutamate. The experimental culture medium (n = 4) contained, in addition, 1 m*M* unlabeled glutamate.

Statistically significant differences by Student's *t* test from the control are indicated: [a] $p < 0.01$, [c] $p < 0.05$.

[b] Values were corrected for loss of glycogen during ethanol washes; glycogen recovery was 86 ± 6%. Total label is given in dpm/mg of protein.

[d] Specific activity of glycogen given in dpm/nmol.

[e] As no glutamate is normally present in the culture medium, the specific activity is approximately that of the tracer itself, $5.5 \times 10^6$ dpm/nmol.

Therefore, G6P levels were measured in astrocyte cultures incubated with 1 m*M* glutamate or MSO (Fig. 5). Although variable in degree, increases in the G6P levels of the glutamate- and MSO-treated cultures were significant.

## DISCUSSION

The cardinal finding of this study is that the glycogen content of astrocytes in primary culture increases when the cells are incubated with millimolar concentrations of glutamate or aspartate. As previously reported (Swanson et al., 1989), 1 m*M* MSO also caused a marked increase in glycogen content (Fig. 1), although longer incubation periods were required. The effect appears to be specific to these compounds, as no increase was seen after incubation with other amino acids or with other metabolic intermediates (Fig. 2).

Glutamate and aspartate both function as excitatory neurotransmitters (Watkins and Evans, 1981), and both are avidly taken up by astrocytes (Schousboe et al., 1977; Hertz, 1982). Although several neuromodulators are known to affect glial glycogen metabolism (Coopersmith and Leon, 1987; Cambray-Deakin et al., 1988; Magistretti, 1988), the data shown in Fig. 4 suggest that the effects of glutamate and aspartate result from uptake rather than receptor activation. Once taken up, aspartate and glutamate may be interconverted through the action of glutamate–oxaloacetate transaminase and subsequently metabolized by common pathways (Hertz, 1982; Yu et al., 1982).

The effects of incubation time and initial glutamate concentrations in the media are shown in Fig. 3. It is probable that the added glutamate was substantially cleared from the medium over the 4-h incubation period; at a $V_{max}$ of 10.4 nmol/min/mg of protein (Yu et al., 1986), 0.6 mg of protein from astrocytes in 3 ml of medium would reduce the glutamate concentration by up to 0.125 m*M*/h. Thus, the lack of effect seen at 4 h using initial glutamate concentrations of <0.1 m*M* may reflect normalization of media and astrocyte glutamate levels during the incubation, rather than an actual dose–response effect.

One possible route by which rapid glutamate metabolism could elevate glycogen levels is via incorporation of glutamate into glycogen through gluconeogenesis. Although gluconeogenesis is generally thought to have a negligible role in brain metabolism, the formation of glycogen from glutamate, lactate, and other nonglucose precursors has been reported in brain slice preparations (Ide et al., 1969; Phillips and Coxon, 1975), and increased gluconeogenesis has been proposed as a mechanism for the astrocyte glycogen accumulation induced by MSO (Hevor et al., 1985). To assess the relative incorporation of glutamate and glu-

**TABLE 2.** *Effects of glutamate and MSO on astrocyte glucose utilization*

| | 2DG recovered in cells (dpm of $^3$H/μg of protein) |
|---|---|
| Control | 98.4 ± 4.6 |
| Glutamate (1 m*M*) | 46.3 ± 4.47[a] |
| MSO (1 m*M*) | 56.8 ± 5.87[a] |

Data are mean ± SEM values from one of two experiments with similar results (n = 4 for each condition). Cultures were preincubated with either 1 m*M* glutamate for 90 min or 2 m*M* MSO for 24 h. The specific activities of media recovered from the first and third washes were <3 and <0.04%, respectively, that of the incubation media.

[a] $p < 0.001$ versus the control by Student's *t* test.

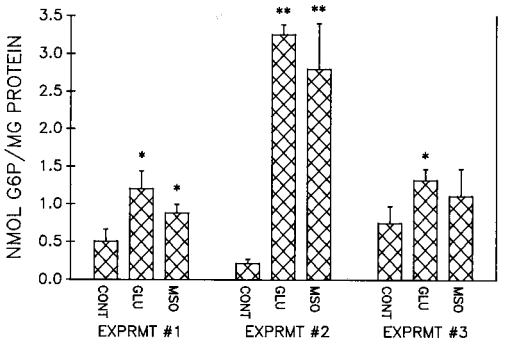


**FIG. 5.** G6P content of cultures incubated with 1 m*M* glutamate (4 h) or MSO (24 h). Data are mean ± SEM (bars) values (n = 4 for each condition). Significant differences by Student's *t* test from the control (CONT) are indicated: $*p < 0.05$, $**p < 0.001$.

cose into glycogen in the present study, astrocytes were incubated with [U-$^{14}$C]glutamate plus [3-$^{3}$H]glucose. The label at C3 for glucose was chosen to minimize labeling of substances other than glycogen, as >95% of the $^{3}$H on [3-$^{3}$H]glucose is lost during glycolysis (Katz and Rognstad, 1976).

Addition of 1 m*M* unlabeled glutamate to the medium caused an increase in glycogen content as well as an increase in $^{3}$H incorporation into glycogen (Table 1). These results indicate increases in exogenous glucose incorporation into glycogen, but the relative changes in glycogen synthesis and utilization rates cannot be assessed without knowledge of the patterns of glycogen metabolism. A "last on, first off" pattern, for example, would be kinetically quite different than a random synthesis and degradation pattern (Youn and Bergman, 1987).

In contrast to the [$^{3}$H]glucose labeling of glycogen, $^{14}$C incorporation was essentially undetectable either with or without 1 m*M* unlabeled glutamate (Table 1). Although the intracellular specific activities of glucose and glutamate are not strictly comparable, it is clear that the >65% increase in glycogen content of cell cultures incubated with glutamate cannot be accounted for by a flux of glutamate carbon into glycogen. In addition, the very low $^{14}$C dpm recovered in glycogen implies a negligible rate of gluconeogenesis in these cells, as glutamate carbon is known to enter the Krebs cycle rapidly (Yu et al., 1982).

To the extent that astrocyte glycogen acts as a local glucose reserve, glycogen levels would be expected to fall during periods of increased astrocyte glucose utilization and rise (within limits) during periods of decreased glucose demand. During rapid uptake and metabolism of glutamate, the requirement for glucose may be decreased as ATP and Krebs cycle intermediates are generated from glutamate. This possibility was examined with a modification of the 2DG method of Sokoloff et al. (1977). The experimental design was similar to that of Newman et al. (1988), in which a "square wave" pulse of label is delivered. Washes with unlabeled medium allow egress of unphosphorylated [$^{3}$H]2DG from the cells, such that the amount of retained $^{3}$H provides a relative index of the glucose utilization rates. As seen in Table 2, incubation with either 1 m*M* glutamate or 1 m*M* MSO caused significant reductions in astrocyte glucose utilization.

An alternative method for assessing glucose utilization in vitro is by measurement of $CO_2$ production from [$^{14}$C]glucose. The 2DG method was used for the present experiments because a substantial fraction of the glucose utilized by astrocytes is metabolized to lactate and other products other than $CO_2$ (Hertz et al., 1988).

MSO is known to have numerous effects on metabolism, some of which may represent secondary or compensatory changes (Van den Berg and Van den Velden, 1970; Sellinger et al., 1984; Hevor et al., 1985; Ratnakumari et al., 1985). It is noteworthy, however, that MSO-induced glycogen accumulation is seen only in astrocytes and not in other neural tissue or in liver (Hevor et al., 1985). As brain glutamine synthetase is restricted primarily to astrocytes, these observations suggest that the effect of MSO on astrocyte glycogen content may be directly related to the inhibition of this enzyme. The reported rate of glutamine synthetase activity in cultured astrocytes, ~13 nmol/min/mg of protein (Yudkoff et al., 1988), is not far removed from the observed rates of glucose utilization, 18–27 nmol/min/mg of protein (Hertz et al., 1988; Swanson et al., 1989). The nearly complete inhibition of glial glutamine synthetase by MSO (Rao and Meister, 1972) prevents the normal flow of glutamate into glutamine (Benjamin and Quastel, 1975; Yudkoff et al., 1988) and may thereby result in a significantly increased flux of endogenous glutamate into the Krebs cycle. This result could explain the observed parallel effects of glutamate and MSO on astrocyte glycogen and glucose metabolism.

Glutamate may enter the Krebs cycle through the action of glutamate dehydrogenase, and aspartate may enter through the action of aspartate aminotransferase or via glutamate through the glutamate–oxaloacetate transaminase reaction. The available evidence suggests that the activities of these enzymes are sufficient to allow glutamate and aspartate to replace glucose as substrates for astrocyte energy metabolism (Hertz, 1982; Yu et al., 1982; Hertz et al., 1988). The extremely rapid uptake of aspartate and glutamate by astrocytes (Hertz, 1982; Yu et al., 1986) may explain why effects on glycogen content were seen with these compounds but not with other amino acids, pyruvate, lactate, or $\alpha$-ketoglutarate (Fig. 2).

Glutamate itself is not known to regulate directly either glycolysis or glycogen metabolism. However, assuming constant metabolic demands, the entry of glutamate-derived $\alpha$-ketoglutarate into the Krebs cycle would be expected to result in a proportionate decrease in the entry of glucose-derived pyruvate. The major regulatory enzyme of glycolysis, phosphofructokinase, may be inhibited as a result of the anaplerotic reactions

of glutamate and aspartate, e.g., by the resulting production of citrate or ATP (Lowry and Passonneau, 1966). Inhibition of phosphofructokinase would, in turn, elevate G6P levels with resulting regulatory effects on both glycogen metabolism and glucose utilization (McIlwain and Bachelard, 1985).

The G6P levels measured in three batches of astrocytes incubated with glutamate and MSO are shown in Fig. 5. Although absolute G6P concentrations varied considerably among the batches, relative increases in G6P content were found in the cultures incubated with glutamate or MSO. The increased G6P levels may be the direct cause of the increased glycogen accumulation and decreased glucose utilization observed under these conditions.

Preliminary studies in our laboratory have shown that some glutamate analogues cause a striking *decrease* in glycogen content and an increase in glucose utilization. These analogues include L-$\alpha$-adipate, L-homocysteate, and D-glutamate, all compounds known to be taken up rapidly by astrocytes. Whether these compounds interfere with normal L-glutamate metabolism or affect G6P levels has not been determined.

In summary, an increase in astrocyte glycogen content and a decrease in glucose utilization were observed after incubation with 1 m*M* L-glutamate or MSO. Although the glycogen accumulation was blocked by preventing glutamate uptake, the increased glycogen was not derived from glutamate carbon but rather from exogenous glucose. Elevated G6P levels were found in the glutamate- and MSO-treated cells, a result suggesting a possible mechanism by which these agents may influence glucose and glycogen metabolism.

Although the glutamate concentrations used in this study were higher than are likely to occur in vivo, several inferences can be drawn from these findings. First, astrocytes may preferentially metabolize glutamate in place of glucose. This conclusion was also suggested by the work of Hertz et al. (1988), by comparison of the relative rates of oxidation of the two substrates. During periods of rapid neuronal release of glutamate, this substitution could serve two functions: It could facilitate clearance of glutamate from the extracellular space, and it could spare the available extracellular glucose for neuronal metabolism.

Astrocyte glycogen accumulation occurs in vivo during anesthesia, hibernation, and after neuronal injury. Conversely, brain glycogen content falls during seizures (for review, see Ibrahim, 1975). The inverse correlation found here between astrocyte glycogen content and astrocyte glucose utilization rate supports the tenet that glycogen content in vivo may reflect astrocyte metabolic demand. A coupling of glial energy utilization to neuronal activity has been suggested by other studies (Ibrahim, 1975; Swanson et al., 1987), and the responsiveness of glial glycogen metabolism to various neuromodulators provides a means of local coupling between neuronal activity and glial glycogen metabolism (Magistretti, 1988).

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