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Decreased Glutamate Metabolism in Cultured Astrocytes in the Presence of Thiopental

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ABSTRACT. The effect of thiopental on glutamate metabolism was studied by ^{13}C magnetic resonance spectroscopy. Cerebral cortical astrocytes were incubated with 0.5 mM [U-^{13}C]glutamate for 2 hr in the presence of 0.5 or 1 mM thiopental. Labeled glutamate, glutamine, aspartate, and glutathione were observed in cell extracts, and glutamine, aspartate, and lactate in the medium. Not only present in the medium was uniformly labeled glutamate, but also glutamate derived from the tricarboxylic acid (TCA) cycle, and thus glutamate release could be detected. The amounts of [U-^{13}C]glutamate and unlabeled glucose taken up by astrocytes were unchanged in the presence of 0.5 mM thiopental and decreased to about 50% and 80%, respectively when the concentration was increased to 1 mM. The amounts of most metabolites synthesized from [U-^{13}C]glutamate were unchanged in the presence of 0.5 mM thiopental, but decreased [U-^{13}C]glutamine, [U-^{13}C]aspartate, and [U-^{13}C]lactate were observed in the 1 mM group. Surprisingly, the amounts of [1,2,3-^{13}C]glutamate, [2,3-^{13}C]aspartate, and [3,4-^{13}C]aspartate (2nd turn via the TCA cycle) were unchanged. However, this was not the case for [1,2-^{13}C]lactate and [2,3-^{13}C]lactate. Such variations indicate cellular compartmentation, possibly caused by a heterogeneous glutamate concentration within the cells affecting TCA cycle turnover rates differently. BIOCHEM PHARMACOL **58**;6:1075–1080, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. astrocytes; thiopental; glutamate metabolism; MR spectroscopy; compartmentation

General anesthetics are central nervous system depressants and may act by binding to only a small number of targets in the central nervous system [1]. Barbiturates, intravenously injected anesthetics, have been shown to depress cerebral respiration and decrease the cerebral utilization of glucose in glycolytic pathways [2]. Interaction with the $GABA_A$** receptors at the barbiturate binding site can prolong the opening of the Cl^- channel (for review see [3]). Barbiturates have also been shown to inhibit the net uptake of potassium into cultured astrocytes [4], reduce the fractional sodium channel open-time in a voltage independent manner [5], and interact with glutamate receptors such as kainate and quisqualate receptors on cultured cortical neurons [6]. The free aqueous EC_{50} concentrations of pentobarbital and thiopental for general anesthesia were calculated to be 50 and 25 μM [1]. It is, however, difficult to relate these to clinically valid cerebral concentrations. Pentobarbital concentrations of 0.25 to 1 mM were used in previous studies on cultured astrocytes in the absence of GABA [7–9]. The present study was conducted using 0.5 and 1 mM thiopental. It should be noted that high extracellular concentrations of glutamate can be neurotoxic. The effects of extracellular glutamate are terminated by uptake into neurons or astrocytes, where astrocytes are responsible for a major part of glutamate uptake in the brain [10]. Specific transporters on neuronal and astrocytic membranes for removal of extracellular glutamate have been identified [11–13]. One of these, GLAST, was shown to be the predominant transporter in cultured astrocytes [14, 15].

The effects of barbiturates on metabolism have been studied extensively in human subjects and in laboratory animals. Radioactive labeled compounds such as [U-^{14}C]glucose and [U-^{14}C]glutamate have been used in rat brain [2] and cultured astrocytes [7], respectively. ^{13}C MRS is a unique tool for studying [U-^{13}C]glutamate metabolism in astrocytes, since quantification of isotopomers gives detailed information about metabolic pathways and compartmentation. Thus, cells were incubated with [U-^{13}C]glutamate in the presence or absence of thiopental in the present study. The labeling patterns in compounds present in the incubation media as well as ethanol extracts of the cells were subsequently determined. Since glutamate is uniformly labeled, detection of incorporation of label into metabolites is unambiguous due to ^{13}C-^{13}C spin-spin coupling patterns. Thus, the role of thiopental in the utilization and metabolism of [U-^{13}C]glutamate and glucose in astrocytes could be analyzed.

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** *Abbreviations:* GABA, γ-aminobyturate acid; FBS, fetal bovine serum; and TCA, tricarboxylic acid.

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MATERIALS AND METHODS

Materials

Plastic tissue culture dishes were purchased from Nunc A/S, FBS from Seralab Ltd., and culture medium from GIBCO BRL, Life Technologies. NMRI mice were purchased from Møllegaard Breeding Center. [U-^{13}C]glutamate (99% enriched) and 99.9% D_2O (deuterium oxide) were from Cambridge Isotopes Laboratories, sodium thiopental from Abbott, and ethyleneglycol from Merck. All other chemicals were of the purest grade available from regular commercial sources.

Cell Cultures

All animal procedures were conducted according to national regulation. Cerebral cortical astrocytes were cultured as described earlier [16]. Briefly, prefrontal cortex was taken from newborn mice and passed through Nitex nylon netting (80-μm pore size) into Dulbecco's minimum essential medium (DMEM) containing 20% (v/v) FBS. Culture dishes 15 cm in diameter were used. The medium was changed two days after plating and subsequently twice a week, gradually changed to 10% FBS. Experiments were performed on two- to three-week-old cultures. The medium was removed and replaced by DMEM without glutamine and FBS, containing 0.5 mM [U-^{13}C]glutamate and 3 mM glucose. Sodium thiopental was added to the medium of some cultures to a final concentration of 0.5 or 1 mM. After 2 hr, the medium was removed and cells were washed with 0.9% saline and extracted with 70% ethanol (v/v), followed by centrifugation at 4000 *g* for 10 min. The supernatants and media were lyophilized and stored at −20°. Cellular protein in the ethanol pellets was determined after dissolving in 1 M KOH at 37° for 30 min, using the Pierce BCA (bicinchoninic acid) protein assay with BSA as standard.

MR Spectroscopy

Proton-decoupled 125.5 MHz ^{13}C MR spectra were obtained on a Bruker DRX-500 spectrometer. Samples were redissolved in D_2O containing 0.15% ethyleneglycol as an internal standard. Spectra were accumulated using a 35° pulse angle, 25 kHz spectral width with 64 K data points. The acquisition time was 1.307 sec, and a 2.5-sec relaxation delay was used. The number of scans was typically 2000 for medium and 8000 for cell extract. Some spectra were also broad-band-decoupled only during acquisition to avoid nuclear Overhauser effects (NOE). From several sets of spectra, factors for the NOE of different atoms were obtained and applied to all spectra.

Data Analysis

Relevant peaks from glutamate, glutamine, aspartate, lactate, and glutathione in MR spectra were integrated, and the amounts were quantified from the integrals of the peak areas, using ethyleneglycol as an internal standard. Results are presented as means ± SEM. Differences between groups were analyzed statistically with one-way ANOVA followed by post hoc test, and $P < 0.05$ was considered as significant. Since glucose C-1 and lactate C-2 singlets in the spectra could not be derived from [U-^{13}C]glutamate, they represent the 1.1% natural abundant ^{13}C. The total amount of glucose and lactate was calculated using the amount of singlet divided by 1.1%. The amount of [U-^{13}C]glutamate and glucose removed from the medium by astrocytes during the incubation time was calculated as: the amount added to the medium minus the amount left in the medium divided by the amount of protein. The distribution of [U-^{13}C]glutamate into different pathways was calculated as follows: '% directly to glutamine': [U-^{13}C]glutamine divided by [U-^{13}C]glutamate removed from the medium as percent; '% intracellular [U-^{13}C]glutamate': intracellular [U-^{13}C]glutamate divided by [U-^{13}]glutamate removed from the medium as percent; '% via TCA cycle': sum of all the labels except [U-^{13}C]glutamine synthesized from [U-^{13}C]glutamate in media and cell extracts divided by [U-^{13}C]glutamate removed from the medium as percent; '% other pathways': [U-^{13}C]glutamate removed from the medium minus the sum of all the labels synthesized from [U-^{13}C]glutamate in media and cell extracts divided by [U-^{13}C]glutamate removed from the medium as percent. This last term was calculated indirectly given that the ^{13}C label from [U-^{13}C]glutamate not observed in the spectra is consumed for other pathways, including energy production in the astrocytes.

RESULTS

Typical spectra from cultured cortical astrocytes after incubation with [U-^{13}C]glutamate in the presence of thiopental are shown in Fig. 1 (cell extract, bottom; cell culture medium, top). As seen from the spectra, glutamate was metabolized in cultured astrocytes to a great extent. Labeled glutamine, aspartate, and glutathione synthesized from [U-^{13}C]glutamate are clearly seen in the spectrum from cell extract, whereas in the spectrum of medium, in addition to the added [U-^{13}C]glutamate, labeled glutamine, aspartate, and lactate are also observed. The schematic presentation of the distribution of label in different metabolites from the TCA cycle is shown in Fig. 2. After uptake by astrocytes, [U-^{13}C]glutamate can either be converted to [U-^{13}C]glutamine directly by glutamine synthetase (E.C. 6.3.1.2), or enter the TCA cycle after conversion to α-ketoglutarate for energy production and/or the synthesis of other metabolites. [U-^{13}C]oxaloacetate is formed after several steps, and [U-^{13}C]aspartate can be synthesized thereafter. [U-^{13}C]lactate can be derived from [U-^{13}C]oxaloacetate and [U-^{13}C]malate. In the presence of unlabeled glucose, unlabeled pyruvate can be converted to acetyl-coenzyme A. The condensation of labeled oxaloacetate and unlabeled acetyl-coenzyme A leads to the synthesis of [1,2,3-^{13}C]glutamate and [1,2,3-^{13}C]glutamine via TCA cycle interme-

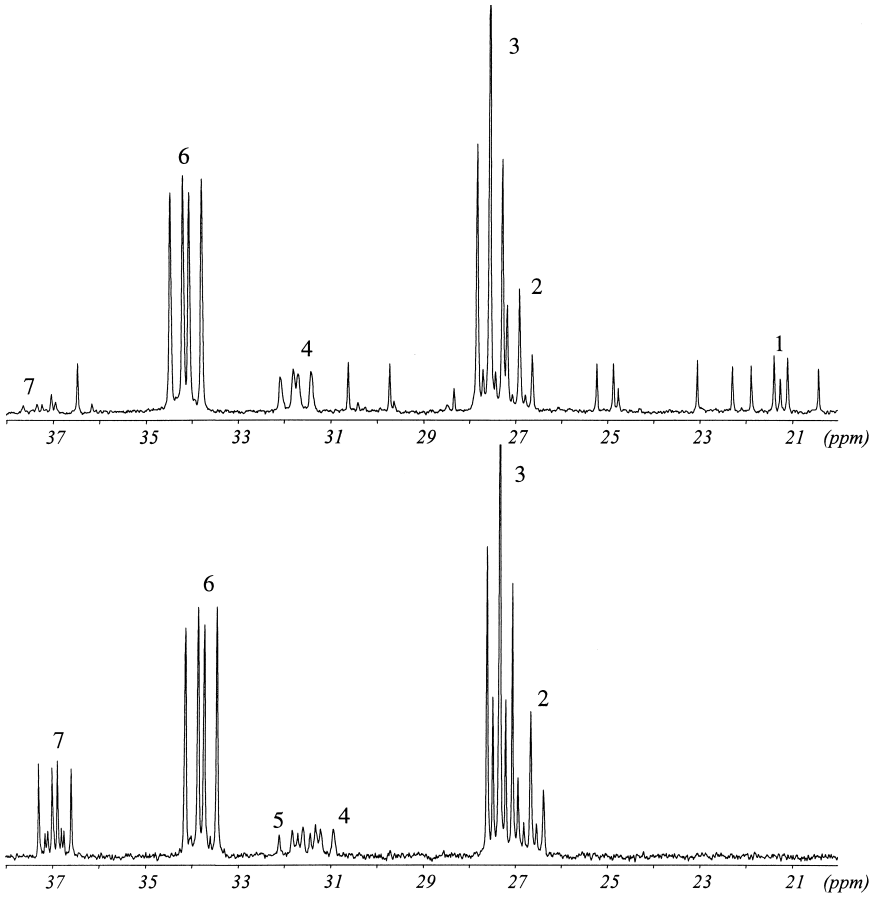

FIG. 1. ^{13}C MR spectra from cultures of cerebral cortical astrocytes. Cell cultures were incubated for 2 hr with [U-^{13}C]glutamate in the presence of 1 mM thiopental. Medium (top), cell extract (bottom). Peak assignment: 1, lactate C-3; 2, glutamine C-3, 3, glutamate C-3; 4, glutamine C-4; 5, glutathione; 6, glutamate C-4; 7, aspartate C-3.

diates. If the label stays in the TCA cycle for another turn, [1,2-^{13}C]-/[3,4-^{13}C]aspartate and [1,2-^{13}C]-/[3-^{13}C]lactate can be formed. [2,3-^{13}C]lactate and [2,3-^{13}C]aspartate can only be formed via pyruvate recycling (for detailed description see Håberg *et al.* [17]). By analyzing the different labeling patterns, [U-^{13}C]glutamine synthesized directly from [U-^{13}C]glutamate can be distinguished from [1,2,3-^{13}C]glutamine formed via the TCA cycle. The former has two ^{13}C atoms as neighbors at the C-3 position and was observed as two doublets. These doublets appeared as triplets due to the equal coupling constants ($J_{2,3} = J_{3,4} =$ 34.5 Hz). The latter, however, has only one ^{13}C atom as neighbor and was observed as a doublet. The same applies to the labeling pattern at the C-3 position in glutamate. At the C-2 position (not shown in Fig. 1), lactate synthesized via the TCA cycle from [U-^{13}C]glutamate can be distinguished from lactate formed from glycolysis: the former was seen as two doublets due to two ^{13}C neighbors, whereas the latter was a singlet from the 1.1% natural abundant ^{13}C. Thus, by quantification of various metabolites and their isotopomers from both cell extracts and media, [U-^{13}C]glutamate metabolism in astrocytes was studied in detail.

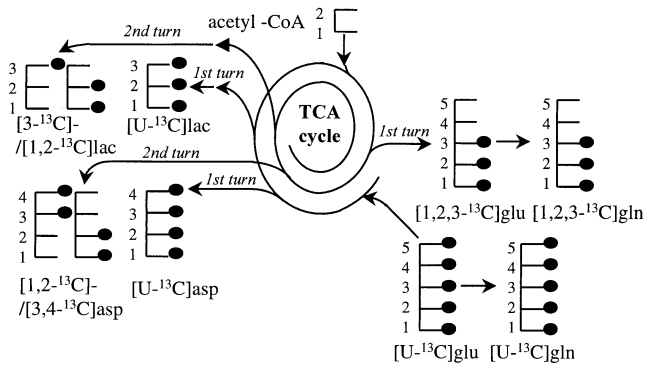

FIG. 2. Schematic representation of possible isotopomers arising from [U-^{13}C]glutamate in astrocytes. ● represents ^{13}C; asp, aspartate; glu, glutamate; gln, glutamine; lac, lactate.

In the medium, the amount of [U-^{13}C]glutamate removed was unchanged in the 0.5-mM thiopental group, but decreased to less than 50% in the 1-mM group compared to control (Table 1). The amount of glutamine, aspartate, and lactate released from astrocytes was unchanged in the 0.5-mM thiopental group, and decreased when 1 mM thiopental was used. The amount of glutamate released, after synthesis via the TCA cycle, was similar in all groups. The amount of [U-^{13}C]glutamine and [1,2,3-^{13}C]glutamine in the medium was decreased slightly in the presence of 0.5 mM thiopental. While the amount of [U-^{13}C]glutamine, directly from [U-^{13}C]glutamate, was decreased further in the presence of 1 mM thiopental, [1,2,3-^{13}C]glutamine derived from the TCA cycle was maintained at the same level as in the 0.5-mM thiopental group. As unlabeled glucose was present in the medium, lactate derived from

TABLE 1. Content of ^{13}C (nmol/mg protein) in metabolites from lyophilized cell media of cortical astrocytes after incubation with [U-^{13}C]glutamate under various conditions

	Control (N = 6)	0.5 mM thiopental (N = 6)	1 mM thiopental (N = 4)
Glucose*†	8027.9 ± 399.5	7310.1 ± 428.0	6496.1 ± 276.2‡
Lactate†	3888.8 ± 330.7	3541.8 ± 405.5	2163.6 ± 124.7‡§
[U-^{13}C]lactate	177.8 ± 19.7	136.2 ± 17.8	78.0 ± 3.6‡§
[1,2-^{13}C]lactate	14.0 ± 2.0	12.1 ± 2.0	6.0 ± 0.3‡
[2,3-^{13}C]lactate	17.4 ± 2.0	13.2 ± 2.2	5.5 ± 0.8‡§
[U-^{13}C]aspartate	31.0 ± 3.6	26.6 ± 2.4	15.6 ± 1.3‡§
[U-^{13}C]glutamate*	2271.9 ± 71.8	2123.5 ± 40.9	1027.5 ± 32.0‡§
[1,2,3-^{13}C]glutamate	79.0 ± 9.3	78.1 ± 10.8	64.0 ± 2.8
[U-^{13}C]glutamine	307.7 ± 11.8	272.7 ± 8.0‡	196.2 ± 6.2‡§
[1,2,3-^{13}C]glutamine	45.6 ± 1.5	38.6 ± 1.7‡	35.8 ± 1.4‡

All cultures were incubated with [U-^{13}C]glutamate (0.5 mM) and 0, 0.5, or 1 mM sodium thiopental for 2 hr as described in Materials and Methods. The C-3 resonance was used for ^{13}C MR determination except for lactate and glucose, where the C-2 and C-1 resonances were used, respectively. Superscripts indicate statistical differences as determined by ANOVA followed by post hoc test for multiple comparisons ($P < 0.05$ was considered significant).

* The amount removed from the medium by astrocytes.

†The total concentration is obtained by the singlet concentration divided by 1.1%.

‡Significantly different from controls.

§Significantly different from 0.5-mM thiopental group.

glycolysis could also be observed. Compared to the control group, the amount of glucose removed from the medium and lactate synthesized from glucose was unchanged in the presence of 0.5 mM thiopental, but decreased when the concentration was increased to 1 mM. The ratio of lactate to glucose was, however, unchanged in all groups.

In the cell extracts, the amount of aspartate, glutamine, glutamate, and glutathione was unchanged in the presence of 0.5 mM thiopental and decreased in the 1-mM group (Table 2). The amount of [1,2,3-^{13}C]glutamate from the TCA cycle and glutamine directly from [U-^{13}C]glutamate showed a slight increase in the 0.5-mM thiopental group, but was the same as control when the concentration of thiopental was doubled. Glutamine and aspartate synthesized via the TCA cycle, i.e. [1,2,3-^{13}C]glutamine and [3,4-^{13}C]aspartate in Table 2, were unchanged under all conditions.

Since the amount of glutamate removed from the medium was decreased more than 50% in the 1-mM thiopental group, less label was available for astrocytic metabolism. To evaluate relative changes in metabolic pathway preference for [U-^{13}C]glutamate, the effects of thiopental on the distribution of glutamate to the different pathways are presented as percent of the total amount of glutamate removed from the medium in Table 3. In the control and 0.5-mM thiopental groups, about 15% of glutamate was converted directly into glutamine, and 19% was consumed for the synthesis of other metabolites. However, in the 1-mM group, more was used for the synthesis of glutamine directly (23.8%) and metabolites via the TCA cycle (30%), but less was consumed for other processes including energy production (Table 3). The ratio between conversion directly into glutamine and other metabolites via the TCA cycle was, however, still unchanged in both groups.

TABLE 2. Content of ^{13}C (nmol/mg protein) in metabolites from lyophilized cell extracts of cortical astrocytes after incubation with [U-^{13}C]glutamate under various conditions

	Control (N = 6)	0.5 mM thiopental (N = 6)	1 mM thiopental (N = 4)
[U-^{13}C]aspartate	52.8 ± 5.5	46.9 ± 4.2	30.2 ± 1.0*†
[2,3-^{13}C]aspartate	4.8 ± 0.4	4.2 ± 0.3	3.2 ± 0.2*
[3,4-^{13}C]aspartate	5.0 ± 0.4	5.3 ± 0.4	4.6 ± 0.3
glutathione	30.4 ± 4.3	29.5 ± 2.7	18.2 ± 0.3*†
[U-^{13}C]glutamate	188.2 ± 15.6	213.3 ± 12.3	144.4 ± 3.4*†
[1,2,3-^{13}C]glutamate	37.7 ± 2.7	45.5 ± 1.9*	39.9 ± 0.8
[U-^{13}C]glutamine	48.9 ± 4.1	59.9 ± 2.7*	47.7 ± 1.3†
[1,2,3-^{13}C]glutamine	8.7 ± 1.0	10.1 ± 0.8	10.1 ± 0.4

All cultures were incubated with [U-^{13}C]glutamate (0.5 mM) and 0, 0.5, or 1 mM sodium thiopental for 2 hr as described in Materials and Methods. The C-3 resonance was used for ^{13}C MR determination except for glutathione, where the C-4 glutamate resonance was used. Superscripts indicate statistical differences as determined by ANOVA followed by post hoc test for multiple comparisons ($P < 0.05$ was considered significant).

*Significantly different from controls.

†Significantly different from 0.5-mM thiopental group.

TABLE 3. Metabolism of [U-^{13}C]glutamate in cultured astrocytes

	Control (N = 6)	0.5 mM thiopental (N = 6)	1 mM thiopental (N = 4)
% directly to glutamine	15.7 ± 0.6	15.3 ± 0.4	23.8 ± 0.6*†
% intracellular [U-^{13}C]glutamate	8.2 ± 0.5	9.8 ± 0.6*	14.1 ± 0.2*†
% via TCA cycle	21.9 ± 1.0	19.2 ± 1.5	30.3 ± 0.7*†
% other pathways	54.2 ± 1.7	54.5 ± 1.8	31.8 ± 1.5*†

See Materials and Methods for description of calculation. Superscripts indicate statistical differences as determined by ANOVA followed by post hoc test for multiple comparisons as follows ($P < 0.05$ was considered significant).

*Significantly different from controls.

†Significantly different from 0.5-mM thiopental group.

DISCUSSION

Effect of Thiopental on Glucose

Unlabeled glucose (3 mM) was present in the medium under all experimental conditions, and the amount of glucose and lactate in the medium could be quantified due to natural abundance of ^{13}C (1.1%). Thus, information concerning the effects of thiopental on glucose metabolism was obtained. The amount of glucose removed from the medium and lactate synthesized from glucose was unchanged with 0.5 mM thiopental, but decreased more than 50% in the 1-mM group. This agrees with results reported by Strang *et al.*, where glucose uptake and lactate synthesis were also decreased more than 50% in rat brain by phenobarbitone (250 mg/kg body weight) [18]. Taken together, this might indicate that the metabolic effects of barbiturates occur predominantly on astrocytes. These results could be due to decreased glucose uptake, decreased glucose consumption, and/or decreased conversion of pyruvate to lactate. Since the ratio of lactate to glucose was unchanged between groups, inhibition may take place at a stage common to these pathways, as discussed in detail by Strang *et al.* [18]. The amount of glucose inside the cells was decreased in the 1-mM group, and decreased energy production would thus be expected. This was indeed the case in cultured astrocytes, where carbon dioxide formation in the presence of 0.5 mM pentobarbital was unchanged from [U-^{14}C]glucose [7]. Furthermore, at an increased concentration (1 mM pentobarbital), oxygen consumption was unchanged, but decreased $^{14}CO_2$ production was observed under similar experimental conditions [8].

Effect of Thiopental on Glutamate

GLUTAMATE UPTAKE. Extracellular glutamate concentration is tightly regulated and coupled to neuronal excitation. Generally, extracellular glutamate concentration in the brain is very low (50 μM), but might rise to mM levels in the vicinity of glutamatergic neurons during neuronal activation and pathological conditions. In the present study, the amount of glutamate removed from the medium was unchanged during incubation with 0.5 mM thiopental, in agreement with the study of Miyazaki *et al.*, where unchanged glutamate uptake with pentobarbital concentrations ranging from 0.03 to 0.3 μM was observed [9]. However, with 1 mM thiopental, glutamate uptake was decreased more than 50%. It is well known that barbiturates can affect cell membrane protein, such as $GABA_A$ receptors (for reviews see [3]), and thus could eventually interfere with intracellular ion homeostasis [4] and glutamate metabolism and therefore decrease sodium-dependent glutamate uptake. It should be noted that this reduction in glutamate uptake might exert a protective effect under conditions where the glutamate transporters are reversed, such as during ischemia. Decreased glutamate release was observed after ischemia in the presence of thiopental in gerbils [19], which is in agreement with this hypothesis. That thiopental indeed exerts protective effects has been shown by reduced clinical expression of cerebral emboli in cardiopulmonary bypass patients [20] and improved energy metabolism during ischemia in gerbils [21].

GLUTAMATE METABOLISM AND COMPARTMENTATION. After entering the cells, glutamate can be converted directly into glutamine or peptides such as glutathione or be metabolized through the TCA cycle for energy production and synthesis of various metabolites (for review see [22]). The amount of most metabolites synthesized from [U-^{13}C]glutamate was unchanged in the presence of 0.5 mM thiopental, but decreased [U-^{13}C]glutamine and [U-^{13}C]aspartate were observed in the 1-mM group which might be due to a decreased [U-^{13}C]glutamate uptake. Surprisingly, the amount of [1,2,3-^{13}C]glutamate, [2,3-^{13}C]aspartate and [3,4-^{13}C]aspartate (2nd turn via the TCA cycle) was unchanged, which indicates that the metabolic pool which these amino acids are derived from is not affected by the decreased amount of ^{13}C label and is different from the pool where [U-^{13}C]aspartate (1st turn via the TCA cycle) is formed. Compared to the control, the percent of glutamate removed from the medium converted directly into glutamine and via the TCA cycle was increased in the presence of 1 mM thiopental. This might indicate that other metabolic pathways for glutamate, which might be active at a high glutamate concentration such as peptide synthesis, are decreased or abolished when the amount of glutamate is lowered. Like aspartate and glutamine, the synthesis of lactate from glutamate via the TCA cycle (both the first turn and the second turn) was also decreased in the 1-mM group. In contrast to aspartate, the ratio of lactate released to glutamate removed from the medium was unchanged. These results might be explained by metabolic compartmentation, with a heterogeneous glutamate concentration within the cells affecting turnover rates differently in different areas. Mitochondrial compartmentation has previously been suggested (for review, see [23]). The mitochondria close to the membrane might have an easier access to exogenous glutamate and could be affected when the glutamate concentration is changed, whereas mitochondria farther away might have a relatively constant supply of glutamate and could maintain unchanged turnover. It was indeed shown in an early study that glutamate metabolism is tightly coupled to glutamate concentration: The synthesis of labeled aspartate and lactate only occurred at a [U-^{13}C]glutamate concentration above 0.2 mM [24].

RELEASE. As stated above, glutamine, aspartate, and lactate can be synthesized from glutamate in astrocytes. These metabolites have further been shown to be released into the extracellular space [25], and such release is crucial to maintaining the normal level of neuronal TCA cycle intermediates, since neurons lack the main anaplerotic enzyme, pyruvate carboxylase (E.C. 6.4.1.1), which is located in astrocytes [26, 27]. The release of aspartate and lactate was unchanged in the 0.5-mM thiopental group, but decreased in the 1-mM group. This decrease might be explained by the fact that less glutamate was taken up and thus less label was available. Surprisingly, the release of [1,2,3-^{13}C]glutamate was unchanged in either group, also

reflected in the unchanged intracellular concentration, whereas the release of glutamine, synthesized both directly from glutamate and via the TCA cycle, was decreased in the 0.5-mM and 1-mM groups. Together with an unchanged amount of intracellular glutamine (increased [U-^{13}C]glutamine in 0.5 mM), this might indicate that the glutamine transporters are affected by thiopental and/or compartmentation, where not all glutamine is equally available for release. In the present study, the amount of glutamine released from astrocytes was decreased in the presence of thiopental. However, Yu *et al.* reported unchanged glutamine synthesis based on its concentration in the medium [7]. This discrepancy might be due to different calculation methods and experimental conditions, such as duration of incubation (15 min vs 2 hr) and glutamate concentration, which is important but was not reported.

In the presence of 1 mM thiopental, less [U-^{13}C]glutamate was removed from the medium by astrocytes, and therefore reduced energy production from glutamate should be expected. This was shown in the study by Yu *et al.*, where unchanged or less CO_2 was produced from [U-^{13}C]glutamate in cultured astrocytes in the presence of 0.5 or 1 mM pentobarbital [7].

The present study shows that the amount of glutamate removed from the medium by astrocytes is decreased in the presence of 1 mM thiopental but that the synthesis of amino acids is decreased to a smaller extent. The differential effects of thiopental on the synthesis of metabolites further support the notion of compartmentation inside astrocytes. As stated above, lower concentrations of thiopental might be sufficient in the presence of GABA, and further experiments will be necessary to explore the effects of thiopental on astrocytes under such conditions.

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