Archives of Biochemistry and Biophysics 536 (2013) 158–163

Contents lists available at SciVerse ScienceDirect

Archives of Biochemistry and Biophysics

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

Osmotic and oxidative/nitrosative stress in ammonia toxicity and hepatic encephalopathy

Boris Görg, Freimut Schliess [1], Dieter Häussinger *

Heinrich-Heine-University Düsseldorf, Clinic for Gastroenterology, Hepatology, and Infectious Diseases, Germany

ARTICLE INFO

Article history:
Available online 6 April 2013

Keywords:
Astrocytes
Protein tyrosine nitration
RNA oxidation
Zinc
NADPH oxidase

ABSTRACT

Hepatic encephalopathy (HE) is a neuropsychiatric complication of acute or chronic liver failure. Currently, HE in cirrhotic patients is seen as a clinical manifestation of a low grade cerebral edema which exacerbates in response to a variety of precipitating factors after an ammonia-induced exhaustion of the volume-regulatory capacity of the astrocyte. Astrocyte swelling triggers a complex signaling cascade which relies on NMDA receptor activation, elevation of intracellular Ca^{2+} concentration and prostanoid-driven glutamate exocytosis, which result in increased formation of reactive nitrogen and oxygen species (RNOS) through activation of NADPH oxidase and nitric oxide synthase. Since RNOS in turn promote astrocyte swelling, a self-amplifying signaling loop between osmotic- and oxidative stress ensues, which triggers a variety of downstream consequences. These include protein tyrosine nitration (PTN), oxidation of RNA, mobilization of zinc, alterations in intra- and intercellular signaling and multiple effects on gene transcription. Whereas PTN can affect the function of a variety of proteins, such as glutamine synthetase, oxidized RNA may affect local protein synthesis at synapses, thereby potentially interfering with protein synthesis-dependent memory formation. PTN and RNA oxidation are also found in *post mortem* human cerebral cortex of cirrhotic patients with HE but not in those without HE, thereby confirming a role for oxidative stress in the pathophysiology of HE.

Evidence derived from animal experiments and human *post mortem* brain tissue also indicates an up-regulation of microglia activation markers in the absence of increased synthesis of *pro*-inflammatory cytokines. However, the role of activated microglia in the pathophysiology of HE needs to be worked out in more detail. Most recent observations made in whole genome micro-array analyses of *post mortem* human brain tissue point to a hitherto unrecognized activation of multiple *anti*-inflammatory signaling pathways.

Introduction

Hepatic encephalopathy (HE) is a neuropsychiatric complication of acute and chronic liver disease. In patients with liver cirrhosis HE is characterized by reversible impairment of motor function, cognitive performance, emotional/affective regulation and behavioral patterns [1], which are related to disturbances of glioneuronal communication, synaptic plasticity, and oscillatory networks in the brain and which result in a broad range of clinical signs and symptoms. Current evidence suggests that HE is the clinical manifestation of low grade cerebral edema due to osmotic astrocyte swelling with increased production of reactive oxygen and nitrogen species [2,3]. Indeed, there is growing evidence that factors involved in the precipitation of HE episodes such as ammonia, benzodiazepines, hyponatremia and inflammatory cytokines trigger a self-amplifying cycle of astrocyte swelling and cerebral oxidative/nitrosative stress, leading to altered patterns of signal transduction and gene expression with post-translational protein modification and RNA oxidation in the brain [2,4]. Ammonia is a key toxin in HE and provides a paradigm for studies on the pathogenic interplay between osmotic and oxidative stress in the brain [2,3]. This article summarizes our current view on the interplay between osmotic and oxidative stress in ammonia toxicity and its role in the pathogenesis of HE.

Ammonia and swelling of astrocytes in culture

Cerebral ammonia detoxification is accomplished *via* the glutamine synthetase-catalysed formation of glutamine from ammonia and glutamate, which is largely confined to the astrocytes in the brain [5], but is also expressed in cultured microglia at the mRNA and protein level [6]. Accordingly, astrocyte cultures were

* Corresponding author. Address: Heinrich-Heine-Universität Düsseldorf, Klinik für Gastroenterologie, Hepatologie, und Infektiologie, Moorenstrasse 5, D-40225 Düsseldorf, Germany. Fax: +49 211 81 18752.
E-mail address: haeussin@uni-duesseldorf.de (D. Häussinger).
[1] Current address: Profil Institut für Stoffwechselforschung, Hellersbergstrasse 9, 41460 Neuss, Germany.

0003-9861/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.abb.2013.03.010

frequently used to study primary ammonia effects relevant to the brain [7,8]. However, cultured astrocytes may not necessarily behave like astrocytes in their *in situ* environment in the brain. Data from astrocyte cultures indicate that ammonia induces astrocyte swelling in a reversible manner, which is sensitive to inhibition of glutamine synthetase [9–12].

Several approaches for assessment of ammonia effects on astrocyte volume have been employed. These include the ^{3}HO-methyl-glucose equilibration method [13], single cell BCECF fluorescence recordings at the isosbestic point [12] and more recently the VivaTome technique [6]. In contrast to the methyl-glucose equilibration technique, the two latter techniques allow to monitor rapid astrocyte volume changes (in the range of seconds) and to study their time course in a single cell, showing that astrocyte swelling in response to ammonia occurs within seconds. By means of the VivaTome technique it was also shown that astrocytes and microglia, but not neurons swell in response to ammonia and that cell swelling of cultured astrocytes treated with HE-relevant factors, e.g. ammonia, TNF-α or diazepam, is also accompanied by swelling of the nucleus [6]. Nuclear swelling has also been reported in cerebrocortical astrocytes in animal models for HE [10] and Alzheimer-type II astrocytes of cirrhotic patients with HE [14]. While nuclear swelling may enhance gene expression through chromatin decondensation [15], its impact on astrocyte function in HE remains to be established.

In addition to intracellular glutamine accumulation, induction of oxidative/nitrosative stress (for further details see [6]) and activation of the Na–K–Cl cotransporter NKCC1 by ammonia [16,17] contributes to osmotic astrocyte swelling by ammonia. Furthermore, *in vitro* data suggest that aquaporin-4, which is the most abundant water channel in the brain and almost exclusively expressed in astrocytes, is an important water entry route in ammonia-treated astrocytes [18].

Astrocyte swelling is accompanied by a decrease of intracellular *myo*-inositol levels, indicating a volume-regulatory response of the astrocytes under hyperammonemic conditions [19,20]. Acute ammonia infusion in rats leads to glutamine synthetase-dependent astrocyte swelling [10,21]. In rats with portocaval anastomosis, acute ammonia infusion increases the brain water content, which significantly correlates with the cerebral blood flow and contributes to the development of brain edema [22]. Pharmacological inhibition of glutamine synthetase not only prevented cerebral glutamine accumulation, but also the increase of brain water and intracranial pressure [23] despite a further increase of plasma ammonia concentration. From these and other studies it has been argued that glutamine and/or astrocyte swelling at least in part mediate ammonia toxicity to the brain [1,2,19,24] and that HE could be considered as primary gliopathy with secondary impact on neuronal function [8,25]. In line with astrocytes being the primary target for ammonia, long-term ammonia exposure of neurons co-cultured with astrocytes resulted in a protection of the neurons against the formation of apoptotic bodies, caspase-3 activation, oxidative stress and collapse of the mitochondrial membrane potential [26]. These observations may indicate that astrocytes can protect neurons at least from irreversible ammonia toxicity. As shown in recent studies on rat cortical cells, which were cultured on microelectrode arrays, the ammonia-induced increase of global network activity, but not the suppression of network synchrony depends on astroglial glutamine synthesis [27].

Astrocyte swelling and low grade cerebral edema in HE in chronic liver disease

Evidence for the presence of a low grade cerebral edema in patients with liver cirrhosis and HE has been presented for the first time in 1994 by *in vivo* proton magnetic resonance spectroscopy (^{1}H-MRS) studies on human brain [19]. This study identified *myo*-inositol and glutamine as organic osmolytes in the human brain, which are released in response to hypoosmolarity/hyponatremia, and characterized their disturbances in HE. Already in cirrhotic patients with subclinical HE the recordings indicated an increase of brain glutamine together with a decrease of brain *myo*-inositol. These data were interpreted to reflect a glial edema with partial compensation by a volume-regulatory release of *myo*-inositol. In line with this, a subsequent study using quantitative water mapping by magnetic resonance imaging (MRI) directly demonstrated increased brain water content in cirrhotic patients with HE [28].

Similar results were obtained using the portocaval anastomosis rat model of chronic HE. Here, increased brain glutamine levels are accompanied by decreased brain *myo*-inositol and taurine levels but, importantly, the overall content of the brain osmolytes determined in this study remained unchanged suggesting a compensatory mechanism, which under these conditions might prevent the development of an overt brain edema [29,30].

Since all HE-relevant factors were shown to trigger astrocyte swelling, the concept has been introduced that at least some of the pathogenic events triggered by HE-precipitating factors integrate at the level of swelling-induced alterations of astrocytic functions [19,24].

Astrocyte swelling and oxidative/nitrosative stress

There is substantial evidence from animal and cell culture studies for an important role of oxidative/nitrosative stress in the pathogenesis of HE. Ammonia, inflammatory cytokines, benzodiazepines and hyponatremia induce the rapid formation of reactive nitrogen and oxygen species (RNOS) through *N*-methyl-D-aspartate (NMDA)-receptor and Ca^{2+}-dependent mechanisms in cultured astrocytes and in rat brain *in vivo* [2,3,12,31–34]. Under these conditions, NMDA receptor (NMDA-R) activation is thought to result from a depolarization-induced removal of the Mg^{2+}-blockade, which is further amplified by subsequent prostanoid- and Ca^{2+}-dependent astroglial vesicular glutamate release and autocrine NMDA-R stimulation [1–3] (Fig. 1). In line with this, ammonia activates phospholipase A_2 [35] and triggers prostanoid formation in cultured astrocytes [36,37]. There is a close relationship between astrocyte swelling and oxidative stress involving a self-amplifying cycle: astrocyte swelling induces oxidative stress through NMDA receptor- and Ca^{2+}-dependent mechanisms on the one hand and on the other, NMDA receptor activation and oxidative stress trigger astrocyte swelling [2,3]. Activation of NADPH oxidase and of Ca^{2+}/calmodulin-dependent isoforms of nitric oxide synthase in response to hypoosmotic astrocyte swelling or ammonia exposure are major sources of the early ROS and NO formation [12,38]. Ammonia and hypoosmotic swelling trigger in astrocytes an activating serine-phosphorylation of $p47^{phox}$, which is critical for NADPH oxidase activation. Upstream events of hypoosmolarity-induced $p47^{phox}$ phosphorylation are NMDA receptor activation, a Ca^{2+} signal, sphingomyelinase activation and activation of protein kinase Cζ, for which $p47^{phox}$ is a known substrate [12].

Ammonia not only triggers RNOS formation in astrocytes [4,32] but also in microglia [13,36,39] and cerebral endothelial cells [40]. Thus, RNOS formation in ammonia-treated microglia [39] or cerebral endothelial cells [40] could contribute to astrocyte swelling in HE.

Ammonia and inflammation also act synergistically on RNOS formation in peripheral immune cells such as neutrophil granulocytes [41], which may explain systemic oxidative stress in some animal models for HE [42].