CMLS, Cell. Mol. Life Sci. 62 (2005) 2295–2304
1420-682X/05/202295-10
DOI 10.1007/s00018-005-5089-0

CMLS Cellular and Molecular Life Sciences

Review

The pathophysiologic basis of hepatic encephalopathy: central role for ammonia and inflammation

D. Shawcross and R. Jalan*

Institute of Hepatology, University College London, 69-75 Chenies Mews, London WC1E 6HX (United Kingdom), Fax: +44 2073800405, e-mail: r.jalan@ucl.ac.uk

Received 4 March 05; received after revision 20 June 05; accepted 29 June 05
Online First 15 September 2005

Abstract. Hepatic encephalopathy (HE) is a neuropsychiatric syndrome associated with both acute and chronic liver dysfunction. It defines prognosis in acute liver injury in which patients can succumb with brain oedema and intracranial hypertension. In cirrhosis, it occurs insidiously, causing a range of neuropsychiatric disturbances. For over a century, we have known that ammonia is important in its pathogenesis and astrocytes are the cells that have been most commonly found to be affected neuropathologically. In this review we centre on the story of the 'sick astrocyte', focusing on the molecular pathogenesis of HE and the important role that inflammation has on its modulation. We describe new developments in this area with respect to potential targets for future therapies.

Key words. Hepatic encephalopathy; ammonia; inflammation; astrocyte; cytokines.

The foundation stones of hepatic encephalopathy

Hepatic Encephalopathy (HE) was first described by Nencki, Pavlov and Zaleski in the 1890s when they observed that dogs developed a behavioural syndrome following the formation of a surgical shunt diverting blood away from the portal vein into the inferior vena cava (Eck's fistula) [1]. This was nicknamed the [6]meat intoxication syndrome[9] and subsequent nitrogen balance studies demonstrated raised ammonium salts in the urine [2]. After ammonia ingestion, the dog became comatose and died. The brain ammonia content was four times that found in normal animals. The researchers concluded that the cerebral disorders observed in these dogs with portacaval fistulas could be attributed to ammonia following the failure of its conversion to urea in the liver [3].
Some 50 years later, Gabuzda et al. [4] noted that patients with cirrhosis and ascites treated with cation-exchange resins that absorbed sodium but released ammonium ions developed episodic HE. Phillips et al. [5] then went on to describe the behavioural alterations and a syndrome of impending hepatic coma in cirrhotics given certain nitrogenous substances. The severity of HE was then shown to broadly relate to the blood ammonia level in two large studies [6, 7].

Definitions and syndromes

HE is a neuropsychiatric syndrome which is associated with liver dysfunction and has quantitatively and qualitatively distinct features relating to its severity. It defines the prognosis in acute liver injury in which up to 30% of patients succumb from brain herniation due to brain oedema and intracranial hypertension. In cirrhosis (chronic liver dysfunction), it occurs more insidiously causing a range of neuropsychiatric disturbances which include psychomotor dysfunction, impaired memory, increased reaction time, sensory abnormalities and poor concentration. In its severest forms, patients may develop

* Corresponding author.

confusion, stupor, coma and death [8]. In minimal HE, the changes in mental function are subtle and may be observed in patients with no overt clinical evidence of encephalopathy [9]. The neuropsychological features of minimal HE are suggestive of a disorder of executive functioning. This primarily affects selective attention and psychomotor speed [10], which has a huge impact on health-related quality of life and has been shown to reduce the ability to drive [11]. Acute-on-chronic liver failure defines a group of patients that have chronic liver disease and in these patients a severe precipitating event such as sepsis, gastrointestinal bleeding (increased ammonia load) or the creation of portosystemic shunting (increased ammonia load) provides a pathophysiologic framework in which the patients with a chronic 'phenotype' can appear clinically indistinct from those with acute liver failure [12] (fig. 1).

Neuropathology

In acute liver failure, astrocytes swell and patients develop cytotoxic brain oedema. This observation has been replicated in cultured astrocytes exposed to high concentrations of ammonia [13]. In chronic liver disease, astrocytes show the characteristic morphological features of Alzheimer type II astrocytosis. Astrocytes exhibit a large swollen nucleus, prominent nucleolus, margination of the chromatin pattern and marked enlargement of the cytoplasm associated with isolated proliferation of cytoplasmic organelles which has also been replicated in astrocytes exposed chronically to ammonia [14]. There is also an alteration in the expression of key astrocytic proteins including the peripheral-type benzodiazepine receptor [15], glutamate transporters [16] and glial acidic fibrillary protein [17].

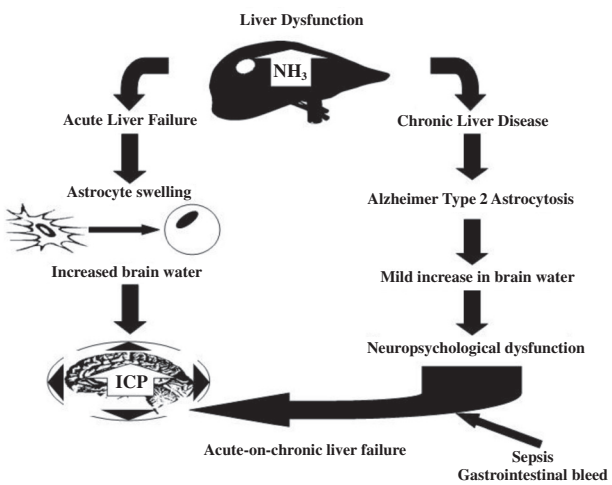

Figure 1. The cell that is most important in the pathogenesis of HE is the astrocyte. This diagram shows that acute and chronic liver dysfunction both result in increased brain water and that an acute insult on a background of chronic liver disease can result in acute cerebral oedema and raised intracranial pressure.

The central nervous system (CNS) neurovascular unit is a dynamic structure consisting of vascular endothelial cells, pericytes, and closely juxtaposed astrocytes and neurons. Contact and communication between these cells modulates cerebral blood flow and influences the permeability properties of the blood brain barrier [18]. The blood brain barrier remains anatomically intact in HE, but studies using positron emission tomography reveal an increase in the permeability-surface area to ammonia with increasing severity of disease [19]. A recent study has shown that the removal of astrocytes from culture resulted in increased permeability to small tracers across the brain endothelial cell monolayer and an opening of the tight junctions which was not accompanied by the loss of tight junction proteins such as claudin and occludin [20].

Pathogenesis of hepatic encephalopathy

Ammonia has been thought to be central in the pathogenesis of HE for the past 100 years, and most of the current therapies are based on modulating the circulating levels of this neurotoxin. Disturbances in neurotransmission due to increased γ-aminobutyric acid (GABA) (increased neuroinhibition) [21], reduced glutamate (reduced neuroexcitation) [22] and increased endogenous benzodiazepines and neurosteroids [23, 24] have been thought to be important, but current data suggest that at least some of these effects may be mediated by ammonia [25]. Altered cerebral blood flow is thought to be important, but it is not clear whether this is mediated through ammonia or secondary to superimposed inflammation. There is emerging literature suggesting that inflammation and its mediators may be important in the pathogenesis of HE. In this review we will focus primarily on the roles of ammonia and inflammation in the pathogenesis of HE.

The ammonia hypothesis

Important direct evidence showing that ammonia is taken up by the brain in patients with liver disease and hyperammonemia was provided by Lockwood et al [26]. Using positron emission tomography with ^{13}N-ammonia, he elegantly demonstrated that uptake of ammonia into the brain of patients with HE was significantly higher than in healthy volunteers and that the arterial concentrations of ammonia may increase the uptake of ammonia in the brain through an increase in the permeability of the blood brain barrier to ammonia [19]. In experimental animals with acute liver failure, brain ammonia flux may be up to 45-fold higher than normal [27]. In acute liver failure, arterial ammonia levels of >150 μmol/l predict a greater likelihood of dying from brain herniation [28].

Ammonia-glutamine brain swelling hypothesis

In the presence of liver dysfunction, urea synthesis is impaired and the brain acts as a major ammonia detoxification pathway. Astrocytes, which provide physical and nutritional support for neurons, also eliminate ammonia by the synthesis of glutamine through amidation of glutamate. Accumulation of glutamine in astrocytes, induced by hyperammonemia, produces osmotic stress and causes the astrocytes to swell [29]. Evidence of an increase in brain water in minimal HE has been provided in humans through studies using magnetic resonance imaging which show decreased magnetisation transfer ratio, indicating increased brain water. This was shown to correlate with neuropsychological function, and the abnormality was reversed by liver transplantation [30]. More recently, hyperammonemia induced by oral administration of an amino acid solution in patients with cirrhosis was shown to result in significant deterioration in neuropsychological function, an increase in brain glutamine levels and a reduction in the magnetic transfer ratio, suggesting an increase in brain water [31]. This study provided further support for the ammonia-glutamine brain water hypothesis of HE. The effect of hyperammonemia is likely to be determined by the ability of the astrocytes to maintain osmotic equilibrium by losing osmolytes such as myo-inositol in response to the ammonia-induced increase in glutamine [32]. Therefore, patients with co-existing hyponatremia [33] or chronic hyperammonemia, which result in depleted myo-inositol stores, may be more sensitive to the effects of a sudden increase in ammonia levels (fig. 2a, b).

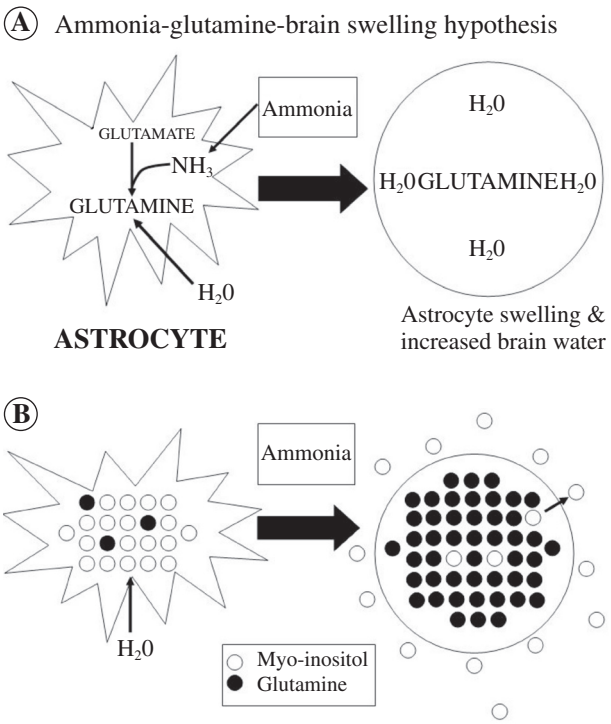

Figure 2. (a) A diagrammatic representation of the ammonia-glutamine-brain swelling hypothesis. Astrocytes are the site of ammonia detoxification in the brain and eliminate ammonia by the synthesis of glutamine through amidation of glutamate. The glutamine is retained, and its osmotic effect within the astrocyte causes it to take up water, causing it to swell. (b) A diagrammatic representation to show the role of myo-inositol as an osmotic regulator within the astrocyte. As glutamine accumulates within the astrocyte, myoinositol exits to try to redress the osmotic balance within the cell and prevent cerebral oedema.

Direct ammonia toxicity

Ammonia, when present in high concentrations, has the potential to adversely affect the central nervous system, particularly through modulation of inhibitory and excitatory neurotransmission [34].

In acute liver failure, the manifestation of hyperammonemia may be excitatory in nature and result in seizures associated with increased synaptic release of glutamate. Glutamate is the major excitatory brain neurotransmitter and results in the subsequent overactivation of the ionotropic glutamate receptors, the N-methyl-D-aspartate receptors. Acute exposure to ammonia in astrocyte cultures results in cytosolic alkalinization, leading to calcium-dependent release of glutamate. Furthermore, a deregulation of glutamate release from astrocytes by ammonia could contribute to glutamate dysfunction consistently observed in the animal models of acute HE [35].

In chronic liver disease, however, there appears to be a shift in the balance between inhibitory and excitatory neurotransmission towards a net increase in inhibitory neurotransmission. This may be due to a downregulation of glutamate receptors, resulting in decreased glutaminergic tone. In patients with HE, cerebral glutamate is decreased and downregulation of glutamate binding sites on the postsynaptic neurons and astrocytes occurs, resulting in decreased neuroexcitation [36]. Furthermore, chronic hyperammonemia inactivates the glutamate transporter (GLT-1) in astrocytes [37].

For over 25 years now, it has been observed that there is increased GABAergic tone in patients with HE. GABA is the predominant inhibitory neurotransmitter in the brain, and it was hypothesised that GABA accumulated in liver failure and crossed the blood brain barrier [21]. This hypothesis has not been confirmed in some of the more recent studies. Recent hypotheses suggest that hyperammonemia may modulate the observed increase in GABAergic tone in liver disease. Hyperammonemia has been shown to inhibit astrocytic GABA uptake, increase neuronal chloride currents by a direct action on the $GABA_A$ receptor complex, and potentiate the binding of $GABA_A$ and central benzodiazepine receptor agonists to the $GABA_A$ receptor complex [23, 38]. Recent studies have suggested that alterations in brain GABA content or receptor complex function may not be as important as the presence of neurosteroids with GABA agonist properties, which may explain the increased GABAergic tone in HE [39].

In rat models of chronic liver failure and hyperammonemia, it has been shown that ammonia impairs the glutamate-nitric oxide-cGMP pathway, which is activated by the NMDA receptor and is important in learning. Moreover, chronic treatment with sildenafil, an inhibitor of the phosphodiesterase that degrades cGMP, normalises the function of the pathway and restores learning ability in rats with portacaval shunts or with hyperammonemia. This may well explain why patients with minimal HE have impairment in short-term memory [40].

Ammonia and brain metabolism

Ammonia at millimolar concentrations also has the potential to impair brain energy metabolism, particularly as it is known to inhibit the tricarboxylic acid cycle enzyme ketoglutarate dehydrogenase [41]. However, brain energy metabolism does not appear to be impaired in chronic liver disease until the very late stages, when isoelectric electroencephalography traces become abnormal [42]. Nevertheless, cerebral spinal fluid lactate is increased in patients with HE [43] and in animal models of chronic liver disease and ammonia-precipitated encephalopathy [44].

Using nuclear magnetic resonance studies in the hepatic devascularised rat model of acute liver failure, Zwingmann et al. [45] demonstrated 2–4.5-fold increase in total brain glutamine and lactate in the early pre-coma stages of HE. In the more severe coma stage associated with brain oedema there was a further significant increase in brain lactate. ^{13}C isotopomer analysis showed a selective increase of de novo synthesis of lactate from [1-^{13}C] glucose resulting in a 2.5-fold increased fractional ^{13}C enrichment in lactate [2-^{13}C] Glutamine, synthesised through the astrocytic enzyme pyruvate carboxylase, increased 10-fold in the pre-coma stages of HE, but there was no further increase in the coma stages. ^{13}C-label incorporation into [4-^{13}C] glutamate, synthesised mainly through neuronal pyruvate dehydrogenase, was selectively reduced in the coma stages, whilst brain GABA synthesis remained unchanged. These observations indicate that increased brain lactate synthesis and impaired glucose oxidative pathways are important in the pathogenesis of brain oedema in acute liver failure.

Altered gene expression

Acute liver failure results in altered expression of several genes in the brain which code for important proteins involved in brain function and are summarized in table 1. These proteins include the glucose (GLUT-1) and glutamate (GLT-1) transporters, the astrocytic structural protein glial fibrillary acidic protein, the peripheral-type benzodiazepine receptor and aquaporin IV, which is a water-channel transmembrane protein. Loss of expression of GLT-1 results in increased extracellular glutamate. The monoamines serotonin and noradrenaline are also increased extracellularly following post-translational modifications to their receptors [46]. This altered monoaminergic function may also be responsible for the early neuropsychiatric symptoms of HE [47]. The increased expression and activation of the peripheral-type benzodiazepine receptor results in altered brain excitability. As a mitochondrial protein, it plays an important role in the maintenance of astrocytic energy metabolism and uptake of cholesterol, resulting in the synthesis of neurosteroids which have potent neuroinhibitory properties [48]. Increased expression of aquaporin IV may be important in regulation of water transport and hence alter brain water [49], and aquaporin IV knockout mice show reduced brain oedema and improved neurological outcome compared with wild-type mice in models of brain oedema [50]. Portacaval anastomosis in the rat results in increased gene expression of the constitutive neuronal isoform of nitric oxide synthase in the brain. This may contribute to altered mental states through disturbances in cerebral blood flow [51].

Table 1. A summary of the main astrocytic genes which are up- and downregulated in acute liver failure.

Upregulation	Downregulation
Peripheral-type benzodiazepine receptor (PTBR)	GLT-1 (glutamate transporter)
GLUT-1 (glucose transporter)	Glial fibrillary acidic protein (GFAP)
Neuronal nitric oxide synthase (nNOS)	GLYT-1 (glycine transporter)
Aquaporin IV	

Cerebral blood flow

Cerebral blood flow is closely coupled to neuronal activity and is modified by afferent projection fibres that release vasoactive neurotransmitters in the perivascular region, principally on the astrocyte endfeet that outline cerebral blood vessels, enabling cerebral blood flow autoregulation. Cerebral vasoconstriction induced by increased calcium in astrocytic endfeet is generated through the phospholipase A2-arachidonic acid pathway and 20-hydroxyeicosatetraenoic acid production [52].

In acute liver failure, there is a loss of cerebral autoregulation, altered reactivity to carbon dioxide and cerebral hyperemia. Arterial concentrations of ammonia, its delivery to the brain and its metabolic rate are significantly higher in patients with acute liver failure and intracranial hypertension, confirming the important role of ammonia in the pathogenesis of intracranial hypertension, although the underlying mechanisms are unclear [53]. The development of increased blood brain volume leads to a rise in intracranial pressure, and this may facilitate the movement of water across the blood brain barrier in an osmoti-

cally altered brain [54]. As the astrocyte is involved in the normal coupling of neuronal activity with cerebral blood flow [55], alterations in astrocyte function either directly or indirectly by increased brain ammonia concentrations are likely to affect cerebral blood flow. When methods are introduced to inhibit brain glutamine synthesis, with methionine-sulfoximine [56], a rise in cerebral blood flow is prevented. This observation supports the view that increased cerebral blood flow may be important in initiating brain swelling in this model. However, in humans with acute liver failure, the increase in cerebral blood flow is a later event. Patients with acute liver failure that have severe encephalopathy and mildly increased intracranial pressure often have normal cerebral blood flow. Marked increases in intracranial pressure are associated with a concomitant rise in cerebral blood flow [53].

In contrast, in chronic liver disease, the predominant picture is one of cerebral vasoconstriction [57]. Regional differences in cerebral blood flow are also seen. Regional cerebral blood flow corresponds to functional impairment of the frontal cortex and cingulate gyrus [58]. Administration of an oral amino acid load to patients with cirrhosis that results in increased ammonia is associated with a reduction in regional cerebral perfusion in the temporal lobes, left superior frontal gyrus and right parietal and cingulate gyrus, and deterioration in memory tests [59].

The role of inflammatory mediators in modulating the manifestation of HE

As is clear from the previous discussion, overwhelming evidence supports the ammonia hypothesis of HE. However, in clinical practice a consistent correlation between the concentration of ammonia in the blood and the symptoms of HE cannot be confirmed. Sepsis is a frequent precipitating factor for the development of HE, supporting the view that additional inflammation may play a role in the pathogenesis of HE (fig. 3). There is a growing body of evidence supporting the role of inflammation in increasing the susceptibility of the brain to the effects of hyperammonemia. This concept and current views on its pathophysiologic basis will now be reviewed.

Cytokines, astrocytes and the blood brain barrier

It is clear from the discussion so far that astrocytes are the key cells involved in the pathogenesis of HE, as they have been found to be the cells that are most commonly seen to be affected neuropathologically. Astrocytes secrete a full repertoire of cytokines and neurotrophic factors to neurons, consistent with their neurosupportive role. As the astrocytes are one of the main components involved in the formation of the blood brain barrier and in controlling cerebrovascular tone, it is conceivable that astrocyte swelling, which is a feature of HE, is likely to affect the function of the blood brain barrier.

In the brain, activated microglial cells and astrocytes produce cytokines in response to injury or inflammation. One of the early cytokines to be released is tumour necrosis factor α (TNF-α). TNF-α is involved in the induction of the cytokines interleukin (IL)-1 and IL-6 [60]. It has been shown in vitro that the integrity of the blood brain barrier is compromised by IL-1β, which is mediated through the cyclooxygenase pathway within the endothelial cells [61]. In astrocyte cultures, interferon γ (IFN-γ) upregulates inducible nitric oxide synthase (iNOS) [62]. The effect of TNF-α on human brain microvesicular endothe-

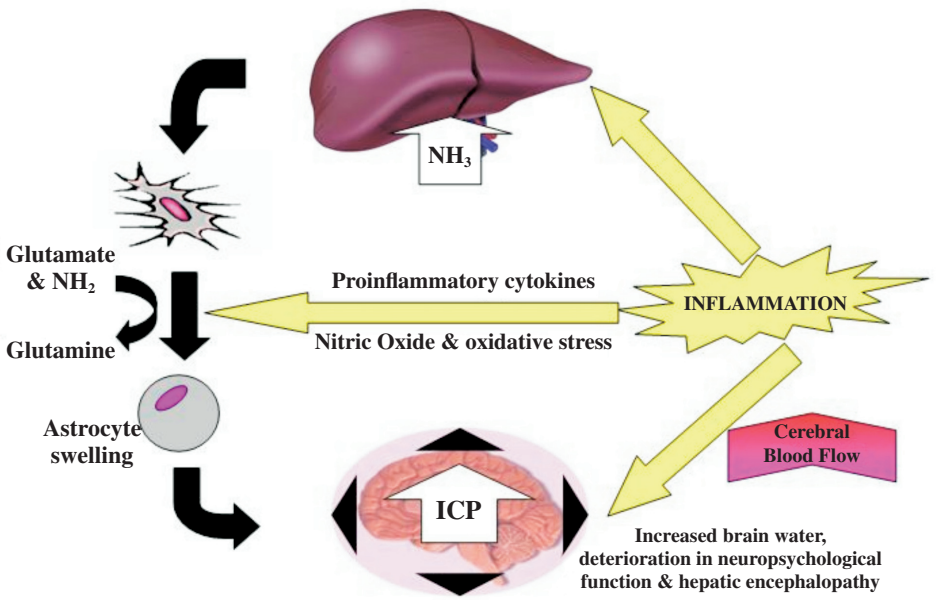

Figure 3. A diagrammatic representation of the relationship between inflammation and cerebral blood flow on the development and manifestation of HE. Astrocytes are the site of ammonia detoxification in the brain and eliminate ammonia by the synthesis of glutamine through amidation of glutamate. The resultant astrocytic swelling leads to increased brain water and raised intracranial pressure.

lial cell permeability has the potential to compromise the blood brain barrier function [63].

There is considerable evidence that the peripheral immune system can signal the brain to elicit a response during infection and inflammation. This response involves the expression of proinflammatory cytokines such as IL-1β, TNF-α and IL-6, both in the periphery and the brain. This can occur through direct entry of cytokine into the brain across the blood brain barrier by a storable transport mechanism, the interaction of cytokine with circumventricular organs such as the orgnum vasculosum of the lamina terminalis and area postrema [64] and activation of afferent neurons of the vagus nerve [65]. Sharshar et al. [66] showed that in patients dying with septic shock, neuronal and glial apoptosis occurs within the brain autonomic centres, which are strongly associated with iNOS expression in the endothelial cells. This may alter glutamatergic neurotransmission [67] and increase the number of peripheral-type benzodiazepine receptors, which may alter cellular osmotic homeostasis [68]. Cytokines may also modulate ammonia diffusion, and it has been shown that TNF-α and IL-6 increase fluid phase permeability and ammonia diffusion in CNS-derived endothelial cells [69]. Brain endothelial cells have receptors for IL-1β and TNF-α. These can transduce signals which can culminate in the intracerebral synthesis of nitric oxide and prostanoids [64]. Perivascular cells of macrophage origin may be a target for these cytokine effects [70].

Stimulation of astrocytes in culture with lipopolysaccharide results in upregulation of iNOS whitch culminates in increased production of nitric oxide [71]. A similar phenomenon is observed with ammonia. Schleiss et al. have provided evidence of protein tyrosine nitration in ammonia-treated cultured astrocytes and also in vivo in brains of rats treated with an ammonia load [72]. Furthermore, the addition of ammonia to astrocyte cultures generates reactive oxygen species, a process involving the synthesis of glutamine. Treatment of astrocytes with glutamine also increases free radical production [73]. If phosphate-activated glutaminase is inhibited, free radical production is blocked, suggesting that ammonia released by the hydrolysis of glutamine may be a factor [74, 75]. As most of the glutamine in astrocytes is metabolised by mitochondrial phosphate-activated glutaminase, breakdown of glutamine could result in release of high levels of ammonia in mitochondria, which is likely to increase reactive oxygen species.

Peroxynitrite is formed from the combination of superoxide and nitric oxide. Peroxynitrite in the presence of carbon dioxide can modify tissue proteins to form nitrotyrosine, which may mediate nitric oxide-induced blood brain barrier damage [76]. An increase in the permeability of the inner mitochondrial membrane to small solutes (mitochondrial permeability transition) results from oxidative and nitrosative stress, and ammonia has been recently shown to induce similar changes in cultured astrocytes [77].

Evidence for the role of inflammation in acute liver failure

Oxidative stress is important in the pathogenesis of ammonia-induced neurotoxicity. In hyperammonemia, free-radical production may be mediated by N-methyl-D-aspartate (NMDA)-receptor activation [78], and ammonia-induced mitochondrial dysfunction [79] could also be a source of reactive oxygen species such as peroxynitrite [76]. Furthermore, antioxidants have beneficial effects in experimental animal models of HE and hyperammonemia [80, 81]. As previously mentioned, hypothermia improves the outcome in acute liver failure. Hypothermia reduces free radical production, which may account for some of its beneficial effects [82]. Takada et al. [83] showed in a pig model of acute liver failure that animals administered lipopolysaccharide and amatoxin intraportally developed more pronounced intracranial hypertension than animals given amatoxin alone, even though ammonia concentrations were similar in both groups. In another pig model of acute liver failure induced by hepatic devascularisation, improvement in the severity of HE following treatment with albumin dialysis occurs independently of changes in ammonia [84]. Albumin dialysis probably achieves this effect through reduction in oxidative stress and restoring nitric oxide metabolism. These observations support a role for mechanisms other than ammonia in the pathogenesis of HE.

Studies in patients with acute liver failure have shown rapid progression to severe HE in those with evidence of a systemic inflammatory response, suggesting a possible link between inflammation and HE [85, 86]. In addition, in patients with acetaminophen-induced acute liver failure, infection and/or the resulting systemic inflammatory response was shown to be an important factor in contributing to deterioration in the severity of HE. [87]. In the advanced stages of HE in acute liver failure, when patients have uncontrolled intracranial pressure, the brain produces cytokines such as TNF-α, IL-1β and IL-6 [88, 89].

In addition, it is likely that one of the mechanisms through which inflammation exerts its deleterious effect is by inducing alterations in cerebral blood flow through its effects on the expression of the NOS proteins. Accordingly, Aggarwal et al. [90] showed that patients with high intracranial pressure have elevated cerebral blood flow. More recently we showed a direct correlation between the severity of inflammation and increased cerebral blood flow observed in acute liver failure [53]. Further evidence supporting the role of inflammation is derived from experimental interventions that reduce brain swelling by altering cerebral blood flow and inflammatory responses.

In patients with uncontrolled intracranial hypertension and acute liver failure, moderate hypothermia reduces brain flux of TNF-α, IL-1β and IL-6 and cerebral blood flow contributing to reduction in intracranial pressure [81, 91]. Removal of the necrotic liver in acute liver failure reduces proinflammatory cytokines such as TNF-α, IL-1β and IL-6 and cerebral blood flow, resulting in a reduction in the severity of intracranial hypertension [92].

Evidence for the role of inflammation in cirrhosis

A distinctive postmortem finding in human brains with HE is the presence of Alzheimer type II astrocytosis [13], which characteristically contains increased amounts of lipofuscin pigment that consists of peroxidized lipids compatible with oxidative damage [93]. Nitric oxide has also been implicated in the pathogenesis of HE. Nitric oxide synthase activity has been shown to be elevated in experimental models of HE [94], and increased brain nitric oxide production was shown in portacaval-shunted rats given ammonia infusions [56] with evidence of nitrotyrosine accumulation in astrocytes (fig. 4) [95]. Studies in portacaval shunted rats administered an ammonia load have demonstrated a rise in cerebral blood flow that parallels the increase in intracranial pressure and correlates directly with brain water content [56, 96].

Direct evidence for the role of inflammation in modulating the severity of HE in patients was observed in a recent study. In cirrhosis, the presence of minimal HE and its severity were independent of the severity of liver disease and plasma ammonia concentrations, but markers of inflammation were significantly higher in those with minimal HE compared with those without [97]. Furthermore, we showed a significant deterioration of neuropsychological test scores following induced hyperammonemia during the inflammatory state, but not after its resolution, suggests that inflammation and its mediators may be important in modulating the cerebral effect of ammonia in liver disease [98]. Also, it has been shown that changes in regional cerebral blood flow lead to differences in cerebral ammonia uptake [58]. Cerebral blood flow is higher in the basal ganglia and cerebellum, which correlates with increased ammonia extraction [59].

Conclusions

In conclusion, we have described the current new perspectives in the molecular pathogenesis of HE. The importance of the role of ammonia has been highlighted with respect both to its direct neurotoxicity and on brain swelling through its detoxification to glutamine in the astrocyte, the cell most often implicated in the pathogenesis of HE. In fact one might say that the key to understanding the pathogenesis of HE is to explore the story of the 'sick astrocyte'. We have also reviewed the factors that we believe are critical in modulating the manifest symptoms of HE, the most important of which is the synergistic role of inflammation in modulating the cerebral effects of ammonia. Furthermore, the production of reactive oxygen species and increased protein tyrosine nitration may alter astrocyte function and contribute to or precipitate episodes of HE.

The above description of the possible mechanisms involved in the pathogenesis of HE has led to new thoughts on possible therapeutic interventions. Targets such as nitric oxide, oxidative stress and inflammation have been utilized by hypothermia [91], albumin infusion and use of extracorporeal detoxification devices [99]. The cytokine milieu can be altered by the use of probiotics [100], and a better understanding of the role of cytokines may lead to the development of anti-cytokine strategies. Prostanoids can be targeted with cyclooxygenase inhibitors [101]. Better understanding of the role of individual organs

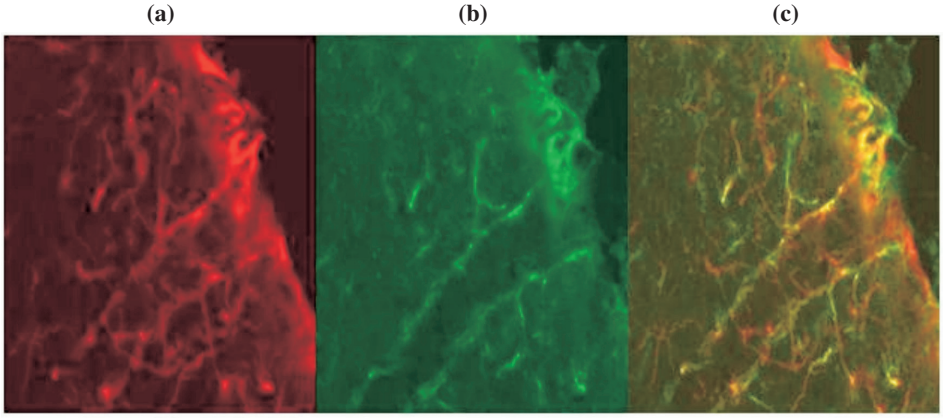

Figure 4. Slides showing staining for nitrotyrosine in the cerebral cortex of a portacaval shunted rat receiving an ammonia infusion (courtesy of Professor Andres Blei, Northwestern University Feinberg School of Medicine, Chicago and published in Seminars in Liver Disease 2003; 23: 259–269). **(A)** shows glial fibrillary acidic protein (GFAP)-positive astrocytes in the cerebral cortex; **(B)** shows nitrotyrosine staining in astrocytes; and **(C)** reveals the overlap of GFAP and nitrotyrosine staining in cortical astrocytes.

involved in ammonia and amino acid metabolism will allow targeted therapies aimed at individual organs. We have so far only uncovered the tip of the iceberg in terms of understanding the operative mechanisms involved, but very much look forward to moving this exciting area of research forward over the next few years.

Acknowledgement. We thank all the members of the Liver Failure Group and our collaborators for their helpful advice and discussion. We would like to especially thank Professor Andres Blei for providing Figure 4 in the manuscript.

1 Hahn M., Massen O., Nencki M., Pavlov I. (1893) Die Eck'sche fistel zwischen der unteren hohlvene und der pfortader und ihre folgen fur den organismus. Arch. Exp. Pathol. Pharm. **32:** 161–210
2 Nencki M. and Zaleski J. (1895) Ueber die Bestimmung des Ammoniaks in Thierischen Fluessigkeiten und Geweben. Arch. exp. pathol. pharm. **36:** 385–396
3 Nencki M., Pawlow J., Zaleski J. (1896) Ueber den ammoniakgehalt des blutes under der organe und die harnstoffbildung bei den saugethieren. Arch. exp. pathol. pharm. **37:** 26–51
4 Gazuba G. J. Jr, Phillips G. B., Davidson C.S. (1952) Reversible toxic manifestations in patients with cirrhosis of the liver given cation-exchange resins. N. Engl. J. Med. **246(4):** 124–130
5 Phillips G., Schwartz R., Gabuzda G., Davidson C. (1952) The syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. N. Engl. J. Med. **247:** 239–246
6 Sherlock S. (1958) Pathogenesis and management of hepatic coma. Am. J. Med. **24(5):** 805–813
7 Stahl J. (1963) Studies of the blood ammonia in liver disease. Its diagnostic, prognostic, and therapeutic significance. Ann. Intern. Med. **58:** 1–24
8 Ferenci P., Lockwood A., Mullen K., Tater R., Weissenborn K., Blei A. et al. (2002) Hepatic encephalopathy – definition, nomenclature, diagnosis and quantification: final report of the Working Party at the 11th World Congress of Gastroenterology, Vienna, 1998. Hepatology **35:** 716–721
9 Amodio P., Del Piccolo F., Marchetti P., Angeli P., Iemmolo R., Caregaro L. et al. (1999) Clinical features and survivial of cirrhotic patients with subclinical cognitive alterations detected by the number connection test and computerized psychometric tests. Hepatology **29(6):** 1662–1667
10 Amodio P., Montagnese S., Gatta A., Morgan M. Y. (2004) Characteristics of minimal hepatic encephalopathy. Metab. Brain Dis. **19(3–4):** 253–267
11 Schomerus H., Hamster W., Blunck H., Reinhard U., Mayer K., Dolle W. (1981) Latent portasystemic encephalopathy. I. Nature of cerebral functional defects and their effect on fitness to drive. Dig. Dis. Sci. **26(7):** 622–630
12 Sen S., Williams R., Jalan R. (2002) The pathophysiological basis of acute-on-chronic liver failure. Liver Suppl. **2:** 5–13
13 Norenberg M. (1988) Hepatic Encephalopathy: studies with astrocyte cultures. In: The Biochemical Pathology of Astrocytes. Norenberg M., Hertz L. and Schousboe A. (eds.). Alan R. Liss, New York; pp. 451–464
14 Butterworth R.F, Giguere J.F., Michaud J., Lavoie J., Layrargues G.P. (1987) Ammonia: key factor in the pathogenesis of hepatic encephalopathy. Neurochem. Pathol. **6(1–2):** 1–12
15 Desjardins P., Bandeira P., Rao V. L., Butterworth R. F. (1999) Portacaval anastomosis causes selective alterations of peripheral-type benzodiazepine receptor expression in rat brain and peripheral tissues. Neurochem. Int. **35(4):** 293–299
16 Chan H, Butterworth RF. Evidence for an astrocytic glutamate transporter deficit in hepatic encephalopathy. Neurochem. Res. **24(11):** 1397–1401
17 Belanger M., Desjardins P., Chatauret N., Butterworth R. F. (2002) Loss of expression of glial fibrillary acidic protein in acute hyperammonemia. Neurochem. Int. **41(2–3):** 155–160
18 McCarty J. H. (2005) Cell biology of the neurovascular unit: implications for drug delivery across the blood-brain barrier. Assay Drug Dev. Technol. **3(1):** 89–95
19 Lockwood A., Yap E., Wong W. (1991) Cerebral ammonia metabolism in patients with severe liver disease and minimal hepatic encephalopathy. J. Cereb. Blood Flow Metab. **11:** 337–341
20 Hamm S., Dehouck B., Kraus J., Wolburg-Buchholz K., Wolburg H., Risau W. et al. (2004) Astrocyte mediated modulation of blood-brain barrier permeability does not correlate with a loss of tight junction proteins from the cellular contacts. Cell Tissue Res. **315(2):** 157–166
21 Bassett M. L., Mullen K. D., Scholz B., Fenstermacher J. D., Jones E. A. (1990) Increased brain uptake of gamma-aminobutyric acid in a rabbit model of hepatic encephalopathy. Gastroenterology **98(3):** 747–757
22 Michalak A., Rose C., Butterworth J., Butterworth R. F. (1996) Neuroactive amino acids and glutamate (NMDA) receptors in frontal cortex of rats with experimental acute liver failure. Hepatology **24(4):** 908–913
23 Schafer D. F., Fowler J. M., Munson P. J., Thakur A. K., Waggoner J. G, Jones E. A. (1983) Gamma-aminobutyric acid and benzodiazepine receptors in an animal model of fulminant hepatic failure. J. Lab. Clin. Med. **102(6):** 870–880
24 Ahboucha S., Butterworth R. F. (2004) Pathophysiology of hepatic encephalopathy: a new look at GABA from the molecular standpoint. Metab. Brain Dis. **19(3–4):** 331–343
25 Butterworth R. F. (2002) Pathophysiology of hepatic encephalopathy: a new look at ammonia. Metab. Brain Dis. **17(4):** 221–227
26 Lockwood A. H., McDonald J. M., Reiman R. E., Gelbard A. S., Laughlin J. S., Duffy T. E. et al. (1979) The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammonemia. J. Clin. Invest. **63(3):** 449–460
27 Dejong C., Kampman M., Deutz N., Soeters P. (1992) Cerebral cortex ammonia and glutamine metabolism during liver insufficiency-induced hyperammonemia in the rat. J. Neurochem. **59:** 1071–1079
28 Clemmesen J., Larsen F., Kondrup J., Hansen B., Ott P. (1999) Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology **29:** 648–653
29 Haussinger D., Kircheis G., Fischer R., Schleiss F., vom Dahl S. (2000) Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low grade oedema? J. Hepatol. **32:** 1035–1038
30 Cordoba J., Alonso J., Rovira A., Jacas C., Sanpedro F., Castells L. et al. (2001) The development of low-grade cerebral oedema in cirrhosis is supported by the evolution of 1H-magnetic resonance abnormalities after liver transplantation. J. Hepatol. **35:** 598–604
31 Balata S., Olde Damink S., Ferguson K., Marshall I., Hayes P., Deutz N. et al. (2003) Induced hyperammonemia alters neuropsychology, brain MR spectroscopy and magnetization transfer in cirrhosis. Hepatology **37:** 931–939
32 Shawcross D. L., Balata S., Olde Damink S. W., Hayes P. C., Wardlaw J., Marshall I. et al. (2004) Low myo-inositol and high glutamine levels in brain are associated with neuropsychological deterioration after induced hyperammonemia. Am. J. Physiol. Gastrointest. Liver Physiol. **287(3):** G503–G509
33 Cordoba J., Gottstein J., Blei A. (1998) Chronic hyponatremia exacerbates ammonia-induced brain oedema in rats after portacaval anastomosis. Hepatology **29(4):** 589–594
34 Szerb J. C., Butterworth R. F. (1992) Effect of ammonium ions on synaptic transmission in the mammalian central nervous system. Prog. Neurobiol. **39(2):** 135–153

35 Rose C., Kresse W., Kettenmann H. (2005) Acute insult of ammonia leads to calcium-dependent glutamate release from cultured astrocytes: an effect of pH. J. Biol. Chem.

36 Michalak A., Rose C., Butterworth J., Butterworth R. F. (1996) Neuroactive amino acids and glutamate (NMDA) receptors in frontal cortex of rats with experimental acute liver failure. Hepatology **24(4):** 908–913

37 Albrecht J., Jones E.A. (1999) Hepatic encephalopathy: molecular mechanisms underlying the clinical syndrome. J. Neurol. Sci. **170(2):** 138–146

38 Jones E.A. (2003) Potential mechanisms of enhanced GABA-mediated inhibitory neurotransmission in liver failure. Neurochem.Int. **43(4–5):** 509–516

39 Ahboucha S., Pomier-Layrargues G., Butterworth R. F. (2004) Increased brain concentrations of endogenous (non-benzodiazepine) GABA-A receptor ligands in human hepatic encephalopathy. Metab. Brain. Dis. **19(3–4):** 241–251

40 Erceg S., Monfort P., Hernandez-Viadel M., Rodrigo R., Montoliu C., Felipo V. (2005) Oral administration of sildenafil restores learning ability in rats with hyperammonemia and with portacaval shunts. Hepatology **41(2):** 299–306

41 Lai J. C., Cooper A. J. (1986) Brain alpha-ketoglutarate dehydrogenase complex: kinetic properties, regional distribution and effects of inhibitors. J. Neurochem. **47(5):** 1376–1386

42 Hindfelt B., Plum F., Duffy T. (1977) Effect of acute ammonia intoxication on cerebral metabolism in rats with portacaval shunts. J. Clin. Invest. **59(3):** 386–396

43 Yao H., Sadoshima S., Fujii K., Kusuda K., Ishitsuka T., Tamaki K. et al. (1987) Cerebrospinal fluid lactate in patients with hepatic encephalopathy. Eur. Neurol. **27(3):** 182–187

44 Therrien G., Giguere J. F., Butterworth R. F. (1991) Increased cerebrospinal fluid lactate reflects deterioration of neurological status in experimental portal-systemic encephalopathy. Metab. Brain Dis. **6(4):** 225–231

45 Zwingmann C., Chatauret N., Leibfritz D., Butterworth R. F. (2003) Selective increase of brain lactate synthesis in experimental acute liver failure: results of a [H-C] nuclear magnetic resonance study. Hepatology **37(2):** 420–428

46 Desjardins P., Belanger M., Butterworth R. F. (2001) Alterations in expression of genes coding for key astrocytic proteins in acute liver failure. J. Neurosci. Res. **66(5):** 967–971

47 Bergeron M., Reader T. A., Layrargues G. P., Butterworth R. F. (1989) Monoamines and metabolites in autopsied brain tissue from cirrhotic patients with hepatic encephalopathy. Neurochem. Res. **14(9):** 853–859

48 Norenberg M. D. (1996) Astrocytic-ammonia interactions in hepatic encephalopathy. Semin. Liver Dis. **16(3):** 245–253

49 Rama Rao K. V., Chen M., Simard J. M., Norenberg M. D. (2003) Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. Neuroreport **14(18):** 2379–2382

50 Manley G. T., Binder D. K., Papadopoulos M. C., Verkman A. S. (2004) New insights into water transport and edema in the central nervous system from phenotype analysis of aquaporin-4 null mice. Neuroscience **129(4):** 983–991

51 Rao V., Butterworth R. (1998) Neuronal nitric oxide synthase and hepatic encephalopathy. Metab. Brain Dis. **13:** 175–189

52 Mulligan S. J., MacVicar B. A. (2004) Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature **431(7005):** 195–199

53 Jalan R., Olde Damink S. W., Hayes P. C., Deutz N. E., Lee A. (2004) Pathogenesis of intracranial hypertension in acute liver failure: inflammation, ammonia and cerebral blood flow. J. Hepatol. **41(4):** 613–620

54 Vaquero J., Chung C., Blei A. T. (2004) Cerebral blood flow in acute liver failure: a finding in search of a mechanism. Metab. Brain Dis. **19(3–4):** 177–194

55 Magistretti P. J., Pellerin L., Rothman D. L., Shulman R. G. (1999) Energy on demand. Science **283(5401):** 496–497

56 Master S., Gottstein J., Blei A. T. (1999) Cerebral blood flow and the development of ammonia-induced brain edema in rats after portacaval anastomosis. Hepatology **30(4):** 876–880

57 Guevara M., Bru C., Gines P., Fernandez-Esparrach G., Sort P., Bataller R. et al. (1998) Increased cerebrovascular resistance in cirrhotic patients with ascites. Hepatology **28(1):** 39–44

58 Ahl B, Weissenborn K, van den HJ, Fischer-Wasels D, Kostler H, Hecker H et al. Regional differences in cerebral blood flow and cerebral ammonia metabolism in patients with cirrhosis. Hepatology 2004;40(1):73–9.

59 Jalan R., Olde Damink S., Lui H., Glabus M., Deutz N., Hayes P. et al. (2003) Oral amino acid load mimicking haemoglobin results in reduced regional cerebral perfusion and deterioration in memory tests in patients with cirrhosis of the liver. Metab. Brain Dis. **18:** 37–49

60 Fong Y., Tracey K. J., Moldawer L. L., Hesse D. G., Manogue K. B., Kenney J. S. et al. (1989) Antibodies to cachectin/tumor necrosis factor reduce interleukin 1 beta and interleukin 6 appearance during lethal bacteremia. J. Exp. Med. **170(5):** 1627–1633

61 de Vries H. E., Blom-Roosemalen M. C., van Oosten M., de Boer A. G., van Berkel T. J., Breimer D. D. et al. (1996) The influence of cytokines on the integrity of the blood-brain barrier in vitro. J. Neuroimmunol. **64(1):** 37–43

62 Falsig J., Latta M., Leist M. (2004) Defined inflammatory states in astrocyte cultures: correlation with susceptibility towards CD95-driven apoptosis. J. Neurochem. **88(1):** 181–193

63 Didier N., Romero I. A., Creminon C., Wijkhuisen A., Grassi J., Mabondzo A. (2003) Secretion of interleukin-1beta by astrocytes mediates endothelin-1 and tumour necrosis factor-alpha effects on human brain microvascular endothelial cell permeability. J: Neurochem: **86(1):** 246–254

64 Licinio J., Wong M. (1997) Pathways and mechanisms for cytokine signalling of the central nervous system. J. Clin. Invest. **100:** 2941–2947

65 Hosoi T., Okuma Y., Nomura Y. (2002) The mechanisms of immune-to-brain communication in inflammation as a drug target. Curr. Drug Targets Inflamm. Allergy **1(3):** 257–262

66 Sharshar T., Gray F., Lorin de la Grandmaison G., Hopkinson N., Ross E., Dorandeau A. et al. (2003) Apoptosis of neurons in cardiovascular autonomic centres triggered by inducible nitric oxide synthase after death from septic shock. Lancet **363:** 1799–1805

67 Hu S., Sheng W., Ehrlich L., Peterson P., Chao C. (2000) Cytokine effects on glutamate uptake by human astrocytes. Neuroimmunomodulation **7:**153–159

68 Oh Y. J., Francis J. W., Markelonis G. J., Oh T. H. (1992) Interleukin-1-beta and tumor necrosis factor-alpha increase peripheral-type benzodiazepine binding sites in cultured polygonal astrocytes. J. Neurochem. **58(6):** 2131–2138

69 Duchini A., Govindarajan S., Santucci M., Zampi G., Hofman F. M. (1996) Effects of tumor necrosis factor-alpha and interleukin-6 on fluid-phase permeability and ammonia diffusion in CNS-derived endothelial cells. J. Investig. Med. **44(8):** 474–482

70 Schiltz J. C., Sawchenko P. E. (2003) Signaling the brain in systemic inflammation: the role of perivascular cells. Front. Biosci. **8:** s1321–s1329

71 Korcok J., Wu F., Tyml K., Hammond R. R., Wilson J. X. (2002) Sepsis inhibits reduction of dehydroascorbic acid and accumulation of ascorbate in astroglial cultures: intracellular ascorbate depletion increases nitric oxide synthase induction and glutamate uptake inhibition. J. Neurochem. **81(1):** 185–193

72 Schliess F., Gorg B., Fischer R., Desjardins P., Bidmon H. J., Herrmann A. et al. (2002) Ammonia induces MK-801-sensitive nitration and phosphorylation of protein tyrosine residues in rat astrocytes. FASEB J. **16(7):** 739–741

73 Murthy C. R. K., Bai G., Dombro R. S. and Norenberg M. D. (2000) Ammonia-induced swelling in primary cultures of rat astrocytes: Role of free radicals. Soc. Neurosci. Abstr. **26:** 1893

74 Sonnewald U., Westergaard N., Jones P., Taylor A., Bachelard H. S., Schousboe A. (1996) Metabolism of [U-13C5] glutamine in cultured astrocytes studied by NMR spectroscopy: first evidence of astrocytic pyruvate recycling. J. Neurochem. **67(6):** 2566–2572
75 Yudkoff M., Nissim I., Pleasure D. (1988) Astrocyte metabolism of [15N]glutamine: implications for the glutamine-glutamate cycle. J. Neurochem. **51(3):** 843–850
76 Tan K. H., Harrington S., Purcell W. M., Hurst R. D. (2004) Peroxynitrite mediates nitric oxide-induced blood-brain barrier damage. Neurochem. Res. **29(3):** 579–587
77 Rama Rao K. V., Jayakumar A. R., Norenberg M. D. (2003) Induction of the mitochondrial permeability transition in cultured astrocytes by glutamine. Neurochem. Int. **43(4–5):** 517–523
78 Marcaida G., Felipo V., Hermenegildo C., Minana M. D., Grisolia S. (1992) Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. FEBS Lett. **296(1):** 67–68
79 Rao K. V., Norenberg M. D. (2001) Cerebral energy metabolism in hepatic encephalopathy and hyperammonemia. Metab. Brain Dis. **16(1–2):** 67–78
80 Guerrini V. H. (1994) Effect of antioxidants on ammonia induced CNS-renal pathobiology in sheep. Free Radic. Res. **21(1):** 35–43
81 Jalan R., Olde Damink S. W., Deutz N. E., Hayes P. C., Lee A. (2004) Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. Gastroenterology **127(5):** 1338–1346
82 Globus M. Y., Alonso O., Dietrich W. D., Busto R., Ginsberg M. D. (1995) Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J. Neurochem. **65(4):** 1704–1711
83 Takada Y., Ishiguro S., Fukunaga K., Gu M., Tangiguchi H., Seino K.-I. et al. (2001) Increased intracranial pressure in a porcine model of fulminant hepatic failure using amatoxin and endotoxin. J. Hepatol. **34:** 825–831
84 Sen S., Rose C., Davies N., Williams R., Butterworth R., Revhaug A. and Jalan R. (2003) Albumin dialysis reduces brain water and intracranial pressure in acute liver failure: a randomised controlled study in a pig model. Hepatology **38:** 540 A
85 Rolando N., Wade J., Davalos M., Wendon J., Philpott-Howard J., Williams R. (2000) The systemic inflammatory response syndrome in acute liver failure. Hepatology **32:** 734–739
86 Vacquero J., Polson J., Chung C., Helenowski I., Schiodt F., Reisch J. et al. (2003) Infection and the progression of hepatic encephalopathy in acute liver failure. Gastroenterology **125:** 755–764
87 Jalan R., Olde Damink S. W., Hayes P. C., Deutz N. E., Lee A. (2004) Pathogenesis of intracranial hypertension in acute liver failure: inflammation, ammonia and cerebral blood flow. J. Hepatol. **41(4):** 613–620
88 Jalan R., Olde Damink S. W. M., Lee A., Hayes P. C. and Williams R. (2003) Brain production of inflammatory cytokines in patients with acute liver failure and uncontrolled intracranial hypertension. Hepatology **38:** 548A
89 Jalan R., Olde Damink S. W. M., Sen S., Mookerjee R. P, Hayes P. C. and Deutz N. E. P. (2004) Arginine, citrulline and nitric oxide metabolism in patients with liver failure and the effect of intervention with hypothermia, MARS and transplantation. Hepatology **40:** 636 A
90 Aggarwal S., Kramer D., Yonas H., Obrist W., Kang Y., Martin M. et al. (1994) Cerebral hemodynamic and metabolic changes in fulminant hepatic failure: a retrospective study. Hepatology **19(1):** 80–87
91 Jalan R., Olde Damink S., Deutz N., Lee A., Hayes P. (1999) Treatment of uncontrolled intracranial hypertension in acute liver failure with moderate hypothermia. Lancet **354:** 1164–1168
92 Jalan R., Pollok A., Shah S. H., Madhavan K., Simpson K. J. (2002) Liver derived pro-inflammatory cytokines may be important in producing intracranial hypertension in acute liver failure. J. Hepatol. **37(4):** 536–538
93 Brunk U. T. (1989) On the origin of lipofuscin; the iron content of residual bodies, and the relation of these organelles to the lysosomal vacuome. A study on cultured human glial cells. Adv. Exp. Med. Biol. **266:** 313–320
94 Rao V. L., Audet R. M., Butterworth R. F. (1995) Increased nitric oxide synthase activities and L-[3H]arginine uptake in brain following portacaval anastomosis. J. Neurochem. **65(2):** 677–678
95 Vaquero J., Chung C., Cahill M. E., Blei A. T. (2003) Pathogenesis of hepatic encephalopathy in acute liver failure. Semin. Liver Dis. **23(3):** 259–269
96 Larsen F., Gottstein J., Blei A. (2001= Cerebral hyperemia and nitric oxide synthase in rats with ammonia-induced brain edema. J. Hepatol. **34:** 548–554
97 Shawcross D. L. and Jalan R. (2004) Systemic inflammation determines baseline and ammonia-induced neuropsychological deterioration in cirrhosis. Hepatology **40 (4 Suppl. 1):** A310
98 Shawcross D., Davies N., Williams R., Jalan R. (2004) Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis. J. Hepatol. **40(2):** 247–254
99 Sen S., Davies N. A., Mookerjee R. P., Cheshire L. M., Hodges S. J., Williams R. et al. (2004) Pathophysiological effects of albumin dialysis in acute-on-chronic liver failure: a randomized controlled study. Liver Transpl. **10(9):** 1109–1119
100 Liu Q., Duan Z. P., Ha dK., Bengmark S., Kurtovic J., Riordan S. M. (2004) Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. Hepatology **39(5):** 1441–1449
101 Clemmesen J. O., Hansen B. A., Larsen F. S. (1997) Indomethacin normalizes intracranial pressure in acute liver failure: a twenty-three-year-old woman treated with indomethacin. Hepatology **26(6):** 1423–1425