Short report

Aspartic acid aminotransferase activity is increased in actively spiking compared with non-spiking human epileptic cortex

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SUMMARY Increased concentration of the excitatory neurotransmitter aspartic acid in actively spiking human epileptic cerebral cortex was recently described.^{2,3} In order to further characterise changes in the aspartergic system in epileptic brain, the behaviour of aspartic acid aminotransferase (AAT), a key enzyme involved in aspartic acid metabolism has now been examined. Electro-corticography performed during surgery was employed to identify cortical epileptic spike foci in 16 patients undergoing temporal lobectomy for intractable seizures. Patients with spontaneously spiking lateral temporal cortex (n = 8) were compared with a non-spiking control group (n = 8) of patients in whom the epileptic lesions were confined to the hippocampus sparing the temporal convexity. Mean activity of AAT in spiking cortex was significantly elevated by 16-18%, with aspartic acid concentration increased by 28%. Possible explanations for the enhanced AAT activity include increased proliferation of cortical AAT-containing astrocytes at the spiking focus and/or a generalised increase in neuronal or extraneuronal metabolism consequent to the ongoing epileptic discharge. It is suggested that the data provide additional support for a disturbance of central excitatory aspartic acid mechanisms in human epileptic brain.

We have recently reported an increase in the concentration of two excitatory amino acid neurotransmitters, aspartic acid and glutamic acid, in actively spiking lateral temporal cortex excised for neurosurgical therapy of focal epilepsy.^{2,3} These changes may be especially relevant with respect to the pathophysiological processes in human focal cortical epilepsy in view of the experimental evidence that activation of central aspartic acid/glutamic acid systems may induce, in some brain areas, both seizure activity and concomitant neuronal damage.^{4,5} Taken together, these findings support the notion that sei-

zures and seizure-mediated brain damage are associated with excessive activation of excitatory amino acid receptors.

In order to clarify further the status of the aspartic acid neuronal system we have now examined in biopsied human epileptic brain the behaviour of aspartic acid aminotransferase (AAT, EC 2.6.1.1), a key enzyme involved in both aspartic acid as well as glutamic acid metabolism.⁶ This enzyme catalyses the reversible conversion of oxaloacetic acid and glutamic acid to aspartic acid and alpha-ketoglutarate. We found a significant increase in AAT activity together with above-normal levels of aspartic and glutamic acid in spiking vs. non-spiking epileptic temporal cortex.

Patients and methods

Patients

Electrocorticography with surface and depth electrodes was

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Aspartic acid aminotransferase activity in epilepsy

employed during surgery to identify areas of focal epileptic activity in 16 patients undergoing temporal lobectomy for the therapy of intractable seizures. Antiepileptic drugs were partially or completely withdrawn to enhance the recording of epileptic activity at the time of operation which was performed under local anaesthesia. Spontaneously active widespread epileptic activity was present in eight patients with lateral temporal cortical foci (S1 to S8, see table). In eight other cases (N9 to N16), the epileptic activity recorded (approximately 30 minutes duration) did not involve the surface of the temporal lobe, but was restricted to the hippocampus. By anatomical and therapeutic necessity these superficial nonspiking regions were also included in the standard "en bloc" temporal lobectomy for intractable seizures. Tissue was excised from either the middle or superior temporal gyrus (Brodmann areas 21, 22, and 38) within anterior 5–6 cm of temporal cortex. The surgical specimens were immediately placed on ice and from one to six samples (approximately 200 mg) carefully dissected from blood vessels and extraneous tissue, wrapped in aluminium foil, frozen on dry ice within 15 minutes of excision, and immediately stored at -80°C . After excision of tissues for biochemical analysis adjacent regions of cortex and white matter were fixed and stained by the Kluver-Barrera and Cajal Gold chloride sublimate techniques for neurons and glia respectively. As shown in the table the mean age of the patients with actively

spiking cortex was not significantly different from that for the non-spiking patients ($p > 0.05$, Student's two-tailed test). The type (see table) and duration of the anticonvulsant medication were similar for both patient groups.

All biochemical determinations were conducted blind.

Enzyme and amino acid assays

For the assay of total (cytosolic and mitochondrial) AAT, biopsied brain was homogenised in 600 volumes of 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5% Triton X-100. All incubation mixtures contained 600 μl homogenate, 100 μl of potassium phosphate buffer (0.41 M, pH 7.5) and 10 μl of 12.5 mM pyridoxal-5-phosphate. For assays in the direction of aspartic acid formation incubation mixtures also contained 190 μl of 0.313 M potassium glutamate, which included 0.2 μCi of ^{14}C -glutamate (New England Nuclear) and either 100 μl of 30 mM oxaloacetic acid (Sigma) or (for blanks) water. In the direction of aspartic acid degradation incubation mixtures contained 190 μl of 0.215 M potassium aspartate which included 0.2 μCi ^{14}C -aspartate (New England Nuclear) and either 100 μl of 0.2 M potassium alpha-ketoglutarate or (for blanks) 100 μl of water. After a 10 minute incubation at 37°C the reaction was stopped by adding 100 μl of 2N HCl. The ^{14}C -labelled alpha-ketoglutarate formed (aspartic acid formation) or ^{14}C -labelled oxaloacetate formed (aspartic acid

Table Patient information, aspartic acid aminotransferase and amino acid values: spiking vs. non-spiking temporal cortex

Spiking								
Patient No.	Age (yr)	Sex	AED	Histopathology	AAT activity		Aspartic acid	Glutamic acid
					Formation	Degradation		
S1	41	M	PHT	++	14.3	4.0	14.5	107.5
S2	17	M	CBZ	+	16.9	5.2	15.1	115.4
S3	22	F	PHT	++	16.1	5.2	14.2	101.4
S4	16	F	CBZ	+++	15.0	4.5	13.2	84.0
S5	39	M	CBZ	++	14.9	4.8	14.4	107.0
S6	21	F	CBZ	++	15.6	5.3	15.1	114.5
S7	33	F	CBZ	+	14.8	4.7	16.8	109.4
S8	28	F	Nil	+	14.4	4.4	14.6	112.6
Mean, S.E.: 27, 4					15.3, 0.3	4.8, 0.2	14.7, 0.4	106.5, 3.8
Non-spiking								
Patient No.	Age (yr)	Sex	AED	Histopathology	AAT activity		Aspartic acid	Glutamic acid
					Formation	Degradation		
N 9	14	F	Nil	+	11.9	4.5	10.8	88.7
N10	28	M	CBZ	+	13.9	4.1	11.9	90.0
N11	20	M	PHT, PRM	+	12.7	3.7	11.6	76.1
N12	24	M	CBZ	++	13.9	4.3	14.7	88.3
N13	48	F	PHT	+	13.6	4.4	8.1	65.5
N14	28	F	Nil	0	11.3	3.4	11.3	83.2
N15	29	M	CBZ	+	13.0	4.0	10.1	85.2
N16	19	M	CBZ	++	13.5	4.4	9.2	90.7
Mean, S.E.: 26, 4					13.0, 0.4*	4.1, 0.2*	11.0, 0.7†	83.5, 3.3†

* $p < 0.01$. † $p < 0.001$; Student's two-tailed t test.

Abbreviations: AAT, aspartic acid aminotransferase; AED, antiepileptic medication; CBZ, carbamazepine; PB, phenobarbital; PHT, phenytoin; PRM, primidone; Nil, anti-epileptic drug completely eliminated prior to surgery. 0, no evidence of astroglial proliferation, small benign lesion in hippocampus. +, very mild astrogliosis without evidence of neuronal loss; ++, mild to moderate astrogliosis and slight neuronal loss; +++, marked proliferation of fibrous astrocytes with neuronal loss involving all cortical lamina. AAT activity was measured in the direction of aspartic acid formation and degradation. Values are expressed as mmol/g protein/10 min (AAT) and nmol/mg protein (aspartic and glutamic acid).

degradation) were separated from their radioactive precursors by applying incubation mixtures to 5×0.75 cm columns of Dowex 50-X8 resin, H^+ form (Sigma) and eluted with 3.0 ml of water. The eluates were then counted for radioactivity. The enzyme assays were linear with respect to time and protein homogenate concentration over the range studied. Amino acids were quantitated using minor modifications of the procedure of Fernstrom and Fernstrom.⁷

Results

As shown in the table, AAT activity measured in the direction of aspartic acid formation and degradation was significantly increased by 18 and 16% respectively, in spiking vs non-spiking cortex. In spiking cortex concentrations of aspartic and glutamic acid were significantly elevated by 34% and 28% respectively. An analysis of the individual values for AAT revealed little overlap between the spiking and non-spiking groups (especially in the direction of aspartic acid formation) with the majority of the spiking values falling outside the range of the non-spiking levels (see fig). When the biochemical data from all 16 patients were subjected to regression analysis a significant positive correlation was observed between AAT activity measured in the direction of aspartic acid formation and in the direction of degradation ($r = 0.83$; $p < 0.001$). AAT activity was also significantly correlated with concentrations of aspartic acid (formation, $r = 0.65$; $p < 0.005$; degradation, $r = 0.48$; $p < 0.05$) and glutamic acid (formation, $r = 0.66$; $p < 0.005$; degradation, $r = 0.61$; $p < 0.01$).

Specimens from patients with superficial cortical spike foci tended to exhibit a greater degree of cortical astrogliosis and neuronal loss than those from the

patients with deep temporal lesions. Thus five patients in the spiking group showed grade ++ or +++ histopathology vs. only two patients in the non-spiking group.

Discussion

To our knowledge, this study describes, for the first time, the behaviour of AAT in actively spiking human epileptic brain.

Our observation of above normal AAT activity in human epileptic cortex may be related to a generalised increase in neuronal and/or extraneuronal metabolic activity present in the epileptic focus. In this regard, in biochemical studies of human epileptic cortex elevated activities of other enzymes involved in neurotransmitter metabolism, namely, glutamate dehydrogenase,⁸ tyrosine hydroxylase,⁸ cholineacetyltransferase (Kish and Sherwin, unpublished observations), and acetylcholinesterase (refs.^{9, 10}; Kish and Sherwin, unpublished observations) have been demonstrated. In this context, the possibility also has to be considered that the above-normal AAT levels in spiking cortex may be causally related to enhanced astroglial proliferation and activity. In this respect we observed in the 16 patients examined a slight, but statistically significant correlation between the activity of AAT in the direction of aspartic acid formation and extent of the histopathological changes ($r_s = 0.52$, $p < 0.02$, Spearman Rank Order Correlation; $p > 0.05$ for histopathology vs. AAT in direction of aspartic acid degradation, aspartic acid or glutamic acid concentration). The enzyme AAT has been demonstrated to be present in especially high activities in astrocytes (11, cf. ref. 12).

In our study all antiepileptic medication had been partially or completely withdrawn prior to operation. Most of the patients had received carbamazepine monotherapy and in all cases plasma levels had been in the lower therapeutic range for several weeks to permit EEG localisation. However, the possibility that these drugs even in low dosage may have influenced the amino acid and enzyme values cannot be excluded. None had received valproate which the acute administration of high doses in experimental animal studies is reported to result in a reduction in brain aspartate levels.^{13, 14} Aspartic acid concentration in cerebrospinal fluid has been reported to be higher in epileptic patients receiving antiepileptic polytherapy vs. patients without any medication.¹⁵

With respect to functional considerations, much evidence now suggests the involvement of the excitatory neurotransmitter aspartic acid in seizure conditions as well as seizure-related neuronal damage.

Sloviter and Dempster⁴ have provided evidence

Fig Activity of aspartic acid amino transferase in the direction of aspartic acid formation and degradation in actively spiking vs. non-spiking human epileptic cortex.

that in rodents repeated intraventricular injection of aspartic acid produces both seizures and morphological changes in the hippocampus which were qualitatively similar to the changes observed in temporal lobe of epileptic patients. Similarly, Piredda and Gale⁵ have recently demonstrated the ability of aspartic acid to induce bilateral motor seizures when microinjected into the deep prepiriform cortex of the rat. In the experimental literature studies of the behaviour of brain aspartic acid in a variety of animal models of convulsive seizures have produced no consistent pattern with aspartic acid levels reported as being reduced: cobalt focus,¹⁶⁻¹⁹ methoxy-pyridine,²⁰ allylglycine, bicuculline, kainic acid,²¹ benzodiazepine inverse agonists,²² increased: pyridoxal phosphate,^{23,24} sound-induced seizures,²⁵ homocysteine²⁶ or normal: kindling,²⁷⁻²⁹ pentylenetetrazol.³⁰ However, investigations of the influence of experimental epileptic conditions on aspartic acid release have consistently shown enhanced release of this excitatory neurotransmitter.^{19,22,24,29} Additional evidence is derived from pharmacological studies demonstrating anti-convulsant properties of amino acid analogues which preferentially block the electrophysiological responses of aspartic acid and N-methyl-D-aspartate.³¹⁻³³ In this regard it may be especially noteworthy that such excitatory amino acid antagonists have recently been shown to block electrically stimulated bursting of action potentials in biopsied human epileptic cortex.³⁴ Chapman and coworkers¹⁴ reported a strong correlation between the anti-convulsant potency of valproic acid analogues with their ability to reduce brain aspartic acid levels. These experimental data taken together with our present biochemical findings suggest the possibility of enhanced aspartic acid synthesis and neuronal release in actively spiking (but not with convulsions) epileptic human brain. This receives some additional support from the observations of increased aspartic acid levels in cerebrospinal fluid of epileptic patients.^{15,35-37}

Although the magnitude of the AAT elevation was slight (+16-18%) we observed little overlap in enzyme values (especially in the direction of aspartic acid formation) between the two patient groups (see fig). It will therefore be interesting to determine whether in future studies employing this new research paradigm AAT activity can be shown to distinguish reliably between actively spiking vs. non-spiking epileptic temporal cortex.

Finally, we suggest that our biochemical observations implicating the brain aspartic acid system in cortical epilepsy further strengthen the rationale for the clinical testing of centrally acting excitatory amino acid antagonists in human epileptic conditions.³⁸

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