Pathogenetic mechanisms of hepatic encephalopathy

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ABSTRACT

Hepatic encephalopathy (HE) in liver cirrhosis is a clinical manifestation of a low-grade cerebral oedema, which is exacerbated in response to ammonia and other precipitating factors.

This low-grade cerebral oedema is accompanied by an increased production of reactive oxygen and nitrogen oxide species (ROS/RNOS), which trigger multiple protein and RNA modifications, thereby affecting brain function. The action of ammonia, inflammatory cytokines, benzodiazepines and hyponatraemia integrates at the level of astrocyte swelling and oxidative stress. This explains why heterogenous clinical conditions can precipitate HE episodes.

Oxidised RNA species, which are formed in response to oxidative stress, also participate in local postsynaptic protein synthesis in neurons, which is required for memory formation. Although the functional consequences of RNA oxidation in this context remain to be established, these findings bear a potential biochemical explanation for the multiple alterations of neurotransmitter receptor systems and of synaptic plasticity.

Such changes may in part also underlie the pathologically altered oscillatory networks in the brain of HE patients in vivo, as detected by magnetencephalography. These disturbances of oscillatory networks, which in part are triggered by hypothalamic structures, can explain the motor and cognitive deficits in patients with HE.

Current therapeutic strategies aim at the elimination of precipitating factors. The potential of therapies targeting downstream pathophysiological events in HE has not yet been explored, but offers novel potential sites of therapeutic intervention.

Hepatic encephalopathy (HE) defines a frequent neuropsychiatric manifestation of chronic and acute liver disease with disturbances of psychomotor, intellectual, cognitive, emotional/affective, behavioural and fine motor functions of varying severity (for a review, see Häussinger and Blei[1]). Traditionally, HE is graded according to the West Haven criteria, which define HE grades I–IV based on the presence of specific clinical signs and symptoms and their severity.[2] However, patients with cirrhosis present with a continuous severity spectrum of neuropsychological symptoms ranging from entire normality (HE 0) up to obvious deficits. Even in minimal HE (mHE) without obvious clinical symptoms, neuropsychological and neurophysiological testing uncovers deficits which impact on the quality of life and the fitness to drive a motor vehicle.[3–5] Recently, it was demonstrated that the determination of the critical flicker frequency (CFF) allows a rapid and reliable quantification of HE.[5] Depending on the population under study 20–80% of patients with cirrhosis may suffer from minimal or manifest HE.

Pathogenetic models of HE in liver cirrhosis have to explain the rapid kinetics of symptom severity, their reversibility and the fact that HE episodes are exacerbated by heterogeneous precipitating factors. They have also to explain the multiple alteration of neurotransmitter systems in the brains of patients and animal models with HE, as well as the selective alterations in the permeability of the blood–brain barrier. There is consensus that ammonia is a key toxin in HE, which may sensitise the brain to the different precipitating factors.[6 7] Further, there is substantial evidence that astrocytes play an important role in the pathogenesis of HE with consequences for neuronal function.[8] At the neurophysiological level the motor deficits seen in patients with HE are characterised by a pathologically altered oscillatory coupling within the central motor system and the cognitive deficits are assigned to pathologically altered oscillatory activity in higher cognitive brain areas.[9–11]

Whereas in fulminant hepatic failure the development of a clinically manifest cerebral oedema is a well known complication,[12] patients with liver cirrhosis and HE usually show no clinical signs of an overt cerebral oedema or increased intracranial pressure. In 1994, however, in vivo proton magnetic resonance spectroscopy (^{1}H-MRS) studies on the human brain provided the first evidence for the presence of a low-grade cerebral oedema in patients with cirrhosis,[13] when it became clear that the myo-inositol peak in ^{1}H-MRS reflects an osmosensitive pool. Its depletion in the brains of patients with cirrhosis and HE, together with a consistent elevation of the glutamine/glutamate signal, was therefore indicative of the presence of a low-grade brain (glial) oedema in these patients. From this and many in vitro and animal studies, it was hypothesised that the pathogenetic action of ammonia and HE precipitating factors integrates at the level of astrocyte swelling.[13–15]

This article summarises recent insights into the pathogenesis of HE.

ASSESSMENT OF THE SEVERITY OF HEPATIC ENCEPHALOPATHY

Depending on the presence and severity of specific clinical signs and symptoms manifest HE is traditionally classified into four grades according to the West Haven criteria.[2] However, assignment of patients with cirrhosis to HE stages 0–II strongly relies on the subjective impression by

Box 1: Assessment of the severity of hepatic encephalopathy (HE) (for a review, see Häussinger *et al*[16])

- Manifest HE: overt neuropsychiatric symptoms ranging from mild personality changes and impairment of conciousness and orientation to deep coma (grades I–IV)
- Minimal HE: no overt symptoms by deficits in neuropsychological and neurophysiological tests:
  - *Computer–psychometric test batteries* may serve as a "gold standard" for the assessment of mHE, but are not applicable in clinical routine
  - *Paper–pencil tests* are easy to perform but are hampered by training effects, a dependence on age and education, little specificity and sensitivity for detection of mHE. Single paper–pencil test are insufficient to detect or rule out mHE; test batteries such as the psychometric hepatic encephalopathy score (PHES) test are preferred[18]
  - *EEG* shows characteristic generalised slowing increasing with HE severity. EEG may not be sensitive enough for detecting mHE
  - *Evoked potentials* (*EPs*) are obtained by stimulation of a sensory system (visual, accoustic); the patient's attention is not required. Cognitive, event-related (endogenous) potentials including P300 are less dependent on the physical characteristics of the stimulus but require the active cooperation of the patient
  - *Critical flicker frequency* (*CFF*) is an objective and reproducible parameter for assessment of low-grade HE (HE0, mHE, HEI, II)

In order to minimise subjectivity in the assessment of HE, a simplified grading system was suggested[16]

- *"High-grade HE"* summarises patients unable to cooperate and who present with confusion, somnolence or coma; ie, patients formerly categorised as HE III and IV
- *"Low-grade HE"* describes patients with cirrhosis who are cooperative. This group was formerly categorised as having mHE, HE I or HE II. By use of an objective, reproducible and, in physical units, quantifiable neurophysiological measurement technique, the severity of low-grade HE can be precisely described. Such an objective variable may be used for the follow-up of disease evolution and assessment of treatment efficacy in clinical studies

the physician. A considerable proportion of these patients, which appears normal on clinical examination and is therefore not picked up by the West Haven criteria, displays quantifiable neuropsychological deficits when subjected to neuropsychological or neurophysiological testings. This condition has been termed minimal HE (mHE);[2 16] however, so far, no standardisation for the diagnosis of mHE has been achieved. Routinely used paper–pencil test batteries suffer from training and education effects and may exhibit low sensitivity.[5 17 18] The problems in assessing the severity of mild HE also became apparent in a recent re-evaluation of a clinical trial,[18] which showed that about 30% of paper–pencil tests were performed invalidly and that clinical assessment of patients with HE 0–II yielded 30–50% misgradations. In view of this, a new description for the severity of HE in patients with cirrhosis has been proposed recently, which discriminates "low-grade HE" from "high-grade HE" only. High-grade HE describes patients with a low level of conciousness, an inability to cooperate, and a requirement for hospitalisation; it corresponds to HE grades III and IV. Low-grade HE comprises patients with mild HE (mHE, HE I and II, no hospitalisation required).[16] Further specification of low-grade HE severity is made by the use of objective physical parameters, such as latencies of evoked potentials or critical flicker frequency. This proposal takes into account that patients with cirrhosis and HE 0, mHE, HE I or HE II ("low-grade HE") present with a continuous spectrum of symptoms, which ranges from entire normality to overt pathology.[16]

Due to the prognostic and socioeconomic relevance of mHE different neurophysiological, neuropsychological and imaging methods have been used to establish a diagnosis of mHE (for reviews see Ferenci *et al*[2] and Kircheis *et al*[17]). As already mentioned[17 18] the value of most psychometric tests, which are used in clinical routine, is limited by methodological problems, age-dependence, training and education effects, as well as by a lack of standardisation.

Recently, critical flicker frequency (CFF) analysis was introduced as an objective and reproducible assay of HE severity. CFF describes the severity of low-grade HE as a continuum[5 16 17] and defines the frequency at which a proband perceives the apparent transition from fused light to a flickering one. In patients with cirrhosis, CFF decreases in parallel with increasing severity of HE and reliably separates patients with cirrhosis but without HE (HE 0) from those with manifest HE. Patients with mHE, as defined by computer–psychometric test batteries, exhibit flicker frequencies either above or below the threshold of 39 Hz.[5 16 17 19 20] The technique, which has been evaluated by several groups,[19 20] shows little dependence upon age, education or training.[5 20] A comparison of CFF analysis with the results from different psychometric tests demonstrated that a decrease of CFF picks up a broad spectrum of neuropsychological qualities (cognitive, motor and visual functions, and the processing of the visual signal) in an integrated manner.[5 17 20 21] However, it is not yet clear whether abnormal CFF values will reflect disturbances of quality of life and whether CFF threshold analyses are suitable for the follow-up of patients in the course of treatment and in clinical trials.

HEPATIC ENCEPHALOPATHY IN LIVER CIRRHOSIS AND LOW-GRADE CEREBRAL OEDEMA

Ammonia plays a key role in the pathogenesis of HE,[6 7] although blood ammonia levels in patients with cirrhosis do not necessarily correlate with HE severity. This may be explained by the fact that in these patients a heterogenous panel of factors and conditions, including hyponatraemia, inflammatory cytokines and benzodiazepine-type sedatives, can precipitate HE without further increasing ammonia levels.[22]

In the brain ammonia is detoxified by the astrocytes, which are the predominant cellular compartment expressing glutamine synthetase. In vivo ^{1}H-MRS studies on the brain of patients with cirrhosis consistently show an increase of the glutamine/glutamate signal accompanied by a depletion of myo-inositol, which is indicative of a partially compensated glial oedema.[13 15 22–25] The

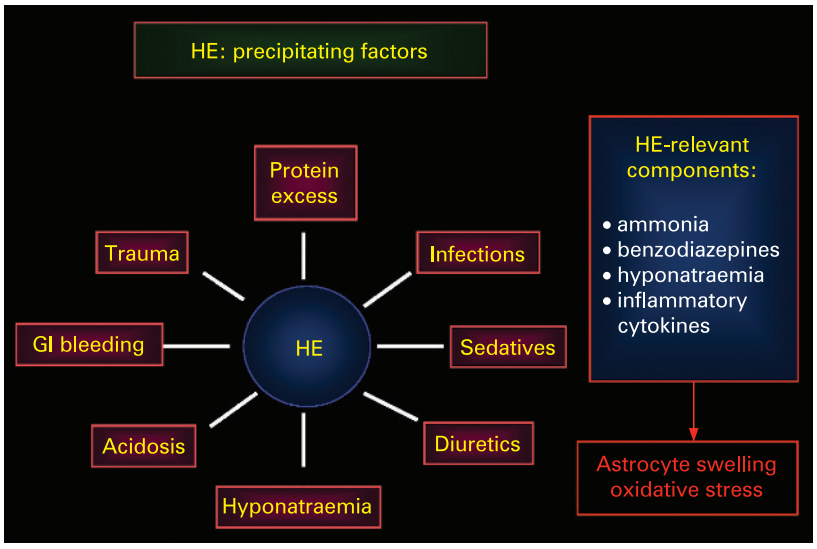

Figure 1 Factors that precipitate hepatic encephalopathy (HE). HE is precipitated by a heterogeneous panel of factors and conditions. All of these contain components that induce astrocyte swelling and increase oxidative stress. Thus, the action of different precipitating factors integrates at least in part at the level of astrocyte swelling and oxidative stress. GI, gastro-intestinal.

presence of a low-grade cerebral oedema in patients with cirrhosis and HE was confirmed by other magnetic resonance techniques, such as magnetisation transfer ratio measurements[26 27] and the recently introduced T1 mapping with partial inversion recovery (TAPIR), which allows quantitative water mapping in the human brain in vivo.[28] Also, patients with non-cirrhotic portal vein thrombosis and minimal HE exhibit magnetic resonance findings consistent with increased brain water[27] and mild brain oedema developed in a recently characterised animal model of hepatic encephalopathy in cirrhosis.[29] Magnetic resonance findings consistent with a low-grade cerebral oedema have been found in patients with cirrhosis who have minimal HE and become more pronounced when HE severity increases,[13 30–33] for example after implantation of a transjugular intrahepatic portosystemic stent[13] or experimentally induced hyperammonaemia.[34] Conversely, these MRS abnormalities can resolve after liver transplantation[33] or successful medical treatment for HE.[35] Quantitative water mapping in the brain, a recently developed technique based on magnetic resonance imaging, has provided direct and quantitative evidence for the presence of low-grade cerebral oedema in patients with HE and allowed the water contents in different brain regions to be detected.[31] The severity of HE as assessed by CFF analyses correlated well with the increase in water in the frontal and occipital white matter, the globus pallidus, the anterior limb of the internal capsule and the putamen,[31] whereas no correlation was found in the grey matter, the thalamus, the posterior limb of the internal capsule, the caudate nucleus and the coronal white matter.[31] However, patients with HE showed a more inhomogeneous water distribution in the thalamus, when compared to healthy controls.

In vitro studies on the mechanisms underlying this low-grade cerebral oedema largely focused on the astrocytes, which undergo so-called Alzheimer type II changes as a morphological counterpart of astrocyte swelling, when exposed to ammonia. Undoubtedly, intra-astrocytic accumulation of osmotically active glutamine in liver cirrhosis and other hyperammonaemic states contributes to the development of the low-grade brain oedema, as evidenced by consistently elevated ^{1}H-MRS glutamine signals in the brains of patients with cirrhosis. Further, methionine sulfoximine, an inhibitor of glutamine synthetase, prevents astrocyte swelling following an ammonia load in experimental animals.[36] However, not only ammonia, but also benzodiazepines, hyponatraemia and inflammatory cytokines can induce astrocyte swelling in vitro (for a review, see Häussinger *et al*[22]). Thus, different neurotoxins, which were repeatedly implicated in the pathogenesis of HE, may synergistically promote astrocyte swelling as one common pathogenetic endpoint. This may explain one key feature of HE in patients with cirrhosis, namely that the syndrome is precipitated by heterogeneous factors, such as bleeding, infections, sedatives, diuretics or electrolyte disturbances (fig 1). Patients who do not have cirrhosis may tolerate such precipitating factors because their astroglial osmolyte pools (eg, myo-inositol, taurine) are not exhausted. However, when the organic osmolytes under hyperammonaemic conditions are largely depleted in order to compensate for glutamine-induced osmotic stress, astrocytes are sensitised to swelling induction by other HE-precipitating factors. In line with this, it was suggested that in patients with cirrhosis brain myo-inositol levels may even act as a predictive marker for the risk of developing HE: low baseline myo-inositol levels were found to predispose for neuropsychological deterioration by an experimental amino acid load.[37] Further, endotoxin produces coma and brain swelling in rats with cirrhosis[38] and induced hyperammonaemia resulted in neuropsychological worsening in patients with cirrhosis with evidence of an inflammatory response due to bacterial infection.[39]

Box 2: Oxidative stress (for a review, see Schliess *et al*[50])

- *Oxidative stress* defines the cell challenge with reactive oxygen and nitric oxide species (RNOS), resulting from their increased production and/or diminished removal due to loss of antioxidative capacity
- *Reactive oxygen* species include the superoxide anion ($O_2^{\bullet -}$), which reacts with hydrogen peroxide to produce the hydroxyl radical (OH). Reactive nitric oxide species include nitric oxide (NO), the nitrogen dioxide radical ($NO_2^{\bullet}$) and peroxynitrite ($ONOO^-$)
- *Molecular consequences of oxidative stress* include the oxidation of proteins and nucleic acids, *S*-nitrosylation of proteins, protein tyrosine nitration and zinc release from metallothionein. A transient oxidative stress is involved in growth factor signalling, whereas sustained oxidative stress may trigger cell injury and apoptosis

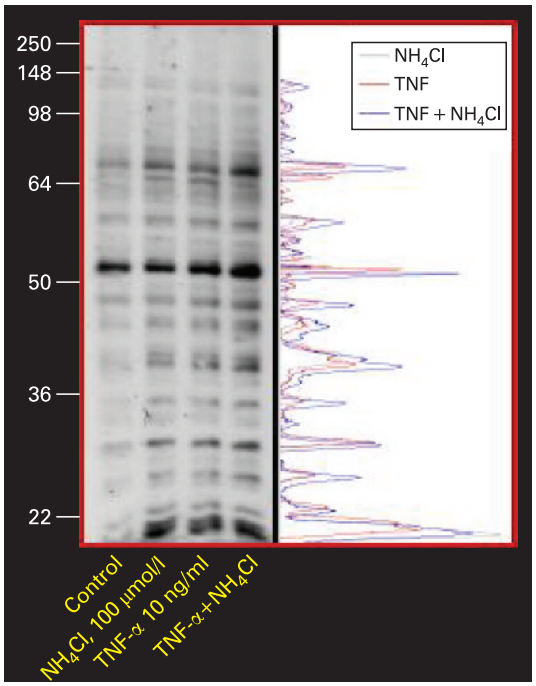

Figure 2 Ammonia and tumour necrosis factor-α (TNF-α) synergistically increase protein tyrosine nitration in cultured rat astrocytes. Rat astrocytes were prepared and cultured as described.[40] Astrocytes remained untreated or were exposed (6 h) to NH_4Cl (100 μmol/l), TNF-α (10 ng/l) and 100 μmol/l NH_4Cl + 10 ng/l TNF-α, respectively. Protein tyrosine nitration was analysed by probing western blots with an anti-3′-nitrotyrosine antibody. Dendrograms of the tracks of the 3′-nitrotyrosine staining are superimposed.

ASTROCYTE SWELLING AND OXIDATIVE STRESS

Although, so far, there have been few human studies concerning the involvement of oxidative stress in HE,[40–43] there is substantial evidence from animal and cell culture studies for an important role of oxidative/nitrosative stress in the pathogenesis of HE. In cultured astrocytes and in rat brain in vivo, ammonia, inflammatory cytokines, benzodiazepines and hyponatraemia induce the rapid formation of reactive oxygen and nitrogen oxide species, including nitric oxide (NO), through the *N*-methyl-D-aspartate (NMDA) receptor and Ca^{2+}-dependent mechanisms.[44–48] NMDA receptor activation under these conditions is thought to result from a depolarisation-induced removal of a Mg^{2+} blockade from the receptor and autocrine amplification of NMDA receptor activation by astroglial glutamate release. There is a close relationship between astrocyte swelling and oxidative stress,[49 50] which makes it difficult to separate both events with regard to pathophysiology of HE. On the one hand, astrocyte swelling induces oxidative stress through NMDA receptor- and Ca^{2+}-dependent mechanisms, while on the other hand, NMDA receptor activation and oxidative stress trigger astrocyte swelling (for a review, see Schliess *et al*[49 50]). This points to an auto-amplificatory signalling loop between astrocyte swelling and oxidative stress.[15] Activation of NADPH oxidase isoforms[48] is the most likely cause for ROS, which are formed in response to astrocyte swelling and ammonia, whereas Ca^{2+}/calmodulin-dependent isoforms of nitric oxide synthase are involved in the swelling-induced NO synthesis.[47]

The recently published "Trojan horse" hypothesis[51] suggests that glutamine, which accumulates in astrocytes during ammonia detoxification, is the "Trojan horse" that actually mediates ammonia toxicity. This idea is essentially based on the observation that glutamine in cultured astrocytes increases oxidative stress in a 6-diazo-5-oxo-L-norleucine (DON)-, histidine- and cyclosporine A-sensitive manner. From this it was concluded that mitochondrial glutamine uptake and subsequent cleavage of glutamine by the phosphate-activated glutaminase elevates mitochondrial ammonia levels, which in turn stimulates ROS production via induction of the mitochondrial permeability transition (MPT).[51–53] Ammonia-induced astrocyte swelling was considered as a consequence of mitochondrial glutamine uptake, glutamine cleavage, MPT induction and ROS formation.[51 53] However, this view is difficult to reconcile with the fact that ammonia in cultured astrocytes induces almost instantaneous ROS/RNOS production[44 48] and astrocyte swelling,[48 54] whereas MPT induction[55] and glutamine accumulation occur thereafter.[56] Both ammonia and hypo-osmotic swelling of cultured astrocytes rapidly increase ROS via activation of NADPH oxidase isoforms,[48] indicating that, at least initially, ammonia and hypo-osmotic astrocyte swelling produce oxidative stress without participation of mitochondria. This does not exclude MPT involvement in ammonia-induced ROS/RNOS production and swelling on a long-term time scale and it is well conceivable that MPT induction is a consequence rather than the cause of astrocyte swelling. Here, NADPH oxidase-dependent ROS production and MPT-dependent ROS will contribute to the autoamplificatory loop mentioned above. On the other hand, in rats, in vivo ROS/RNOS production and brain oedema critically depend on the synthesis of glutamine;[57 58] however, ammonia does not induce MPT in non-synaptic brain mitochondria.[59] This suggests that in vivo astrocyte swelling rather than a glutamine-dependent MTP induction contributes to ROS/RNOS production and ammonia toxicity to the brain.

FUNCTIONAL CONSEQUENCES OF ASTROCYTE SWELLING AND OXIDATIVE/NITROSATIVE STRESS

Although it would be an oversimplification to ascribe all pathogenetic effects of ammonia and HE-relevant toxins to astrocyte swelling and oxidative/nitrosative stress, current evidence suggests at least a major contribution of these events to HE pathophysiology. In almost every cell type under study, changes in cell hydration are an independent regulator of membrane transport, gene expression and metabolism through the activation of osmosensing and osmosignalling pathways (for reviews, see Häussinger and Sies[60]).

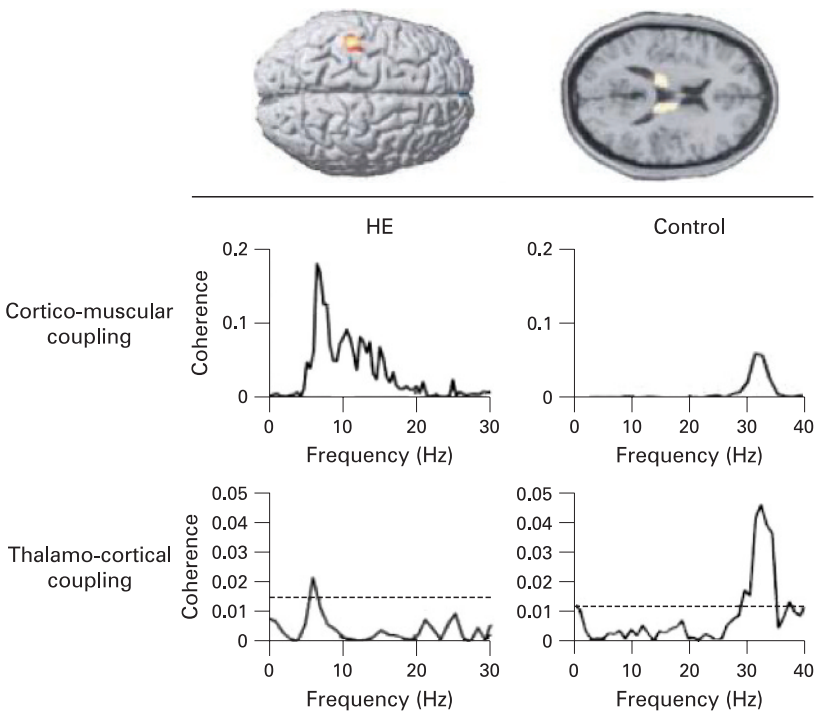

Figure 3 Pathological oscillatory coupling in the motor system of a patient with cirrhosis and hepatic encephalopathy (HE), as assessed by magnetencephalography. Cortico-muscular coupling reveals a high-amplitude peak at the frequency of the tremor in the HE patient. Thalamo-motor cortical coupling in the HE patient occurs exactly at the individual tremor frequency. Apparently, a pathologically rigid and slowed thalamo-cortical drive triggers mini-asterixis in HE. Adapted from Timmermann *et al*.[120]

Also, the function of cultured astrocytes changes in response to transmembrane water shifts. For example, hypo-osmotic astrocyte swelling triggers activation of Erk- and p38-type MAP kinases,[61–64] elevates intracellular Ca^{2+} concentration and stimulates glycogen synthesis,[65] which may account for the increased astroglial deposition of glycogen in animal models of chronic HE.[6] Astrocyte swelling increases the pH in endocytotic vesicles,[66] which may affect receptor/ligand sorting and neurotransmitter processing. Recent data indicate that astrocytes possess powerful mechanisms for local vasoregulation and are key mediators of functional hyperaemia; a process that is triggered by intra-astrocytic Ca^{2+} in a cyclooxygenase-1-dependent manner.[67] This raises the possibility that astrocytic Ca^{2+} signals, which are induced by cell swelling, may impact on cerebral microcirculation. However, nothing is known about the consequences of this potential vascular dysfunction for oxidative stress and brain swelling.

Astrocyte swelling may also predispose to neuronal dysfunction due to impairment of their protective homeostatic functions. Moderate astrocyte swelling in the brain, in vivo, may lower the extracellular fluid volume in the brain, which is encapsulated by rigid bone. This contraction of the extracellular space may decrease diffusion of molecules and increase the accumulation of ions, excitotoxic transmitters and neurotoxic metabolites, thereby contributing to neuronal injury.[68 69]

Astrocyte swelling leads to a depletion of taurine, which acts not only as an organic osmolyte and antioxidant, but also has neuromodulatory properties. In line with this, taurine was shown to rescue hippocampal long-term potentiation from ammonia-induced impairment.[70] Taurine is also involved in the osmoregulation of vasopressin secretion and taurine transporter-deficient mice exhibit an impaired ability to increase water excretion by the kidneys.[71] Thus, cerebral taurine deficiency could provide an interesting link between HE and hypothalamic vasopressin secretion. Hyponatraemia triggers astrocyte swelling but under conditions of taurine depletion it no longer inhibits hypothalamic vasopressin secretion. Finally, taurine deficiency due to taurine transporter knockout results in disinhibition of striatal network activity due to modulation of $GABA_A$ receptor signalling, which can be rescued by taurine supplementation.[72] Thus, taurine depletion in response to astrocyte swelling plays a role in cerebral ammonia toxicity and HE pathophysiology which is not yet fully recognised.

Further important consequences for brain function arise from the production of reactive oxygen and nitrogen oxide species (ROS/RNOS), which is triggered in response to astrocyte swelling and HE-relevant neurotoxins. Whereas astrocytes are relatively resistant towards oxidative/nitrosative stress, astroglia-derived radicals can affect the respiratory chain of neighbouring neurons[73] and may contribute to the compromised brain energy metabolism and neuronal transmission in HE. Furthermore, ammonia, tumour necrosis factor-α (TNF-α), benzodiazepines and osmotic astrocyte swelling trigger a nitric oxide-dependent mobilisation of zinc, probably from metallothionein and other proteins that complex Zn^{2+} via cysteine thiols.[74 75] The activities of multiple enzymes and trancription factors such as the metal response element-binding transcription factor MTF-1 and the specificity protein SP1 are regulated in a Zn^{2+}-dependent manner.[76] Further, Zn^{2+} is a negative modulator of $GABA_A$ receptor-mediated currents[77] and affects synaptic plasticity. Thus, its potential mobilisation from proteins in response to nitrosative stress may augment GABAergic neurotransmission. Although the pathophysiological consequences of Zn^{2+} mobilisation in response to nitrosative stress are far from being understood, one consequence is a protein kinase C-dependent translocation of SP1 to the nucleus.[74] Sp1 participates in the regulation of the expression of the peripheral benzodiazepine receptor (PBR).[78] This protein, which is upregulated in HE,[79 80] not only plays a role in mitochondrial permeability transition and mediates the oxidative stress response towards benzodiazepines[46] but is also involved in the synthesis of neurosteroids, such as allopregnanolone and allotetrahydrodeoxy corticosterone.[81 82] Such neurosteroids have a positive $GABA_A$-receptor modulatory activity and were identified in the brain from patients with hepatic coma.[80 81] This could provide another explanation for the

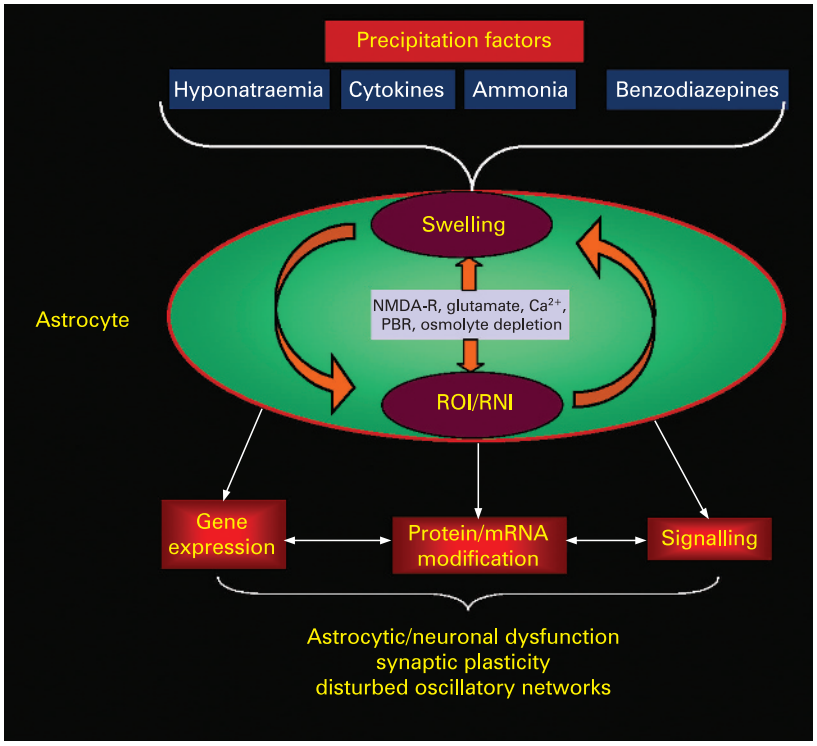

Figure 4 Low-grade cerebral oedema in hepatic encephalopathy (HE). Ammonia induces astrocyte swelling which is, in part, counteracted by a volume-regulatory osmolyte depletion but can be aggravated by a heterogenous set of precipitating factors. Astrocyte swelling involves activation of the *N*-methyl-D-aspartate (NMDA) receptor and the generation of reactive nitrogen oxide species, which again forwards astrocyte swelling. In this way an autoamplificatory loop is generated which produces signals changing astrocyte function at multiple levels including covalent modifications of proteins and RNA. As a consequence, glioneuronal communication and synaptic plasticity are impaired, resulting in a disturbance of oscillatory networks which finally account for the HE symptoms. Adapted from Häussinger.[15] PBR, peripheral benzodiazepine receptor; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates.

increased GABAergic tone found in patients with HE.[83] Recent evidence suggests that the PBR could be involved in astrocyte swelling and the increase of mitochondrial permeability transition induced by ammonia.[84] Apart from PBR upregulation other potential HE-relevant effects of Zn^{2+} may include a decrease in glutamate uptake and a rise in cGMP. The latter may counteract a disturbance of the glutamate–NO–cGMP pathway, which was suggested to play a role in the pathogenesis of cognitive defects in HE animal models.[85] In line with this the phosphodiesterase inhibitor sildenafil restored learning ability in rats with portocaval anastomosis and hyperammonaemia.[85 86] However, it must be kept in mind that sildenafil also exerts antioxidative properties[87] and downregulates expression of the NADPH oxidase regulatory subunit $p47^{phox}$.[88] Interestingly, Zn^{2+} (see Noh and Koh[89]) like hypo-osmolarity and ammonia[48] stimulates ROS production by NADPH oxidase in astrocytes, indicating that Zn^{2+} may be another player in the self-amplifying cycle between astroglial swelling and ROS/RNOS formation.

Box 3: Treatment of hepatic encephalopathy (for a review, see Häussinger and Blei[1])

- Non-absorbable disaccarides, antibiotics, probiotics, fermentable fibre (bowel cleansing)
- Zinc, L-ornithine aspartate (stimulation of urea and glutamine synthesis)
- Flumazenil (antagonising the benzodiazepine receptor)
- Branched-chain amino acids (oral)
- Artificial liver support

OXIDATIVE STRESS, NITRIC OXIDE, AND PROTEIN TYROSINE NITRATION

Another consequence of the ROS/RNOS formation, which is induced by astrocyte swelling, is the tyrosine nitration of proteins. This covalent modification of astrocytic proteins occurs in vitro not only in response to ammonia,[45] benzodiazepines[46] or inflammatory cytokines,[90] but also after experimental astrocyte swelling,[47] indicating that astrocyte swelling is sufficient to induce protein tyrosine nitration. These effectors act synergistically with respect to protein tyrosine nitration[46] (fig 2), indicating that there is synergism between hyperammonaemia and inflammation in precipitating HE symptoms[29 38 39 91 92] is also reflected at the level of tyrosine nitration. Peroxynitrite is involved in ammonia-, cytokine- and swelling-induced tyrosine nitration,[45 47 90] whereas benzodiazepine-induced tyrosine nitration is peroxynitrite-independent and involves the activation of the PBR.[46] Protein tyrosine nitration in astrocytes is also found in vivo in ammonia-, diazepam- or lipopolysaccharide-intoxicated or portocaval shunted rats.[45 46 90] Astrocytes located near the blood–brain barrier exhibit especially high levels of nitrotyrosine, with potential consequences for blood–brain barrier permeability. In a mouse model of experimental allergic encephalomyelitis the increase of blood–brain barrier permeability is sensitive to the peroxynitrite scavenger uric acid,[93–95] which also prevents ammonia-induced protein tyrosine nitration.[45 50] Thus, protein tyrosine nitration may affect transastrocytic substrate transport, which would correspond to the known selective alterations of blood–brain barrier permeability in HE. Tyrosine nitration involves distinct proteins only, such as glutamine synthetase, the PBR, glyceraldehyde-3-phosphate dehydrogenase and the extracellular signal regulated kinase Erk-1. Whereas tyrosine nitration of glutamine synthetase affects the catalytic centre of the enzyme and is associated with its inactivation,[45 96 97] the functional consequences of PBR tyrosine nitration are unclear. Although protein tyrosine nitration interferes with protein function and signal transduction, its role in the pathogenesis of hepatic encephalopathy remains to be established. Inactivation of glutamine synthetase by protein tyrosine nitration under hyperammonaemic conditions may even protect the brain from excessive glutamine accumulation and astrocyte swelling and the possibility must be considered that protein

tyrosine nitration represents only a surrogate marker for other pathogenetically more important alterations. Nevertheless, inhibition of ammonia-induced protein tyrosine nitration by NMDA receptor antagonists, inhibitors of glutamine synthetase or NOS is associated with an amelioration of ammonia toxicity in animals.[98–101]

OXIDATIVE STRESS AND RNA OXIDATION

A fascinating and novel perspective on the pathogenesis of HE arises from the recent discovery that ammonia, TNF-α, benzodiazepines and hypo-osmotic swelling can induce RNA oxidation in cultured astrocytes and brain slices.[101] Here, reactive oxygen species hydroxylate guanosine to produce 8-oxo-7,8-dihydro-2′-guanosine (8OHG), which is detected not only in astrocytes but also in the cytosol of neurons from ammonia-intoxicated rats.[101] Importantly, 8OHG immunoreactivity was identified in granular structures along the dendrites and in postsynaptic dendritic regions in association with the RNA-binding splicing protein NOVA-2. These findings suggest that HE-associated oxidative stress modifies RNA species, which participate in the granular RNA transport along the dendrites. Such neuronal RNA granules are heterogeneous and can contain all elements required for local postsynaptic protein synthesis, which is controlled by synaptic signals (for reviews, see Görg *et al*,[101] Schuman *et al*,[102] Steward and Schuman,[103] Kiebler and Bassell,[104] and Sossin and DesGroseiller[105]). Local protein synthesis plays a major role for synaptic plasticity, as reflected by late-phase long-term potentiation (L-LTP), and is required for learning and the formation of long-term memory (for reviews, see Kandel,[106] Sutton and Schuman,[107] and Lynch[108]). RNA oxidation in response to HE-relevant conditions (eg, ammonia, TNF-α, benzodiazepines and hyponatraemia) is apparently a selective process and among the RNA species being oxidised the mRNA for the glutamate uptake system GLAST and ribosomal (r) RNA were identified, whereas no oxidation of mRNAs coding for the $GABA_B$ receptor subunit 1 and actin was found.[101] The implications of RNA oxidation for ammonia neurotoxicity and HE are currently unknown; however, there is good evidence that rRNA and mRNA oxidation compromise translation accuracy and efficacy resulting in the formation of defective or unstable proteins.[109–112] Oxidation of astroglial GLAST mRNA by ammonia may partly explain the ammonia-induced decrease of GLAST expression and glutamate uptake in cultured astrocytes[113 114] and contribute to the known disturbances in glutamatergic neurotransmission in HE.[115] To what extent RNA oxidation contributes to the multiple derangements of neurotransmitter receptor systems in HE (for a review, see Häussinger and Blei[1]) is currently unknown. Neuronal RNA oxidation was associated with mild cognitive impairment as an early stage of Alzheimer disease.[116] Cognitive impairment without neuronal degeneration is also a hallmark of HE and may involve a disturbed protein synthesis-dependent late phase long-term potentiation (L-LTP), learning and memory consolidation due to the oxidation of postsynaptically translated mRNA species. In line with this, learning ability is disturbed in rats fed with a hyperammonaemic diet[117] and rats with portocaval anastomosis.[118] LTP is impaired in mouse brain slices exposed to ammonia[70] or TNF-α.[119] However, to what extent the oxidation of locally translated mRNA species contributes to the L-LTP impairment under these conditions remains to be established. Nonetheless, RNA oxidation in response to the oxidative stress as induced by HE-relevant neurotoxins can provide a mechanistic link between cell swelling and oxidative stress on the one hand and alterations of synaptic plasticity on the other.

HEPATIC ENCEPHALOPATHY AND DISTURBANCE OF OSCILLATORY NETWORKS

Magnetoencephalography (MEG) allows the non-invasive assessment of information processing in the human brain with high spatial and temporal resolution. This technique was employed, together with electromyography, in patients with cirrhosis and HE in order to obtain insight into the neurophysiological basis of mini-asterixis, ie, a postural tremor of varying frequency (6–12 Hz), which is observed in patients with low-grade HE. The studies revealed a stronger cortico-muscular coherence with a shift to lower frequencies, when compared to controls, indicating a pathologically slowed and synchronised motor cortical drive[120 121] (fig 3). The extent of these changes correlates with the severity of HE and are apparently triggered by altered thalamo-cortical oscillatory coupling.[120] Thus, neurotoxin- and hydration-sensitive thalamic structures may act as pacemakers for an abnormally low-frequent and rigid thalamo-cortical and cortico-muscular coupling and could explain some motor deficits in HE patients. Interestingly, the HE severity-dependent slowing of the cortical motor drive went in parallel with the impairment of perception of oscillatory visual stimuli, as offered during the determination of critical flicker frequency.[121] These observations suggest that different systems (visual and motor) within the human brain react to a similar extent with impairment to process oscillatory neuronal activity when HE deteriorates. This prompted the speculation that slowed oscillatory communication in different subsystems is a key mechanism of encephalopathic brain dysfunction explaining the broad variety of clinical deficits.[121]

PATHOGENETIC MODEL

It remains to be established whether the functional sequelae of astrocyte swelling, oxidative stress and other actions of neurotoxins, which were mainly studied in in vitro or experimental animals, also apply for the humans with cirrhosis. If this were true, the emerging pathogenetic model for HE (fig 4) would offer novel sites for treatment beyond

those currently available, which are predominantly directed towards precipitating factors. Potential sites of intervention may be NMDA-receptor inhibition, antioxidant strategies, phosphodiesterase inhibitors, neurosteroid antagonists or anti-inflammatory strategies. However, none of these has yet been tested in humans. In the model depicted in fig 4 ammonia triggers astrocytic glutamine accumulation resulting in a compensatory depletion of osmolytes, such as taurine and myo-inositol. Exhaustion of the volume-regulatory capacity of astrocytes predisposes the brain to the induction of swelling by HE-precipitating factors, which synergistically promote a low-grade cerebral oedema. This occurs without a clinically overt increase in intracranial pressure, but the hydration increase is sufficient to trigger multiple alterations of astrocyte function and gene expression in part through oxidative stress-dependent modifications of proteins and RNA. A mutual amplification of astroglial swelling and oxidative stress creates an autoamplificatory signalling loop and the action of various HE-precipitating factors integrates at least in part at this level. Oxidative stress also affects glioneuronal communication and induces RNA oxidation in neurons with impact on synaptic plasticity. As a result, disturbances of oscillatory cerebral networks occur, which finally account for the symptoms of HE.

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Pathogenetic mechanisms of hepatic encephalopathy

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