β-endorphin, somatostatin, and prolactin levels in cerebrospinal fluid of epileptic patients after generalised convulsion

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SUMMARY The possible role of different peptidergic systems in the postictal stage of human epilepsy was studied by measuring β-endorphin, somatostatin, and prolactin levels by radioimmunoassay of cerebrospinal fluid (CSF) from nine epileptic patients. The first sample was taken within 2 hours after generalised tonic-clonic convulsion, and the second sample was obtained interictally after 1–4 days without any kind of clinically observable seizures. β-endorphin was elevated postictally (p = 0.044) compared with interictal levels. SLI and PROL were similar in both samples. The present study suggests that in humans β-endorphin is released into CSF during generalised seizures. This may indicate that neurons containing β-endorphin are activated during a seizure.

Epilepsy is a manifestation of abnormal cerebral irritability, where the interictal stage progresses to seizure and further to status epilepticus or to the postictal stage often seen as postictal depression. The neurophysiological disturbance is often associated with diffuse histopathological, neuropsychological, and neuroradiological changes, suggesting both an organic and a functional basis for disturbance in multiple neurotransmitter or neuromodulator systems that regulate neuronal irritability.1–4 Several neuropeptides are thought to play a role in the modulation of seizure activity.5 Peptides localised in limbic structures, such as endogenous opiates and somatostatin, are increased in amygdala kindling, an animal model that mimics human complex partial epilepsy.6 7 The release of these peptides into the CSF after a generalised convulsion has been reported in animal models of epilepsy.8 9 The study of Tortella and Long suggests that an endogenous opiate-like substance may mediate postictal depression after generalised seizure.8 The role of somatostatin in the seizure is, however, not known. β-endorphin and somatostatin in cerebrospinal fluid (CSF) are obviously of cerebral origin. In contrast, prolactin in CSF seems to be of peripheral origin.10 Epileptic seizures elevate the prolactin levels in plasma secondarily to limbic activation.11

The aim of the present study was to further elucidate the role of different peptidergic systems in human epilepsy. We measured β-endorphin, somatostatin, and prolactin levels in the CSF of nine epileptic patients after a generalised convulsion and compared these values with the peptide levels of the same patients at the interictal stage.

Patients and methods

Nine patients with epileptic tonic-clonic convulsions were included in the study after informed consent of the patients and the approval of Ethics Committee of the Vaajasalo Epilepsy Centre. Details of the clinical data for these patients are presented in table 1. Patients were under their usual anticonvulsant therapy at the time of sampling. The drug levels were within therapeutic range. The first sample of CSF was taken within 2 hours after the beginning of a generalised tonic-clonic convulsion. The second sample was taken 1–4 days after the last clinically observed epileptic seizure of any kind. After lumbar puncture, the CSF (4 ml) was divided into aliquots, frozen immediately with dry ice and stored at −20°C until used. The peptide levels of both the postictal and interictal samples were assayed at the same time.

β-endorphin-like immunoreactivity (β-EP) in CSF was measured by radioimmunoassay as described by Jolkkonen et al.12 In brief, the antiserum (B3) used was a gift from Dr Wiegant (Rudolf Magnus Institute, Utrecht, The Netherlands), radioactive β-endorphin was prepared using the Iodogen method, and β-endorphin for standard curves and
iodination was purchased from Organon International BV (Oss, The Netherlands). For radioimmunoassay all reagents were diluted in 0-05 M phosphate buffer, pH 7.4, containing 0-25% bovine serum albumin. The assay was run dis-equilibrated; standard or sample and 50 µl antiserum were incubated for 20 h at 4°C and incubation was continued for another 20 h after adding 50 µl tracer. The reaction was stopped by adding 50 µl bovine serum and 1 ml 20% polyethylene glycol 6000. The sensitivity of assay was 2-7 pmol/l. The within-assay and between-assay coefficients of variation for pooled CSF were both 15%. The CSF dilution curves were parallel to the standard curve.

Somatostatin-like immunoreactivity (SLI) was measured using a commercial radioimmunoassay kit (Immunonuclear Corporation, Minnesota, USA) as described previously. A commercial radioimmunoassay kit (HPRLK-PR) and standard (SB-PROLK-STD) from CIS (France) were used for determination of prolactin (PROL) in CSF. The volume of CSF and standard was 300 µl. The sensitivity of assay was 13.4 mIU/l. The within-assay and between-assay coefficients of variation for pooled CSF were 10-1% and 7-2%, respectively. The CSF dilution curves were parallel to the standard curve.

Significance of the difference in peptide levels between the postictal and interictal samples was evaluated by the Wilcoxon matched-pairs test.

### Results

According to the postictal and interictal levels of the peptides studied (table 2), β-EP was elevated postictally (p = 0.044). Postictal β-EP was higher (p = 0.046) in samples taken 1–2 h (n = 4) after the seizure, compared to the β-endorphin level in samples taken 1 h after seizure (n = 5). The interictal levels of β-endorphin in these groups did not differ. Compared to the interictal levels, SLI and PROL were unchanged after the generalised convulsion.

### Discussion

In the present study we found a slight increase in β-EP in human CSF after generalised tonic-clonic convulsion. SLI and PROL were unchanged. All our patients had a long history of epilepsy. Eight of nine patients had seizures of partial onset with secondary generalisation. These data suggest that the limbic areas are affected during the seizures. The effect of anticonvulsant medication on the release of neuropeptides is not completely understood. In this study

### Table 1  Clinical data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Duration of epilepsy (years)</th>
<th>Type of epilepsy</th>
<th>Ethiology of epilepsy</th>
<th>Interictal EEG</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>31</td>
<td>24</td>
<td>PG</td>
<td>nk</td>
<td>irritative</td>
<td>CBZ, VPA, PHB</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>54</td>
<td>44</td>
<td>CPS</td>
<td>nk</td>
<td>irritative</td>
<td>CBZ, VPA</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>33</td>
<td>33</td>
<td>CPS</td>
<td>nk</td>
<td>irritative</td>
<td>CBZ, VPA, CLN, PHE</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>47</td>
<td>45</td>
<td>CPS</td>
<td>encephalitis</td>
<td>irritative</td>
<td>CBZ, PHB, PHE</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>36</td>
<td>33</td>
<td>CPS</td>
<td>birth asphyxia</td>
<td>irritative</td>
<td>CBZ, CLN</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>36</td>
<td>34</td>
<td>CPS</td>
<td>nk</td>
<td>irritative</td>
<td>CBZ, CLN, PHE</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>41</td>
<td>37</td>
<td>CPS</td>
<td>nk</td>
<td>irritative</td>
<td>PHE, PRI</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>24</td>
<td>14</td>
<td>CPS</td>
<td>nk</td>
<td>irritative</td>
<td>CBZ, VPA, CLN, PHE</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>20</td>
<td>19</td>
<td>CPS</td>
<td>nk</td>
<td>no irritation</td>
<td>CBZ, PHE</td>
</tr>
</tbody>
</table>

Abbreviations: M (male), F (female), PG (primary generalised), CPS (complex partial seizures with secondary generalisation), nk (not known), irritative (spikes, spike-and-wave bursts, or irritative focal activity), CBZ (carbamazepine), VPA (valproate), PHB (phenobarbital), PHE (phenytoin), CLN (clonazepam), PRI (primidone).

### Table 2  Levels of β-EP, SLI, and PROL in cerebrospinal fluid in postictal and interictal samples from epileptic patients

<table>
<thead>
<tr>
<th>Time of CSF tapping after GC</th>
<th>All patients (n = 9)</th>
<th>≤1 h (n = 5)</th>
<th>1–2 (n = 4)</th>
</tr>
</thead>
</table>
<ref>β-EP (pmol/l):</ref> |                      |             |             |
postictal                   | 16.4 ± 3.1**         | 14.6 ± 2.9  | 18.7 ± 2.6* |
interictal                  | 15.2 ± 2.8           | 14.3 ± 2.2  | 16.3 ± 2.3  |
SLI (pmol/l):               |                      |             |             |
postictal                   | 14.9 ± 5.5           | 13.2 ± 4.2  | 17.1 ± 6.9  |
interictal                  | 14.7 ± 6.6           | 11.8 ± 3.6  | 18.4 ± 8.1  |
PROL (mIU/l):               |                      |             |             |
postictal                   | 49.8 ± 15.3          | 56.7 ± 17.4 | 41.4 ± 6.9  |
interictal                  | 46.2 ± 10.8          | 51.0 ± 12.3 | 40.5 ± 6.0  |

Abbreviations: β-EP (β-endorphin-like immunoreactivity), SLI (somatostatin-like immunoreactivity), PROL (prolactin-like immunoreactivity), n (number of patients), GC (generalised tonic-clonic convulsion). Values are expressed as mean ± standard deviation. **p < 0.05, *p = 0.068 (Wilcoxon matched-pairs test) compared with interictal value.
the anticonvulsant medication was kept constant during the interval between the two samples.

Neuronal tracts that contain β-endorphin project from the arcuate nucleus to several periventricular and middle regions.14 Intracerebroventricular injection of β-endorphin into rats induces epileptic seizures, which are mediated primarily by limbic structures.15 Previous studies of opioid peptides have suggested that these peptides play a role in postictal depression and in elevation of the seizure threshold.16 Our finding that in humans β-endorphin increased in CSF after generalised convulsion (GC) agrees with previous findings in experimental epilepsy, where increased release of β-endorphin into CSF was reported.8 We still need to study how our finding is connected functionally to postictal phenomena.

Somatostatin is a neuropeptide localised in both cortical and limbic structures.17 It has both excitatory and inhibitory effects on neuronal firing and behaviour, depending on the dose and the area of the brain studied.18–20 Somatostatin has been suggested as playing a role in the development of kindling.21 We recently showed increased release of somatostatin into the CSF of rats after pentylenetetrazol-induced generalised convulsion.9 In the present study we could not demonstrate postictal release of somatostatin into the CSF of human subjects. In rats the postictal release of SLI into cisternal CSF occurred in 5 minutes and disappeared rapidly during the next 30 minutes. In humans the degradation of somatostatin by peptidases after its release may affect the levels of SLI in CSF. The time required for the transport of somatostatin from the cerebral level to the lumbar space may also be critical, and this should be considered in future studies when the time after seizure is selected for lumbar puncture. The present data suggest, however, that the postictal measurement of SLI in human lumbar CSF has little value for evaluating the role of somatostatin in generalised convulsions.

After primary or secondary generalised convulsions, in humans the plasma level of prolactin increases in 20 minutes.22 After intravenous stimulation of hypophyseal prolactin release by thyrotropin-releasing hormone (TRH) in the rhesus monkey Kalin et al found that the peak PROL in plasma occurred within 15–30 minutes after stimulation.23 The peak for PROL in CSF was seen 90 minutes after administration of TRH. In the present study prolactin levels did not differ in samples taken either 1 or 1–2 hours postictally. This finding indicates that, although the retrograde transport of prolactin from the hypophyseal area or penetration from plasma into CSF might be increased after a generalised seizure, this is not reflected in the postictal samples of lumbar CSF.

In conclusion, results of the present study suggest that in humans β-endorphin is released into the CSF during generalised convulsions. This may indicate that tracts containing β-endorphin participate in seizure generation.

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