

## Amino acid composition of brain cysts: levels of excitatory amino acids in cyst fluid fail to predict seizures

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### Abstract

A recent study describing two epileptic patients with brain cysts has suggested that elevated concentrations of excitatory amino acids in cysts may play a role in induction and maintenance of epileptogenesis [Epilepsy Res. 28 (1997) 245]. Here, we report that only in 3 out of 22 patients with brain cysts undergoing brain surgery cyst fluids displayed highly increased amounts of the excitatory amino acids aspartate and/or glutamate. Two of these patients experienced epileptic seizures prior to neurosurgical intervention. Thus, highly increased excitatory amino acid levels are present only in a subset of patients with brain cysts. Our observation that one patient with highly increased glutamate and aspartate concentrations in the cyst did not display seizures or typical epileptiform potentials in the EEG questions that these excitatory amino acids in the cyst fluid are directly involved in epileptogenicity. This patient displayed an increased level of adenosine in the cyst fluid, which is known to have anticonvulsant properties and might provide protection from seizures. In summary, there is no evidence for a close correlation between excitatory amino acids in brain cysts and the occurrence of seizures.

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**Keywords:** Seizures; Cyst; Aspartate; Glutamate; Amino acids; Adenosine

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## 1. Introduction

Cystic lesions of the brain are characterized by a disruption of brain parenchyma, which is replaced by fluid. They may occur in benign conditions, when associated with a clear fluid or with malignant diseases, when they are usually filled with yellow proteinaceous fluid. Based on pathogenesis, Go et al. (1993) suggested the following classification: (1) cysts containing cerebrospinal fluid (CSF)-like fluid, (2) cysts with fluid-secreting walls and CSF-like content, such as arachnoid cysts, (3) cysts associated with dysgenesis, (4) cysts with a lining of non-neuronal epithelium, (5) cysts associated with gliomas and other tumors, and (6) cysts of infectious origins. Cysts may compress adjacent tissue and thus cause neurological symptoms and signs. In clinical practice, however, more often brain cysts without signs of compression are observed in epileptic patients, raising the question whether the cyst content may be relevant for epileptogenesis. In a retrospective analysis of 867 patients of whom 17 had arachnoid cysts, only in 4 patients the seizure focus was adjacent to the cyst, suggesting that arachnoid cysts are often incidental findings in epileptic patients (Arroyo and Santamaria, 1997). A similar conclusion was recently reached by Yalcin et al. (2002), who reported that seizure type and EEG abnormalities corresponded to arachnoid cyst location in only one out of eight patients. In the first study to report on the amino acid composition of brain cysts (Hajek et al., 1997), two epileptic patients, one with ganglioglioma WHO grade I and one 15 years post-hemispherectomy for suspected Rasmussen's encephalitis have been described who had increased concentrations of glutamate and aspartate. Since these amino acids, which are agonists at NMDA receptors, are implicated in induction and maintenance of epileptogenesis (Do et al., 1991; Chapman et al., 1996), it was hypothesized that the increased concentrations of excitatory neurotransmitters in the cysts might play a role in the development of seizures. In order to evaluate whether increased levels of excitatory amino acids are a common phenomenon in epileptic or non-epileptic patients with brain cysts, we analyzed the amino acid content in 22 consecutive patients with brain cysts undergoing neurosurgery.

## 2. Patients and methods

From 22 consecutive patients with brain cysts undergoing neurosurgery at the University Hospital, Zürich, cyst fluid was harvested and kept at  $-80^{\circ}\text{C}$ . The free amino acids were analyzed chromatographically. Normal values for amino acids in the CSF can be found in Blau et al. (2003). Adenosine levels in the cyst fluid were determined in an enzyme-coupled bioluminescent assay (Boison et al., 1999).

The protein content of the samples was determined in the routine diagnostics laboratory at the Institute for Clinical Chemistry at the University Hospital, Zurich. Age, gender, histopathological diagnosis, localization, the presence or absence of seizures pre- and postoperatively, and the use of antiepileptic drugs (AED) are listed in Table 1. The age of the patients ranged from 11 to 75 years (mean: 43.6 years; 10 males, 12 females).

The times of the last seizure prior to resection sampling of fluids varied from 5 to 10 min (patients 11, 15, 20) via 8 h (patient 6), 18 h (patient 19), 2 weeks (patients 5, 16), more than 2 weeks (patients 1, 2) to 4 months (patient 16) and 5 months (patient 22).

## 3. Results

The content of individual amino acids and of the total protein of the cyst fluids of the 22 patients included in this study were determined. Compared with normal values for total protein in the cerebrospinal fluid (0.15–0.45 g/l), all patients displayed an enhanced protein content (see Table 2). The amino acid levels show some correlations with the underlying pathology (Table 2). In the cyst fluid from our patient with an arachnoid cyst associated with a pituitary adenoma (patient 17), only 1 out of 23 amino acids determined was elevated. When the cysts were associated with low-grade tumors, like ganglioglioma WHO grade I (patients 10 and 22), glioneuronal tumor consistent with dysembryoplastic neuroepithelial tumor (DNT) grade I (patient 5), and plexus papilloma grade I (patient 4), between 5 and 8 amino acids were elevated. In contrast, in our six patients with glioblastoma multiforme WHO grade IV (patients 8, 9, 15, 16, 18, 21), the levels of between 20 and 23 amino acids were elevated. Likewise, in the cysts associated with

Table 1  
Synopsis of clinical patient data

	Age at ex. (years)	Cyst	CSF	ECoG-epileptogenicity interict./ict. Date of Surg.	Diagnosis (grading: WHO I-IV)	Localization	Seizures (type and time*) AEDs		f-up (mo) Engel	Remarks
							Preop.	Postop.		
Patient 1: m, M.F.	31	+		+++/0 23-07-98	Fibrillary astrocytoma II	L F-T-P	Yes: foc. sensorimotor (>2 weeks) PH, DIAZ, DISTRA	Yes CBZ, DIAZ	28:IV 31:III	Tu-resection with ECoG (12-01-00)
Patient 2: m, D.C.	35	++		+++/0 28-07-98	St. p. head trauma, parenchymal defect, perifocal gliosis	R T-P-(F)	Yes: foc. motor (>2 weeks) CBZ, PH	Yes CBZ, PH	18:III	Resection TP + antCCT
Patient 3: f, P.N.	65	++		– 17-08-98	Metastasis mamma carcinoma	R T-O, putamen	No No	No PH	3 –	MRI Preop.
Patient 4: m, L.B.	11	++		– 30-07-98	Intraventricular plexus papilloma I with parietal cyst	L P	Yes: foc. with sec. gen. (not known: no seizure within last 24 h) No	Yes PH	? –	No follow-up
Patient 5: f, A.R.D.	15	+		+++/0 16-10-98	Glioneuronal tumor (DNT I)	L Tpole	Yes: cps with rare sec. gen. (2 weeks) CBZ	No CBZ	37:IA	
Patient 6: m, H.-J.E.	52	+		+++/0 12-10-98	Focal cortical dysplasia	L T-O	Yes: cps (8 h) Polytherapy	Yes Polytherapy*	10:III 40:II	
Patient 7: f, N.E.	15	+		– 22-10-98	Ganglioglioma I	R Tpole	No No	No No	5 –	Multiple sclerosis (Azothiaprim; Interferon)
Patient 8: m, A.S.	67	+		– 20-11-98	Glioblastoma multiforme IV	R basal ganglia	No No	No CBZ	? –	No follow-up
Patient 9: f, U.B.	62	++		– 01-12-98	Glioblastoma multiforme IV	R T	No PH	Yes PH, CBZ, CLB	15:IV	Radio- and chemotherapy
Patient 10: m, B.S.	40	+++	+	+++/0 14-10-99	Ganglioglioma I	R T	Yes: cps with rare sec. gen. (2 weeks) CBZ	No CBZ	32:IC	
Patient 11: f, C.R.	35	+		+++/2 foc. seizures (ton-clon R arm and leg) introduced by intraop. electrical stimulation 25-10-99	Anaplastic astrocytoma III	LC	Yes: foc. sensorimotor (10 min) PB*, CLON	Yes PB*, CLON	6:II 30:III	1st Surg.: 17-07-90 (astrocytoma II); 2nd Surg.: 03-11-94 (anaplastic astrocytoma III); 3rd Surg.: 25-10-99 (anaplastic astrocytoma III); 4th Surg.: 18-05-00 (GBM IV); 5th Surg.: 01-07-02 (GBM IV); St. p. radiation and chemotherapy
Patient 15: f, E.K.	42	+	+	+/2 foc. ton seizures (R arm) introduced by intraop. electrical stimulation 11-01-00	Glioblastoma multiforme IV	L F-preC	Yes: foc. sensorimotor (10 min) PH, CBZ	Yes BB, PB, CBZ; LAM, LORAZ	19:III	St. p. Op astrocytoma II (25-06-90); radiotherapy (1999); Re-op. (2000), shunt, chemotherapy

Table 1 (Continued)

	Age at ex. (years)	Cyst	CSF	ECoG-epileptogenicity interict./ict. Date of Surg.	Diagnosis (grading: WHO I–IV)	Localization	Seizures (type and time*) AEDs		f-up (mo) Engel	Remarks
							Preop.	Postop.		
Patient 16: m, W.J.	59	+	–	– 16-12-99	Glioblastoma multiforme IV	L preC	Yes: foc. motor (4 months) VAL, CLB	Yes VAL, CLB	3:III	St. p. Op GBM (06-08-99); radiotherapy, Re-op., chemotherapy
Patient 17: m, D.F.	59	+	–	– 01-12-99	Pituitary adenoma, arachnoid cyst L-F	L F-T	No No	No No	24 –	St. p. partial tu-removal (19-11-99); St. p. Re-op. (01-12-99); St. p. cysto-peritetal shunt L (21-01-00)
Patient 18: f, R.S.	44	+	+	– 25-01-00	Glioblastoma multiforme IV	L F	No No	? PB	?	No follow-up
Patient 19: f, P.R.	56	+	–	– 22-02-00	Gemistocytic astrocytoma II	L F	Yes: foc. motor with sec. gen. (22 and 18 h) VAL	Yes VAL, CLB	17:II 29:I	Biopsy 06-11-96
Patient 20: f, C.A.	37	+ Sang	–	+/1 foc. ton seizure (R arm) introduced by intraop. electrical stimulation 25-02-00	Oligoastrocytoma WHO III with anaplastic portions	L C	Yes: foc. motor with/without sec. gen. (5 min) Polytherapy*	Yes Polytherapy*	12:IV	Finally GBM [MRI 09-02-01]
Patient 21: m, B.B.	46	+	–	– 03-03-00	Glioblastoma multiforme IV	L T	No PH	No CBZ	21 –	Postop. radiotherapy; Re-op. (23-08-01); chemotherapy
Patient 22: f, G.B.	47	+	+	++/0 30-03-00	Ganglioglioma grade I	R F (g. front. Med.)	Yes: 1 sec. gen. seizure (5 months) CBZ	No PB*	9:IA	
Patient 23: f, D.W.	53	+	–	– 02-05-95	Anaplastic "Klarzell" ependymoma III	L F (g. front. Med.)	No BP	No PB*	48:IA	
Patient 24: f, A.B.	75	+	–	– 28-04-00	Urothel-carcinoma metastasis	R P	No No	No PH	6 9	Postop. radiotherapy
Patient 25: m, R.H.	14	+	–	– 11-05-00	Gemistocytic astrocytoma II	R P-O	No No	No DP	6	

Abbreviations: ex., examination; Op, Surg., surgery; St. p., status post; Re-op., another surgery; Preop., before surgery; Postop., after surgery; intraop., intraoperative; ECoG, electrocorticography; antCCT, anterior corpus callosum section; Tu, tu, tumor; GBM, glioblastoma multiforme; f-up (mo) Engel, follow-up in months, classification of seizure outcome according to Engel classes I–IV [I free of disabling seizures; IA, aura- and seizure-free; II rare seizures; III, worthwhile improvement; IV, no worthwhile improvement, including worsening]; m, male; f, female; L, left; R, right; F, frontal; C, central; preC, precentral; T, temporal; P, parietal; O, occipital; Tpole, temporal pole; g. front. med., middle frontal gyrus; AEDs, antiepileptic drugs; polytherapy, more than two AEDs; CBZ, Carbamazepine; PH, Phenytoin; PB, Phenobarbital; PB\*, Barbexaclonum = Maliasin<sup>®</sup>; VAL, Valproate; LAM, Lamotrigine; DIAZ, Diazepam; CLON, Clonazepam; CLB, Clobazam; LORAZ, Lorazepam; DISTRA, Distraeurin; Sang, contaminated by blood.

Table 2  
Free amino acids ( $\mu\text{mol/l}$ ), total protein (g/l), and adenosine (ng/ml) in cyst fluid

	Tau	Asp	Thr	Ser	Glu	Gln	Asp	Pro	Gly	Ala	Cit	AABA	Val	Cys	Met	Ile	Leu	Tyr	Phe	Orn	Lys	His	Arg	Protein	Adenosine
Patient 1: M.F.	<b>20</b>	2	43	<b>51</b>	Sp	<b>1092</b>	n.d.	n.d.	8	6	<b>8</b>	Sp	37	n.d.	<b>13</b>	<b>17</b>	22	<b>21</b>	14	<b>39</b>	9	22	9	<b>1.81</b>	1
Patient 2: D.C.	<b>22</b>	n.d.	29	<b>39</b>	12	628	Sp	6	<b>32</b>	29	Sp	6	17	2	4	4	12	8	<b>19</b>	20	10	6	<b>12.16</b>	n.d.	
Patient 3: P.N.	<b>31</b>	<u><b>59</b></u>	<b>200</b>	<b>202</b>	<u><b>766</b></u>	410	n.d.	373	<b>369</b>	<b>562</b>	<b>19</b>	<b>41</b>	<b>307</b>	10	<b>42</b>	<b>95</b>	<b>186</b>	<b>89</b>	<b>89</b>	<b>32</b>	<b>321</b>	<b>92</b>	<b>141</b>	<b>51.12</b>	<b>13.3</b>
Patient 4: L.B.	12	n.d.	49	<b>86</b>	n.d.	<b>1504</b>	n.d.	n.d.	6	14	<b>7</b>	<b>8</b>	<b>34</b>	n.d.	<b>12</b>	5	13	<b>26</b>	<b>25</b>	5	21	<b>32</b>	<b>38</b>	<b>1.2</b>	3.6
Patient 5: A.R.D.	6	n.d.	42	<b>68</b>	Sp	<b>1043</b>	n.d.	n.d.	7	23	Sp	6	<b>41</b>	n.d.	<b>20</b>	13	24	13	14	4	26	<b>24</b>	18	<b>31.24</b>	4.1
Patient 6: H.-J.E.	<b>36</b>	<u><b>94</b></u>	41	<b>70</b>	<u><b>215</b></u>	<b>1139</b>	n.d.	n.d.	<b>29</b>	18	4	<b>8</b>	<b>43</b>	3	7	9	24	<b>19</b>	15	4	11	<b>25</b>	17	<b>1.69</b>	n.d.
Patient 7: N.E.	<b>25</b>	<b>4</b>	<b>192</b>	<b>200</b>	7	<b>2348</b>	<b>37</b>	135	<b>32</b>	<b>262</b>	<b>13</b>	<b>31</b>	<b>229</b>	1	<b>49</b>	<b>55</b>	<b>115</b>	<b>111</b>	<b>94</b>	<b>26</b>	<b>176</b>	<b>103</b>	<b>111</b>	<b>48.18</b>	3.8
Patient 8: A.S.	<b>39</b>	<b>16</b>	<b>222</b>	<b>153</b>	33	<b>943</b>	<b>53</b>	323	<b>425</b>	<b>966</b>	<b>31</b>	<b>32</b>	<b>414</b>	1	<b>53</b>	<b>118</b>	<b>303</b>	<b>134</b>	<b>148</b>	<b>93</b>	<b>364</b>	<b>136</b>	<b>74</b>	<b>49.66</b>	6
Patient 9: U.B.	<b>27</b>	<b>6</b>	<b>199</b>	<b>150</b>	Sp	<b>1253</b>	<b>125</b>	284	<b>358</b>	<b>867</b>	<b>32</b>	<b>49</b>	<b>359</b>	5	<b>59</b>	<b>101</b>	<b>247</b>	<b>129</b>	<b>144</b>	<b>101</b>	<b>336</b>	<b>136</b>	<b>170</b>	<b>45.39</b>	0.8
Patient 10: B.S.	9	<b>14</b>	39	<b>54</b>	28	675	Sp	n.d.	<b>54</b>	45	Sp	5	34	4	5	7	21	13	11	<b>20</b>	41	20	12	<b>29.55</b>	<b>8.7</b>
Patient 11: C.R.	<b>64</b>	<b>10</b>	<b>219</b>	<b>147</b>	33	<b>1438</b>	<b>62</b>	184	<b>273</b>	<b>266</b>	<b>32</b>	<b>37</b>	<b>277</b>	3	<b>46</b>	<b>58</b>	<b>162</b>	<b>95</b>	<b>100</b>	<b>46</b>	<b>216</b>	<b>132</b>	<b>118</b>	n.d.	n.d.
Patient 15: E.K.	<b>26</b>	3	<b>125</b>	<b>94</b>	7	<b>1219</b>	<b>26</b>	120	<b>115</b>	<b>108</b>	<b>19</b>	<b>14</b>	<b>155</b>	8	<b>21</b>	<b>33</b>	<b>76</b>	<b>46</b>	<b>50</b>	<b>25</b>	<b>89</b>	<b>58</b>	<b>70</b>	<b>38.11</b>	4.3
Patient 16: W.J.	<b>24</b>	4	<b>148</b>	<b>204</b>	n.d.	<b>1397</b>	<b>55</b>	223	<b>169</b>	<b>353</b>	<b>37</b>	<b>36</b>	<b>209</b>	9	<b>35</b>	<b>52</b>	<b>111</b>	<b>94</b>	<b>79</b>	<b>41</b>	<b>267</b>	<b>102</b>	<b>97</b>	<b>14.2</b>	3.2
Patient 17: D.F.	7	Sp	14	25	n.d.	410	n.d.	n.d.	8	29	n.d.	n.d.	11	n.d.	3	4	13	9	8	6	35	7	16	<b>0.9</b>	0
Patient 18: R.S.	<b>23</b>	6	<b>142</b>	<b>120</b>	17	<b>1225</b>	<b>42</b>	255	<b>169</b>	<b>494</b>	<b>36</b>	<b>26</b>	<b>213</b>	n.d.	<b>29</b>	<b>59</b>	<b>137</b>	<b>74</b>	<b>72</b>	<b>32</b>	<b>245</b>	<b>94</b>	<b>72</b>	<b>31.04</b>	<b>5.8</b>
Patient 19: P.R.	<b>31</b>	5	<b>89</b>	<b>112</b>	9	<b>1540</b>	12	33	<b>29</b>	<b>73</b>	<b>7</b>	<b>16</b>	<b>186</b>	n.d.	<b>21</b>	<b>48</b>	<b>137</b>	<b>50</b>	<b>63</b>	<b>19</b>	<b>107</b>	<b>69</b>	<b>37</b>	<b>40.02</b>	3
Patient 20: C.A.	<b>842</b>	n.d.	<b>351</b>	<b>463</b>	<u><b>1264</b></u>	<b>1443</b>	<b>147</b>	532	<b>1406</b>	<b>1456</b>	<b>72</b>	<b>54</b>	<b>348</b>	n.d.	<b>78</b>	<b>109</b>	<b>286</b>	<b>139</b>	<b>128</b>	<b>119</b>	<b>312</b>	<b>124</b>	<b>120</b>	n.d.	n.d.
Patient 21: B.B.	<b>17</b>	3	<b>147</b>	<b>192</b>	Sp	<b>1073</b>	<b>57</b>	220	<b>105</b>	<b>640</b>	<b>20</b>	<b>34</b>	<b>243</b>	1	<b>30</b>	<b>58</b>	<b>150</b>	<b>84</b>	<b>85</b>	<b>42</b>	<b>255</b>	<b>93</b>	<b>91</b>	<b>56</b>	1.8
Patient 22: G.B.	4	n.d.	<b>41</b>	<b>79</b>	n.d.	<b>1354</b>	n.d.	n.d.	2	12	Sp	Sp	31	n.d.	8	6	23	<b>14</b>	<b>20</b>	4	21	<b>30</b>	22	<b>16.72</b>	4.9
Patient 23: D.W.	<b>40</b>	3	<b>91</b>	<b>117</b>	29	<b>1345</b>	<b>23</b>	75	<b>80</b>	<b>163</b>	<b>16</b>	<b>15</b>	<b>188</b>	3	<b>22</b>	<b>43</b>	<b>116</b>	<b>59</b>	<b>58</b>	<b>28</b>	<b>192</b>	<b>77</b>	<b>88</b>	<b>47.64</b>	4.3
Patient 24: A.B.	<b>17</b>	n.d.	<b>80</b>	<b>103</b>	18	582	n.d.	76	<b>147</b>	<b>246</b>	<b>13</b>	<b>18</b>	<b>156</b>	9	<b>20</b>	<b>47</b>	<b>114</b>	<b>46</b>	<b>61</b>	<b>27</b>	<b>206</b>	<b>52</b>	<b>80</b>	<b>48.49</b>	7.2
Patient 25: R.H.	<b>19</b>	<b>11</b>	<b>472</b>	<b>391</b>	n.d.	<b>7416</b>	<b>61</b>	<b>65</b>	<b>21</b>	<b>376</b>	Sp	<b>31</b>	<b>391</b>	n.d.	<b>61</b>	<b>56</b>	<b>181</b>	<b>132</b>	<b>141</b>	<b>46</b>	<b>323</b>	<b>177</b>	<b>165</b>	<b>25.63</b>	1.3

Abbreviations: Tau: taurine, Asp: aspartate, Thr: threonine, Ser: serine, Glu: glutamate, Gln: glutamine, Asn: asparagine, Pro: proline, Gly: glycine, Ala: alanine, Cit: citrulline, AABA:  $\alpha$ -aminobutyrate, Val: valine, Cys: cystine, Met: methionine, Ile: isoleucine, Leu: leucine, Tyr: tyrosine, Phe: phenylalanine, Orn: ornithine, Lys: lysine, His: histidine, Arg: arginine. Sp: traces; n.d.: not done. Bold indicates increased values. The highly increased aspartate and glutamate values in patients 3, 6, and 20 are underlined.

carcinoma metastases (patients 3 and 24), the levels of 19 and 21 amino acids, respectively, were elevated.

Thus, in cysts formed by highly malignant tumors the levels of the great majority of amino acids are altered. Interestingly, only in one of the six glioblastoma multiforme patients the levels of aspartate and glutamate are moderately increased (patient 8). The levels of the excitatory amino acids aspartate and glutamate (see also Fig. 1) appear to be regulated independently of the levels of other amino acids. This view is also supported by the observation that in our patient with focal cortical dysplasia (patient 6), who has very high levels of aspartate and glutamate, the levels of only 10 other amino acids also were elevated. Similarly, in our patient with ganglioglioma grade I and mildly elevated levels of aspartate and glutamate (patient 10), only four other amino acids were elevated. Since the other patient in our study with ganglioglioma grade

I (patient 22) has no detectable aspartate and glutamate levels in the cyst fluid, this indicates that even within a histologically well-defined entity aspartate and glutamate can be either absent or present at a high level.

Of the 22 patients in this study, 12 experienced seizures before the neurosurgical intervention, whereas 10 did not experience seizures. All patients with seizures were on an AED treatment, whereas only one patient without seizures received AEDs preoperatively.

In 3 out of 22 patients, we found very high levels of the excitatory amino acids aspartate and/or glutamate exceeding the normal values for these amino acids in cerebrospinal fluid (Blau et al., 2003; Gjessing et al., 1972; Heiblim et al., 1978 and references cited therein) by at least 10-fold for aspartate and at least 7-fold for glutamate. These include patient 6 (focal cortical dysplasia; aspartic acid 94  $\mu\text{mol/l}$ , glutamic acid 215  $\mu\text{mol/l}$ ); patient 20 (anaplastic oligoastrocytoma WHO grade III; aspartic acid undetectable, glutamic acid 1264  $\mu\text{mol/l}$ ); and patient 3 (metastasis of mamma carcinoma; aspartic acid 59  $\mu\text{mol/l}$ , glutamic acid 766  $\mu\text{mol/l}$ ). The normal range in cerebrospinal fluid for aspartic acid is 0.4–5.2  $\mu\text{mol/l}$  (Blau et al., 2003), for glutamic acid published values range from  $1.7 \pm 0.9 \mu\text{mol/l}$  to  $14.7 \pm 13.3 \mu\text{mol/l}$  (see Gjessing et al., 1972; Heiblim et al., 1978). Of the three patients, patients 6 and 20 experienced seizures before the operation, while patient 3 did not report the occurrence of seizures.

Of the 12 patients with seizures before the operation, 2 had highly increased aspartate and/or glutamate levels (i.e.  $\geq 59 \mu\text{mol/l}$  for aspartate and  $\geq 215 \mu\text{mol/l}$  for glutamate), whereas the other 10 did not. Of the 10 patients without seizures before the operation, 1 had highly increased aspartate and glutamate levels, whereas 9 had not. This indicates that highly increased concentrations of aspartate and glutamate in brain cysts are not necessarily associated with seizures.

When considering values higher than 10  $\mu\text{mol/l}$  for aspartate and 18  $\mu\text{mol/l}$  for glutamate as being increased, a total of nine patients (patients 3, 6, 8, 10, 11, 20, 23, 24, 25) displayed increased aspartic acid and/or glutamic acid levels in the cyst fluid. Of these patients, four had seizures preoperatively, whereas five patients had no seizures. This indicates that more moderate increases in aspartic acid and/or glutamic acid levels

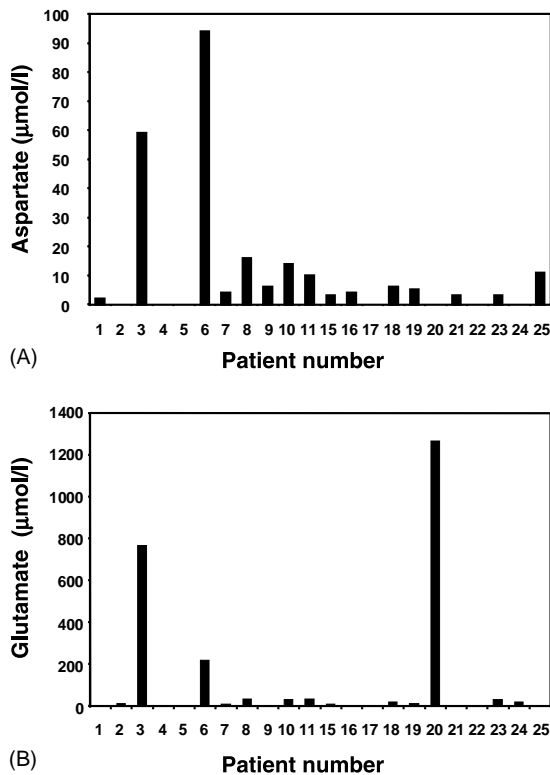


Fig. 1. Aspartate concentration (A) and glutamate concentration (B) in the fluid of brain cysts of 22 patients measured chromatographically.

in the cysts also display no tight association with the presence of seizures.

Glycine is a co-agonist at NMDA receptors and thus has excitatory functions in the CNS. It was increased in the cyst fluid of 17 out of 22 patients. Of the 5 patients without increased glycine levels, 4 had seizures before the operation, whereas 8 of the 17 patients with increased glycine levels experienced seizures before the operation. Thus, the glycine levels appear not to be associated with increased seizure frequency. Of the nine patients with increased levels of aspartate and/or glutamate, all had displayed also increased glycine levels, so that the glycine levels do not represent a factor that would determine the presence of seizures before the operation.

Adenosine is a modulator with an inhibitory action on neuronal activity, which has been shown to regulate seizure susceptibility (During and Spencer, 1992; Dunwiddie and Masino, 2001). Therefore, we also determined adenosine levels in the cyst fluid (Table 2). To set a baseline for our study, we first determined the adenosine levels in the CSF of seven patients with unrelated neurological diagnoses. These values ranged from 2.4 to 5.0 ng/ml adenosine (2.4, 2.9, 3.1, 3.6, 4.1, 4.9, and 5 ng/ml). We then determined the adenosine values in the brain cysts in 18 out of the 22 patients in our study. Only patients 3, 8, 10, and 18 had adenosine levels above baseline, and only in patient 3, who did not display seizure activity prior to surgery, the adenosine level was elevated at least twofold above the upper limit of the range seen in the control patients. Since patient 3 had very high aspartate and glutamate levels in the cyst fluid, it is tempting to speculate that these increased adenosine levels protected this patient from seizures.

#### 4. Discussion

The primary aims of our study were to determine whether high levels of glutamate and/or aspartate occur frequently in brain cysts and whether there is a correlation between increased aspartate or glutamate concentrations in brain cysts and seizure frequency. To this end, the cyst fluid from 22 patients undergoing brain surgery was examined for the content of protein and free amino acids. The cyst fluid of all patients displayed protein values higher than those considered

normal in the CSF. Three patients had dramatically increased levels of aspartate and/or glutamate, but there was no correlation between presence of seizures and these highly increased aspartate and/or glutamate levels. Furthermore, also when considering all aspartate/glutamate levels increased beyond published values for the CSF, there was no apparent correlation between these levels and the occurrence of seizures. Two of the patients (patients 10 and 22) had ganglioglioma WHO grade I, one with moderately increased aspartate and glutamate levels in the cyst fluid, the other one without, indicating no absolute correlation between the histopathological diagnosis and aspartate/glutamate levels. Of the six patients with glioblastoma multiforme grade IV (patients 8, 9, 15, 16, 18, and 21), one had increased aspartate and glutamate levels, whereas the others had not. Thus, this histopathological diagnosis typically appears not to be associated with increased aspartate/glutamate levels in the cyst fluid. Even in the group of patients with glioblastoma multiforme grade IV there is no correlation between aspartate/glutamate levels and seizures before the operation: the only patient with increased aspartate/glutamate levels had no seizures, whereas two patients with normal aspartate/glutamate levels experienced seizures. There is also no obvious association between aspartate/glutamate levels and time span between the onset of disease and operation of the cyst, the size of the cyst, the localization of the cyst, and the occurrence of seizures prior to surgery.

Evidence suggests that excitatory amino acids play a role in the pathophysiology of both experimental and human epilepsy. Chemical kindling by repeated spaced injections of subconvulsive doses of glutamate and aspartate into the amygdala produced progressive seizure development culminating in generalized convulsions, indicating a role for these amino acids in the development and persistence of increased seizure susceptibility (Mori and Wada, 1987). In the genetic rat model of absence epilepsy, GAERS, the basal level of glutamate but not of aspartate, glycine, citrulline, or GABA was significantly increased in the dialysate from ventral hippocampus (Richards et al., 2000). Furthermore, in Shetland dogs with familial idiopathic epilepsy, an increased value for glutamate and aspartate was found in the CSF (Morita et al., 2002). Large and rapid increases of extracellular glutamate

and aspartate were also observed after injection of pilocarpine (Millan et al., 1993). The reduction of the extracellular aspartate and glutamate concentrations in the central nervous system is primarily achieved by excitatory amino acid transporters (EAATs) (for review see Amara and Fontana, 2002). When the widely distributed astrocytic glutamate transporter EAAT2 was knocked out in mice, these mice showed spontaneous lethal epileptic seizures, selective neuronal degeneration in the hippocampus, and increased susceptibility to acute cortical injury (Tanaka et al., 1997). Thus, without the action of this transporter, the glutamate levels rise and cause epilepsy and cell death. It is, however, unknown whether increased levels of aspartate and/or glutamate in patients are related to a dysfunction of EAATs. It is conceivable that the high levels of aspartate and/or glutamate in the cysts are due to insufficient capacity of glutamate transporters either in the cyst epithelium and/or the solid parts of the tumor, thus leading to extracellular accumulation of aspartate and/or glutamate. In fact, in the sporadic form of amyotrophic lateral sclerosis (ALS), a marked reduction of EAAT2 was found in motor cortex and spinal cord (Rothstein et al., 1992, 1995) and an increase of aspartate/glutamate levels in the CSF (Rothstein et al., 1990). So far, no genetic epilepsy has been identified in man with a primary defect involving glutamate transporters (Meldrum et al., 1999). It is noteworthy that in a DNT, which was associated with seizures, the expression of the excitatory amino acid transporter proteins EAAT1 and EAAT2 was found to be weaker than in normal cortex and uneven, which would be consistent with an elevated glutamatergic stimulation being involved in the epileptogenesis (Adamek et al., 2001).

In patients undergoing temporal lobectomy, spiking cortex—as identified by intraoperative electrocorticography—had higher concentrations of glutamate, aspartate, and glycine compared to non-spiking cortex, while the GABA and taurine levels were unaltered. This suggests an association between cortical epileptic activity and elevated concentrations of glutamate, aspartate, and glycine (Sherwin et al., 1988). Glycine acts as a co-agonist at NMDA receptors and thus also has an excitatory function. Further evidence for the involvement of aspartate comes from push–pull perfusion combined with depth electrode recording after electrical stimulation (Do et al., 1991). In complex

partial epilepsy, a sustained increase in extracellular glutamate was observed (During and Spencer, 1993).

It has also been suggested that an increase in the concentration of aspartate in the CSF of patients with epilepsy may in part be related to treatment with AEDs, since patients with AED therapy showed higher values than patients without such therapy (Engelsen and Elsayed, 1984). Furthermore, patients with trigeminal neuralgia also displayed increased aspartate values (Engelsen and Elsayed, 1984). However, one of our patients with a massive increase in aspartate and glutamate levels in the cyst fluid was not on AED therapy, suggesting, if any, a relatively small importance of this factor.

In our study, we also determined the levels of adenosine, which is a natural anticonvulsant, in brain cysts. Interestingly, patient 3, who had a metastasis of a breast cancer in the brain, was seizure-free despite highly increased levels of aspartate and glutamate. Tumor cells are characterized by an elevated purine metabolism to sustain increased DNA synthesis and thus frequently release ATP, which can be converted into adenosine by extracellular apyrases. In cell culture, fast growing cells thus release higher amounts of adenosine into the medium in comparison to slower growing cells. Adenosine can rapidly cross cell membranes via ubiquitously expressed equilibrate adenosine transporters (Cass et al., 1999) and it is thus likely that adenosine concentrations measured within the brain cyst reflect those in the surrounding tissue. It is thus conceivable that this patient was protected from seizures by an increased level of adenosine.

In summary, this study shows that only a limited number of brain cysts contain highly increased levels of aspartate and glutamate and that there is no tight correlation between increased concentrations of aspartate or glutamate in the cyst fluid and the presence of epileptic seizures. Although these findings indicate that the observation of increased concentrations of aspartate and glutamate in the cyst fluid of two epileptic patients described by Hajek et al. (1997) is not necessarily the rule and that there is no tight correlation between aspartate and glutamate concentrations in the cyst and active epilepsy, our data are consistent with a potential contribution of high concentrations of aspartate and glutamate in cysts to epileptogenesis in specific patients.

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