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ORIGINAL INVESTIGATION

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Thiopental and midazolam do not seem to impede metabolism of glutamate in brain-injured patients

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Abstract Increased extracellular glutamate levels are related to glial and neuronal damage. Glutamate-mediated toxicity is limited by glial uptake and metabolic transformation of glutamate to glutamine and the energetic compounds alanine and lactate which are utilized by surrounding neurons. Under in vitro conditions, barbiturates have been shown to reduce glutamate uptake and its further metabolism, possibly impeding metabolic coupling between astrocytes and neurons. The aims were to investigate if under clinical conditions, the barbiturate thiopental reduces important detoxification of glutamate, resulting in lower CSF glutamine, alanine and lactate levels as opposed to patients receiving midazolam. During long-term administration of thiopental and midazolam, pathologically elevated ventricular CSF glutamate levels were associated with significantly increased glutamine and alanine levels up to 14 days after trauma. CSF lactate, however, remained normal. These data suggest that long-term administration of thiopental and midazolam under clinical conditions does not impede enzymatic activities responsible for detoxification and metabolism of glutamate.

Key words Cerebrospinal fluid · Glutamate · Glutamine · Alanine · Lactate · Brain trauma · Midazolam · Thiopental

Introduction

Cerebral metabolic and energetic homeostasis is tightly regulated, as glial and neuronal cells possess various transporters and enzymes of intermediary metabolism (Erecinska and Silver 1990; Tsacopoulos and Magistretti 1996). Energetic stability is required to maintain synaptic activity and to prevent neuronal and glial damage due to uncontrolled release of the possibly excitotoxic transmitter glutamate due to reversal of neuronal transport (Madl and Burgesser 1993) and synaptosomal leakage (Dagani and Erecinska 1987). Glial and neuronal uptake mechanisms are coupled to energy-dependent pump processes which are fueled by ATP derived from glycolysis and mitochondrial oxidative phosphorylation. As transport of glucose via the blood-brain barrier is limited in conditions of acute energy demand (Hu and Wilson 1997), glutamate, lactate, and alanine have also been shown to serve as energetic substrates (Schousboe et al. 1997). Following compensatory glial uptake, glutamate is transformed to glutamine via glutamine synthetase, to alanine via alanine aminotransferase or to α-ketoglutarate, and lactate via the tricarboxylic cycle (Erecinska and Silver 1990) which, in turn, are released to the extracellular space for subsequent neuronal uptake (Dringen et al. 1993; Sonnewald et al. 1993; Westergaard et al. 1993).

Under physiological conditions, glutamate-mediated neuronal activation causes an increase in free lactate (Demestre et al. 1997; Hu and Wilson 1997) related to increased glial aerobic glycolysis (Pellerin and Magistretti 1994; Sibson et al. 1998) and metabolism via the tricarboxylic cycle (Sonnewald et al. 1993; McKenna et al. 1996). Following traumatic brain injury, glutamate is substantially increased within the extracellular space and linked to sustained increase in extracellular lactate concentration (Kawamata et al. 1995). To reduce posttraumatic excitotoxic injury, pharmacological protection is anticipated by applying drugs known to stabilize membranes and reduce brain activity (Biebuyck 1993). Routinely administered barbiturates, however, have been shown to attenuate glial uptake of glutamate (Balcar et al. 1978) and reduce energy and intermediary metabolism in cultured

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astrocytes (Yu et al. 1983; Hertz et al. 1986). Given these effects also to hold true in vivo, glial detoxification and metabolism of traumatically increased glutamate should be reduced. This, in turn, could result in low ventricular CSF glutamine, alanine, and lactate concentrations.

The aims were to investigate long-term changes in glutamate, glutamine, alanine, and lactate in ventricular CSF of severely brain-injured patients treated with the barbiturate thiopental as opposed to patients receiving the benzodiazepine midazolam eventually to unmask drug-induced impeded metabolism of glutamate.

Materials and methods

Patients

From 1994 to 1997, 28 patients (38 ± 7 years, 17–67 years) with severe traumatic brain injury were investigated. All patients were admitted within 12 h after trauma, had a GCS ≤ 9 upon arrival in the emergency room, and received intraventricular catheters to monitor intracranial pressure (ICP) and therapeutically drain CSF. Thereafter, the intubated and mechanically ventilated patients were treated according to a standard protocol (Stocker et al. 1995). They were given either midazolam, if ICP remained controllable (<20 mmHg) or thiopental, whenever ICP became uncontrollable, exceeding 20 mmHg. Midazolam and thiopental were administered continuously. Their dosage was tapered according to changes in ICP (midazolam: 0.2–0.7 mg/kg body weight/h) and in case of barbiturate coma according to the burst-suppression pattern allowing four to six bursts/minute as controlled by electroencephalographic recordings (thiopental: 4–6 mg/kg body weight per hour).

The study protocol was approved by the University Hospital Medical Ethics Board.

Study protocol

CSF samples were collected on days 1, 3, 5, 7, and 14 after trauma. Control lumbar CSF was taken from 20 control patients free of any neuropathology as described previously in detail (Stover et al. 1997).

Biochemical parameters

Amino acids (glutamate, glutamine, alanine, serine) were analyzed by high performance liquid chromatography (HPLC) with orthophthaldialdehyde (OPA) pre-column derivatisation as previously described in detail (Stover et al. 1997). CSF lactate was measured using an automatic lactate analyzer with immobilized L-lactate oxidase (YSI Incorporated; Yellow Springs, Ohio, USA).

Statistical analysis

Results of amino acids and lactate are given as means ± SEM. Neurochemical parameters in CSF were compared for significant differences within and between groups of brain injured patients under midazolam and thiopental using analysis of variances (ANOVA) Kruskal-Wallis and Dunn's tests or ranked-sum test for non-parametric data, where appropriate. Mutual dependencies between the different parameters were evaluated by linear regression analysis. Differences between the groups were rated significant at $P < 0.05$.

Results

CSF glutamate, glutamine, alanine, serine, and lactate (Table 1)

Ventricular CSF glutamate, glutamine, and alanine concentrations of 28 brain-injured patients were significantly increased compared to 20 lumbar control CSF samples at all investigated time points. Ventricular CSF lactate and serine levels, however, did not differ from control values.

Under thiopental, CSF glutamate was significantly decreased from 27 ± 4 (day 1) to 10 ± 2 μM (day 3), remaining at significantly lower values compared to patients receiving midazolam. Under midazolam, CSF glutamate increased steadily, reaching maximum values by day 7 (23 ± 2 μM), and returned to initial values by day 14 (16 ± 2 μM).

Under thiopental, CSF glutamine levels were significantly decreased from 754 ± 77 (day 1) to 546 ± 71 μM (day 3), parallel to the observed decrease in glutamate,

Table 1 CSF concentrations of glutamate, glutamine, alanine, serine, and lactate in severely brain-injured patients receiving either midazolam or thiopental. Differences within (#) and between the groups (§) are significant ($P < 0.05$)

	Controls	Day 1	Day 3	Day 5	Day 7	Day 14
Glutamate	1.3 ± 0.1					
Midazolam		16 ± 3	17 ± 6	19 ± 3	23 ± 2 #	16 ± 2
Thiopental		27 ± 4 §	10 ± 2 #§	12 ± 2 #§	12 ± 2 #§	11 ± 2 #
Glutamine	514 ± 25					
Midazolam		671 ± 92	665 ± 72	688 ± 71	747 ± 66 #§	677 ± 55
Thiopental		754 ± 77#	546 ± 71	589 ± 74	556 ± 68	598 ± 81
Alanine	18 ± 2					
Midazolam		62 ± 16	63 ± 12	59 ± 11	41 ± 8	46 ± 5
Thiopental		60 ± 12	66 ± 16	58 ± 10	53 ± 9	52 ± 10
Serine	50 ± 4					
Midazolam		50 ± 9	44 ± 7	51 ± 10	44 ± 7	47 ± 8
Thiopental		45 ± 8	43 ± 9	45 ± 7	49 ± 5	45 ± 6
Lactate	1.6 ± 0.2					
Midazolam		1.8 ± 0.2	1.8 ± 0.2	1.6 ± 0.2	1.7 ± 0.3	1.6 ± 0.2
Thiopental		1.6 ± 0.3	1.3 ± 0.3	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.3

followed by stable values thereafter. With midazolam, CSF glutamine increased parallel to the changes in glutamate, reaching maximal values by day 7 (747 ± 66 μM).

CSF alanine levels remained fairly stable during long-term administration of thiopental and midazolam at significantly increased values without any difference between the investigated two groups of brain-injured patients. CSF alanine did not show any parallel increases to glutamate as seen for glutamine.

Relationship between glutamate, glutamine, alanine, and lactate in CSF

Regression analysis of CSF glutamate, glutamine, alanine, and lactate only revealed a mutual dependency between glutamine and glutamate under thiopental and midazolam administration, suggesting that glutamine may be increased by glutamate in both groups (thiopental: $n = 60$; $r = 0.69$; midazolam: $n = 78$; $r = 0.71$; $P < 0.001$).

Discussion

Analysis of ventricular cerebrospinal fluid of severely brain-injured patients under the influence of long-term administration of thiopental and midazolam revealed significantly elevated CSF glutamate, glutamine, and alanine levels up to and including 14 days after trauma. These data are suggestive of preserved enzymatic transformation of increased glutamate to glutamine and alanine, which does not seem influenced by administered thiopental or midazolam.

Elevated amino acids within cerebral and spinal extracellular space will follow their gradients resulting in increased CSF levels (Shimada et al. 1993), whenever balancing uptake mechanisms fail. Therefore, amino acids as measured in CSF may serve to estimate ongoing cerebral activity and pathology.

Under physiological conditions only a minute fraction of glutamate which is stored intracellularly within the millimolar range is found within the extracellular space of the central nervous system, ranging from 1 to 3 μM (Storm-Mathisen et al. 1992). A tight energy-dependent regulation of this gradient is indispensable in preventing abnormal release of this excitotoxin. Energy depletion, in turn, is associated with pump failure and sustained excitotoxicity (Madl and Burgesser 1993). Despite the abundant presence of glucose transporters within the central nervous system (Vanucci et al. 1997), it has been demonstrated that glucose alone is not sufficient in supplying energy under conditions of increased neuronal activity (Hu and Wilson 1997). To prevent cellular perturbation due to inadequate supply of blood glucose metabolic coupling between glia and neurons (Tsacopoulos and Magistretti 1996) is able to furnish energetic compounds. For this, glial glycogen is degraded, resulting in increased endogenous glucose levels (Swanson 1992) and glutamate is transformed to α-ketoglutarate, alanine and lactate which, in turn, may fuel glial and neuronal energy consuming processes (Erecinska and Silver 1990).

The balancing clearance of elevated glutamate concentration from the extracellular space by glia is crucial in preventing evolving tissue damage (Rosenberg and Aizenman 1989), as the risk for continuous neuronal excitation is reduced and energy substrates are generated. Under in vivo conditions in non-traumatized animals, intracerebral application of glutamate is dose-dependently cleared from the extracellular space (Alexander et al. 1997) by active uptake mechanisms linked to increased lactate production (Demestre et al. 1997).

Astrocytes are more resistant to noxious influences than neurons, and a shift of glutamate accumulation from neurons to glia has been demonstrated under pathological conditions (Torp et al. 1991), which is attributed to preserved high-affinity uptake of glutamate by glia cells (Swanson 1992) and maintained enzymatic activity (Sher and Hu 1990). Brain injury has been shown to cause a rapid increase in production and activity of glutamine synthetase occurring in parallel to increased extracellular glutamate levels (Petito et al. 1992) and resulting in a sustained production of glutamine, as seen in the present study and as reported by others (Andiné et al. 1991; Palmer et al. 1993). Activities of glutamine synthetase and alanine aminotransferase do not seem impeded by long-term application of thiopental or midazolam, as ventricular CSF glutamine is increased together with CSF glutamate in the investigated brain-injured patients and since CSF alanine levels remain significantly elevated compared to control CSF as long as pathologic CSF glutamate concentrations are present. These in vivo findings are in agreement with in vitro studies showing preservation and even up-regulation of glial enzymatic activity during long-term application of barbiturates (Roth-Schechter et al. 1979; Yu et al. 1983).

The observed alterations in ventricular CSF glutamine and alanine levels seem to reflect changes within intermediary metabolism and do not appear related to a damaged blood-brain/CSF barrier, since ventricular CSF serine, an amino acid without any transmitter and metabolic function within the central nervous system, remains unchanged in brain-injured compared to control patients.

The most astonishing finding of the present study was the missing increase in CSF lactate levels despite significantly elevated CSF glutamate concentrations. As shown in animal experiments, pathologic lactate levels should parallel the massively increased extracellular glutamate concentrations, reflecting glutamate-mediated activation of energy consuming processes

(Kawamata et al. 1995; Demestre et al. 1997; Hu and Wilson 1997) which can be blocked by specific glutamate-receptor antagonists (Kawamata et al. 1995). Successful pharmacological neuroprotection related to administered midazolam and thiopental in terms of attenuation of glutamate-mediated activation of energy consuming processes could explain the absent increases in ventricular CSF lactate. In addition, drug-independent intact glial and neuronal uptake of lactate with its subsequent metabolism (Nedergaard and Goldman 1993) could also account for the normal CSF lactate levels. Under these routine clinical conditions, it is impossible to assess the rate of release and uptake processes contributing to changes in ventricular CSF amino acid and lactate levels.

One major setback of the present study, however, is the inability in defining origin, transport mechanisms and metabolic fate of the investigated amino acids as sampled from ventricular CSF in brain-injured patients. Under in vivo conditions, more detailed answers concerning brain metabolism can only be obtained by the sophisticated analytical method of NMR spectroscopy (Michaelis et al. 1991), which is superior to the approach chosen in the present study. The metabolic fate of glutamate, however, can only be assessed by applying radioactively labeled precursors (Sibson et al. 1998) which, in turn, limits its application in clinical routine. At the bed-side of severely brain-injured patients, NMR spectroscopy is an impractical method. Therefore, estimation of ongoing processes is restricted to the practical but less accurate analysis of changes in ventricular CSF amino acid concentrations.

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