

Short report

Putative amino acid transmitters in lumbar cerebrospinal fluid of patients with histologically verified Alzheimer's dementia

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SUMMARY Concentrations of individual free amino acids were determined in lumbar cerebrospinal fluid (CSF) from patients with various complaints including histologically verified Alzheimer's dementia. Glycine and glutamine in the CSF of Alzheimer's dementia samples were lower than that of control samples. Only the concentration of glutamic acid in Alzheimer's dementia patients correlated with psychological measures. The reduction in glycine concentration was not specific for Alzheimer's dementia.

Studies on neurotransmitters in Alzheimer's disease have recently concentrated on monoamines, in particular acetylcholine. This has laid emphasis on the probable role in memory mechanisms of ascending tracts to the neocortex.¹ Indices of several types of neurons intrinsic to the cortex are unaltered so not all neurons seem to be affected.^{2,3} Much less information, however, is available for major descending pathways from the cortex. This is because of the difficulty in examining excitatory amino acid transmitter candidates in human brain.⁴ To provide further information about these substances, lumbar cerebrospinal fluid (CSF) from biopsy-verified examples of Alzheimer disease, as well as several groups of control and other demented patients, have been analysed for the content of free amino-acids.

Materials and methods

An Alzheimer group of 11 patients (five male, six female) were all of presenile age (61 ± 5 , 53-69 years, \pm SD) and had plaque and tangle formation of an intensity consistent with a diagnosis of that disorder in biopsy specimens

obtained by diagnostic craniotomy. Neuroactive drugs were withdrawn at least 1 week before sampling the CSF. Psychological measurements made on nine patients were as previously described;⁵ mild, moderate and severe examples of the disease were represented. Another 22 patients were chronic epileptics, receiving anticonvulsant drugs, described elsewhere.⁶ Twelve epileptic patients (10 male, two female; 40 ± 7 , 33-51 years) were considered to be demented; 10 patients (nine male, one female; 39 ± 11 , 20-54 years) were not demented. The control samples, from 23 patients, consisted of two groups. Group A (six male, six female) was of similar age (62 ± 6 , 53-71 years) to the Alzheimer group and group B (five male, six female) was of similar age (39 ± 12 , 23-62 years) to the epileptics. Group A patients had either peripheral neuropathy (four cases), demyelinating disease (two cases), spastic paraparesis, brain stem stroke, spondylosis, progressive ataxia, retinitis pigmentosa or traumatic syrinx (one case each). Group B were all undergoing investigation for a variety of peripheral and spinal cord complaints but had no evidence of neurological disease or disc lesions.

Spinal fluid (4-10 ml) was removed from the lumbar subarachnoid space following a 24 hour fast and frozen at -70°C until analysis. The CSF was mixed with nor-leucine internal standard, deproteinised and chromatographed on an autoanalyser, as previously described⁷ except that centrifugation was at 12,000 g and the supernatant fraction was left overnight at -20°C . The concentrations of aspartic acid, threonine, serine, glutamic acid, glutamine, glycine, alanine, leucine, isoleucine, valine, methionine, tyrosine, phenylalanine, histidine and lysine were determined.

Statistical comparisons were by analysis of variance and

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Concentrations of individual free amino acids in lumbar CSF

Amino acid	Controls (n = 23)	Epileptic patients		Histologically verified Alzheimer's disease (n = 11)
		Non-demented (n = 10)	Demented (n = 12)	
Transmitter candidate				
Aspartic acid (nmol/ml)	3.8 ± 1.8	2.2 ± 1.1	2.6 ± 2.3	3.7 ± 1.8
Glutamic acid (nmol/ml)	28.3 ± 10.3	25.8 ± 5.1	24.1 ± 6.4	27.1 ± 1.8
Glycine (nmol/ml)	16.4 ± 5.3	10.8 ± 4.9*	11.0 ± 3.5*	12.5 ± 3.2*
Non-Transmitter				
Glutamine (nmol/ml)	497 ± 147	715 ± 145*	733 ± 165*	389 ± 79†
Isoleucine (nmol/ml)	9.1 ± 3.2	7.7 ± 3.0	7.2 ± 2.1	7.7 ± 1.6
Serine (nmol/ml)	31.1 ± 9.3	30.1 ± 8.9	26.7 ± 7.3	31.6 ± 11.2
Threonine (nmol/ml)	36.6 ± 19.3	35.8 ± 12.9	40.9 ± 14.0	36.7 ± 7.3

Data is mean (± SD)

*Significantly different from control.

†Significantly different from control and epilepsy.

(ANOVA and Least Significant Difference test, null hypothesis being rejected at $p < 0.05$).

either the Student Newman-Keuls test or the Least Significant Difference test, significance being accepted at $p < 0.05$. Correlations were examined using Kendall's correlation (two tailed) statistic.

Results

Comparison of the concentrations of all amino acids in the various groups showed that only aspartic acid, glutamic acid, glycine, glutamine, isoleucine, serine and threonine in CSF from the two control groups were not significantly different (ANOVA and Student Newman-Keuls test). Therefore, data for these amino acids in the groups A and B were pooled (controls, table). CSF amino acid concentrations were independent of patient gender and age, except for threonine which correlated with age in the controls (Tau = 0.34, $n = 23$, $p < 0.02$).

The table shows that in comparison with control subjects, the glycine content in CSF of both Alzheimer and epileptic patients was reduced by 24–34%. The glutamine content of Alzheimer CSF was reduced by 22% whereas in the epileptic patients the concentration was increased by some 50%. Aspartic acid, glutamic acid, isoleucine, serine and threonine were unchanged.

The performance of Alzheimer patients ($n = 9$) on the Token test (TT)⁸ and a continuous visual reaction time test (RT)⁵ significantly ($p < 0.01$) correlated with the content of glutamic acid in the CSF (Tau = -0.63 and 0.71 for TT and RT, respectively). Glutamic acid content and either dementia⁵ or IQ (WAIS, full scale) scores showed similar trends ($p < 0.1$, Tau = 0.46 and -0.46, for dementia score and IQ respectively). None of the other 14 amino acids in Alzheimer CSF showed a trend towards correlation with the psychological measures (Tau = 0.06, -0.15, -0.17 and 0.35 for aspartic

acid content and dementia score, IQ, TT and RT, respectively; Tau = 0.03, 0.12, -0.03 and -0.03 for glycine and dementia score, IQ, TT, and RT, respectively). The IQ scores for the epileptics ($n = 22$) significantly correlated with the content of only valine (Tau = -0.43, $p < 0.02$) and methionine (Tau = -0.37, $p < 0.05$); for the putative amino acid transmitters: Tau = -0.13 (glutamic acid), 0.02 (aspartic acid) and 0.08 (glycine).

Discussion

For CSF amino acids little information is available, particularly for Alzheimer disease.⁹ Preliminary analysis of ventricular CSF has shown that no significant differences exist between the amino acid content of lumbar and ventricular fluid with the exception of glutamine (higher in lumbar fluid) and serine and glycine (both higher in ventricular fluid).¹⁰ Although glycine may be a transmitter in the corticohypothalamic projection it is likely that glycine in lumbar fluid originates also from glycinergic neurons in the spinal cord. Thus the present finding of a reduced concentration of glycine in the CSF of histologically verified Alzheimer patients may be a consequence of either decreased release from the spinal cord and brain or increased outflow from CSF. The change in CSF glycine is apparently neither specific for Alzheimer's disease nor associated with the degree of psychological impairment so the relevance of this change is unclear.

The observation that the aspartate content of CSF is neither reduced in Alzheimer's disease nor correlated with psychological measurements is perhaps surprising because of a recent report of a reduction in aspartate concentration in Alzheimer neocortex obtained at necropsy.¹¹ Aspartate releasing neurons

have been particularly difficult to investigate in Alzheimer's disease¹² so it is unknown whether the transmitter function of this amino acid is altered.

It is difficult to be sure of the relevance of the correlations of amino acids with cognitive scores, when the concentrations do not differ between control and dementia groups. However, the correlations between the glutamate content of CSF and psychological measurements are significant for only the Alzheimer group. Similarly, the glutamine content of CSF is reduced only in Alzheimer's disease. This suggests that glutamate metabolism is altered in Alzheimer's disease and possibly that glutamergic neurons are not spared in this disorder. These neurons may possess serotonin₂ receptors,¹³ which are reduced in Alzheimer's disease.¹⁴ Other evidence indicates that descending cortical glutamergic neurons are involved¹⁵ whereas intrinsic cortical glutamergic cells appear to be spared.¹² As methods improve for studying these neurons and their connections it should prove possible to test directly hypotheses that these cells are involved in memory mechanisms.^{16,17}

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