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High extracellular concentration of excitatory amino acids glutamate and aspartate in human brain abscess

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ABSTRACT

Brain abscesses often cause symptoms of brain dysfunction, including seizures, suggesting interference with normal neurotransmission. We determined the concentration of extracellular neuroactive amino acids in brain abscesses from 16 human patients. Glutamate was present at 3.6 mmol/L (median value, range 0.5–10.8), aspartate at 1.0 mmol/L (range 0.09–6.8). For comparison, in cerebroventricular fluid glutamate was ~0.6 μmol/L, and aspartate was not different from zero. The total concentration of amino acids was higher in eight patients with seizures: 66 mmol/L (median value, range 19–109) vs. 21 mmol/L (range 4–52) in eight patients without seizures ($p = 0.026$). The concentration of aspartate and essential amino acids tryptophan, phenylalanine, tyrosine, leucine, and isoleucine was higher in pus from patients with seizures ($p \leqslant 0.040$), whereas that of glutamate was not ($p = 0.095$). The median concentration of the non-proteinogenic, inhibitory amino acid taurine was similar in the two groups, 0.7–0.8 mmol/L (range 0.1–6.1). GABA could not be detected in pus. The patient groups did not differ with respect to abscess volume, the cerebral lobe affected, age, or time from symptom onset to surgery. Seven patients with extracerebral, intracranial abscesses had significantly lower pus concentration of glutamate (352 μmol/L, range 83–1368) and aspartate (71 μmol/L, range 22–330) than intracerebral abscesses ($p < 0.001$). We conclude that excitatory amino acids glutamate and aspartate may reach very high concentrations in brain abscesses, probably contributing to symptoms through activation of glutamate receptors in the surrounding brain tissue.

1. Introduction

Brain abscesses often cause symptoms of brain dysfunction, including seizures (Yang and Zhao, 1993; Kilpatrick, 1997; Sellner and Trinka, 2013), suggesting interference with normal neurotransmission. Several factors could contribute to these symptoms, including the inflammatory reaction that accompanies the infection (Kielian, 2004), the opening of the blood–brain barrier, which allows influx of plasma components into the brain (Lo et al., 1994), and the distortion of anatomy that accompanies a space-occupying lesion in the brain (Ebeling and Reulen, 1995). Several studies on the content of brain abscesses showed high concentrations of essential amino acids in the pus (Chang et al., 1998; Grand et al., 1999; Garg et al., 2004; Lai et al., 2005; Himmelreich et al., 2005; Chiang et al., 2009; Pal et al., 2010; Hsu et al., 2013). With respect to seizure-generation, these findings were potentially interesting if they also applied to neuroactive amino acids, especially glutamate, the major excitatory neurotransmitter of the human brain (Fonnum, 1984; Traynelis et al., 2010), whose extracellular concentration is normally kept in the low micromolar range or below (Danbolt, 2001; Eide and Stanisic, 2010). Excessive glutamate-mediated neurotransmission is believed to be a cause of epileptic seizures (Zaczek et al., 1981; Meldrum et al., 1983; During and Spencer, 1993; Eid et al., 2008). However, in the quoted studies on pus analysis was done with ^{1}H magnetic resonance spectroscopy, which was either not used to identify individual amino acids (Pal et al., 2010), or could not identify (or distinguish between) glutamate and glutamine (Chang et al., 1998; Grand et al., 1999; Garg et al., 2004; Lai et al., 2005; Himmelreich et al., 2005; Chiang et al., 2009; Hsu et al., 2013). In contrast to glutamate, glutamine is not excitatory, and its extracellular concentration in the brain is several hundred micromoles per liter (Kanamori and Ross, 2004). Therefore, to be able to evaluate a role for glutamate and other

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neuroactive amino acids in abscess-related symptoms, the various amino acids must be separated during analysis. Further, the above mentioned studies on pus did not distinguish between amino acids in extracellular and intracellular compartments. This issue is important, because only extracellular neuroactive amino acids would be expected to affect surrounding neurons. We therefore analyzed pus from brain abscesses after centrifugation to obtain data on extracellular levels. For amino acid analysis we used high performance liquid chromatography (HPLC), which identifies glutamate and several other amino acids unambiguously (Sandberg and Corazzi, 1983). We compared amino acid levels in intracerebral abscesses to those obtained in extracerebral, intracranial abscesses (empyema), and to the levels in cerebroventricular fluid from patients who underwent cerebroventricular drainage; the latter samples would represent a more normal extracellular fluid of the brain. Finally, we hydrolyzed brain tissue from pig's frontal lobe to get an impression of proteolysis as a potential source of free amino acids in brain abscesses.

2. Materials and methods

2.1. Patients

The study was approved by the Regional Ethics Committee for Medical Research in the southern and eastern part of Norway, and all participants (or their parents, in the case of children) gave informed, written consent. During three years (2011–2014) pus was collected at surgery from 16 patients with intracerebral abscess and from 7 patients with extracerebral, intracranial abscess (empyema). Patients underwent craniotomy and pus evacuation, but the abscess walls were not removed. The pus was aspirated into a polypropylene syringe, cooled on ice, and rapidly centrifuged at 3000×*g* for 10 min at 4 °C. Cerebroventricular fluid (centrifuged as above) was from 10 patients undergoing drainage of the cerebral ventricles as part of the treatment for increased intracranial pressure. After centrifugation supernatants were frozen at −20 °C until analysis, which took place within 0–3 days. Prior to surgery patients underwent MRI or CT scans of the brains to identify abscess localization and size. Abscess volume was calculated from the radius in the *x*, *y*, and *z* planes, using the ellipsoid formula, $4/3\ \pi \cdot r_x \cdot r_y \cdot r_z$ (Mistry et al., 2013).

2.2. Amino acid analysis

Supernatants from centrifuged pus were mixed 1:1 with α-aminoadipate, 2 mmol/L, as an internal concentration standard, and the mixture was diluted 1:100 with sodium azide, 150 mmol/L. Cerebroventricular fluid was mixed 1:1 with α-aminoadipate, 400 μmol/L in sodium azide, 300 mmol/L, for the analysis of glutamine, or with α-aminoadipate, 20 μmol/L in sodium azide, 300 mmol/L, for the analysis of other amino acids. Further handling of the samples was done with an Agilent 1100 series HPLC (Santa Clara, CA, USA) equipped with a fluorescence detector, following the method of Sandberg and Corazzi (1983). Ten μL of the sample-α-aminoadipate mixture was added to 5 μL of o-phthaldialdehyde reagent. This reagent was o-phthaldialdehyde (Sigma, St. Louis, MO, USA), 75 mmol/L, in a mixture of 10% methanol (vol/vol) in sodium borate 0.4 mol/L, pH 11, supplemented with mercaptoethanol, 140 mmol/L. After one minute 2.5 μL NaH_2PO_4, 2 mol/L, pH 4.5, was added to the sample-α-aminoadipate-o-phthaldialdehyde mixture, and 15 μL of the resulting mixture was injected onto a C18 reverse phase column with 250 mm length, 3 mm inner diameter, and 5 μm particle size. The mobile phase was 75% sodium phosphate buffer (50 mmol/L, pH 5.25) and 25% methanol, changing gradually to 25% sodium phosphate buffer and 75% methanol over 40 min before reverting to 75% sodium phosphate buffer and 25% methanol over 5 min. Flow rate was 600 μL/min. Amino acids were eluted at the following time points (in minutes): aspartate 10.3, asparagine 12.8, glutamate 13.4, serine 14.8, glutamine + histidine 15.5, α-aminoadipate 16.8, threonine + glycine 18.4, arginine 19.2, taurine 20.2, tyrosine 21.3, alanine 22.1, GABA 24.1, tryptophan 26.9, methionine 28.0, valine 28.8, phenylalanine 29.2, isoleucine 31.2, leucine 32.5, and lysine 35.6. The method does not detect cysteine or proline. Amino acid concentrations were calculated using α-aminoadipate as an internal concentration standard. The detection limit for aspartate, which was not always found in cerebroventricular fluid, was 0.01 μmol/L. Equi-osmolar solutions of amino acids at 0.01–10 mmol/L were run to obtain correction factors for differences in response from the various amino acids.

2.3. Hydrolysis of pigs' brains

Pigs' brains ($N = 4$) were obtained at a local slaughterhouse within 5 min after slaughtering. The brains were cooled on ice, and a piece of cortical gray matter from the frontal pole and a piece of the underlying white matter were dissected out, weighed, and transferred to a 1.8 mL Eppendorf tube. HCl, 6 N, with α-aminoadipate, 2 mmol/L, was added at 40 μL per mg brain tissue (Damm et al., 2010). Tubes were put on a heating block (100 °C). After 20 h tubes were cooled on ice, centrifuged at 5000×*g* for 5 min, and supernatants were neutralized with NaOH, 5 mol/L, before amino acid analysis as described above. Amino acid concentrations in fully hydrolyzed brain tissue were calculated from the internal concentration standard (α-aminoadipate), correcting for the dilution by HCl.

2.4. Data presentation and statistics

Data from human samples are given as median values with minimum and maximum values. In box-and-whisker plots data are given as median value with the lower and upper ends of the box representing the first and third quartile. Whiskers represent minimum and maximum values. Groups were compared using the Student's *t*-test or the Mann–Whitney *U* test, depending on whether data had normal distribution or not, according to the D'Agostino-Pearson normality test. Relative risk of seizures with increasing pus concentrations of amino acids was calculated by Poisson regression analysis, adjusting for abscess volume and number of days from symptom onset to surgery. Correlations were analyzed by Pearson's method. Values from hydrolyzed pigs' brains are given as mean ± SD values. A p-value < 0.05 was considered significant.

3. Results

3.1. Patient characteristics, symptoms, and treatment

Median age of patients with intracerebral abscess was 56 years (range 2.5–84; Table 1). A likely predisposing condition or extracerebral origin of the infection could be identified in 8 out the 16 patients, including focal infections and immunosuppression with chemotherapy (Table 1). Median time from symptom onset to surgery was 10 days (range 4–56). Abscesses were found in all the lobes of the cerebrum, including 8 in frontal lobes, 2 in temporal lobes, 3 in parietal lobes, and 1 in an occipital lobe. In one patient the abscess was localized in the right parieto-occipital area. One patient had a single cerebellar abscess. Two patients had more than one abscess; one patient had one abscess in a frontal lobe and one in cerebellum; only the former is included here; another patient had 4 abscesses in the right hemisphere; only one in the frontal