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Neurotransmitters in cerebrospinal fluid reflect pathological activity

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Abstract. The excitatory transmitters glutamate and aspartate become toxic whenever their extracellular levels are increased because of neuronal, glial and endothelial impairment. Taurine, a volume-regulating amino acid, is released upon excitotoxin-induced cell swelling. Our aim was to investigate if glutamate and aspartate in cerebrospinal fluid (CSF) reveal neuropathology in neurological patients, and if taurine unmasks glutamate-mediated toxicity. Glutamate and aspartate are doubled in viral meningitis, acute multiple sclerosis (MS) and myelopathy compared with control subjects and patients with peripheral facial nerve palsy. These levels do not coincide with a disturbed blood–brain barrier, as estimated by the albumin ratio, are independent of their precursors (glutamine, asparagine) and are not associated with cell lysis. Taurine is significantly increased in meninigitis, acute MS, and myelopathy, suggesting glutamate-mediated toxicity. Analysis of transmitters in lumbar CSF can be used to identify patients with cerebral and spinal pathology who might benefit from specific receptor-modulating agents.

Keywords. CSF, glutamate, monitoring, lactate dehydrogenase, neurotransmitters.

Introduction

Glutamate and aspartate are of crucial importance in the pathophysiology of various neurological diseases associated with glial and neuronal death [1–6]. Disturbance of the blood–brain barrier [7,8], functional and metabolic impairment of astrocytes and neurons [9,10], and cell lysis [11] sustain the elevation of extracellular glutamate and aspartate and cause cerebral or spinal vasogenic and cytotoxic oedema [12,13]. Taurine, a potent volume-regulating amino acid, is released upon cell swelling in a counter-regulatory homeostatic mechanism [14]. Like glycine, it also exerts inhibitory actions by modulating excitable membranes, thus attenuating glutamate-mediated neuronal excitation [15]. In case of glutamate-induced cell swelling, taurine is released [16], eventually serving as a marker for excitotoxin-related damage. Possible cell destruction leading to increased CSF levels of excitatory and inhibitory transmitters is determined by measuring lactate dehydrogenase (LDH), a known marker for cell death and cell lysis.

In clinical practice, the administration of glutamate receptor-modulating agents might be of benefit only to those patients who also exhibit increased CSF excitotoxin levels. Therefore, the aim of this study was to clarify if changes in glutamate and aspartate levels in CSF reveal ongoing pathological processes in different neurological diseases, possibly exhibiting pathognomonic values and if taurine might unmask glutamate-mediated toxicity.

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Materials and methods

Patients

Patients admitted to the Department of Neurology, Staedtisches Klinikum Ludwigshafen, Germany, presenting with aseptic meningitis, chronic but presently silent and acute multiple sclerosis, myelopathy, acute thrombotic cortical infarction without haemorrhagic components, new-onset grand mal seizures, normal-pressure hydrocephalus and peripheral facial nerve palsy underwent clinically indicated diagnostic lumbar puncture.

The patients with aseptic meningitis presented with meningism, lymphocytosis and negative bacteriological examination. In four cases, the viral aetiology could be identified serologically (two cases of herpes zoster and two cases of herpes simplex).

The diagnosis of MS was based on the clinical course of the disease, neurological, neurophysiological and radiological examiniations. In addition, all patients exhibited intrathecal IgG synthesis with oliclonal IgG production.

The heterogeneous group of myelopathy consists of

Table 1. Age and sex in patients presenting with different neurological diseases

Patients (*n*)	Age (years)	Gender	
		Women	Men
Controls (20)	36 ± 5	12	8
VII palsy (5)	26 ± 2	3	4
Silent MS (14)	33 ± 2	14	–
Acute MS (21)	25 ± 3	14	6
Viral meningitis (14)	38 ± 3	6	8
Myelopathy (18)	54 ± 3	7	11
Stroke (8)	55 ± 5	3	5
NPH (6)	65 ± 2	2	4
Epilepsy (4)	46 ± 5	–	4

VII palsy, facial nerve palsy; MS, multiple sclerosis; NPH, normal pressure hydrocephalus.

different diseases of the spinal cord, for example cervical and lumbar stenosis, diabetic myelopathy and viral myelitis. Patients with psychiatric disorders, migraine and patients suffering from cerebral primary and secondary tumours were excluded. CSF samples free of blood were saved from spinal taps, which otherwise would have been discarded. Lumbar puncture was performed approximately within 2 h after admission to the emergency room or the neurological ward. The time-point of beginning of symptoms was not considered in this study and serial analysis of neurochemical parameters was not performed. Accompanying medical diseases (e.g. diabetes and hypertension) were not considered as exclusion criteria for the present investigations.

Controls

Control CSF samples were taken from patients presenting with cephalgia or cervical or lumbar back pain in whom routine clinical, neurochemical, neurophysiological and neuroradiological evaluations (radiograph, computerized tomography/magnetic resonance imaging, evoked potentials) excluded any underlying disease.

Biochemical parameters

Albumin in CSF and serum was measured by routine automated laser photometry. The albumin ratio (Q_A = albumin in CSF/albumin in serum) was used to quantitate the disturbance of the blood–brain barrier (BBB) [17]. The BBB was considered to be intact whenever the Q_A was below 0·007. Values between 0·007 and 0·01 and between 0·01 and 0·02 represent mild and moderate BBB damage respectively. Values exceeding 0·02 demonstrate a severely disturbed BBB.

CSF glutamate and aspartate, their complementary, non-toxic forms glutamine and asparagine, the inhibitory transmitter glycine and the volume-regulating amino acid taurine were analysed by high-performance liquid chromatography (HPLC). According to our standardized protocol, all investigated CSF samples were taken from the last of four vials (1 mL each) collected in every patient for routine analysis of albumin, glucose, lactate and cell count. Within 5 min after collection, CSF samples were deproteinized with perchloric acid (6%), mixed with potassium carbonate (1 mol L^{-1}), centrifuged and stored at −70°C until analysis. HPLC using a BioRad model 2700 Solvent Delivery System chromatograph (Munich, Germany) was linked to a Biotronik fluorescence detector (Maintal, Germany), set at 330 nm (excitation) and 450 nm (emission wavelength). Stationary phase was a Spherisorb C_{18} column (3 μm particle size), 125 × 4 mm (GROM, Herrenberg-Kayh, Germany). Mobile phases were (a) stock buffer mixed with 1% tetrahydrofuran and 5% acetonitrile (pH 7); and (b) stock buffer–acetonitrile (50/50) (pH 7·2). Stock buffer consisted of 1·724 g L^{-1} sodium dihydrogen phosphate and 1·77 g L^{-1} disodium hydrogen phosphate (40:60). After injection, a stepped gradient at a flow rate of 0·6 mL min^{-1} was applied (0–5 min 100% a, 5–52 min 0–95% b, 52–60 min 95–0% b). Before injection, CSF samples were mixed with an equal amount of ortho-phthaldialdehyde (OPA) and incubated for 2 min. A standard mixture of the amino acids of interest was analysed as an external standard before measuring CSF samples. Sample peak areas were compared with areas of corresponding standard amino acids of known concentration, which allowed calculating the measured sample concentration.

LDH in CSF was analysed using ELISA with an enzymatic colorimetric assay to quantitate cell lysis and cell death (cytotoxicity detection kit, Boehringer Mannheim). In a first step, lactate dehydrogenase transforms lactate to pyruvate, reducing NAD^+ to NADH and H^+. In a second step, H^+ is transferred to tetrazolium salt forming formazan. The dark-red colour of formazan allows estimation of its concentration at an absorption wavelength of 490 nm. The amount of formazan correlates directly with the activity of LDH liberated from lysed cells.

Statistical analysis

Results of amino acids and LDH are given as μmol L^{-1} and U L^{-1} ± SEM respectively. Neurochemical parameters in CSF were compared for significant differences between groups of patients with different neurological diseases using the Kruskal–Wallis and Dunn tests or the ranked-sum test for non-parametric data, where appropriate. Mutual dependencies between the excitotoxins and taurine and the Q_A were evaluated by linear regression. Differences between the groups were rated significant at $P < 0{\cdot}05$.

Results

Patient data (Table 1)

According to clinical manifestation, multiple sclerosis is predominantly encountered in young women, whereas infarctions, myelopathy and normal-pressure hydrocephalus most commonly involve older patients, with a nearly equal distribution among women and men.

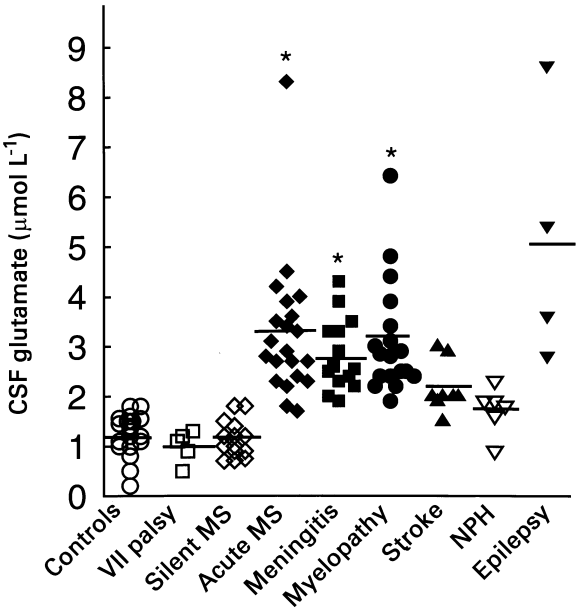

Figure 1. Distribution of CSF glutamate in patients suffering from peripheral facial nerve palsy (□), silent (◇) and acute MS (◆), meningitis (■), myelopathy (●), focal cerebral infarction (▲), normal pressure hydrocephalus (▽), and epilepsy (▼). CSF glutamate concentrations are significantly elevated compared with patients (○) with no evident neuropathological findings (*$P < 0{\cdot}001$).

CSF glutamate (Fig. 1, Table 2)

CSF glutamate levels are significantly elevated in patients suffering from acute MS, viral meningitis, myelopathy and cortical infarctions compared with patients lacking objective signs of neurological disease, those with peripheral facial nerve palsy and compared with individuals with known but presently silent MS. Patients suffering from normal-pressure hydrocephalus and experiencing grand mal seizures for the first time also have higher glutamate levels.

CSF aspartate (Table 2)

CSF aspartate is significantly increased in patients presenting with acute neurological impairment because of MS and myelopathy compared with control subjects, patients with peripheral facial nerve palsy, and silent MS. CSF aspartate is also elevated in patients with focal cerebral infarctions, postictally and in those with normal-pressure hydrocephalus.

CSF taurine (Table 2)

CSF taurine levels are significantly increased in patients suffering from acute MS, viral meningitis, myelopathy and focal cortical infarctions when compared with controls. Postictally and with normal pressure hydrocephalus, higher CSF taurine values are encountered, however, without attaining significance. CSF taurine remains unchanged in silent MS and facial nerve palsy.

CSF glycine (Table 2)

CSF glycine is significantly increased in patients presenting with acute MS, viral meningitis, myelopathy and cortical infarctions compared with controls, patients with chronic but presently silent MS, and peripheral facial nerve palsy.

Relationship of CSF glutamate and taurine

Correlating CSF taurine with CSF glutamate concentrations reveals glutamate to increase taurine levels significantly in patients suffering from acute MS (CSF taurine $= 12{\cdot}5 + 2{\cdot}5 \times$ glutamate; $n = 21$; $r = 0{\cdot}74$; $P < 0{\cdot}001$), viral meningitis (CSF taurine $= 3{\cdot}0 + 6{\cdot}3 \times$ glutamate; $n = 14$; $P = 0{\cdot}007$) and myelopathy (CSF taurine $= 13{\cdot}5 + 4{\cdot}4 \times$ glutamate; $n = 18$; $r = 0{\cdot}73$; $P < 0{\cdot}001$).

CSF glutamine and asparagine (Table 2)

Glutamine and asparagine are non-toxic precursors of glutamate and aspartate that are produced by enzymatic transformation. Despite significantly increased CSF glutamate and aspartate levels, glutamine and asparagine values remain unchanged in CSF. There is no correlation between excitatory transmitters and their precursors.

CSF serine (Table 2)

CSF levels of serine lacking any neurotransmitter function within cerebral and spinal structures remain unchanged in all investigated subgroups.

CSF lactic dehydrogenase (LDH) (Table 2)

Compared with control subjects, LDH levels in CSF are not significantly increased in patients with underlying neurological diseases that are related to cerebral or spinal structures. Furthermore, there is no correlation between CSF amino acid and LDH levels.

CSF amino acids and blood–brain barrier damage

The extent of a disturbed blood–brain barrier possibly contributing to an increase in soluble substances in CSF does not correlate with increased CSF amino acid levels.

Discussion

The analysis of lumbar CSF in patients with cerebral and spinal cord pathology involving neurons and glia reveals significantly increased neurotransmitter levels. Under normal conditions, only a fraction of glutamate and aspartate that are stored intracellularly up to $100\,\text{mmol}\,\text{L}^{-1}$ are found in the extracellular space within the central nervous system, ranging from 1 to $3\,\mu\text{mol}\,\text{L}^{-1}$ [18]. A tight regulation of this gradient is indispensable in preventing any elevation of excitatory

Table 2. CSF levels of excitatory amino acids glutamate and aspartate, their complementary non-active forms glutamine and asparagine, inhibitory transmitter glycine, volume-regulating amino acid taurine, inert amino acid serine, albumin ratio (Q_A), and lactate dehydrogenase (LDH) in various neurological diseases

	Glutamate [μM]	Aspartate [μM]	Glutamine [μM]	Asparagine [μM]	Glycine [μM]	Taurine [μM]	Serine [μM]	Q_A	LDH [U L^{-1}]
Controls (20)	1·3 ± 0·1	0·15 ± 0·01	574 ± 25	5·2 ± 0·9	12·6 ± 1·4	12 ± 1	50·2 ± 3·7	0·005 ± 0·0005	12·9 ± 2·2
VII palsy (5)	1·0 ± 0·1	0·08 ± 0·01	570 ± 54	7·6 ± 1·3	9 ± 1·9	12 ± 1·4	45·6 ± 4·7	0·005 ± 0·0007	10·4 ± 3·3
silent MS (14)	1·2 ± 0·1	0·12 ± 0·02	467 ± 47	4·3 ± 1	12·7 ± 2·1	12 ± 1	52·3 ± 4·2	0·005 ± 0·0006	8·5 ± 1·6
acute MS (21)	3·3 ± 0·3 *	0·25 ± 0·02 *	528 ± 22	4·5 ± 1	25·1 ± 2·6 *	21 ± 1 *	50·3 ± 3·5	0·007 ± 0·0007	12·9 ± 2·5
meningitis (14)	2·8 ± 0·2 *	0·19 ± 0·03	587 ± 35	5·4 ± 1·1	28·9 ± 3·4 *	21 ± 1·8 *	51·5 ± 7·3	0·008 ± 0·001	12·3 ± 2·1
myelopathy (15)	3·1 ± 0·3 *	0·25 ± 0·02 *	597 ± 54	5·5 ± 1	35·6 ± 3·7 *	27 ± 1·8 *	60·1 ± 9·4	0·009 ± 0·001	19·7 ± 4·7
stroke (8)	2·2 ± 0·2 *	0·20 ± 0·01 *	655 ± 31	4·7 ± 1·8	28·9 ± 3·3 *	20 ± 1·4 *	56·2 ± 6·3	0·006 ± 0·001	15·1 ± 3·2
NPH (6)	1·7 ± 0·2 *	0·20 ± 0·04 *	615 ± 48	5·1 ± 1·8	35·2 ± 7·8 *	19 ± 2·5 *	57·5 ± 4·1	0·006 ± 0·0007	no samples
epilepsy (4)	5·0 ± 1·8 *	0·17 ± 0·05	629 ± 84	6·8 ± 5·2	41·4 ± 9·2 *	24 ± 7 *	59·1 ± 12·7	0·01 ± 0·003	no samples

Results compared with controls and patients presenting with peripheral facial nerve palsy are significant *$P<0·05$.
VII palsy, facial nerve palsy; MS, multiple sclerosis; NPH, normal pressure hydrocephalus.

amino acids (EAA) that may become harmful because of intensified excitation and activation of neurons and glia [19]. Activated second-messenger cascades increase intracellular calcium levels, generate free radicals, produce acidosis, increase energetic consumption and cause cellular swelling, resulting in neuronal and glial death [19–22]. EAA-mediated cell death will result in a further release of otherwise compartmentalized excitotoxins, increasing extracellular levels. Thus, a self-sustaining vicious circle is generated, leading to widespread neuronal and glial damage [23]. Whenever amino acids are elevated in cerebral and spinal extracellular space and glial uptake mechanisms are not sufficient to clear potentially toxic substances, these transmitters will follow their gradient and lead to an increase in CSF [24,25]. Thus, elevated CSF values allow estimation of ongoing cerebral and spinal pathology. Lumbar CSF amino acid levels correspond to those in the ventricular system [26] and therefore CSF from lumbar puncture can reveal cerebral damage. Increases in excitatory and inhibitory neurotransmitters in CSF could occur passively because of concomitant neuronal vesicular release. However, mutual dependency of glutamate and taurine as found in patients suffering from acute MS, viral meningitis and myelopathy suggests functional interactions, as shown under *in vitro* conditions [16]. Increased glutamate levels overstimulate neurons and astrocytes, inducing cell swelling. This, in turn, might lead to a counter-regulatory release of volume-regulating and inhibitory transmitters with the aim of maintaining cellular homeostasis [27] and possibly preventing spreading of neuronal and glial damage. Whereas taurine is an inhibitory and neuromodulating transmitter within both brain and spinal cord, glycine is known to have different functions according to its location. Within the brain, glycine is an important co-activator of glutamate at NMDA receptors and is considered to potentiate glutamate-induced cytotoxicity [28,29] by increasing inflow of glutamate-dependent calcium [30]. Within the spinal cord, glycine is the predominant inhibitory transmitter [31]. Increased CSF glycine levels in patients with diseases involving the brain (MS, cortical infarctions, seizures, normal pressure hydrocephalus) might depict supported glutamate-mediated excitotoxicity. In myelopathy, increased CSF glycine may reflect counter-regulatory neuronal inhibition. Overall, the damaged blood–brain barrier as estimated by the albumin ratio does not contribute to elevated CSF amino acid levels [32], which is stressed by the finding that serine without any transmitter function within the central nervous system remains unchanged in all subgroups and is underlined by normal CSF amino acid levels in patients with peripheral facial nerve palsy. Thus, an unbalanced release and decreased uptake of amino acids within cerebral and spinal structures could account for the observed increases in neurotransmitter levels in CSF of patients suffering from acute MS, meningitis [33,34], ischaemia [1,35–38], epilepsy [39,40], myelopathy [3,41,42], and normal-pressure hydrocephalus [43,44].

The analysis of amino acids in plasma would be helpful to rule out any passive increase because of a damaged blood–brain barrier but would not attenuate the pathophysiological importance of elevated excitotoxin levels in CSF. Unchanged glutamine and asparagine levels in CSF and an absent inverse correlation make an increase of glutamate and aspartate levels due to enzymatic transformation unlikely. Increases in CSF amino acids as a result of cell lysis and cell death is ruled out by unchanged LDH levels in all patient subgroups and by the absence of a correlation between transmitters and LDH. The exclusion of simple amino acid overflow of CSF due to a damaged blood–brain barrier, enzymatic transformation and cell lysis, stresses the complexity of underlying pathomechanisms in each investigated group.

Conclusions

Inexpensive and simple analysis of amino acids in the CSF of patients with different neurological diseases is capable of unmasking patients with increased excitatory and inhibitory neurotransmitter levels. The exact pathophysiological mechanisms remain to be clarified. There is, however, substantial evidence that an increased release and an impaired uptake of amino acids in cerebral and spinal structures could account for pathologically elevated transmitter levels in CSF. Identifying patients with increased glutamate and aspartate levels in clinical routine could aid in deciding who might profit from specific glutamate receptor blocking or modulating agents.

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