CONFERENCES PROCEEDINGS

Pathogenesis of hepatic encephalopathy

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Abstract  Hepatic encephalopathy (HE) is a neuropsychiatric syndrome during the course of acute or chronic liver disease. It is functional in nature, potentially reversible and precipitated by rather heterogeneous factors. At the neurophysiological level HE is characterized by a low frequent cortico-cortical electrical coupling, which may explain the cognitive deficits and a low frequent corticomuscular coupling, which may explain the fine motor deficits. Current evidence suggests that HE is the consequence of a low grade chronic glial edema with subsequent alterations of glioneuronal communication. Different factors, such as ammonia, benzodiazepines, inflammatory cytokines can induce or aggravate astrocyte swelling, which results in the activation of osmosignaling cascades, protein modifications, alterations in gene expression and neurotransmission. Among the protein modifications nitration of critical tyrosine residues in glial proteins may play an important role. Several proteins, which are nitrated in response to ammonia, benzodiazepines, hypoosmotic astrocyte swelling or inflammatory cytokines have been identified, including glutamine synthetase and the peripheral type benzodiazepine receptor.

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INTRODUCTION

The pathogenesis of HE involves the action of neurotoxins, such as ammonia, and several phenomena, including alterations in neurotransmission, blood brain barrier permeability or energy metabolism, have been described.¹⁴ However, it remained unclear whether such phenomena are of pathogenetic relevance or merely reflect epiphenomena. Considerable advances in the understanding of HE pathogenesis were made with modern cell and molecular biology techniques and by the use of modern imaging and neurophysiological techniques. Previous hypotheses about the pathogenesis of HE were unable to explain all the facets of this clinical syndrome. Clearly, pathogenetic models have to explain the functional nature and reversibility of HE symptoms, and their precipitation by heterogeneous factors, such as infections, diuretics, sedatives, trauma, bleeding or high protein intake. Furthermore, pathogenetic considerations must address the neurophysiological level, morphology, cell and molecular biology and the clinical experience.

NEUROPHYSIOLOGICAL LEVEL

Hepatic encephalopathy symptomatology is characterized by cognitive and fine motor deficits of varying severity. New aspects on the understanding of the pathogenesis of postural tremor in HE came from magnetencephalographic studies in comparison with electromyographic measurements.⁵ These studies showed that the tremor in HE is of cortical origin and is due to a shift of corticomuscular coherence to low frequencies. A similarly low frequent coherence was found for cortico-cortical coupling, which may explain the cognitive deficits in HE (Timmermann, unpubl. data, 2002).

ASTROCYTE SWELLING IN HEPATIC ENCEPHALOPATHY

In HE, the neurons appear morphologically normal, whereas astrocytes exhibit signs of Alzheimer type II degeneration. Such Alzheimer type II changes can be
induced experimentally in cultured astrocytes upon exposure to ammonia. This prompted the idea that hepatic encephalopathy is a primary disorder of glial cells, with neuronal dysfunction being the consequence. Astroglycerol-phosphorylcholine are depleted in order to counteract astrocyte swelling in HE.

These findings prompt us to view hepatic encephalopathy as a consequence of a low grade chronic glial edema with important consequences for glioneuronal communication.

FUNCTIONAL CONSEQUENCES OF LOW-GRADE ASTROCYTE SWELLING: CELL AND MOLECULAR BIOLOGY

In all cell types studied so far, the cellular hydration state was identified as an independent signal which regulates cell function and gene expression (for reviews see,19,20). Multiple osmosignaling pathways have been identified which provide the link between cell hydration and cell function.21 Thus, small increases in astrocyte water content, as they occur in HE, already have important functional consequences despite the absence of clinically overt increases of intracranial pressure. Indeed, complex alterations of astrocyte function in response to astrocyte swelling, hyperammonemia and other precipitating factors have been identified and include activation of osmosignaling cascades, covalent modification of astrocytic proteins and gene expression. Swelling of astrocytes activates extracellular signal-regulated protein kinases (Erks)13 (i.e. members of the MAP-kinase family with multiple functions) in a phosphatidylinositol-3-kinase-dependent way, elevates intracellular calcium concentration22 up-regulates the peripheral type benzodiazepine receptor (PBR)23 affects multiple ion channels and amino acid transport.14 Furthermore, astrocyte swelling increases the pH in endocytotic vesicles24 through an Erk-dependent osmosignaling pathway (R Fischer and D Haussinger, unpubl. data, 2000). Given the important role of a low endosomal pH for receptor/ligand sorting, the marked endosomal alkalization in response to astrocyte swelling is expected to affect receptor densities and neurotransmitter processing. The increased deposition of glycogen in astrocytes in animal models of chronic HE9 may also be explained by cell swelling, because swelling of hepatocytes increases glycogen synthesis and inhibits glycogenolysis (for review see,29). Increased expression of PBR in response to astrocyte swelling augments the synthesis of neurosteroids, which are potent modulators of neuronal GABA<sub>A</sub> receptor activity.17 Thus, the interaction between astrocyte swelling, PBR expression and increased neurosteroid synthesis may explain the increased GABA<sub>A</sub>-ergic tone found in HE.

Interestingly, astrocyte swelling and ammonia at HE-relevant concentrations induce a rapid nitration of critical tyrosine residues in astrocytic proteins.25 Protein tyrosine nitration is also found in vivo in response to ammonia injection or institution of a portocaval anastomosis.25 Ammonia-induced protein tyrosine nitration is Ca<sup>2+</sup>-dependent, involves NMDA receptor activation, nitric oxide synthase induction and is prevented by NMDA receptor antagonists, methionine sulfoximine and antioxidants. Among the proteins being tyrosine-nitrated in response to ammonia, glutamine synthetase and the PBR were identified. Although the pathogenic role of tyrosine nitration remains to be established, several observations suggest its pathogenic relevance.

(1) NMDA receptor antagonists and methionine sulfoximine inhibit tyrosine nitration29 and are known to ameliorate ammonia toxicity in vivo.26-28

(2) All factors known to precipitate HE in vivo, such as ammonia, astrocyte swelling, benzodiazepines and inflammatory cytokines can induce protein tyrosine nitration.25

(3) Ammonia-induced protein tyrosine nitration is most pronounced in astrocytes constituting the blood–brain barrier and selective alterations in blood–brain barrier permeability are a hallmark of HE.4
Thus, current knowledge suggests that at least several key findings in HE can be explained as a result of an increased astrocyte hydration and ammonia effects.

**CURRENT PATHOGENETIC MODEL**

In view of the above, one major pathogenetic event in the development of HE in chronic liver disease is an increase in astrocyte hydration, that is a low-grade cerebral edema without a clinically overt increase in intracranial pressure, but sufficient to trigger multiple alterations of astrocyte function. Ammonia and other HE-precipitating factors may act through astrocyte swelling, but further effects are not ruled out. Astrocyte swelling leads to complex alterations in astrocyte function finally resulting in a disturbance of glioneuronal communication and the clinical picture of HE. One of these latter events may be the shift of neuronal electrical coherence patterns to abnormally low frequencies, as these latter events may be the shift of neuronal electrical coherence patterns to abnormally low frequencies, as detected by recent magnetencephalography studies. As astrocyte swelling is induced not only by ammonia, but also by hyponatremia, benzodiazepines and inflammatory cytokines, such a model would explain why rather heterogeneous conditions (e.g. bleeding, electrolyte disturbances, sedatives, infections) can precipitate HE in the cirrhotic patient. Thus, multiple factors act synergistically on the common pathogenetic endpoint (i.e. glial swelling with its functional consequences). As stated previously non-cirrhotics may tolerate such precipitating factors without developing HE symptoms, because their osmolyte systems for counteraction of cell swelling are not exhausted. In cirrhosis, however, organic osmolytes are largely depleted in order to compensate for glial glutamine accumulation and there may be little room for action of these volume-regulatory mechanisms against further challenges of cell volume. Thus, the $^1$H-MRS findings in non-encephalopathic cirrhotics may describe an early, still compensated stage of astrocyte volume homeostasis with few consequences yet for astrocyte hydration and function. This situation, however, can decompensate rapidly in response to precipitating factors, and hydration-dependent alterations of glial function will become clinically apparent. This labile situation may explain the rapid kinetics of HE episodes and why severe brain edema with fatal outcome can occasionally develop in endstage cirrhosis. Thus similarities exist with respect to the pathogenesis of HE in chronic liver disease and acute liver failure, but differences in the kinetics, extent and counterregulation of glial swelling may be responsible for differences in the clinical picture of HE in acute and chronic liver disease, respectively.

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