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Does glutamate mediate brain damage in acute encephalitis?

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CEREBROSPINAL fluid (CSF) amino acid neurotransmitter concentrations in 23 patients with acute encephalitis were compared with those in patients with acute brain infarction, multiple sclerosis and controls. The concentration of glutamate was significantly higher in encephalitis (5.2 ± 6.7 μmol/l) and stroke patients (9.6 ± 14.2 μmol/l) than in MS patients (1.6 ± 0.9 μmol/l) and controls (1.7 ± 0.8 μmol/l; $p < 0.001$). The concentration of glycine was significantly higher in encephalitis (11.0 ± 4.7 μmol/l) than in stroke (7.6 ± 3.2 μmol/l) and MS patients (6.3 ± 2.1 μmol/l) or controls (5.6 ± 1.8 μmol/l; $p < 0.002$). Taurine levels were significantly lower in encephalitis patients than in the other groups ($p = 0.04$). The correlation of high glutamate levels with poor outcome was almost significant (Kendall tau 0.63, $p = 0.06$). Our observations suggest that exicitotoxic neurotransmission may play an important role in the series of events that lead to neuronal damage in encephalitis.

Key words: Brain damage; Encephalitis; Glutamate; Multiple sclerosis; Stroke

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Introduction

Excitatory neurotransmitters such as glutamate and aspartate excite neuronal depolarisation,[1,2] which is postulated to cause neuronal destruction during epilepsy[3,4] ischemia and subarachnoidal haemorrhage.[5–8] Glutamate is also believed to be an important proximal mediator in the cascade of events leading to brain damage in stroke, subarachnoidal haemorrhage and migraine.[7–10] The pathological role of glutamate in stroke has been studied clinically, and several drugs with glutamine antagonistic properties have been tested for presumed neuroprotective action.[11–15] In animal models of focal ischemia, and in a few clinical studies, increased levels of aspartate, taurine, glycine and glutamate have been demonstrated in the brain or in the CSF.[16–18]

By binding to the NMDA or AMPA receptors, glutamate causes ion fluxes, particularly calcium influx across the cell membrane. The activation of the NMDA receptor complex requires also the presence of glycine,[19,20] but elevated glycine levels do not necessarily cause abnormal neuronal function.[21] However, blocking of the modulatory glycine binding site of the NMDA complex may protect from the toxic action of glutamate or glycine.[19,22]

We recently presented data showing that in herpes simplex encephalitis the neuronal damage as shown by [^{123}I]iomazenil scans occurs relatively early during the course of disease, whereas hyperperfusion indicating inflammation of the parenchyma lasts considerably longer.[23] There is as yet no explanation for this sequence of events. We postulated that early inflammatory reactions in the brain release excitatory amino acids, causing early neuronal damage in encephalitides. Therefore we studied the concentrations of excitatory and inhibitory amino acid neurotransmitters in the CSF of patients with acute encephalitis and compared these data with other types of acute and chronic CNS damage.

Patients and Methods

Patients with encephalitis: We studied 23 patients with acute encephalitis (age 48.1 ± 15.9 years, eight males and 15 females). There were six patients with herpes encephalitis, two patients with zoster encephalitis, two with adenovirus encephalitis, one patient with tuberculous encephalitis and 12 patients whose virological diagnosis could not be established. The diagnosis of encephalitis was established as described elsewhere.[23,24] All CSF samples were taken within the first 48 h after admission to hospital and not later than 4 days after the first mental or neurological symptoms. All patients with encephalitis were treated with acyclovir for 10–14 days. Twelve patients had epileptic seizures, which were treated by i.v. diazepam. None of the patients were given other antiepileptics at the time of the first lumbar

puncture. The state of consciousness, number of epileptic seizures, presence of focal neurologic signs (including focal neuroradiological findings), fever and headache were recorded and correlated with the CSF results. Outcome was measured in terms of activities in daily living using the Rankin score. Neuropsychological evaluation including the Wechsler Adult Intelligence Scale (WAIS) and the Wechsler Memory Scale (WMS) was performed on 13 patients.[25,26]

Disease-positive controls: We also studied the CSF of 10 patients with newly diagnosed multiple sclerosis (MS; age 33.3 ± 9.4 years, four males and six females) and nine patients with acute brain infarction (age 65.4 ± 14.0 years, three males and six females) with either large middle cerebral artery area infarction ($n = 8$) or basilary artery thrombosis ($n = 1$) as disease-positive controls. In the stroke cohort the CSF was sampled within 24 h after the onset of ischemic symptoms.

Normal controls: Thirty-eight normal controls (age 41.4 ± 12.2, 16 males and 22 females) were chosen from patients referred to our hospital's neurological out-patient clinic for ruling out demyelinating disease or neuroborreliosis. These patients had normal findings in both neuroradiological and laboratory investigations. Informed consent was obtained for an extra 2 ml CSF sample to be taken after the routine diagnostic CSF.

CSF samples were stored in 2 ml Eppendorf tubes, frozen immediately, and kept at −70°C until analysis. None of the samples were contaminated with blood and all patients were normally ventilated at the time of lumbar puncture.[27,28]

CSF concentrations of glutamate, glycine, and taurine, aspartate, as well as 32 other amino acids in the CSF were measured by an automatic amino acid analyser (Biochrom 20, Pharmacia LKB Biochrom Ltd. Cambridge, England). Those amino acids that exceeded the detection limit are given in Table 1. Unfortunately, this system is not sensitive enough to detect GABA or N-acetylaspartate at the concentrations present in the CSF.

Statistics: To avoid the problems involved in multiple testing we used multiple analysis of variance (MANCOVA) with the age and CSF protein concentration (because the function of the blood brain barrier may affect the transmitter concentrations) as covariates. The CSF glucose level was initially included as a covariate, but as it had no effect on the results this covariate was discarded. To determine which group means were statistically significantly different, the Duncan *post hoc* test was used. Correlations were calculated using nonparametric Kendall Tau statistics.

Table 1. Mean CSF concentrations (μmol/l) of 23 amino acids and *p* levels,for the main effects in multiple analysis of variance with covariates.

Amino acid	Encephalitis ($n = 23$)	MS ($n = 10$)	Infarction ($n = 9$)	Normal controls ($n = 36$)	*p*
Alanine	39.9 ± 14.7	29.4 ± 8.3	44.1 ± 18.0	33.2 ± 8.0	0.42
α-Amino adipinic acid	0.5 ± 1.1	1.1 ± 1.1	0.6 ± 0.5	0.8 ± 1.1	0.49
α-Amino butyrate	5.3 ± 3.9	2.2 ± 1.1	4.0 ± 1.9	2.1 ± 1.1	0.09
Arginine	17.6 ± 5.8	17.0 ± 4.8	16.2 ± 4.2	20.7 ± 3.9	0.006
Asparagine	13.8 ± 11.5	6.7 ± 2.0	7.1 ± 2.1	7.1 ± 2.0	0.66
Citrulline	1.8 ± 1.4	0.8 ± 1.0	1.8 ± 1.1	1.3 ± 1.3	0.62
Glutamate	5.2 ± 6.7	1.6 ± 0.9	9.6 ± 14.2	1.7 ± 0.8	0.03
Glutamine	469.3 ± 72.8	364.8 ± 48.0	431.6 ± 65.6	437.3 ± 55.1	0.003
Glycine	11.0 ± 4.7	6.3 ± 2.1	7.6 ± 3.2	5.6 ± 1.8	0.009
Histidine	14.1 ± 3.9	12.0 ± 1.9	13.8 ± 3.3	13.4 ± 2.5	0.35
Isoleucine	4.4 ± 4.4	3.5 ± 1.8	7.1 ± 5.3	3.9 ± 2.1	0.19
Leucine	13.6 ± 9.5	9.9 ± 3.6	17.2 ± 10.2	12.0 ± 3.5	0.51
L-Methylhistidine	1.4 ± 2.9	1.0 ± 1.3	1.4 ± 2.2	0.8 ± 1.6	0.81
Lysine	26.0 ± 8.2	22.0 ± 4.8	27.1 ± 7.5	28.1 ± 6.3	0.008
Methionine	2.6 ± 1.9	2.1 ± 0.6	1.9 ± 0.8	2.4 ± 1.1	0.33
Ornitine	5.3 ± 2.7	4.2 ± 0.8	5.0 ± 1.3	5.2 ± 1.4	0.51
Phenylalanine	12.5 ± 4.2	8.4 ± 2.1	13.7 ± 3.8	10.0 ± 2.2	0.21
Phosphoserine	2.0 ± 0.7	1.7 ± 0.5	2.3 ± 0.7	2.0 ± 0.7	0.62
Phosphoethanolamine	5.2 ± 2.4	4.8 ± 1.5	5.0 ± 2.2	6.0 ± 2.7	0.41
Serine	22.3 ± 7.6	22.7 ± 2.7	25.2 ± 6.1	27.1 ± 4.8	0.06
Taurine	7.7 ± 3.1	7.8 ± 1.8	9.7 ± 3.6	9.1 ± 1.6	0.003
Threonine	30.7 ± 12.4	22.7 ± 5.2	29.2 ± 9.5	33.5 ± 11.0	0.07
Tyrosine	9.0 ± 3.3	6.7 ± 3.1	10.8 ± 3.8	9.2 ± 2.4	0.20
Valine	22.1 ± 11.0	14.5 ± 5.9	24.8 ± 10.9	17.4 ± 5.1	0.39
Covariate: age	49.6 ± 17.3	33.6 ± 9.5	65.4 ± 14.0	41.0 ± 12.2	
Covariate: CSF protein (mg/l)	809 ± 500	404 ± 154	478 ± 211	354 ± 126	

Ethics: The extra CSF sampling for both the controls as well as the patients was approved by the ethics committee of the Department of Neurology of the Helsinki University Central Hospital.

Results

The concentrations of transmitters in the CSF and the p levels of the main effect in the multiple analysis of variance are given in Table 1.

Multiple ANOVA suggested highly significant differences between the groups (Rao's $R = 2.14$, $p = 0.00005$). Thus we tested the main effects and found the concentrations of arginine ($p = 0.006$), glutamate ($p = 0.028$), glutamine ($p = 0.003$), glycine ($p = 0.009$), lysine ($p = 0.008$) and taurine ($p = 0.003$) to be significantly different in at least one of the diagnostic groups. These differences were then tested using the Duncan *post hoc* analysis.

The concentration of glutamate was significantly higher in acute encephalitis and in stroke than in the other groups ($p < 0.001$ for all comparisons, see Table 1 for values) but the concentrations in encephalitis and stroke did not differ significantly. The concentration of glycine was significantly higher in the encephalitis group than in the other groups ($p < 0.002$ for all comparisons, see Table 1 for values). The concentration of taurine was significantly lower in the encephalitis and multiple sclerosis groups ($p = 0.035$ and $p = 0.044$, respectively, see Table 1 for values). The distributions of CSF concentrations of glutamate, glycine and taurine are illustrated in Fig. 1.

The arginine concentration was somewhat higher in the control group than in the other groups. Glutamine and lysine concentration were significantly lower in the MS group. These changes were considered irrelevant for this study.

The CSF concentration of glutamate did not correlate significantly with any clinical variable. However there was an almost significant correlation with outcome ($Tau = 0.63$, $p = 0.06$). Among the seven patients with the highest glutamate concentrations there were two cases of adenovirus encephalitis, two cases of herpes encephalitis, one case of zoster encephalitis and only two unidentified cases. The two herpes encephalitis cases with the highest glutamate concentrations had the highest grade of disability among the encephalitis patients at discharge, although there was no difference in the delay of antiviral medication. Glutamate concentrations did not correlate with epileptic seizures. There were no correlations of glutamate levels and neuropsychological test results in the 13 patients tested.

High CSF concentrations of glycine were related to a more favorable outcome measured by the Rankin

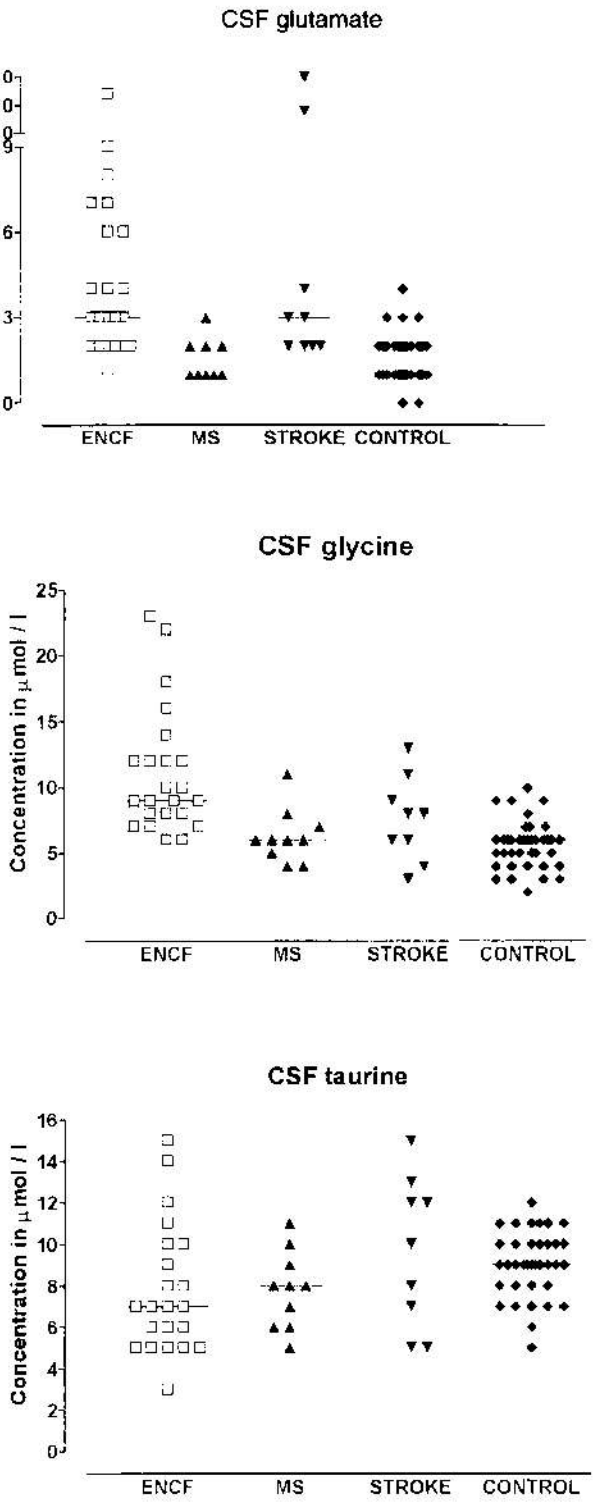

FIG. 1. CSF glutamate, glycine, and taurine concentrations in 23 patients with encephalitis, 10 patients with newly diagnosed multiple sclerosis, 9 patients with acute stroke and 38 healthy controls.

score (Tau = 0.35, $p = 0.023$), and absence of headache at admission (Tau = 0.41, $p = 0.008$).

High CSF concentrations of taurine coincided with epileptic seizures (Tau = 0.38, $p = 0.011$) and absence of fever at admission (Tau = 0.48, $p = 0.002$). Low concentrations of taurine were significantly associated with an unfavorable Glasgow Coma Scale score (Tau = 0.33, $p = 0.041$) and the presence of focal hyperperfusion in the initial HMPAO SPECT scan (Tau = 0.35, $p = 0.029$).

Discussion and Conclusion

We present here evidence of increased glutamate release in encephalitis. The glutamate concentration in encephalitis was elevated similarly as in ischemic stroke where excessive glutamate release is considered a major cause of neuronal damage. The CSF concentrations of several other amino acids than glutamate and glycine were statistically not significantly different in any of the groups. Thus, our findings cannot be explained by brain parenchyma damage *per se*.

There were no significant changes in the CSF concentrations of most of the analysed amino acids† (Table 1). Therefore the elevation of CSF amino acid concentrations is not caused by the disruption of the blood–brain barrier either. We expected to observe a significantly higher glutamate concentration in stroke than in encephalitides due to the extensive acute neuronal damage in brain infarction and the postulated crucial role of glutamate in cerebral ischemia;[5] however the concentrations of glutamate were comparable in stroke and in encephalitis. Since the tissue damage in our patients with encephalitis was less extensive than in those with large MCA area infarctions, yet the glutamate concentrations were comparable, we conclude that the glutamate concentration increase in the CSF in encephalitis is not caused by focal neuronal damage alone. Rather it may indicate that significant amounts of glutamate is released by an early inflammatory response in encephalitis. The mechanism of this neuronal stimulation and release of glutamate could involve liberation of proinflammatory cytokines. e.g. interleukin-1 or interleukin 6, which have been shown to facilitate excitation of neuronal membranes.[29,30]

Release of glutamate may rapidly kill neurons due to shortage of ATP in ischemic brain, where vascular occlusion prevents a compensatory perfusion increase. In encephalitis, however, the blood supply to the brain is not significantly decreased, but the infected tissue may even increase its perfusion.[24] This hyperperfusion in focal encephalitis may be caused by the increased metabolic demand caused by glutaminergic neuronal excitation. Although the neuronal damage in encephalitis occurs rapidly, clinical observation is that the ischemic damage occurs even more rapidly. One reason could be that the neurons tolerate excitotoxicity better in a hyperperfused ATP-rich environment. The association of poor clinical outcome with high CSF glutamate levels, although not quite statistically significant (Tau = 0.29, $p = 0.06$), and the clustering of acutely more severe cases in the high glutamate group supports such an interpretation.

Glutaminergic pathways are abundant in the temporal lobes. Especially in the hippocampus and amygdala excitotoxic neuronal damage may cause severe memory disturbances.[3] In the subgroup of 13 patients assessed neuropsychologically, we could not find significant correlations with glutamate concentrations, however. This may be due to the small group size and the heterogeneity of the tested patients. Seven of them were nearly intact, five had clear memory disturbance and one was psychometrically untestable. The role of glutamate for long-term potentiation and long-term depression i.e. in the development and function of memory is presently actively studied.[31,32] Should glutamate also have a role in the neuronal damage in focal encephalitis especially in herpes encephalitis, it could in part explain why patients with herpes encephalitis are characteristically prone to developing memory disturbances and other cognitive temporal lobe symptoms.

The role of glycine in neuronal damage is not very well understood. Glycine is essential for the neurotoxic action of glutamate through the NMDA-channel, and glycine site blocking agents may eventually prove beneficial in restricting neurological damage caused by excessive neuronal excitation by glutamate. We found that high glycine levels correlated with a more favorable outcome. It would be tempting to assume that in encephalitis the increase in glycine concentration would reflect a compensatory attempt to counteract the action of glutamate.

Lowered concentrations of taurine in the CSF have been previously reported in various diseases. In our patients the CSF taurine concentration was significantly lower in those with encephalitis and multiple sclerosis than in stroke and in the controls. Taurine is considered an inhibitory amino acid transmitter, but its actions are unclear in the adult human.[13,33] Since taurine levels correlated with the initial neurological condition (GCS) the role of taurine in acute neurological deterioration should be further studied.

Caution must be used when trying to study neurochemical mechanisms by sampling CSF from as peripheral a source as the lumbar spinal fluid. The lumbar CSF concentrations of amino acids may not accurately reflect the concentrations in the ventricles or the interstitial space.[34] The best method for

measuring interstitial transmitter concentrations is microdialysis. However, there is no other ethically acceptable method of investigating the amino acid transmitters in encephalitis than lumbar CSF sampling. However, we believe that our results are important, as the changes in the amino acid concentrations in lumbar CSF probably correlate with those in the interstitial fluid of the brain. As glutamate release inhibitors and NMDA receptor antagonists are currently under clinical tests, our data raise important therapeutic considerations in encephalitis and may even make out a case for a clinical trial.

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