Archives of Biochemistry and Biophysics 536 (2013) 97–100

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Editorial

Hepatic encephalopathy: Clinical aspects and pathogenetic concept

The present highlight addresses current research aspects regarding hepatic encephalopathy (HE), a neuropsychiatric complication of acute or chronic liver failure. It can also reflect ammonia toxicity in inborn hyperammonemic disorders [1].

Hepatic encephalopathy: clinical aspects

The following comments will largely focus on HE in chronic liver disease, because 97% of HE episodes occur in patients with liver cirrhosis. Depending on the population under study, the prevalence of minimal and manifest HE among cirrhotic patients was reported to be 20–70%. The affliction is characterized by reversible impairment of motor function, cognition, emotional/affective regulation and behavioral patterns (for review see [2]). In clinical routine four severity grades of manifest HE are distinguished depending on the symptomatology (Table 1). However, at low HE grades severity assessment may be subjective, because grade I symptoms can also be found in patients without liver disease. Apart from the manifest forms of HE, there is so-called minimal HE (mHE) which, by definition, does not present overt symptoms and is detectable only by means of neuropsychological tests. Such tests comprise paper–pencil test batteries, computerized psychometric test batteries, recordings of evoked potentials and determination of the critical flicker frequency (CFF) [3,4]. Unfortunately, the most frequently used psychometric test batteries are not standardized, and mHE is currently defined by the test which is used for its diagnosis. In view of this dilemma, it was suggested to collectively denote mHE, HE grade I and II as "low grade HE" with further specification by means of an objective parameter such as CFF or evoked potentials [5]. Even mild forms of HE are thought to impair quality of life and there is concern about the fitness to drive a car in these patients. A study on real driving showed that mHE and HE I patients as groups exhibit impaired driving fitness due to inaccurate actions and attention deficits (mHE patients) and slow reaction times (HE I patients), respectively [6]. However, despite these statistically significant group effects, in the individual patient the presence of mHE or HE I did not necessarily predict inability to drive a car.

In patients with liver cirrhosis, episodes of HE can be triggered by so-called *precipitating factors* such as bleeding, infections, trauma, sedatives, high oral protein intake, metabolic acidosis, sedatives and diuretics. In these patients HE is seen as a clinical manifestation of a low-grade cerebral edema which exacerbates in response to these precipitating factors after an ammonia-induced exhaustion of the volume-regulatory capacity of the astrocyte (Fig. 1). These factors are frequently associated with increased ammonia and/or inflammatory cytokine loads to the brain, electrolyte disturbances (hyponatremia) or direct neurodepressive actions on $GABA_A$ receptors (benzodiazepines). However, HE cannot be ascribed to the disturbance of a single neurotransmitter/receptor system. Instead, multiple alterations of neurotransmitter receptors and their ligands have been described, which may contribute to the pathogenesis of HE. A comprehensive survey about the changes of neurotransmitter receptors in HE is given in the articles by Palomero–Gallagher and Zilles and Sergeeva et al. [7,8]. HE is also accompanied by metabolic changes in the brain, which can be studied by modern brain imaging techniques and are discussed in this Highlight by Keiding and Pavese [9].

Hepatic encephalopathy: pathogenetic model

As mentioned above, current evidence suggests that HE in cirrhotic patients reflects the clinical manifestation of a low grade cerebral edema which exacerbates in response to a variety of precipitating factors (Fig. 1). Evidence for such low grade cerebral edema in HE came from *in vivo* proton magnetic resonance (^{1}H-MRS) studies on human brain and by quantitative water mapping of the human brain *in vivo* [10,11]. HE severity, as assessed by analysis of critical flicker frequency (CFF) correlated well with the water increase in the frontal and occipital white matter, the globus pallidus, the anterior limb of the internal capsule and the putamen, but not in the grey matter, the thalamus, the caudate nucleus and the coronal white matter. However, HE patients showed a more inhomogeneous water distribution in the thalamus, when compared to healthy controls [11]. The cerebral edema accompanying HE is in part due to osmotic astrocyte swelling triggered by an ammonia-induced glutamine accumulation in astrocytes and, probably more important, due to an oxidative/nitrosative stress response [12] which may involve not only an activation of the Na–K–Cl cotransporter 1 (NKCC1) [13], but also responses on antioxidant defense systems, *e.g.*, selenoproteins, in the brain [14]. Astrocyte swelling and oxidative/nitrosative stress are also induced by inflammatory cytokines, benzodiazepines and hypoosmotic conditions and thereby provide a common final pathway of action of different precipitating factors. This explains why rather heterogeneous clinical conditions can precipitate HE episodes. As mentioned above, swelling and oxidative/nitrosative stress are linked to each other in a self-amplifying signaling loop: astrocyte swelling induces oxidative/nitrosative stress and, conversely, oxidative/nitrosative stress induces astrocyte swelling (Fig. 2). Ammonia also triggers the formation of reactive oxygen species in microglia. A detailed analysis of the interaction between astrocyte swelling and oxidative/nitrosative stress is given in [12,15].

Oxidative/nitrosative stress has functional consequences, which are thought to be crucial in the pathogenesis of hepatic encephalopathy. These include protein tyrosine nitration, oxidation of RNA, zinc mobilization and alterations in gene expression and

0003-9861/$ - see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.abb.2013.04.013

Table 1
Severity grades of hepatic encephalopathy.

Low Grade	
Minimal HE (mHE)	
	No overt symptoms, detectable only by psychometric and neurophysiological testing
Manifest HE	
	Grade I: personality changes, short attention span, irritability, disturbed sleep-wake-rhythmicity, mini-asterixis (postural tremor)
	Grade II: lethargy, fatigue, desorientation, asterixis (flapping tremor), forgetfulness
High Grade	
	Grade III: somnolence, stupor
	Grade IV: coma

According to a suggestion [5] mHE and manifest HE grades I and II are summarized as low grade HE with further specification by objective and reproducible parameters (*e.g.*, critical flicker frequency, evoked potentials), whereas high grade HE will comprise HE grades III and IV.

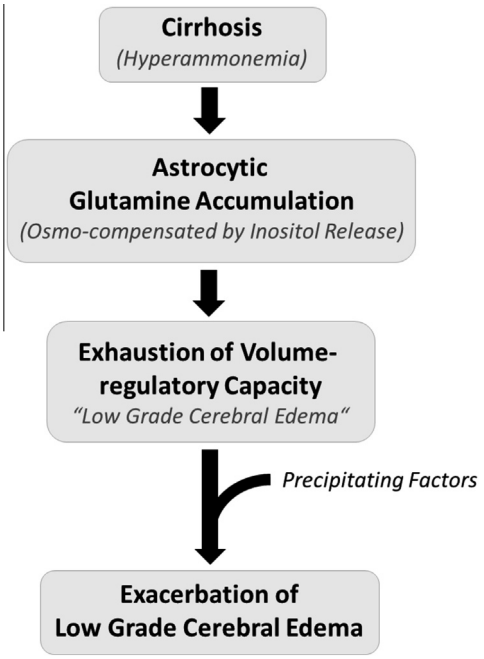

Fig. 1. Low-grade cerebral edema, precipitating factors and induction of hepatic encephalopathy episodes. Liver cirrhosis triggers glutamine accumulation in the astrocytes with subsequent volume regulatory release of organic osmolytes such as *myo*-inositol. Whereas the release of osmolytes attenuates astrocyte swelling ("low-grade cerebral edema"), it lowers the volume-regulatory capacity and makes the astrocyte vulnerable to further volume challenges that are introduced by HE-precipitating conditions. Exacerbation of the low-grade cerebral edema then triggers a self-amplifying cycle between cell swelling and oxidative/nitrosative stress, which is central to the pathophysiology of HE (for details see text; see also Fig. 2).

signal transduction (see [15]). Protein tyrosine nitration is most pronounced in perivascular astrocytes, possibly relating to selective alterations of blood–brain barrier permeability, which have repeatedly been reported in HE. Increased serum levels of 3-nitro-tyrosine were shown to serve as a peripheral biomarker for low grade HE [16,17]. Oxidized RNA was detected in astrocytes and in the cytosol of neurons from ammonia–intoxicated rats. Importantly, oxidized RNA was also found in RNA granules along the dendrites and in postsynaptic dendritic regions. This suggests that HE-associated oxidative stress modifies RNA species, which participate in local postsynaptic protein synthesis. Local postsynaptic protein synthesis plays a major role for synaptic plasticity and is required for learning and the formation of long-term memory [18]. RNA oxidation in response to ammonia, TNF-α, benzodiazepines, and hyponatremia is a selective process, and among the RNA species being oxidized, the mRNAs for the glutamate uptake system GLAST and ribosomal (r) RNA were identified. The implications of RNA oxidation for ammonia neurotoxicity and HE are currently unknown; however, rRNA and mRNA oxidation can compromise translation accuracy and efficacy resulting in the formation of defective proteins [19]. To what extent RNA oxidation contributes to the multiple derangements of neurotransmitter receptor systems in HE and to the clinical picture of HE is currently unknown.

Another consequence of oxidative/nitrosative stress is the mobilization of Zn^{2+} and a protein kinase C-dependent translocation of the transcription factor SP1 to the nucleus, which is involved in the regulation of peripheral benzodiazepin receptor (PBR) expression [20]. This protein is upregulated in HE and may trigger increased synthesis of neurosteroids, such as allopregnanolone and allotetrahydrodeoxy-corticosterone. These neurosteroids can have a positive $GABA_A$-receptor modulatory activity and were identified in the brain from patients with hepatic coma [21]. This could provide another explanation for the increased GABAergic tone found in patients with HE [8]. These neurosteroids also have a high affinity to the G-protein-coupled receptor TGR5 which, in peripheral organs, acts as bile acid receptor but is abundantly expressed also in the brain [22]. Thus, in the brain, TGR5 is a neurosteroid receptor which is expressed in neurons and astrocytes. TGR5 is also found in synapses and triggers the formation of cyclic AMP. A role of TGR5 in the pathophysiology of HE is suggested by the fact that this receptor is strongly downregulated in the brain of cirrhotic patients with HE [22]. Despite increased cerebral NO formation in response to ammonia, chronic moderate hyperammonemia impairs activation of soluble guanylate cyclase in cerebellum, but not in cerebral cortex resulting in an impairment of the glutamate-NO-cGMP pathway, which was hypothesized to contribute to the cognitive impairment in HE [23]. In line with a lowered cGMP signalling, the phosphodiesterase inhibitor zaprinast reversed the ammonia-induced suppression of corticostriatal long-term depression [24]. Molecular aspects on changes of synaptic plasticity in HE are presented by Wen et al. [25].

Importantly, increased levels of oxidative stress markers were dectected in cerebral cortex from patients with liver cirrhosis and hepatic encephalopathy, but not in patients with cirrhosis without HE (see [15]). These findings suggest that cerebral oxidative/nitrosative stress is a feature of hepatic encephalopathy, but not of liver cirrhosis itself. Whole genome array analysis on human brain also demonstrated complex and HE-specific alterations in gene expression profiles [26]. Genes with altered expression pattern in HE were related to oxidative stress, signaling pathways, cell proliferation, apoptosis and microglia activation. Despite an upregulation of genes associated with microglia activation, pro-inflammatory cytokine mRNA profiles remained unchanged in the brain of patients with liver cirrhosis and HE as compared to controls [26]. Interestingly, many genes counteracting pro-inflammatory signaling and inflammatory cytokine expression are upregulated in the cerebral cortex of patients with liver cirrhosis and HE, in line with studies on cultured rat astrocytes. The role