
Factors affecting excitatory amino acid release following severe human head injury

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Recent animal studies demonstrate that excitatory amino acids (EAAs) play a major role in neuronal damage after brain trauma and ischemia. However, the role of EAAs in patients who have suffered severe head injury is not understood. Excess quantities of glutamate in the extracellular space may lead to uncontrolled shifts of sodium, potassium, and calcium, disrupting ionic homeostasis, which may lead to severe cell swelling and cell death. The authors evaluated the role of EAAs in human traumatic brain injury.

In 80 consecutive severely head injured patients, a microdialysis probe was placed into the gray matter along with a ventriculostomy catheter or an intracranial pressure (ICP) monitor for 4 days. Levels of EAAs and structural amino acids were analyzed using high-performance liquid chromatography. Multifactorial analysis of the amino acid pattern was performed and its correlations with clinical parameters and outcome were tested. The levels of EAAs were increased up to 50 times normal in 30% of the patients and were significantly correlated to levels of structural amino acids both in each patient and across the whole group ($p < 0.01$). Secondary ischemic brain injury and focal contusions were most strongly associated with high EAA levels ($27 \pm 22 \mu\text{mol/L}$). Sustained high ICP and poor outcome were significantly correlated to high levels of EAAs (glutamate $> 20 \mu\text{mol/L}$; $p < 0.01$).

The release of EAAs is closely linked to the release of structural amino acids and may thus reflect nonspecific development of membrane micropores, rather than presynaptic neuronal vesicular exocytosis. The magnitude of EAA release in patients with focal contusions and ischemic events may be sufficient to exacerbate neuronal damage, and these patients may be the best candidates for treatment with glutamate antagonists in the future.

Key Words * severe head injury * microdialysis * glutamate * intracranial pressure * outcome * glutamate antagonist

Glutamate is the most widely distributed excitatory neurotransmitter in the mammalian brain.[3,6,10,11,17,22,49] Following the comparatively recent discovery of this role, it has become a focus of major neuroscientific interest because of its potential role in the causation of both acute and chronic neuronal damage. In 1983, Rothman and Olney[46] proposed the "excitotoxic" hypothesis. This hypothesis was based on previous studies that showed the neurotoxic potential of glutamate and its coagonist aspartate in the normal brain and, more recently, in tissue cultures.[2,3,8,9,15,28] At least four glutamate receptor subtypes exist on different parts of mammalian neurons, and now extensive studies have characterized the responses of these receptors to glutamate and its coagonists (including aspartate and glycine).[3,12,17,20,23,26,29,35,46,53,54]

Many of the normal physiological processes of the cortex and the hippocampus, in particular, are thought to depend on this neurotransmitter function of glutamate.[15,30,31,46]

However, when glutamate is present in excessive quantities, it may overactivate specific ion channels, especially the N-methyl-D-aspartate channel. This results in uncontrolled entry of sodium and calcium into cells along with associated potassium egress into the extracellular space. When this process is rapid, it can result in massive accumulation of intracellular calcium with rapid neuronal death--"fast excitotoxicity." [43,50] This produces neuronal histological appearances that are identical to those seen after ischemic events.[1,18,19,21,50,51] When the entry of calcium is slowed, a "delayed neuronal death" can result in the delayed disappearance of neurons, anywhere from 5 to 7 days after the event.[10,44,45]

A potassium shift into the extracellular space will result in rapid swelling of astrocytes, which absorb quantities of potassium to preserve ionic homeostasis.[2,10,28] This process may cause rapid "cytotoxic edema," which is probably a major factor in causation of posttraumatic raised intracranial pressure (ICP).

Recent studies in which the microdialysis technique has been used have shown that large quantities of glutamate are released into the extracellular space in animal models of both neurotrauma and stroke.[3,6,11,22,23,43,49] In these models, a variety of drugs that block the effects of glutamate both presynaptically and at postsynaptic receptor sites have reduced both ischemic brain damage and glutamate release.[5,7,12,20,21,24,25,29,32,33,36,40,53,55,56]

There are now several Phase II and Phase III trials in progress to evaluate the efficacy of glutamate antagonist drugs in both neurotrauma and stroke. However, the pattern of glutamate release after neurotrauma has hitherto only been documented in the cerebrospinal fluid and in small numbers of patients by using the microdialysis technique.[9,10,15,23,44] We have therefore prospectively studied 80 patients by using intracerebral microdialysis to address the following hypotheses: 1) release of excitatory amino acids (EAAs) is a significant event after human traumatic brain injury (TBI); 2) the magnitude of EAA release in these patients is sufficient to account for neuronal damage in accordance with the excitotoxic hypothesis; and 3) the EAA release after trauma may be exacerbated by secondary ischemic events such as low cerebral perfusion (CPP), high ICP, and hypoxic events.

CLINICAL MATERIAL AND METHODS

These studies were approved by the Committee for Conduct of Human Research of the Medical College of Virginia and Virginia Commonwealth University. In 80 consecutive severely head injured patients admitted to the intensive care unit (ICU) at the Medical College of Virginia, from whom consent was obtained for microdialysis and intensive neuromonitoring studies, microdialysis probes were placed within the cerebral cortex in accordance with an established protocol. The patients received standard aggressive management that included rapid removal of intracranial hematomas, ICP monitoring performed by ventriculostomy, and careful management of ICP by means of artificial ventilation, neuromuscular paralysis, mannitol ventricular drainage, and, in a few patients with uncontrolled high ICP, barbiturate infusion or induction of moderate hypothermia. In 63 patients, regional cerebral blood flow (CBF) measurements were made during or immediately before the microdialysis was begun.

Microdialysis Procedure

We used custom-made, commercially produced microdialysis probes, with a molecular weight cutoff of 20,000 D and a 10-mm active dialysis surface. The probes were sterilized using low-temperature, long-cycle ethylene oxide gas and implanted in one of two ways. In patients who had diffuse injuries and who did not undergo craniotomy, the probes were inserted using the same twist-drill hole that had been used for ventriculostomy in the ICU. In 60% of these patients the probe was secured via a triple lumen transcranial

bolt, as described earlier by our group.[58] In patients who had focal mass lesions and who underwent a craniotomy, the probes were placed into the cortex, under direct vision, at the evacuated hematoma site, adjacent to a removed or unremoved contusion (Fig. 1).

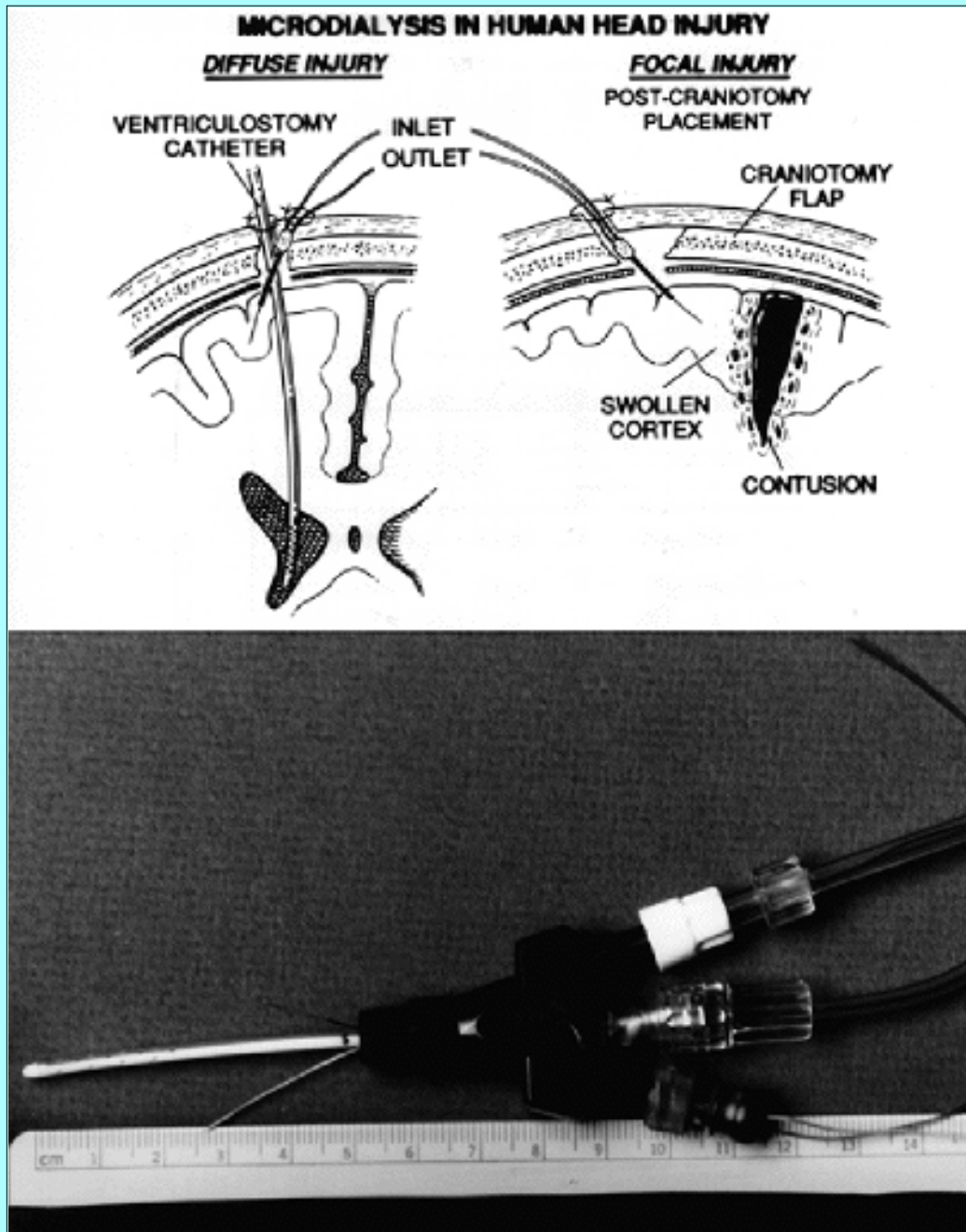


Fig. 1. Upper: Diagram showing insertion techniques used early in the study for placing the microdialysis probe in patients with diffuse and focal injuries. Lower: Photograph of a triple-lumen transcranial bolt for fixation of the microdialysis probe (top), along with an oxygen sensor (lower) used in the patients enrolled later in the study.

The probes were perfused with sterile 0.9% saline at 2 $\mu\text{l}/\text{minute}$ by means of a microdialysis pump. The effluent dialysate was collected using a computer-controlled, automated, refrigerated fraction collector, at 4°C. Fractions (60 μl) were collected every 30 minutes into labeled, sealed glass vials and frozen at -70°C, for later high-performance liquid chromatography (HPLC) analysis. Initially, every microdialysis probe was tested in vitro to measure the total recovery rates for the different analytes tested. Because the probe recovery rates were remarkably constant, this was subsequently performed for approximately every fifth patient only.

Pilot studies indicated that "ischemic events" were an important factor influencing EAA release. Therefore, emergency department and clinical records and ICP, CPP, and regional CBF data were used to construct a five-point scoring system for significant ischemic events (Table 1).

Event & Criteria	Score
hypoxemia PaO ₂ <60 mm Hg	1
hypotension MABP <50 mm Hg†	1
hemispheric CBF <20 ml/100 g-1 min	1
herniation fixed dilated pupil‡	1
CPP <50 mm Hg for >30 min	1

* Individual scores are summed for a total of 0 to 5.
† For 30 minutes or more prior to resuscitation.
‡ In the hemisphere used for microdialysis.

High-Performance Liquid Chromatography Analysis

The concentration of amino acids in the microdialysis samples was measured using HPLC electrochemical detection after o-phthaldialdehyde/thiol derivatization.[34] Briefly, a refrigerated autosampler was used to mix 10 µl of dialysate with 40 µl of an o-phthaldialdehyde/thiol solution. Samples were allowed to react for 15 minutes before injection onto a liquid chromatograph on which derivatized amino acids were separated on an octadecyl-silane (3-µm) C18 reverse-phase column. The mobile phase consisted of 0.1 M phosphate, 2 mM ethylenediamine tetraacetic acid, and 7% acetonitrile at a pH of 5.85. Amino acids were detected on a thin-layer electrochemical cell with a glassy carbon electrode operated at 700 mV. The analog output of the detector cell was digitized and collected by a computer running chromatography software. Peak areas for each amino acid were converted to concentration by comparing them to known standards run at regular intervals.

Statistical Analysis

In all patients, physiological data such as ICP, CPP, mean arterial blood pressure (MABP), and clinical characteristics were logged into a VAX mainframe computer (Neurocore, Medical College of Virginia Database) and these data were time-locked with the biochemical data for excitatory and structural amino acids for analysis. The patients were subdivided on the basis of their glutamate data into three categories: low glutamate, less than 5 µmol/L; intermediate glutamate, 5 to 20 µmol/L; and high glutamate, more than 20 µmol/L. For each of these categories, correlations were sought with the following clinical features: 1) prior ischemic events, according to the ischemia score; 2) computerized tomography (CT) scanning category (hematoma, contusion, and diffuse injury); 3) MABP, ICP, and CPP; and 4) outcome.

For continuously measured variables, the inpatient (within subject) correlations between glutamate and aspartate and structural amino acids were examined.[14] The relationships between glutamate and ICP, CPP, and MABP were tested using rank correlations. The relationships of mean glutamate, aspartate, and threonine with outcome, CT characteristics, and ischemic events were also tested. In addition, "threshold" analyses were performed by classifying patients into high- and low-glutamate groups, (> 20 µmol/L or $\frac{3}{4}$ 20 µmol/L, respectively) and testing the relationship with categorical variables by using a chi-square test.

To test the strength of the correlations among different continuous variables across the entire group of 80

patients, 80 inpatient correlations were first determined for the variables glutamate, aspartate, threonine, ICP, and CPP. Spearman's correlation coefficient was used to represent the degree of the relationship in each patient. Data consisting of 80 independent correlation coefficients were found to be normally distributed and, therefore, a one-sample t-test was used to test the significance of the mean interpatient correlation.

Sources of Supplies and Equipment

The microdialysis probes (CMA 20 Custom Probes), the microdialysis pump (model CMA 15), the refrigerated fraction collector, and the refrigerated autosampler (model CMA 200) were manufactured by CMA Microdialysis (Acton, MA). Rainin Dynamix chromatography software, which was obtained from Rainin Instrument, Inc. (Woburn, MA), was used with a Macintosh computer obtained from Apple Computers (Cupertino, CA) in the HPLC analysis.

RESULTS

No complications were induced by the placement of the probes. In three patients, the probes needed to be removed and replaced due to rupture of the membrane. In one patient, a probe failed to recover analytes. These patients were excluded from the final analysis.

Clinical Characteristics

The clinical characteristics of the 80 patients described in this study are summarized in Table 2. Seventy-six patients were comatose on arrival and the overall mortality rate in the study was 29%. In four patients, microdialysis was begun after they had deteriorated into the coma hours or days after injury.

Characteristics	No. of Patients	Glutamate (%)	
		≤20 mmol/L	>20 mmol/L
sex			
male	56	48	22
female	24	22	8
admission GCS category			
3-4	35	26	18
5-6	11	14	0
7-8	21	22	4
9-11	10	8	4
12-15	3	0	4
w/craniotomy	32	22	18
ischemic events score (0-50)			
0	22	20	8
1	21	22	4
2	10	10	2
3	10	10	2
4	11	6	8
5	6	0	8
outcome†			
favorable	37	40	6
unfavorable	43	18	36

* GCS = Glasgow Coma Scale score.
† p < 0.05.

In 34 patients, microdialysis monitoring was performed at the border zone of a contusion and, in the majority of these cases, the contusion had been resected prior to placement of the microdialysis probe. The microdialysis probe was placed in the nonevocative cortex beneath a hematoma after it had been removed in

14 patients with acute subdural hematomas and in five patients with epidural hematomas.

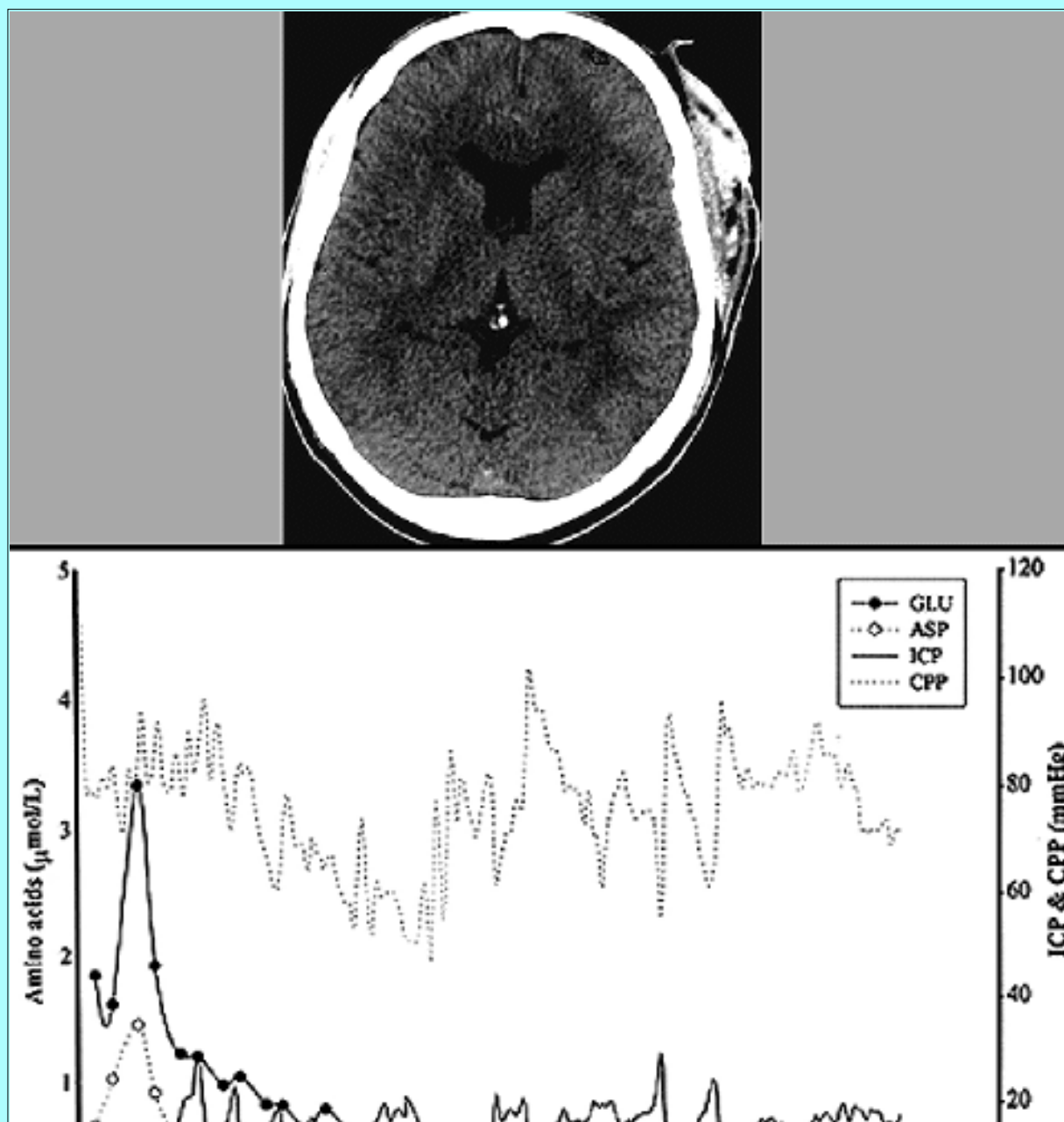
In Vitro Calibration of the Microdialysis Probes and Methodological Errors

The mean recovery rate for glutamate and aspartate was $43 \pm 4\%$; the recovery rate for threonine was $22 \pm 1\%$.

Whenever patients were disconnected from the microdialysis pump, accidentally or to obtain CT scans or be transported to the operating room, the amino acid concentration in the first microdialysis sample obtained after reconnection was found to be 50 to 100% higher than the previous or subsequent value. This is because the first postreconnection samples contained the dialysis fraction that had been standing in the microdialysis probe during the disconnection period. Theoretically, amino acids could equilibrate to 100% of the true extracellular fluid value. These samples were omitted from further data analysis.

Relationship Between Glutamate and Aspartate

When graphs of time-dependent changes in aspartate and glutamate within individual patients were studied, aspartate was seen to follow glutamate variations closely (Fig. 2).



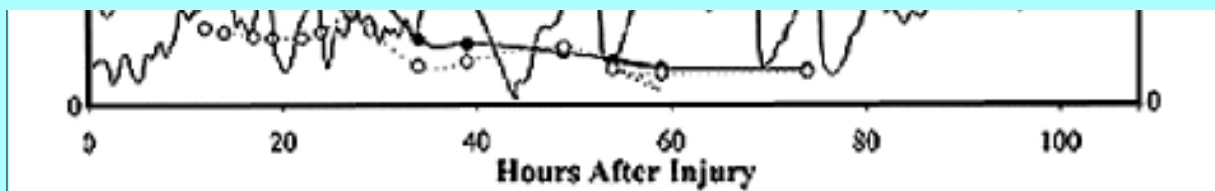


Fig. 2. Data obtained in a 24-year-old patient with diffuse injury. The relationships among the dialysate EAAs (glutamate [GLU] and aspartate [ASP]), ICP, and CPP are shown over time (normal glutamate level < 2 $\mu\text{mol/L}$). Upper: Computerized tomography scan demonstrating the site of the microdialysis probe (arrow). Lower: Graph displaying measurements of GLU, ASP, ICP, and CPP. Note that the EAAs remained in the "normal range" during most of the monitoring period.

The mean correlation coefficient between glutamate and aspartate for the 80 patients studied was 0.683 ± 0.21 ($p < 0.01$). Thus, factors affecting these two EAAs operated together in this patient group. The mean values for glutamate in different injury subtypes, in relation to the normal range reported for humans measured by microdialysis, is shown in Table 3.

CT Finding	No. of Patients	Glutamate (mmol/L)	Aspartate (mmol/L)	Threonine (mmol/L)
diffuse injury	32	14.1 ± 7.7	2.2 ± 1.1	7.3 ± 5.5
contusion	29	27 ± 20	8.3 ± 4.5	9.5 ± 6.5
subdural hematoma	14	18 ± 15	4.5 ± 3.5	9.9 ± 8.0
epidural hematoma	5	3 ± 3	0.4 ± 0.2	6.8 ± 7.8

* Values are expressed as mean \pm standard deviation.

Relationship Between EAAs and Structural Amino Acids

In all patients a structural amino acid, either threonine (71 patients) or valine (nine patients), was measured. Structural amino acids were significantly correlated with EAAs ($r = 0.59$, $p < 0.01$). Although the relationship between structural amino acids and EAAs was remarkably constant within each patient throughout the clinical course, in some patients the former were released in higher concentrations than the latter and in others the reverse occurred. In general, patients with the highest levels of EAAs demonstrated structural amino acids levels that were even higher, whereas in some patients with intermediate levels of EAA release, the levels of EAAs exceeded those of structural amino acids. In no patient did structural amino acids rise or fall without concomitant changes in EAAs or vice versa.

Changes in EAAs During Monitoring

Four general patterns of EAA release could be recognized among these 80 patients. In patients with near-normal EAAs (Group 1), levels of glutamate, aspartate, and threonine tended to decline during the monitoring period, usually falling to within normal range by the end of the first 12 to 18 hours after injury (Fig. 2). These were most often patients in whom there was a normal CT appearance and no secondary ischemic event. In those patients with intermediate levels of EAAs (5-20 $\mu\text{mol/L}$; Group 2), more heterogeneous patterns of change with respect to time were seen. In most of these patients, there was a gradual decline in EAA levels over several days, although the levels of EAAs remained higher than normal for days (Fig. 3). Most of these patients were those who sustained ischemic events or experienced raised ICP during their early postinjury course. In 30% of Group 2 patients, EAA release remained constant and moderately elevated throughout the monitoring period. In none of these patients was a progressive rise in EAAs detected.

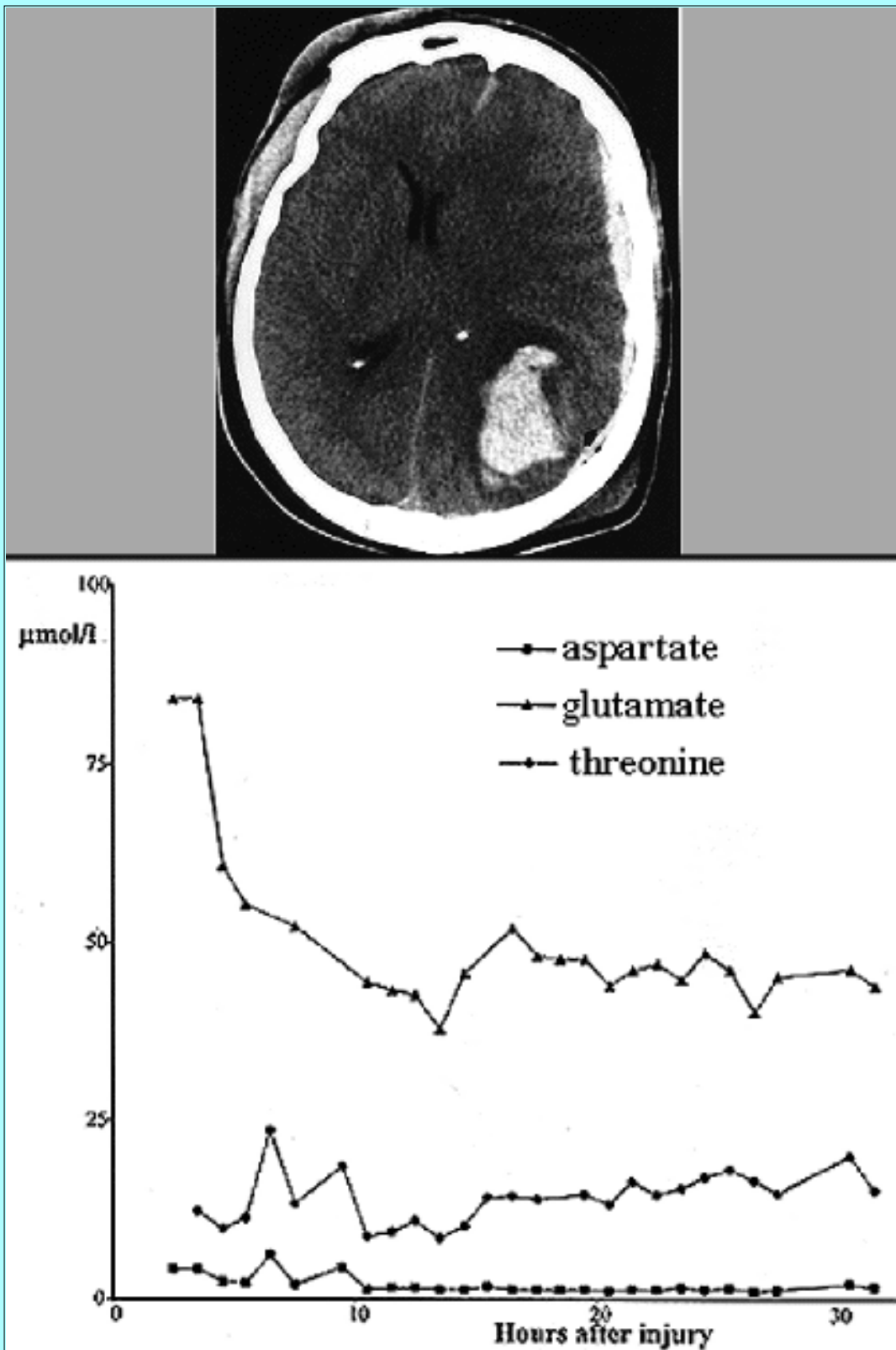


Fig. 3. Data showing typical "decline and stable baseline" pattern of very high EAAs in a patient with focal contusions at the probe placement site. The patient made a good recovery. Upper: Preoperative CT scan showing a right hemisphere subdural hematoma and focal hemorrhagic contusion. The microdialysis probe was placed in position (arrow) after the hemorrhagic contusion was evacuated. Lower: Graph displaying the pattern of dialysate EAA and structural amino acid changes over 30 hours of monitoring. Note that glutamate was approximately 25 times higher than normal, which is 2 $\mu\text{mol/L}$.

Patients with an extracellular fluid glutamate greater than 20 $\mu\text{mol/L}$ (Group 3) demonstrated the most heterogeneous changes in EAAs over time. In 42% of this group, the EAAs were seen to rise progressively and, in most of these patients, elevated ICP were present along with significantly reduced CPP (< 50 mm Hg). In the majority of these patients, death was the outcome. In all these patients, prior ischemic events or elevated ICP was present. Thus, a progressively rising level of EAAs was not seen, except in patients who eventually died. In four patients in the entire series, all members of Group 3, a massive increase in EAAs and structural amino acids was seen superimposed on a relatively constant base line (Fig. 4). In none of these four patients could a hemodynamic explanation for this sudden increase be found. In all four, however, the microdialysis probe was close to a cerebral contusion or intracerebral hematoma. In the remaining 12 patients in Group 3, the EAAs exhibited a progressively downward trend, but remained far above the normal range for several days.

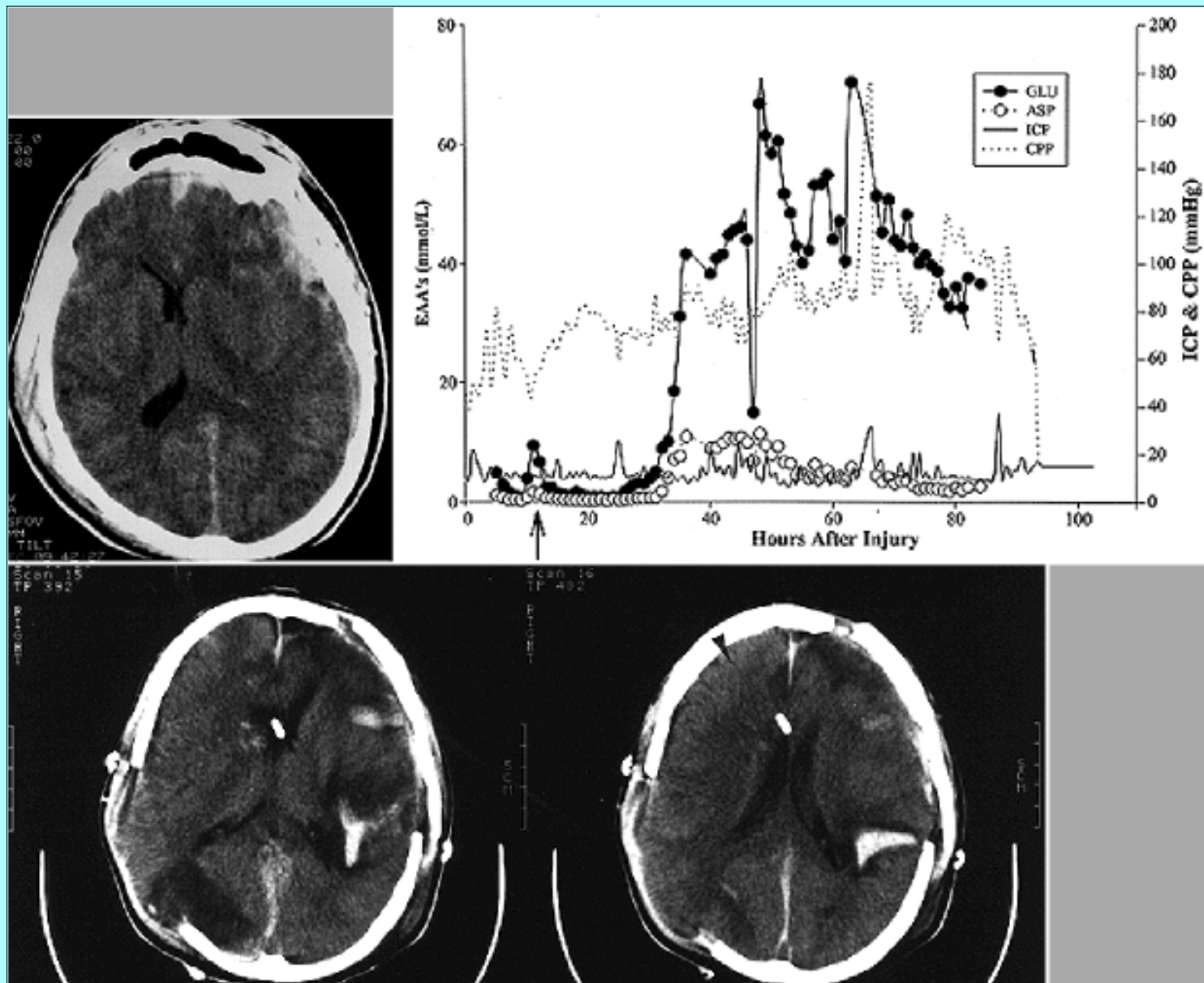


Fig. 4. Data documenting a marked sustained rise in dialysate EAAs in a patient with acute subdural hematoma and delayed epidural and intracerebral hematomas, who underwent two craniotomies. The patient remains vegetative at 3 years postinjury. Upper Left: Initial CT scan showing the acute subdural hematoma. Lower: Postoperative CT scan obtained after second

craniotomy, showing widespread bilateral damage; arrow indicates approximate probe site. Upper Right: Graph showing levels of dialysate EAAs, ICP, and CPP. Note the small (fivefold) increase in glutamate at the time of the epidural hematoma (arrow). There was an unexplained massive delayed EAA rise 32 hours after injury, which was unassociated with ICP, CPP, or PaO₂ changes.

With the exception of the four patients just mentioned, the hour-to-hour variation in levels of EAAs and threonine, as measured in the study, was 10 to 20% above or below baseline. This variability was remarkably constant in all categories of patients. Thus, with the exception of four patients, the magnitude and pattern of EAA release was apparent from the first 12 to 24 hours of microdialysis.

Relationship Between EAAs and Clinical Injury Subtypes

Tables 3 and 4 and Fig. 5 show the relationship between the major head injury CT diagnostic categories and EAA and structural amino acid release.

TABLE 4
EFFECT OF ISCHEMIC EVENTS ON LEVELS OF DIALYSATE GLUTAMATE SUBDIVIDED BY CT LESION CLASSIFICATION*

Secondary Insult	Mean Glutamate (mmol/L)			
	Diffuse Injury	Contusion	SDH	EDH
present	32.6 ± 82.8	33.1 ± 52.2	16.5 ± 22.6	8.7 ± 12.8
absent	9.4 ± 19.7	27.0 ± 39.1	2.9 ± 3.9	4.9 ± 6.7
no. of patients	19	20	20	7

* Values are expressed as means ± standard deviation. Abbreviations: EDH = epidural hematoma; SDH = subdural hematoma.

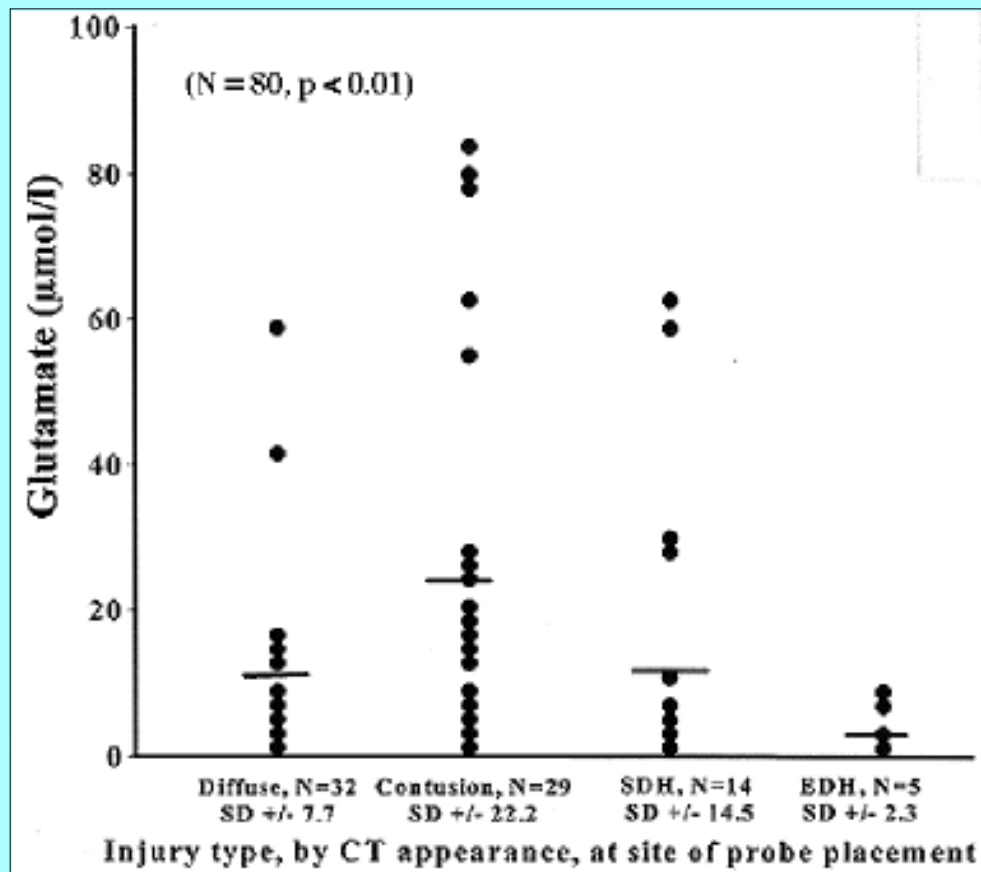
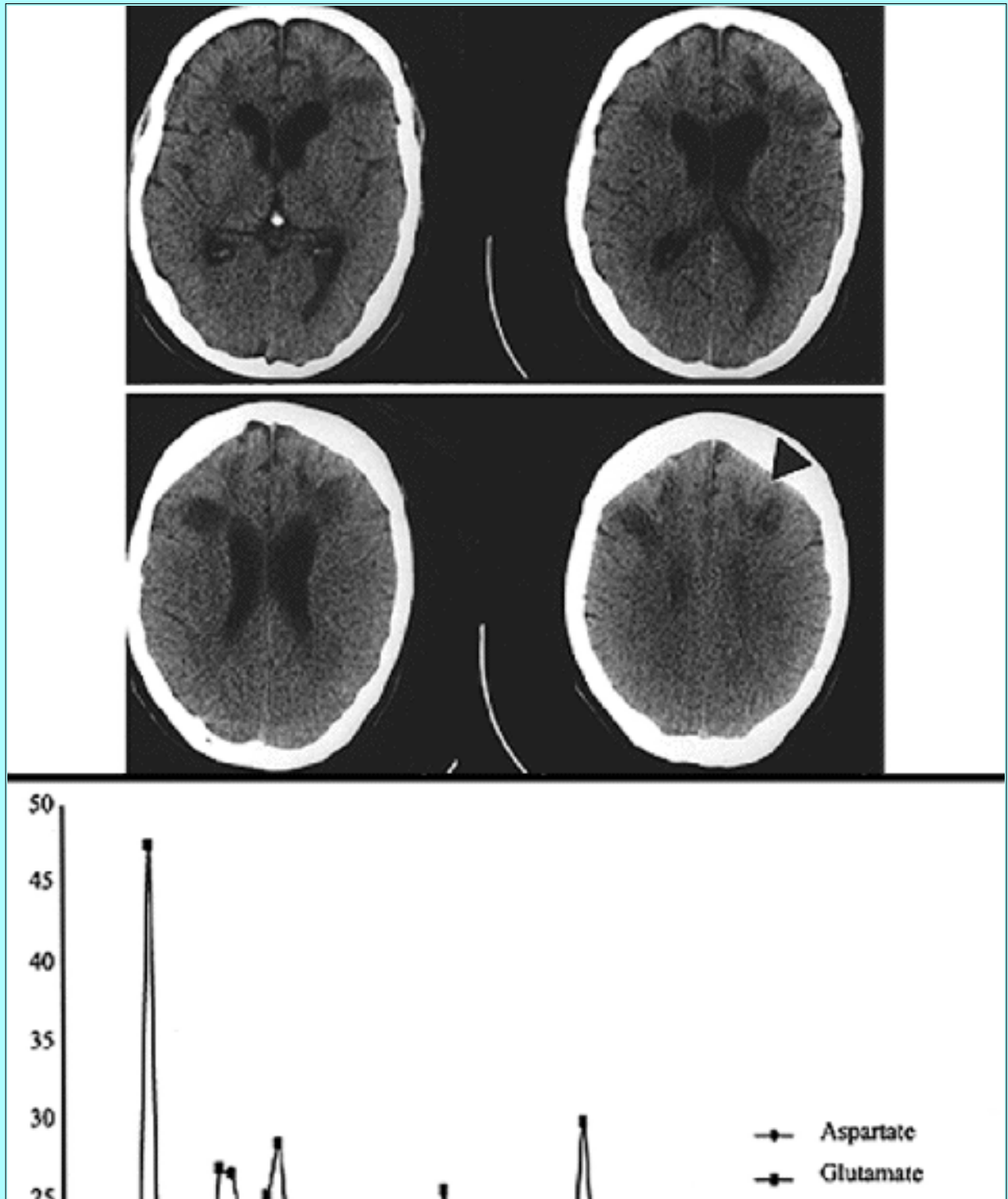


Fig. 5. Scatterplot graph showing the mean levels of dialysate glutamate in the four injury

subtypes classified by CT scanning. SD = standard deviation.

The mean EAA data over time for each patient was used and the mean was obtained again for each diagnostic category (the mean of the mean). It is seen that the mean value for glutamate among patients with diffuse injury (with and without superimposed ischemic events) was $14.1 \pm 7.7 \mu\text{mol/L}$. In patients with subdural or epidural hematomas, levels of EAAs and structural amino acids were not significantly increased when compared with those in patients with diffuse injuries (18 ± 15 and $3 \pm 3 \mu\text{mol/L}$, respectively). In patients with contusions, however, the mean glutamate level was $27 \pm 20 \mu\text{mol/L}$, which was significantly higher than the level for patients with diffuse injuries ($p < 0.01$). When the clinical course was complicated by hypoxic or ischemic events, a marked, significant increase in EAA and structural amino acid release was seen to occur. The relationship between ischemic events and dialysate glutamate is shown in Table 4 and Figs. 6 and 7 (see later discussion of this topic).



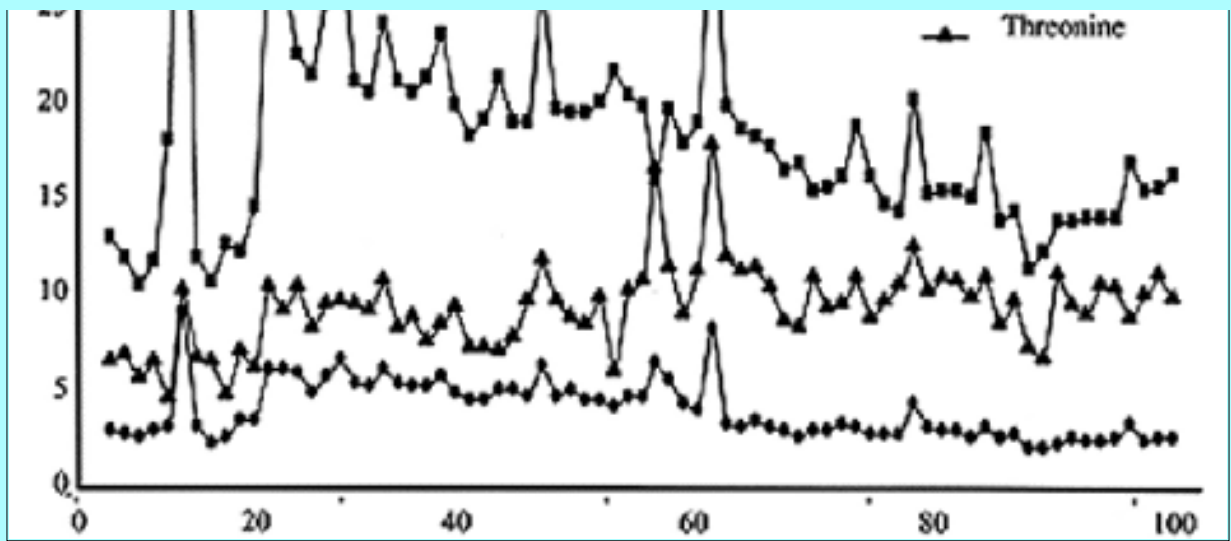


Fig. 6. Data showing the effect of global cerebral ischemia on dialysate EAAs in a 39-year-old patient who sustained bilateral pneumothoraces and retroperitoneal hemorrhage in a "roll-over" motor vehicle accident. The patient was suffering from shock (blood pressure 110/40 mm Hg) and hypoxemia (PaO₂ 55 mm Hg) on arrival in the emergency department. At 1 year the patient had moderate disability. Upper: Computerized tomography scans obtained at 3 months showing a "watershed" infarction (probe site is shown by arrowhead). Lower: Graph showing levels of EAAs, including a 10-fold increase in glutamate over baseline, declining over time.

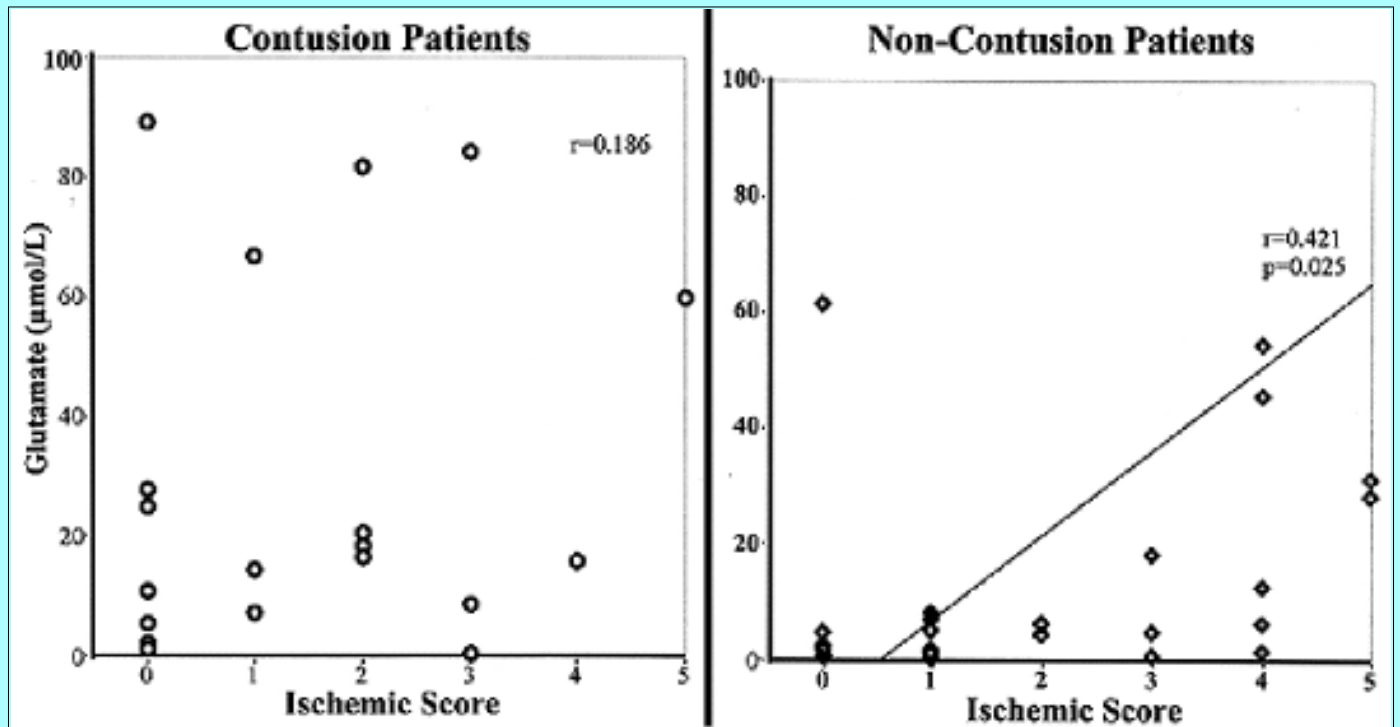


Fig. 7. Graph displaying the relationship between dialysate glutamate and ischemic events in patients with and without focal contusions at the probe site (see Table 1 for description of ischemic events scoring).

Relationship Between EAAs and Clinical Events During Monitoring in the ICU

The only clinical event that could be identified as exerting a significant influence on dialysate amino acids levels during the ICU monitoring was a sustained increase in ICP, such that CPP fell below approximately 40 to 50 mm Hg.

The presence or absence of mild hypoxemia (not below 60 mm Hg), neuromuscular paralysis, ventilator weaning, coma, or fluctuations in core temperature could not be shown to influence amino acid patterns in the dialysate, by inspection of the graphs or by linear regression analysis. However, no patient in this series, except those destined to brain death, developed sustained severe hypoxemia ($\text{PaO}_2 < 50$ mm Hg) while in the ICU, and no patient manifested clinical or subclinical seizures as far as we are aware. In one patient, a large intracerebral and subdural hematoma developed in the hemisphere contralateral to the microdialysis probe and was associated with an increase in ICP to 30 mm Hg. These events did not seem to influence amino acids in the dialysate sampled from the noncompressed hemisphere.

Relationship Between Dialysate EAAs and Ischemic Events

The presence of ischemic events, before commencement of microdialysis or during monitoring, was the only recorded condition that seemed to influence EAAs in the dialysate. Together with the presence of a focal contusion, it was the major cause of an increase in dialysate EAAs and structural amino acids (Table 4). The highest dialysate EAAs were seen in patients with both ischemic events and focal contusion (Fig. 7). However, in the presence of a contusion, the severity of ischemia was not related to the magnitude of EAA release. In patients in whom the dialysis probe was located away from a focal contusion, however, the dialysate glutamate was linearly correlated with the severity of ischemic events ($r = 0.421$, $p = 0.025$; Fig. 7).

Relationship Between Dialysate EAAs and ICP

The relationship between glutamate and ICP for the entire patient group was found to be significant ($r = 0.554$, $p < 0.01$). Patients having the highest overall mean glutamate values displayed the most significant correlation with ICP ($r = 0.659$, $p = 0.0104$) in comparison with the two other glutamate categories (Fig. 8). This is also reflected in the relationship between EAAs and ischemic events (Table 4 and Fig. 7).

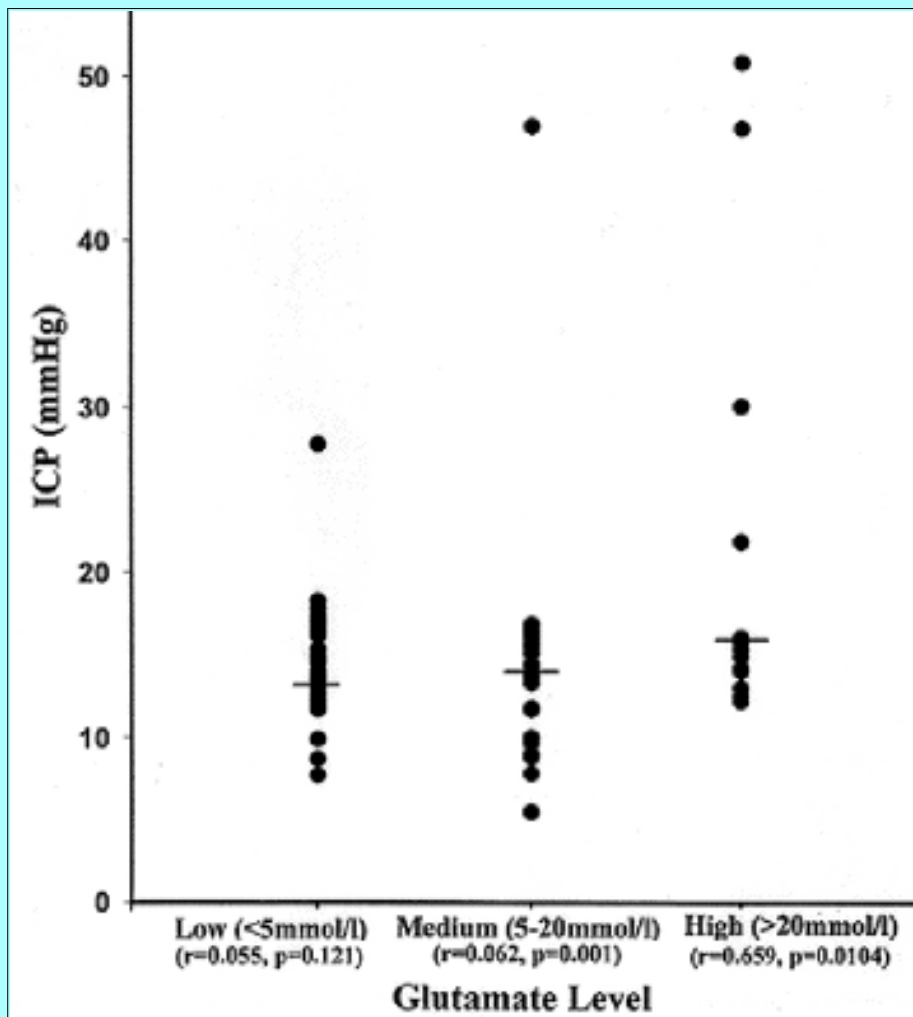


Fig. 8. Scatterplot graph depicting the relationship between mean ICP and dialysate glutamate.

Relationship Between Dialysate EAAs and Outcome

The relationship between patient outcome and dialysate EAA levels is documented in Table 2 and Fig. 9. A significant correlation was found that demonstrated higher glutamate levels in those patients with unfavorable outcomes ($r = 0.33$, $p < 0.05$). Within the high-glutamate group, however, five (6%) of the patients achieved favorable outcomes. Three patients were monitored at the site of contusional damage in the cortex, which has been shown to elevate focal EAA levels dramatically without necessarily compromising the "whole brain" outcome.

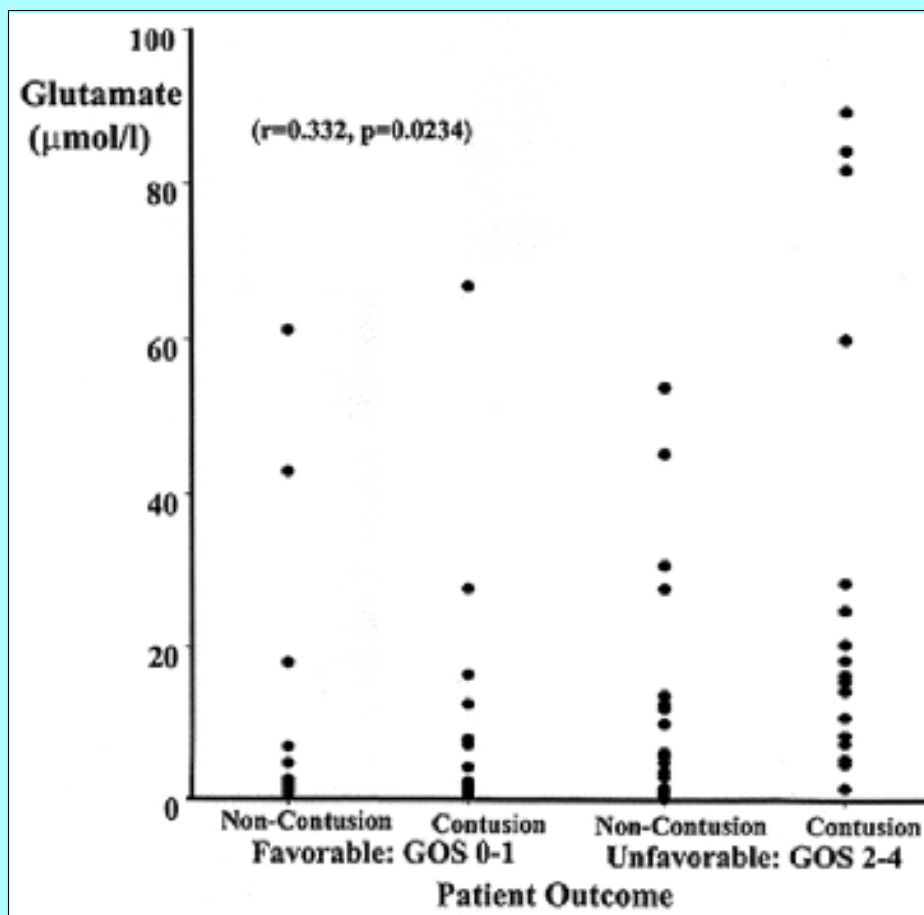


Fig. 9. Scatterplot graph displaying the relationship between dialysate glutamate and outcome for patients in whom the probe was placed in contused and noncontused cerebral tissue. GOS = Glasgow Outcome Scale score.

For the 15 patients in the low-glutamate group who experienced unfavorable outcomes (eight patients died, two are in a vegetative state, and five are severely disabled) without an increase in dialysate EAA levels, the majority experienced secondary or unrelated complications such as pulmonary embolism, deep venous thrombosis, or massive brainstem hematoma, and withdrawal of supportive therapy in the case of an elderly patient in a prolonged vegetative state. These events dramatically worsened these patients' outcomes independently of their EAA levels, measured at a small focal zone.

DISCUSSION

There are five main findings from this study. 1) Dialysate EAAs were increased to strikingly high levels (up to 50 times normal) in 30% of patients, and this also correlated well with structural amino acid changes. 2) The increase in EAAs was seen to persist for the duration of monitoring--up to 4 days in most of these patients. 3) The presence of a focal contusion and primary or secondary ischemic events were the clinical features most strongly correlated with high dialysate EAAs. 4) Raised ICP was significantly more common in patients with high dialysate EAAs. 5) Outcome was worse in patients with high levels of dialysate EAAs.

Contusions in the Cortex

These studies have shown that extracellular amino acids are increased approximately 10 to 20 times higher in contused cortex in comparison with noncontused cortex, and these increases remain throughout the 4-day study period. There is minimal fluctuation in dialysate amino acid levels around contusions over time and the levels are independent of the size of the contusion. These increased levels appear to be independent of changes in ICP or superimposed ischemic events (Fig. 7). Together with the similarity between structural and EAAs,

this finding suggests that nonspecific destructive processes such as development of membrane cell micropores may be occurring in this contused cortex. However, a major increase in extracellular potassium was not seen in the pericontusional tissue, suggesting that astrocytes may be retaining their potassium-buffering capacity (data reported separately).[10,42] Our previous regional blood flow studies have shown profound reductions in CBF, both within contused cortex and in the pericontusional (edema) zone, as detected by low attenuation on CT scanning.[4,38,39,47] Moreover, these studies have also shown no tendency for improved perfusion over time within these pericontusional zones.

We therefore speculate that neuronal disruption by shearing forces at impact when the contusion occurs leads to development of membrane micropores and leakage of amino acids. Glutamate may then contribute to pericontusional edema and ischemia by inducing the opening of neuronal ion channels to promote potassium efflux and sodium influx. This process is likely to result in astrocytic swelling and neuronal shrinkage. Both these findings have been seen in ultrastructural and light microscopic analysis of biopsied peri-contusional tissue in our previous studies.[1,16]

It is noteworthy that the lowest levels of EAAs were seen in the five patients who had epidural hematomas, even though all of these patients were initially in coma after removal of the hematoma. This suggests that transient brain compression caused by epidural hematoma is sufficient to cause coma, yet not sufficient to damage the brain enough to cause EAA release.

The mean EAA level in the 14 patients with acute subdural hematoma was nine times higher than the "normal value" for the mammalian brain. This accords quite closely with findings in our previous studies in the rat model with acute subdural hematoma, but this EAA rise was not significantly higher than in patients with diffuse injury. This is probably because of the heterogeneity of this group of acute subdural hematoma patients: some sustained secondary ischemic events, whereas others improved to emerge from coma in 3 to 4 days, and made a rapid recovery.

Structural Amino Acids, EAAs, and Causes of Release

Some aspects of these studies are consistent with the findings of previous animal studies. Studies conducted by Katayama, et al.,[28] Faden, et al.,[17] and our own previous studies have shown brief, transient elevations of EAAs to two to seven times higher than basal levels, persisting only 10 to 30 minutes after impact, in both fluid percussion injury and subdural hematoma in the rat.[6,8,12]

Animal studies have only assessed events over a few hours before and after impact. In contrast, these human studies have only captured data for the postimpact period, ranging from 3 hours to 6 days after impact. Extrapolating our human data closer to the time of impact, we speculate that human TBI is associated with much higher magnitudes of transient EAA release than those found in animal models. The typical pattern of amino acid efflux seen in patients without significant secondary ischemic events (Fig. 2) and the early fall in EAA levels support the conclusion that an "ictal surge" of EAAs occurs in human TBI, as well as in animal models.

Amino acid levels in certain patients who suffered major secondary insults were up to 50 times higher than normal levels. In most patients who did not have secondary ischemic events, levels of both EAAs and structural amino acids remained constant or declined and fluctuated by only 10 to 20% of the initial values throughout the monitoring period. In those patients who died, however, steadily increasing EAA and structural amino acid levels were usually seen, suggesting ongoing release by one or more mechanisms. Elsewhere, we have reported a close correlation between high glutamate ($> 20 \mu\text{mol/L}$) and regional CBF levels below 18 ml/100 g/minute.[27,57]

These studies have shown massive increases (up to 50-fold) in both EAAs and structural amino acid

concentrations in brain extracellular fluid dialysate in certain categories of patients who have sustained severe head injury, whereas in others, levels of amino acids remain normal or near normal. Although the microdialysis technique is only capable of sampling events within a relatively small volume of cortex (approximately 10-20 mm around the dialysis probe) these studies have shown that the type of intracranial lesion observed on CT scanning and the presence or absence of a significant secondary ischemic event are the major determinants of amino acid release. In contrast, the patterns of amino acid release over time were remarkably constant in individual patients and were not influenced by transient clinical events such as a brief increase in ICP. Increased levels of dialysate amino acids, however, were significantly associated with bad outcome and with prolonged, severely raised ICP in this study.

With regard to the hypothesis we have attempted to test in this study, we have clearly confirmed the first and the third points, namely that EAA release is clearly a major event after human TBI, especially focal contusion, and that EAA release is exacerbated markedly in ischemic secondary events, as seen in animal model studies.[20,32] As yet, however, our data do not allow us to confirm or refute our second hypothesis, namely that high EAA levels cause posttraumatic brain damage. This study does provide three lines of reasoning to support the concept that EAA release after human TBI does cause brain damage in some patients. First, an association was seen between increased EAAs and high ICP (Table 2 and Figs. 4 and 8). This accords with the concept that EAAs open ion channels to cause cell swelling and cell death.[37,42,43,46] Second, in vitro studies show that prolonged exposure of postsynaptic receptors to very high concentrations of glutamate (50-100 mol/L) in mixed cell cultures, for periods as short as a few hours, will kill neurons.[13,20,42] Our studies show that approximately 30% of our patients with severe TBI manifested glutamate levels in the extracellular fluid that were at least double this concentration over many days. Third, the fact that these high dialysate concentrations are sustained for up to several days, in marked contrast with animal models, suggests that glutamate uptake mechanisms in presynaptic dendritic membranes and in astrocytes are nonfunctional, thus allowing such pathologically high EAA levels to persist in the extracellular fluid.[41] The role of neurotrauma in inactivating these "intrinsically neuroprotective" glutamate reuptake mechanisms requires further study.

The clearest implication of this study for future therapy of head-injured patients concerns the use of EAA antagonist drugs. Currently, at least six glutamate antagonists of different types are in clinical testing for different indications, including severe head injury.[5,7,37] However these agents carry an important side-effect profile, including effects such as hallucinations, paranoia, agitation, and sedation. This study suggests that, for the majority of patients in a coma after head injury who have a normal CT scan appearance and have not sustained secondary ischemic events, prolonged therapy with a glutamate antagonist is probably not warranted. A single dose administered as soon as possible after the injury may be sufficient to blunt the consequences of the immediate injury-related surge in EAAs, which has been shown in animal models.[20,21,24,25,32,35,40,44, 48,55] However, it is also clear from this study that patients with focal cerebral contusions and those with secondary ischemic events such as low CPP, raised ICP, hypoxemia, or reduced CBF would benefit from therapy with these agents for at least 4 days or more. Studies focusing on even later time points postinjury are needed to determine the longer time course of these events.

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