Increases in GABA concentrations during cerebral ischaemia: a microdialysis study of extracellular amino acids

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The excitatory amino acids glutamate and aspartate are thought to play an important part in the pathogenesis of neuronal damage and death in acute cerebral ischaemia.^{1–9} In association with reduced oxygen and substrate (glucose) delivery and increased production of protons and lactate, excessive accumulation of these amino acids results in potential pathological activation of receptors and increased calcium entry into cells. Cytoplasm swelling, mitochondrial dysfunction, and membrane disintegration occur, culminating in cell death. These processes were initially demonstrated in animal models and are currently undergoing investigation in humans. Microdialysis is currently being applied to monitor the extracellular chemistry of the brain of neurosurgical patients.^{10–17} Studies in patients with head injury and subarachnoid haemorrhage have disclosed increases of the extracellular concentrations of excitatory amino acids in response to secondary ischaemic events such as intracranial hypertension, systemic hypotension, seizures, and contusions.^{11–13 16 18–20}

By contrast, less attention has been applied to the inhibitory amino acids, notably γ -aminobutyric acid (GABA), despite experimental studies of cerebral ischaemia showing that glutamate and aspartate rises may be accompanied by changes in the concentration of other amino acids^{21–36} and increases in GABA concentrations in the CSF of patients with head injury.³⁷ Although GABA was measured in the first reported human brain microdialysis study performed during thalamotomy³⁸ and changes in GABA concentrations have been shown during seizures,^{16 39} after trauma,⁴⁰ and in lobectomy models of ischaemia,^{41 42} clinical microdialysis studies have concentrated on the excitatory amino acids.

Objectives: Increases in the extracellular concentration of the excitatory amino acids glutamate and aspartate during cerebral ischaemia in patients are well recognised. Less emphasis has been placed on the concentrations of the inhibitory amino acid neurotransmitters, notably γ -aminobutyric acid (GABA), despite evidence from animal studies that GABA may act as a neuroprotectant in models of ischaemia. The objective of this study was to investigate the concentrations of various excitatory, inhibitory and non-transmitter amino acids under basal conditions and during periods of cerebral ischaemia in patients with head injury or a subarachnoid haemorrhage.

Methods: Cerebral microdialysis was established in 12 patients with head injury (n=7) or subarachnoid haemorrhage (n=5). Analysis was performed using high performance liquid chromatography for a total of 19 (excitatory, inhibitory and non-transmitter) amino acids. Patients were monitored in neurointensive care or during aneurysm clipping.

Results: During stable periods of monitoring the concentrations of amino acids were relatively constant enabling basal values to be established. In six patients, cerebral ischaemia was associated with increases (up to 1350 fold) in the concentration of GABA, in addition to the glutamate and aspartate. Parallel increases in the concentration of glutamate and GABA were found ($r=0.71$, $p<0.005$).

Conclusions: The results suggest that, in the human brain, acute cerebral ischaemia is not accompanied by an imbalance between excitatory and inhibitory amino acids, but by an increase in all neurotransmitter amino acids. These findings concur with the animal models of ischaemia and raise the possibility of an endogenous GABA mediated neuroprotective mechanism in humans.

The objective of this study was to apply microdialysis to measure the extracellular concentrations of inhibitory, excitatory, and non-transmitter amino acids in patients at risk of cerebral ischaemia after head injury and subarachnoid haemorrhage. We aimed to establish normal values of amino acids under basal conditions and changes in concentrations during episodes of cerebral ischaemia.

METHODS

Patient selection

The study was approved by the local research ethics committee and consent was obtained from the patient or next of kin. Patients with severe head injury and subarachnoid haemorrhage were eligible. Patients with severe head injury were monitored on the neurointensive care unit. Those with aneurysmal subarachnoid haemorrhage were monitored during aneurysm clipping in the operating theatre, and were preselected on the basis of anticipated difficulty in surgery (for example, giant aneurysms requiring temporary clipping or induced hypotension).

Microdialysis technique

The microdialysis catheter (CMA 70, CMA microdialysis, Stockholm, Sweden) was inserted into the cerebral cortex in conjunction with an intracranial pressure transducer (Codman, Raynham, MA, USA) and Neurotrend (Codman,

Abbreviations: GABA, γ -aminobutyric acid; HPLC, high performance liquid chromatography; CPP, cerebral perfusion pressure; ICP, intracranial pressure

Figure 1 Triple lumen cranial access device transmitting the intracranial pressure transducer, microdialysis catheter and Neurotrend sensor into the cerebral parenchyma (with permission).

Raynham, MA, USA) multiparameter sensor via a specially designed cranial access device (triple bolt; Technicam, Newton Abbot, UK, fig 1) as described previously.⁴³ Microdialysis was performed using Ringer's solution (K^+ 4 mM, Na^+ 147 mM, Ca^{2+} 2 mM, Cl^- 155 mM) in the operating theatre (flow rate 1.0 μ l/min, collection time 10 minutes) and neurointensive care unit (flow rate 0.3 μ l/min, collection time 30–60 minutes). Vials were analysed on line using the CMA600 microdialysis analyser and then stored at -70°C . Selected vials collected both under basal quiescent conditions and during periods of cerebral ischaemia (defined by intracranial hypertension, systemic hypotension, hypoxia) were then analysed for amino acids using high performance liquid chromatography (HPLC).

High performance liquid chromatography analysis

A 1 μ l sample was diluted (1:20) and 5 μ l then mixed with 3 μ l buffer containing norvaline (internal standard) and 3 μ l orthophthaldialdehyde /3-mercaptopropionic acid. The HPLC system consisted of two high pressure pumps with a gradient controller and mixing chamber (Micro-Tech Scientific, Sunnyvale, CA, USA). The amino acids were separated using a C18 column and detected using a Gilson model 122 fluorometer (Gilson, Middleton, WI, USA) fitted with a 100 nl fluoro-booster flow cell.

Analysis of results

The results were analysed by calculating the mean value of amino acid concentrations under basal conditions and changes as a result of ischaemic insults expressed as ratios (ischaemia value/baseline value). Comparison between glutamate and GABA microdialysate concentrations was made using Spearman's correlation analysis.

RESULTS

Patient population

Samples from 12 patients (seven male and five female) were analysed for extracellular amino acids using the HPLC system. Seven patients with severe head injury were monitored on the neurointensive care unit and five patients with subarachnoid haemorrhage were monitored during surgery for clipping of an aneurysm (three of whom had a temporary clip as part of the procedure).

Case illustrations

Illustrative case history 1

An 18 year old man with severe head injury (Glasgow coma score⁴⁴ 3) had a secondary insult with intracranial hypertension and CT showing cerebral swelling and low attenuation areas indicative of cerebral ischaemia. Despite maximum

Figure 2 Severe head injury: illustrative case history 1 demonstrating secondary insult (arrow) with increase in intracranial pressure (ICP) and decrease in cerebral perfusion pressure (CPP) showing decrease in extracellular glucose, increase in lactate and increase in the excitatory amino acids glutamate and aspartate. Note that GABA was undetectable until the insult, then increased concomitantly with glutamate and then decreased to undetectable concentrations. The glutamate and aspartate concentrations did not return to the preinsult baseline but remained increased.

treatment the patient succumbed. Microdialysis showed profound disturbances in metabolism (fig 2) with low glucose and increased lactate concentrations. The amino acid changes are shown in table 1 with increases in glutamate, aspartate, glycine, GABA, methionine and arginine. The concentration of glutamine decreased and there were less pronounced changes in the other amino acids.

Table 1 Microdialysis amino acid analysis in a patient with severe head injury (illustrative case history 1) showing baseline values, values during ischaemic insult, and ratio (ischaemia value/baseline value) (CMA70 microdialysis catheter 10 mm membrane perfused with Ringer's solution at 0.3 µl/min). Valine and histidine were not available in this patient. Controversy surrounds the inhibitory role of glycine and taurine

Amino acid type	Amino acid name	Base line value (µM)	Ischaemia value (µM)	Post- ischaemia value (µM)	Ratio (ischaemia value/baseline value) >1 increase <1 decrease
Excitatory	Glutamate	6.77	228.1	42.8	33.7
	Aspartate	2.32	32.6	10.8	14.1
Inhibitory	GABA	0.06	35.7	0.08	595
	Glycine	16.3	56.0	40.8	3.4
	Taurine	8.6	18.7	13.0	2.2
Non-transmitter	Alanine	10.0	18.7	13.0	1.9
	Arginine	34.6	184.5	135.9	5.3
	Asparagine	5.20	15	11.9	2.9
	Citrulline	1.59	6.9	4.67	4.3
	Glutamine	1738	931.5	1264	0.5
	Methionine	36.8	191.1	59.9	5.2
	Phenylalanine	22.8	78.5	45.6	3.4
	Serine	36.1	86.4	43.4	2.4
	Threonine	24.0	99.3	87.5	4.1
	Tryptophan	0.69	2	0.97	2.9
	Tyrosine	12.3	40.3	33.1	3.3

Illustrative case history 2

A 25 year old man with severe head injury (Glasgow coma score 7) was monitored with a microdialysis catheter placed in the region of a contusion (fig 3). The patient survived with a left hemiparesis (Glasgow outcome score⁴⁵ category severe disability). Microdialysis (fig 3) showed extremely low glucose concentrations (mean 0.01 mM), increased lactate concentrations (mean 5.73 mM) and increased glutamate (mean 62.9 µM, range 17.8–230 µM) and aspartate (mean 20.4 µM, range 10–112 µM) concentrations. There were also increased concentrations of GABA (mean 0.23 µM, range <0.002–1.0 µM), glycine (mean 161 µM, range 35.6–513 µM) and taurine (mean 29.3 µM, range 3.6–150 µM). Other amino acids were essentially unchanged.

Illustrative case history 3

A 41 year old woman presented with World Federation of Neurological Societies⁴⁶ grade II subarachnoid haemorrhage due to rupture of a basilar artery aneurysm. During clipping of this aneurysm a combination of hypotension and hydrocephalus resulted in cerebral ischaemia with parenchymal brain oxygen levels of 2 mm Hg. Microdialysis detected a reduction in brain glucose from 0.78 mM to 0 mM and increase in brain lactate from 0.97 mM to 3.5 mM associated with changes in amino acid concentration (table 2, fig 4). There was a large rise in glutamate and aspartate accompanied by a substantial rise in the concentration of GABA. Changes in concentration of the non-transmitter amino acids varied from no changes to a twofold increase. These changes in amino acid concentrations returned to the baseline after insertion of an external ventricular drain and the patient made a full recovery (Glasgow outcome score category good recovery).

Summary of results

For the patients with head injury, the mean values under basal conditions and values during ischaemia of the major excitatory and inhibitory amino acids (with non-transmitter amino acids for comparison) monitored with a 10 mm membrane microdialysis catheter at 0.3 µl/min on the neurointensive care unit are shown in table 3. The concentration of glutamine was considerably higher than that of the other

amino acids, and the concentration of GABA considerably lower. For the patients with subarachnoid haemorrhage, aneurysm clipping was complicated by an acute episode of ischaemia in three patients. The changes in amino acid concentrations in these patients are shown in table 2.

Of the 12 patients, cerebral ischaemia due to hypotension, intracranial hypertension, or contusions was detected in six. In these patients, characteristic patterns of changes in amino acid concentration were seen. Increased concentrations of glutamate and aspartate were accompanied by large increases in the concentration of GABA. There were less pronounced increases in the concentrations of glycine and taurine. Overall, there was a strong correlation between the concentrations of glutamate and GABA ($r=0.71$ $p<0.005$). The concentrations of the non-transmitter amino acids threonine and valine remained stable or showed modest increases.

DISCUSSION

We have found in this study that during basal quiescent conditions, the concentrations of amino acids tended to be stable. There were consistently higher concentrations of glutamine than the other amino acids. Overall glutamate concentrations were somewhat higher than the value accepted as "normal" for human cerebral extracellular glutamate (2 µM).¹² During cerebral ischaemia, the concentration of glutamate and aspartate increased confirming other clinical microdialysis studies.^{11–13 19 20} Direct comparisons of concentrations between studies are difficult due to variation in flow rates and membrane lengths.⁴⁷ Concentrations of GABA under basal conditions were extremely low (<0.002 µM to 0.02 µM). However, during periods of ischaemia, there were large increases in the concentration of GABA. There was a strong correlation between the concentrations of glutamate and GABA, indicating that, in the human brain, cerebral ischaemia is associated with an increase in both excitatory and inhibitory amino acids.

It is unlikely that the change in either glutamate or GABA can be attributed to concomitant medication. Propofol is used routinely in the operating theatre and neurointensive care unit and has been shown to possess GABA agonist

Figure 3 Severe head injury: illustrative case history 2. (A) CT showing pericontusional location of monitoring probes (arrow); (B) H_2^{15}O PET showing reduced blood flow in the region of contusion (threshold from 0–60 ml/100 mg/min); (C) $^{15}\text{O}_2$ PET showing reduced oxygen metabolism in the region of contusion (threshold from 0–200 $\mu\text{mol}/100\text{g}/\text{min}$); (D) microdialysis results showing increases in glutamate, aspartate and GABA concentrations in the region of the contusion. Note the sustained rise in the lactate/pyruvate ratio.

properties.^{48–53} The rate of administration of propofol remained constant during the episodes of cerebral ischaemia and the pattern of changes which we have found in these patients resemble the findings of animal models of ischaemia which were performed in the absence of propofol.^{21–34}

The origin of increased concentrations of glutamate and other amino acids in association with acute cerebral ischaemia is unclear. Explanations include excessive release of glutamate and impaired reuptake as a consequence of neuronal damage.^{54–58} Microdialysis studies have reported a neuronal origin of GABA with a portion of the basal concentration derived from neurotransmission.^{59–60} The increase in extracellular GABA concentrations during ischaemia may be due to excessive release resulting from membrane damage.³⁷ However, it is possible that the GABA detected is the consequence of increased production and release of GABA in an attempt to counteract the excitatory amino acid surge as an endogenous protective mechanism. This is supported by the difference in the increase in the GABA concentrations compared to the other amino acids and evidence that GABA inhibits glutamate

release via pre-synaptic receptors.⁶¹ This concurs with the data from animal studies that treatments which potentiate the action of GABA reduce neuronal injury.^{62–68} Further studies are, however, required to support this concept of specificity of a neuroprotective mechanism for GABA in humans, particularly to determine whether the increase in GABA is greater than that of the non-transmitter amino acids in the context of GABA, glutamate, and aspartate being the most abundant tissue amino acids.

These findings raise the possibility of a role for GABA agonists or GABA uptake inhibitors in the treatment of patients at risk from cerebral ischaemia—for example, stroke, subarachnoid haemorrhage, and head injury.^{33–69–72} Chlormethiazole has been shown to possess a GABA agonist action and is currently undergoing investigation in patients with stroke.⁷³ Propofol, also shows GABA agonist properties and our results may therefore support the use of this drug in acute cerebral ischaemia.⁴⁸ However, it is becoming increasingly clear that single amino acids cannot be considered in isolation, and the importance of potential endogenous GABA neuroprotection

Table 2 Changes in excitatory, inhibitory, and non-transmitter amino acid concentrations after an acute ischaemic insult in three patients (A, B, C) during aneurysm surgery (CMA70 10 mm catheter perfused with Ringer's solution at 1 $\mu\text{l}/\text{min}$)

Amino acid type	Amino acid name	Patient A concentration (μM)		Patient B concentration (μM)		Patient C concentration (μM) illustrative case 3	
		Baseline $\rightarrow$ ischaemia	Ratio	Baseline $\rightarrow$ ischaemia	Ratio	Baseline $\rightarrow$ ischaemia	Ratio
Excitatory	Glutamate	18 $\rightarrow$ 49	2.7	7.8 $\rightarrow$ 30	3.8	11 $\rightarrow$ 93	8.1
	Aspartate	5.4 $\rightarrow$ 10	1.9	9.6 $\rightarrow$ 14	1.5	7.8 $\rightarrow$ 19	2.5
Inhibitory	GABA	0.08 $\rightarrow$ 0.82	10	0.14 $\rightarrow$ 0.95	6.8	0.01 $\rightarrow$ 14	1350
	Glycine	25 $\rightarrow$ 23	0.9	22 $\rightarrow$ 38	1.7	17 $\rightarrow$ 18	1.1
	Taurine	14 $\rightarrow$ 10	0.7	1.5 $\rightarrow$ 6.5	4.3	10 $\rightarrow$ 18	1.7
Non-transmitter	Alanine	33 $\rightarrow$ 31	0.9	NA		14 $\rightarrow$ 22	1.6
	Arginine	11 $\rightarrow$ 8.7	0.8	NA		9.2 $\rightarrow$ 10	1.1
	Asparagine	3.5 $\rightarrow$ 3.0	0.9	2.8 $\rightarrow$ 6.0	2.1	8.4 $\rightarrow$ 22	2.6
	Citrulline	2.2 $\rightarrow$ 1.6	0.7	1.5 $\rightarrow$ 4.6	3.1	1.5 $\rightarrow$ 1.6	1.0
	Glutamine	171 $\rightarrow$ 119	0.7	87 $\rightarrow$ 199	2.3	151 $\rightarrow$ 240	1.6
	Histidine	18 $\rightarrow$ 12	0.7	5.7 $\rightarrow$ 13	2.3	1.2 $\rightarrow$ 0.01	0.01
	Methionine	2.7 $\rightarrow$ 2.7	1	1.8 $\rightarrow$ 5.3	2.9	3.9 $\rightarrow$ 5.3	1.4
	Phenylalanine	8.0 $\rightarrow$ 6.6	0.8	NA		7.6 $\rightarrow$ 15	2.0
	Serine	14 $\rightarrow$ 14	1.0	32 $\rightarrow$ 51	1.6	17 $\rightarrow$ 15	0.9
	Threonine	29 $\rightarrow$ 40	1.4	10 $\rightarrow$ 25	2.5	7.6 $\rightarrow$ 8.2	1.1
	Tryptophan	2.5 $\rightarrow$ 1.9	0.8	4.9 $\rightarrow$ 14	2.9	3.4 $\rightarrow$ 4.7	1.4
	Tyrosine	7.3 $\rightarrow$ 5.9	0.8	6.3 $\rightarrow$ 12	1.9	4.6 $\rightarrow$ 5.3	1.2
	Valine	32 $\rightarrow$ 29	0.9	15 $\rightarrow$ 44	3.0	21 $\rightarrow$ 24	1.2

The number preceding the arrow represents the baseline value, the number after the arrow represents the value during the ischaemic insult. The ratio is the ischaemia value/baseline value. Note the relatively larger increases in GABA concentration compared with the other amino acids. Patient C is illustrative case history 3. Alanine, arginine, and phenylalanine measurements were not available (NA) in patient B.

may help to explain the lack of efficacy of the phase III clinical trials using glutamate antagonist drugs in head injury.⁷⁴⁻⁷⁸

In conclusion, this study shows that monitoring brain extracellular neurotransmitter amino acid concentrations in patients using microdialysis is feasible and safe. Our results confirm the occurrence of increases in excitatory amino acid concentrations in patients with cerebral ischaemia and show that these increases are associated with increases in the inhibitory amino acids. These findings raise the possibility of an endogenous GABA mediated neuroprotective mechanism and a potential indication for the use of exogenous GABA agonists in the treatment of cerebral ischaemia in stroke, head injury, and subarachnoid haemorrhage. Further investigation using a combined basic experimental and clinical approach is

required to substantiate the nature of a GABA-mediated neuroprotective action, in particular to determine specific patterns and time course of production and release into the extracellular space.

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Figure 4 Subarachnoid haemorrhage aneurysm clipping: illustrative case history 3 showing the time course of change in amino acid concentrations during an acute episode of ischaemia (arrow) demonstrating reversible increases in glutamate, aspartate, and GABA. There were no significant changes in the concentration of the non-transmitter amino acids threonine and valine.

Table 3 Mean (basal conditions) and maximum (ischaemia) values for amino acids in patients with head injury using a CMA70 microdialysis catheter (10 mm membrane) perfused with Ringer's solution at 0.3 $\mu\text{l}/\text{min}$

Amino acid type	Amino acid name	Basal concentration μM	SD	Ischaemia concentration μM
Excitatory	Glutamate	15.5	7.37	230
	Aspartate	4.45	2.29	112
Inhibitory	GABA	0.24	0.36	35.7
	Glycine	69.9	26.1	523
	Taurine	9.0	12.3	172
Non-transmitter	Alanine	99.4	35.6	273
	Arginine	26.9	14.7	203
	Asparagine	25.5	6.78	76.9
	Citrulline	4.24	1.98	27.3
	Glutamine	1379	409.8	3955
	Histidine	65.0	28.9	163
	Methionine	19.8	5.43	170
	Phenylalanine	48.1	20.5	181
	Serine	53.9	20.2	148
	Threonine	72.9	15.8	218
	Tryptophan	9.30	4.43	77.5
	Tyrosine	29.5	12.1	100.3
	Valine	109	50.0	385

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NEUROLOGICAL STAMP.....

Julius Wagner-Jauregg (1857–1940)

Wagner-Jauregg began his medical studies at the University of Vienna in 1874. His career was rooted in the new fields of microscopy and experimental biology. He met and became a lifelong friend of Sigmund Freud. For a short time after leaving the institute he worked in the Department of Internal Medicine at the University of Vienna. He accepted a job in the psychiatric clinic. Even though he had no training in psychiatry or neurology he became greatly interested in the fields.

From 1889 to 1893 he was professor of psychiatry and neurology at the University of Graz. Here he became interested in the relation between cretinism and goitre. He proposed that the disease was due to thyroid deficiency and could be treated by the addition of small amounts of iodide to salt. In 1923 the Austrian government implemented the sale of iodised salt.

In 1883, during residency at the Vienna asylum, Wagner-Jauregg noted that a female patient who had contracted erysipelas experienced a remission of her psychoses. This aroused his interest. In 1890 Robert Koch developed tuberculin, which was considered to be a vaccine supposedly effective against tuberculosis. Wagner-Jauregg injected tuberculin into several patients whose psychotic symptoms were caused by neurosyphilis. He thought that if he gave them tuberculous fever, the fever would arrest the progress of neurosyphilis, on the grounds that the spirochaetes were heat sensitive. By 1909 he was regularly obtaining long term remission of neurosyphilis through the use of tuberculin. He abandoned the experiments because tuberculin was considered to be too toxic. Wagner-Jauregg then returned to the possibility of giving patients a fever with malaria, which unlike other infections had the advantage of being controlled with quinine.

In 1917 he proposed a new treatment for general paresis of the insane, which was then a relatively common disorder. In that year he inoculated nine patients with general paresis of the insane with tertian malaria and later reported that in six of these extensive remissions followed. For this work he received the Nobel prize for medicine or physiology in 1927. His ideas of non-specific therapies to increase the body's defences were in conflict with the "magic bullet" concept advanced by Paul Ehrlich.

Wagner-Jauregg was honoured philatelically by Austria in 1957, the 100th year of his birth (Stanley Gibbons 1289, Scott 615).

L F HAAS