MINIREVIEW

Hepatic Encephalopathy: An Update of Pathophysiologic Mechanisms (44433A)

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Abstract. **Hepatic encephalopathy (HE) is a neuropsychiatric disorder that occurs in both acute and chronic liver failure. Although the precise pathophysiologic mechanisms responsible for HE are not completely understood, a deficit in neurotransmission rather than a primary deficit in cerebral energy metabolism appears to be involved. The neural cell most vulnerable to liver failure is the astrocyte. In acute liver failure, the astrocyte undergoes swelling resulting in increased intracranial pressure; in chronic liver failure, the astrocyte undergoes characteristic changes known as Alzheimer type II astrocytosis. In portal-systemic encephalopathy resulting from chronic liver failure, astrocytes manifest altered expression of several key proteins and enzymes including monoamine oxidase B, glutamine synthetase, and the so-called peripheral-type benzodiazepine receptors. In addition, expression of some neuroneal proteins such as monoamine oxidase A and neuronal nitric oxide synthase are modified. In acute liver failure, expression of the astrocytic glutamate transporter GLT-1 is reduced, leading to increased extracellular concentrations of glutamate. Many of these changes have been attributed to a toxic effect of ammonia and/or manganese, two substances that are normally removed by the hepatobiliary route and that in liver failure accumulate in the brain. Manganese deposition in the globus pallidus in chronic liver failure results in signal hyperintensity on T1-weighted Magnetic Resonance Imaging and may be responsible for the extrapyramidal symptoms characteristic of portal-systemic encephalopathy. Other neurotransmitter systems implicated in the pathogenesis of hepatic encephalopathy include the serotonin system, where a synaptic deficit has been suggested, as well as the catecholaminergic and opioid systems. Further elucidation of the precise nature of these alterations could result in the design of novel pharmacotherapies for the prevention and treatment of hepatic encephalopathy.** [P.S.E.B.M. 1999, Vol 222]

Hepatic Encephalopathy (HE) is a severe neuropsychiatric complication of both acute and chronic liver failure. Depending upon the duration and degree of hepatic dysfunction, HE may present in one of two major forms. Portal-systemic encephalopathy (PSE) accompanies portal-systemic shunting of venous blood that arises either spontaneously due to portal hypertension or surgically following either portacaval anastomosis or transjugular intrahepatic portal-systemic stent shunts (TIPS) aimed at relieving portal hypertension. Neurologically, PSE develops slowly; the onset is often insidious starting with personality changes and altered sleep patterns. Shortened attention span, muscular incoordination, and asterixis (flapping tremor) follow, progressing to stupor and coma. Multiple episodes of PSE are common. PSE frequently results from a precipitating factor such as gastrointestinal bleeding, constipation, or use of a sedative.

Neuropathologically, PSE is characterized by astrocytic (rather than neuronal) changes. Histopathologic studies of brain sections from cirrhotic patients who died in hepatic coma show the presence of astrocytic pathology known as

Studies from the authors' research unit were funded by grants from the Medical Research Council of Canada and the Canadian Liver Foundation.

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0037-9727/99/2222-0099$14.00/0

Alzheimer type II astrocytosis in which astrocytes take on a characteristic swollen shape with a large pale nucleus, a prominent nucleolus, and margination of the chromatin pattern (1, 2). In addition to these morphological changes, astrocytes in PSE manifest altered expression of key astrocyte-specific proteins including glial fibillary acidic protein (GFAP) (1), enzymes such as glutamine synthetase (3) and monoamine oxidase MAO(B) (4), and peripheral-type (mitochondrial) benzodiazepine receptors (5).

Studies using noninvasive techniques continue to provide important clues to the pathogenesis of PSE. For example, Positron Emission Tomography (PET) studies using ^{18}F-fluorodeoxyglucose reveal significant decreases of cerebral glucose utilization in cerebral cortex with concomitant increases in thalamus, caudate nucleus, and cerebellum of cirrhotic patients with mild PSE (6, 7). Cerebral glucose utilization is particularly depressed in the cingulate gyrus of these patients (6–8), a finding that is of particular interest in view of the report that bilateral lesions of this brain structure result in confusion, disorientation, and memory loss (9). These findings suggest that the hypometabolism observed in this brain structure could contribute to the early neuropsychiatric abnormalities frequently observed in cirrhotic patients. Reduced glucose utilization measured using the ^{14}C-deoxyglucose autoradiographic technique has also been demonstrated in the cerebral cortex of rats 4 weeks after end-to-side portacaval anastomosis (10, 11). Furthermore, in aging rats, the glucose utilization deficit following anastomosis was more widespread, involving both frontal and fronto-parietal cortical regions (11), suggesting that the aging brain is more susceptible to portacaval-shunting than is that of the young adult. These findings offer a possible explanation for the increased prevalence of PSE consistently reported in older cirrhotic patients following the portacaval anastomosis or TIPS procedures.

In contrast to PSE, HE in fulminant hepatic failure (FHF) progresses through altered mental status to stupor and coma within hours or days. Seizures are not uncommon (12), and multifocal random muscle twitching is often seen before coma. Mortality rates are high in FHF; death most frequently results from brainstem herniation caused by increased intracranial pressure as a consequence of brain edema. Electron microscopic studies of brain tissue in FHF reveal cytotoxic rather than vasogenic edema (13, 14). Swelling of astrocytes and astrocytic end feet is most frequently observed.

Liver Failure Results in the Accumulation of Neurotoxic Substances in Brain

Liver failure and portal-systemic shunting result in increased blood concentrations of substances that are potentially neurotoxic. Of particular interest at this time are ammonia and manganese.

Ammonia. Evidence for an association between HE and ammonia dates back over a century to the work by Eck that described the effects of portacaval anastomosis in dogs (15). Feeding of meat to Eck-fistula dogs resulted in loss of coordination, stupor, and coma, leading to the suggestion that nitrogenous products were the causative factor in so called meat intoxication in these animals. In 1952, Gabuzda *et al.* (16) attempted to treat ascites in cirrhotic patients with ion-exchange resins that absorbed sodium and released ammonium ions. The treatment resulted in significant reductions in ascitic volume but precipitated severe neurological symptoms that were indistinguishable from PSE.

Arterial blood ammonia concentrations are frequently elevated in patients with all forms of HE, and studies in experimental animal models of acute and chronic liver failure reveal blood and brain ammonia concentrations in the low millimolar range (17). Further evidence consistent with accumulation of toxic levels of ammonia in human HE is provided by the results of recent studies using Positron Emission Tomography (PET) and $^{13}NH_3$ (18). Such studies demonstrate an increase in the cerebral metabolic rate for ammonia (CMRA) (i.e., the rate at which ammonia is taken up and metabolized by brain). Typical PET scans using $^{13}NH_3$ in a patient with chronic liver disease and mild PSE compared to an age-matched control subject are shown in Figure 1.

Furthermore, the PET studies revealed that the increased CMRA in patients with PSE was accompanied by an increase in the permeability/surface area product, a measure of blood-brain barrier permeability suggesting that, in chronic liver failure, the barrier becomes increasingly permeable to ammonia. This apparent ease with which ammonia moves from blood to brain in patients with chronic liver disease and the resulting increase in brain/blood concentration ratio, offers an explanation for 1) the hypersensitivity of cirrhotic patients to ammoniagenic conditions such as ingestion of a high protein diet or gastrointestinal bleeding and 2) the imperfect correlation between the degree of neurological dysfunction and blood ammonia concentrations in these patients.

Ammonia exerts a deleterious effect on cerebral function by both direct and indirect mechanisms (Table I).

Concentrations of ammonia in the millimolar range (equivalent to those reported in brain in experimental liver failure) impair postsynaptic inhibition in cerebral cortex, brainstem, and spinal cord preparations by blocking chloride extrusion from the postsynaptic neuron (19) thus rendering the inhibitory neurotransmitter ineffective. Following portacaval-shunting, postsynaptic inhibition in the cerebral cortex develops increased sensitivity to an acute ammonia load (19), a situation having clinical parallels, namely the increased susceptibility of patients who undergo surgical portacaval anastomosis or TIPS for the treatment of portal hypertension to ammoniagenic conditions such as a protein load or gastrointestinal hemorrhage.

Millimolar concentrations of ammonia also inhibit excitatory neurotransmission. An example of this is provided by the report that synaptic transmission from Schaffer collaterals to CA1 hippocampal neurons is reversibly de-

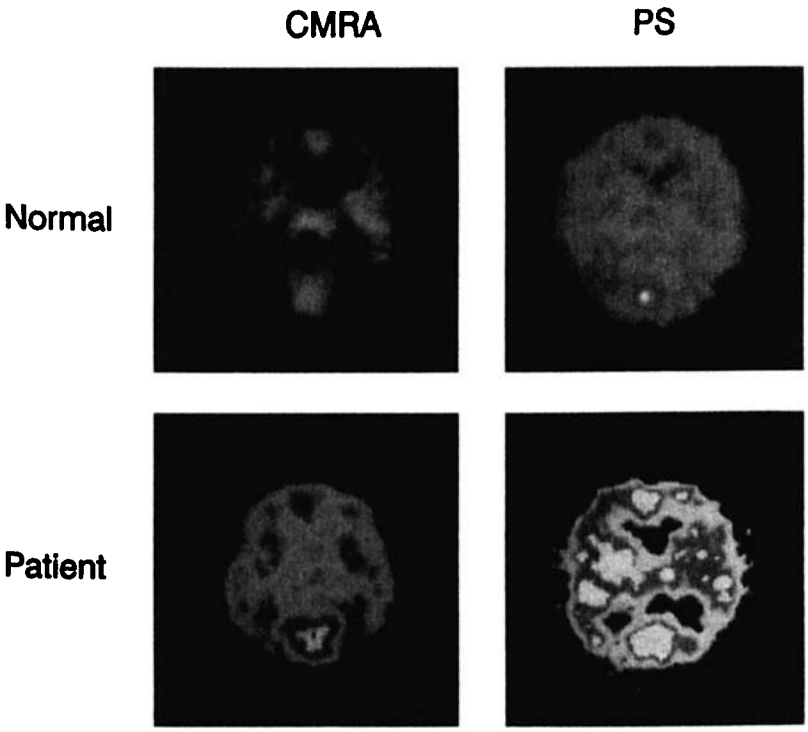

Figure 1. PET images of cerebral metabolic rate for ammonia (CMRA) and permeability/surface area product (PS) in a normal subject compared to images from a cirrhotic patient with mild PSE. Mean values for groups of $n = 5$ controls and patients were: Arterial ammonia: controls 30 ± 7 μ*M*, PSE patients 62 ± 20 μ*M*, $P < 0.01$; CMRA: controls 0.35 ± 0.15 μmol/100 g/min, PSE patients 0.91 ± 0.36 μmol/100 g/min, $P < 0.01$; PS: controls 0.13 ± 0.03 ml/g/min, PSE patients 0.22 ± 0.07 ml/g/min, $P < 0.05$. [Adapted from Ref. 18.]

Table I. Effects of Ammonia on Brain Function

Electrophysiological effects of the ammonium ion
Effects on the inhibitory postsynaptic potential (IPSP)
Effects on glutamatergic neurotransmission (postsynaptic)
Effects on brain energy metabolism
Inhibition of α-ketoglutarate dehydrogenase
Effects on astrocytic function
Decreased expression of the glutamate transporter GLT-1
Increased expression of "peripheral-type" benzodiazepine receptors
Alzheimer type II astrocytosis
Effects on the glutamate neurotransmitter system
Direct postsynaptic effects
Impaired neuron-astrocytic trafficking of glutamate
Inhibition of glutamate uptake
Altered glutamate receptors
Effects medicated by formation of glutamine in brain
Cytotoxic brain edema
Increased uptake of aromatic amino acids
Other effects
Stimulation of L-arginine uptake, nNOS expression

pressed by 1 m*M* ammonia (20). Furthermore, the firing of CA1 neurons evoked by iontophoretic application of glutamate is inhibited by ammonia, suggesting that ammonia decreases excitatory synaptic transmission by a direct postsynaptic action. Further details of the nature of the direct effects of ammonia on inhibitory and excitatory neurotransmission may be found in two review articles (19, 21).

Several studies have suggested that increased brain ammonia concentrations may be causally related to the phenomenon of brain edema in FHF. For example, increased brain water has been described in dogs with urease-induced hyperammonemia (22) and in rats following ammonium acetate infusions (23). Treatment of isolated cerebral cortical slices with ammonia in concentrations equivalent to those reported in brain in experimental FHF resulted in significant swelling and in concomitant reductions of inulin space (24). Cerebral edema in both experimental and human FHF is cytotoxic (rather than vasogenic) in nature with astrocytic swelling being consistently reported (25, 14). Swelling of cerebral cortical astrocytes was observed following ammonia infusions to primates (26), and exposure of primary cultures of astrocytes to millimolar concentrations of ammonia also resulted in significant swelling (27). Ammonia infusions in rats after portacaval anastomosis resulted in brain edema of a sufficient magnitude to raise intracranial pressure (28). Ammonia-induced cell swelling appears to be mediated *via* a metabolite of ammonia rather than ammonia *per se*. Ammonia removal in brain depends on glutamine synthesis *via* the astrocytic enzyme glutamine synthetase.

Some studies have provided evidence of a significant correlation between the rise in brain glutamine and brain water concentrations in rats receiving ammonia infusions (22, 23). It was suggested that the ammonia-induced increase in brain water content was mediated by the osmotic effects of increased cellular (astrocytic) glutamine in these animals (23). Consistent with this possibility, treatment of hyperammonemic animals with methionine sulfoximine, an inhibitor of glutamine synthesis, was shown to prevent the increases of both brain glutamine and water in these animals (23). Under normal physiological conditions, glutamine transport participates in the regulation of water movement in the brain (29). Studies in postmortem brain tissue from patients who died in FHF revealed significantly increased

glutamine concentrations (30), and brain glutamine concentrations were increased 6-fold in experimental ischemic liver failure (31) in parallel with increasing water content of brain tissue in these animals. More recently, a dose-dependent reduction of glutamine levels was again observed following treatment with methionine sulfoximine, this time in portacaval-shunted rats administered ammonium salts. However, brain water content did not decrease proportionately (28), indicating that brain edema in hyperammonemia may not be due solely to increased cellular glutamine accumulation.

Ammonia, if present in sufficiently high concentrations, has the potential to cause cerebral energy failure. Addition of millimolar concentrations of ammonia to brain mitochondrial preparations results in inhibition of α-ketoglutarate dehydrogenase (32), a rate-limiting tricarboxylic acid cycle enzyme. Moreover, chronic liver failure results in increased brain concentrations of lactate (33), and CSF lactate concentrations are increased in direct correlation with deterioration of neurological function in subacute PSE resulting from ammonia treatment of portacaval-shunted rats (34). These findings suggest that ammonia-precipitated PSE, such as that observed in cirrhotic patients following ingestion of protein or a gastrointestinal bleed, may result in a transient impairment of brain energy metabolism. Increased CSF lactate has been reported in cirrhotic patients with PSE (35). Increased lactate production most likely results from decreased entry of pyruvate into the tricarboxylic acid cycle following ammonia-induced inhibition of α-ketoglutarate dehydrogenase. Administration of ammonium salts to portacaval-shunted rats ultimately results in coma and decreased brain ATP content (33). However, this energy deficit is only apparent at late stages (prolonged coma); animals with severe encephalopathy prior to the coma stage do not manifest significant reductions in brain ATP content suggesting that, in this experimental animal model of PSE, a cerebral energy deficit is a late-stage phenomenon.

Manganese. Magnetic Resonance Imaging (MRI) consistently shows signal hyperintensity in the globus pallidus on T_1-weighted images in over 80% of cirrhotic patients (36–40) (Fig. 2).

Pallidal signal hyperintensity is correlated with the presence of extrapyramidal symptoms (36, 37) but not with overall PSE grade (38) nor neuropsychologic test scores (36, 37). Histopathologic evaluation of brain tissue from cirrhotic patients who had manifested T_1-weighted MRI signal hyperintensity revealed Alzheimer type II astrocytosis (36, 38). An angiographic study revealed large portal-systemic collateral vessels originating from the superior mesenteric vein in all nine patients who manifested pallidal MR signal hyperintensities whereas only 2 of 17 patients with normal MRI showed this angiographic pattern. This result suggests that pallidal MR signal hyperintensity in cirrhotic patients is the consequence of portal-systemic shunting (39).

Manganese is excreted by the hepatobiliary route (41). Blood manganese concentrations are increased during the active phase of acute hepatitis as well as in posthepatitic cirrhosis, and a significant correlation exists between blood manganese and activities of liver enzymes in patients with

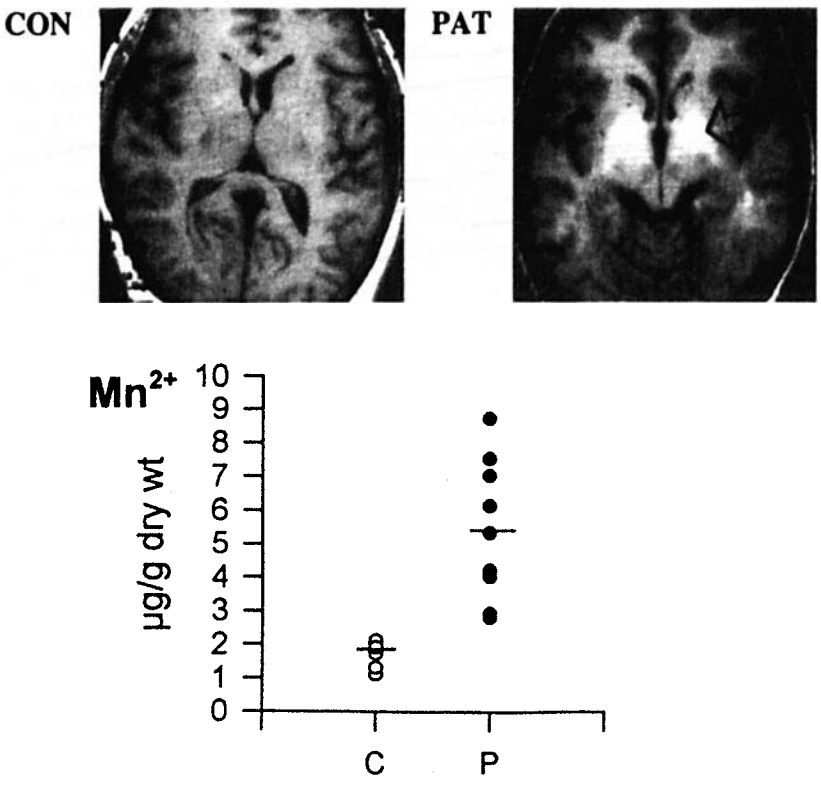

Figure 2. (Upper panel) Magnetic Resonance signal hyperintensity in the globus pallidus of a cirrhotic patient (arrow) (PAT) with subclinical HE compared to an age-matched control (CON). (Lower panel) Manganese concentrations in the globus pallidus obtained at autopsy from eight cirrhotic patients (P) who died in hepatic coma are significantly elevated compared to controls (C). [Adapted from Ref. 40.]

hepatitis (42) or cirrhosis (37). Blood manganese concentrations are consistently increased in cirrhotic patients with pallidal T_1-weighted MR signal hyperintensities (37, 43), and similar pallidal MR signal hyperintensities have been described in a patient with Alagille's Syndrome, an autosomal dominant disorder characterized by cholestasis, intrahepatic bile duct paucity, end-stage liver disease, and increased blood manganese (44). MR signal hyperintensities in pallidum have also been reported in patients during total parenteral nutrition (where it was suggested that the MR signal hyperintensities were the result of manganese deposition in brain) (45), and in cases of industrial manganese poisoning (46).

Direct measurement in pallidal samples obtained at autopsy from cirrhotic patients who died in hepatic coma revealed several-fold increases of manganese concentrations (47, 48) (Fig. 2). Similar increases of brain manganese are observed in experimental animals with chronic liver impairment and portal-systemic shunting (49). Prolonged exposure of humans to manganese produces extrapyramidal symptoms, and repeated administration of manganese to nonhuman primates results in T_1-weighted MR signal hyperintensity in the globus pallidus (50) similar to that observed in cirrhotic patients. In recent studies, manganese exposure has been found to decrease glutamate uptake in cultured astrocytes (51) and increase the expression of the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (52), suggesting that this metal may influence both the glutamatergic system and cerebral energy metabolism in HE. The high capacity of astrocytes to accumulate manganese (53) suggests that its uptake into these cells may play a role in the development of Alzheimer type II astrocytes, the neuropathologic hallmark of HE. In support of this concept, manganese intoxication in nonhuman primates has been shown to result in Alzheimer type II astrocytosis (54), indicating that exposure to manganese could (in addition to ammonia) contribute to the changes in astrocyte function and morphology that are characteristic of PSE.

Compromised Brain Energy Metabolism Versus Neurotransmission Failure as the Cause of Early Hepatic Encephalopathy

Brain Energy Metabolism. The cerebral metabolic rate (CMR) for both oxygen and glucose are reduced in PSE. Such reductions parallel the onset of overt clinical symptoms and are directly proportional to the deterioration of neurological status. Decreased brain glucose utilization in early PSE is most probably the result of decreased energy demand (i.e., reduced neuronal activity in brain in PSE results in decreased energy needs and consequently reduced glucose consumption). Studies in experimental PSE demonstrate that severe neurological impairment precedes decreases in brain levels of high-energy phosphates (33) suggesting that PSE is, at least until late (terminal) stages, the consequence of reduced neuronal activity as a result of neurotransmission failure rather than primary energy failure in the brain. However, in rats administered ammonia acutely, phosphocreatine and ATP levels were reported to be selectively lowered in the reticular activating system (55). This occurrence may be a contributing factor in the later development of loss of consciousness and the onset of coma. A similar effect on labile energy metabolites was observed in cultured astrocytes exposed to ammonia in the absence of dibutyryl cyclic AMP (56). The issue of cerebral energy metabolism in relation to HE is facilitated by the availability of NMR techniques using ^{31}P. In animal models of FHF, using ^{31}P-NMR, no significant reductions of high-energy phosphates were apparent in moderately encephalopathic (but not comatose) animals (57). Studies using ^{31}P-NMR have also been undertaken in cirrhotic patients with mild PSE; again, as in the animal studies, no significant alterations of cerebral high-energy phosphates were observed (58).

Alterations of Multiple Neurotransmitter Systems. In common with other metabolic and degenerative disorders of the CNS, HE is characterized by deficits in several neurotransmitter systems in the brain. Studies in autopsied brain tissue from patients with acute or chronic liver disease who died in hepatic coma together with material from appropriate animal models demonstrate significant alterations of glutamatergic and monoaminergic mechanisms in the brain. In addition, there is new, indirect evidence suggesting a role for the endogenous opioid neurotransmitter system in the pathogenesis of PSE.

Glutamate. Glutamate is an important CNS metabolite and the major excitatory neurotransmitter in the mammalian brain. The key steps in neurotransmitter glutamate synthesis, release, and inactivation are shown in a simplified schematic manner in Figure 3. Glutamate released from the presynaptic nerve terminal is inactivated mainly by uptake into the perineuronal astrocyte where it is rapidly transformed into glutamine *via* the action of glutamine synthe-

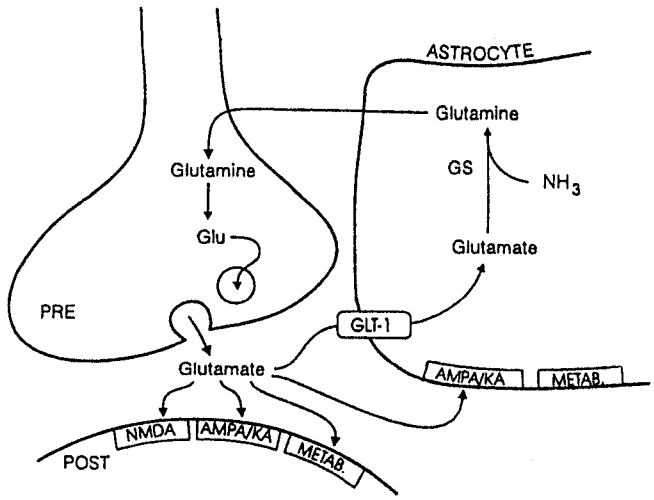

Figure 3. Simplified schematic diagram of the localization of the glutamate transporter GLT-1 and of glutamate receptor subtypes (NMDA, AMPA/KA, METAB) on astrocytic and neuroneal elements implicated in glutamatergic neurotransmission. Glu: glutamate; PRE: presynaptic neuron; POST: postsynaptic neuron.

tase (GS). The glutamine formed is then either recycled to the presynaptic neuron, or diffused into the CSF. As previously discussed in this review article, CSF and brain glutamine concentrations are invariably increased in human HE (30, 59) consistent with exposure of brain to increased concentrations of blood-borne ammonia in liver failure.

Exposure of the astrocyte to low millimolar concentrations of ammonia *in vivo* results in important functional and pathological changes. Such changes include reduced expression of key enzymes such as glutamine synthetase (60) and monoamine oxidase MAO-B (4) and, decreased capacity of the astrocyte glutamate transporter GLT-1 (61), a system essential for the inactivation of glutamate in the synapse. In 1990, Schmidt *et al.* (62), demonstrated a dose-dependent inhibition in the high-affinity uptake of D-aspartate (a nonmetabolizeable analog with high affinity for the L-glutamate transport system) into rat hippocampal slices exposed to blood extracts from PSE patients. Moreover, the relative potency of D-aspartate uptake inhibition correlated significantly with ammonia concentrations in blood extracts from these patients. Previous studies using both *in vitro* and *in vivo* techniques had also demonstrated significant alterations of glutamate transport in brain in both acute and chronic liver failure. For example, electrically stimulated Ca^{2+}-dependent release of glutamate (i.e., glutamate released from nerve terminals) in superfused hippocampal slices from portacaval-shunted rats is significantly increased (63), and *in vivo* release of glutamate from cerebral cortex of rats using the "cortical cup" approach was also found to be increased following portacaval-shunting (64). In addition, release of glutamate from cerebral cortex was reportedly increased in portacaval-shunted animals using *in vivo* cerebral microdialysis (65). Based on these findings of altered neuronal release and astrocyte uptake, it was proposed that PSE is the consequence of impaired neuron-astrocytic trafficking of glutamate (66).

In studies on experimental FHF in the rat following either ischemic or toxic liver injury, increased CSF glutamate levels have been reported (67), and *in vivo* microdialysis approaches have consistently demonstrated increased extracellular glutamate (68, 69) in the brains of rats with FHF. Increased extracellular brain glutamate concentrations described in these studies were significantly correlated both with the severity of neurological impairment and with the degree of hyperammonemia in rats with FHF due to hepatic devascularization (69). In view of reports of decreased capacity for glutamate uptake by astrocytes exposed to pathophysiologic concentrations of ammonia (70), it was suggested that the finding of increased extracellular glutamate in brain in experimental FHF was the consequence of diminished uptake into perineuronal astrocytes rather than increased release *per se* of glutamate from the presynaptic nerve terminal. Further studies involving glutamate transport have been facilitated by the recent cloning and sequencing of a family of genes coding for a family of high affinity glutamate transporters (71). One such transporter, GLT-1, is expressed by astrocytes throughout the central nervous system. Two recent studies have described a loss of GLT-1 protein and gene expression in the cerebral cortex of animal models of FHF (61, 72).

Three major subtypes of glutamate receptor in the CNS are defined according to their coupling to ion channels and their affinity for the glutamate receptor agonist, N-methyl-D-aspartate (NMDA). The three subtypes are 1) the NMDA receptor, an ionotropic receptor with high affinity for NMDA; 2) the non-NMDA receptor (previously named the AMPA/kainate subtype), also ionotropic; and 3) the metabotropic glutamate receptor. The cellular localization of these sites are shown in a simplified manner in Figure 3. Several reports have described significant effects of acute or chronic liver diseases on brain glutamate receptors. Although, in all cases, liver failure resulted in a loss of glutamate receptor sites (66), the subclass of receptor implicated was dependent upon the type of liver failure (acute vs. chronic). An earlier study showed decreased densities of total glutamate binding sites in the brains of rabbits with galactosamine-induced FHF (73). This was followed by a report of a selective loss of NMDA sites in the brains of portacaval-shunted rats (74). On the other hand, non-NMDA receptor densities were found to be significantly reduced in the brains of dogs with congenital PSE (75) as well as in several regions of the brains of rats with ischemic liver failure (76). However, the precise cellular localization of the non-NMDA receptor changes (astrocytic or neuronal) in both acute and chronic liver failure remains to be established.

Serotonin. Many of the neuropsychiatric symptoms of early PSE such as altered sleep patterns are signs that have classically been attributed to modifications of serotonin(5HT) neurotransmission. CSF concentrations of L-tryptophan are increased in cirrhotic patients in hepatic coma (77) and increased concentrations of the 5HT metabolite, 5-hydroxyindoleacetic acid(5HIAA), have consistently been reported in both CSF and brain tissue from patients (77, 78) and experimental animals (79, 80) with severe encephalopathy resulting from chronic liver failure. More recently, studies in autopsied brain tissue from cirrhotic patients who died in hepatic coma reveal increased activities of the 5HT-metabolizing enzyme monoamine oxidase (MAO-A) (4) suggesting that increased 5HT oxidation rather than increased 5HT turnover could be the explanation for the consistent finding of increased brain concentrations of 5HIAA in PSE. These findings would suggest that chronic liver disease results in a 5HT synaptic deficit. However, *in vivo* cerebral microdialysis studies in rats with either acute (81) or chronic (82) liver failure did not reveal any significant alterations of extracellular 5HT in brain. On the other hand, ammonia-precipitated coma in rats with portacaval anastomoses did result in a transient increase of 5HT in brain extracellular space (82). Whether this effect of ammonia was mediated *via* an effect on the release process *per se* or on the 5HT uptake system awaits further study. Post-

synaptic $5HT_2$ binding sites are increased in the hippocampus of cirrhotic patients who died in hepatic coma (83) suggesting up-regulation of these sites consistent with a 5HT synaptic deficit in this brain region. Alterations in $5HT_1$ receptor binding has also been reported in the brains of portacaval-shunted rats (84). Additionally, a positive effect of the nonselective 5HT receptor antagonist methysergide on motor activity was observed in rats with thioacetamide-induced acute liver failure (85), suggesting that serotonergic processes play a role in some of the behavioral characteristics of HE. Clinically, treatment of patients with subclinical PSE with a 5HT reuptake inhibitor, an action which would lead to increased synaptic concentrations of 5HT, did not result in worsening of neuropsychiatric symptoms (86); and treatment with the postsynaptic 5HT receptor antagonist ketanserin leads to precipitation of encephalopathy in some cirrhotic patients (87). These anecdotal clinical reports are also consistent with the notion that a hypoactivity of the 5HT system could be implicated in the pathogenesis of certain clinical manifestations of PSE.

Catecholamines. Neuromuscular abnormalities including extrapyramidal symptoms such as tremor and rigidity form part of the clinical syndrome of PSE. By analogy with the extrapyramidal symptoms in Parkinson's disease, a neurodegenerative disorder characterized neurochemically by a nigrostriatal dopamine deficit, it has therefore been suggested that the extrapyramidal signs and symptoms in HE may be the consequence of abnormalities of dopamine neurotransmission. Studies in autopsied brain tissue from cirrhotic patients who died in hepatic coma revealed increased brain concentrations of the dopamine metabolite homovanillic acid (HVA) (77). Furthermore, HVA concentrations were increased in the brains of rats following portacaval anastomosis (88) suggesting increased dopamine turnover in the brains of these animals. Evidence in favor of a dopamine lesion in human PSE is provided by the report of decreased densities of postsynaptic dopamine D_2 binding sites in the globus pallidus of cirrhotic patients who died in hepatic coma (89).

Central noradrenergic systems may also be implicated in the pathogenesis of HE, particularly in FHF. Hepatectomy (90), liver devascularization (91), as well as thioacetamide-induced toxic liver injury (92) in the rat all result in decreased brain concentrations of norepinephrine (NA). Hepatectomy results in increased concentrations of NA in ventriculocisternal perfusates (93), and *in vivo* cerebral microdialysis studies have revealed significant increases in extracellular brain concentrations of NA at precoma and coma stages of encephalopathy in rats with experimental FHF (81). Additional studies demonstrated a loss of NA transporter sites in cortical and subcortical brain structures from these animals, at coma stages of encephalopathy in rats in experimental FHF accompanied by a selective loss of α_1 and β_1 subclasses of NA receptor sites (81). On the basis of these findings, it was proposed that alterations of the NA system could be responsible for many of the neuropsychiatric symptoms encountered in PSE.

GABA. Originating in the early 1980s, a concept was introduced which suggested that increased inhibitory neurotransmission brought about by GABA played a role in the impaired motor function and decreased consciousness that are characteristic features of HE (94–96). A major proposal considered that gut-derived GABA, by virtue of its diminished removal by liver and its entry into brain *via* a defective blood-brain barrier, could contribute to the neural inhibition characteristic of HE (97). Much of the evidence in favor of this hypothesis was obtained using the galactosamine hepatotoxicity animal model of FHF (98). However, studies in human PSE yielded negative results; no significant alterations of GABA or GABA-related enzymes or receptors were apparent in the brains of these patients (3, 59, 99). On the other hand, the possibility that agonists of the central benzodiazepine receptor component of the $GABA_A$ receptor complex could potentiate the effects of GABA by acting as endogenous benzodiazepines has gained attention. This led to the isolation and partial characterization of substances from brain extracts from humans and experimental animals with acute or chronic liver failure (100, 101). Based on these findings, administration of antagonists of the central benzodiazepine receptor might be expected to reduce the increased GABAergic tone observed in HE by displacing such endogenous agonists from the receptor binding sites. In a subsequent controlled clinical trial of the benzodiazepine receptor antagonist flumazenil, in grade IV HE patients, a subgroup of patients was found to manifest amelioration of neurological symptoms (102). However, there was no clear correlation between the clinical response to the benzodiazepine antagonist and the presence of benzodiazepine receptor ligands in the blood of these patients (103), suggesting that the beneficial effects of flumazenil were not the result of the inhibition of the action of blood-borne substances with benzodiazepine receptor agonist properties in these patients. In addition, the effect of flumazenil was transient and incomplete, suggesting that other factors may also contribute to the pathogenesis of HE. Such factors might include toxins such as ammonia and manganese, as well as modifications of other neurotransmitter systems. At present, the precise mechanism responsible for the beneficial effect of benzodiazepine antagonists in this subgroup of patients with severe PSE remains to be established.

The Opioid System. The endogenous opioid system of the brain may also be implicated in the mediation of some of the neuropsychiatric effects of chronic liver disease on CNS function. Cirrhotic patients are hypersensitive to morphine (104), and portacaval-shunting in the rat results in increased pain sensitivity (105), a phenomenon in which the endogenous opioid system is known to be implicated. Increased plasma levels of the endogenous opioid metenkephalin have been reported in patients with primary bil-

iary cirrhosis (106), and brain extracts from experimental animals with chronic liver failure contain modified concentrations of β-endorphin (107, 108). β-Endorphin, an endogenous opioid peptide with potent analgesic properties, is synthesized mainly in neurons of the arcuate nucleus of the hypothalamus with axons projecting to various parts of the brain including nuclei involved in pain modulation and memory function (109) as well as regions involved in the mediation of the positive reinforcing effects of several drugs of abuse, including ethanol (110). Portacaval-shunted rats drink significantly more ethanol in a free-choice drinking paradigm, a behavior that starts within one week of shunting (111), and autoradiographic studies of the brains of these animals reveal region-selective alterations of μ and δ opioid receptor sites (112). Furthermore the ethanol preference due to portacaval-shunting was found to be significantly attenuated following treatment with the opioid antagonist naloxone (111). Based upon these findings, it was suggested that ethanol preference following portacaval-shunting was the result of modifications of the endogenous opioid system. Extrapolation of these findings to humans would suggest the intriguing possibility that the development of significant liver disease in alcoholics could result in exacerbation of drinking as a result of activation of the brain opioid system.

Other Systems Involved in Hepatic Encephalopathy

The Peripheral-Type Benzodiazepine Receptor and Neurosteroid Production. The peripheral-type benzodiazepine receptor (PTBR) is a hetero-oligomeric protein located on the outer mitochondrial membrane. Although initially thought to be confined to peripheral tissues such as adrenals and kidney, it is now well established that the PTBR is also localized in the central nervous system, but, unlike the central benzodiazepine receptor subtype, is not allosterically coupled to $GABA_A$ receptors. Rather, PTBRs are concentrated on the outer mitochondrial membrane of astrocytes. Densities of PTBRs are increased in autopsied brain tissue from cirrhotic patients who died in hepatic coma (5), in the brains and kidneys of rats following portacaval anastomosis (113, 114), as well as in the brains and peripheral tissues of mice with chronic hyperammonemia resulting from deficiency of a urea cycle enzyme (115). These findings suggest that the increased PTBRs in HE are the consequence of exposure to increased concentrations of ammonia. Consistent with this possibility, it was subsequently demonstrated that treatment of cultured astrocytes with millimolar concentrations of ammonia results in increased binding of PTBR ligands (116, 117). More recently, exposure of cultured astrocytes to manganese has also been shown to result in increased binding of PTBR ligands (118). These findings suggest that the increased binding sites for PTBR ligands previously reported in brain in experimental and human PSE are the consequence of exposure to ammonia and/or manganese.

The role of increased PTBRs in the pathogenesis of HE is unclear. Their mitochondrial localization suggests a role for the PTBR in oxidative metabolism. However, an additional role for these receptors that is gaining attention focuses on the production of neurosteroids. Initial suggestions of an involvement of neurosteroids in HE (119, 120) were followed by reports showing that diazepam binding inhibitor (DBI), an endogenous neuropeptide, acts on the PTBR to stimulate steroid synthesis *via* an action on cholesterol transport across the mitochondrial membrane (121) (Fig. 4). Certain neurosteroids such as 3α-hydroxy-5α-pregnane-20-one and 3α-21-dihydroxy-5α-pregnane-20-one bind with high affinity to the $GABA_A$ receptor for which they are positive modulators (i.e., they amplify the action of GABA and may also gate the chloride channel of the $GABA_A$ receptor in the absence of GABA (122)). Other neurosteroids such as pregnenolone sulfate and dehydroepiandrosterone sulfate exhibit negative modulatory effects on these receptors (123, 124). Pregnenolone sulfate also modulates NMDA glutamate receptors in a positive manner but has negative effects on glycine and AMPA/kainate receptors (125–127). These differential effects of neurosteroids may alter neuroneal excitability and therefore contribute to dysregulation of the functional integrity of the brain. In addition, a new report provides evidence that neurosteroids are capable of influencing ammonia-induced swelling in astrocytes (128). Although the specific target of neurosteroids may be the $GABA_A$ receptor, other evidence suggests that these agents and related hormones may cause morphological changes (129, 130), influence differentiation of the cell (131), and downregulate gliosis and cell proliferation (132) in the astrocyte. The recent finding that treatment of mice with the neurosteroid tetrahydroprogesterone leads to the development of Alzheimer type II changes in astrocytes (133) is consistent with these reported effects and provides evidence that increasing PTBRs may, by enhancing neurosteroid production, lead to the development of this patho-

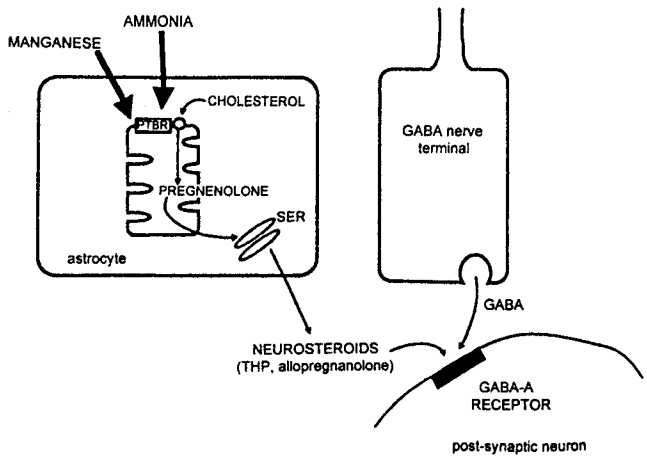

Figure 4. Activation of the peripheral-type benzodiazepine receptor (PTBR) due to ammonia or manganese results in increased mitochondrial cholesterol transport and the production of neurosteroids some of which are positive allosteric modulators of GABAergic transmission and may thus contribute to the pathogenesis of HE. SER: smooth endoplasmic reticulum; THP: tetrahydroprogesterone.

logical hallmark of HE. In addition, DBI concentrations are elevated in the CSF of patients with chronic liver disease with PSE, and a positive correlation is observed between CSF DBI and severity of encephalopathy in these patients (134). Since DBI is stored in selected populations of astrocytes in the central nervous system, where it coexists with the PTBR (135) and since both DBI content and PTBR receptor densities are increased in brain in experimental and human PSE, it is possible that PTBR activation, by facilitating the production of specific neurosteroids, may be an important element in the pathogenesis of HE.

Nitric Oxide. In 1991, Vallance and Moncada (136) proposed a hypothesis that the free radical nitric oxide was implicated in the hyperdynamic circulation associated with cirrhosis. More recently, evidence in support of an involvement of nitric oxide (NO) in HE has emerged. A generalized increase in activities of nitric oxide synthase (NOS), the enzyme responsible for NO production, has been demonstrated in the brains of rats following portacaval anastomosis (137). Increased neuronal NOS protein and mRNA were subsequently reported in the brains of rats following portacaval anastomosis (Fig. 5) (138). Increased NO production in brain could be responsible for oxidative stress as well as the alterations of cerebral perfusion reported in both humans and experimental animals with chronic liver failure. Hyperammonemia has been shown to lead to increased production of the superoxide free radical and decreased activities of several antioxidant enzymes in brain (139). In a preliminary report, NOS activities were also found to be increased in both hyperammonemic mice and animals with acute liver failure produced by thioacetamide (140). Other studies have determined that L-nitroarginine, an NOS inhibitor, prevents the ammonia-induced increase in superoxide production and changes in antioxidant enzymes as well as toxicity following exposure to ammonia (141, 142). Furthermore, NO is capable of increasing glutamate release at the synaptic cleft (143), which may have an important role in the increased extracellular levels of glutamate reported in experimental FHF (68, 69). The intracellular availability and transport of L-arginine (the obligate substrate for NOS) are also important in the regulation of NOS activity, and studies have shown that L-arginine uptake by brain synaptosomal preparations from portacaval-shunted rats increased in parallel to the increased NOS activities (137). L-arginine uptake was also stimulated in synaptosomes from rats with thioacetamide-induced HE (144), in hyperammonemic animals (in the absence of liver failure), and by exposure of these preparations to millimolar concentrations of ammonia *in vitro* (145). Exposure of cultured astrocytes to ammonia leads to increased L-arginine uptake and NOS expression (146, 147). Synaptosomal preparations from animals with thioacetamide-induced HE also show increased activities of enzymes associated with the arginine-glutamate shunt, suggesting that arginine may also act as a precursor for glutamate. Both L-arginine and glutamate are known to be depleted in acute and chronic HE, respectively (59, 148). It is possible that increased L-arginine uptake in astrocytes may indicate the existence of a similar pathway in which glutamate is replenished following ammonia-induced increases in glutamine production.

Therapeutic Implications

There can be little doubt that ammonia, by both direct and indirect mechanisms, plays a major role in the pathogenesis of HE in both acute and chronic liver failure. Standard ammonia-lowering treatments such as lactulose and neomycin aimed at reducing ammonia production in the gut are still widely used. In the cirrhotic patient, two key ammonia detoxifying enzymes responsible for the production of urea and glutamine (carbamylphosphate synthetase and

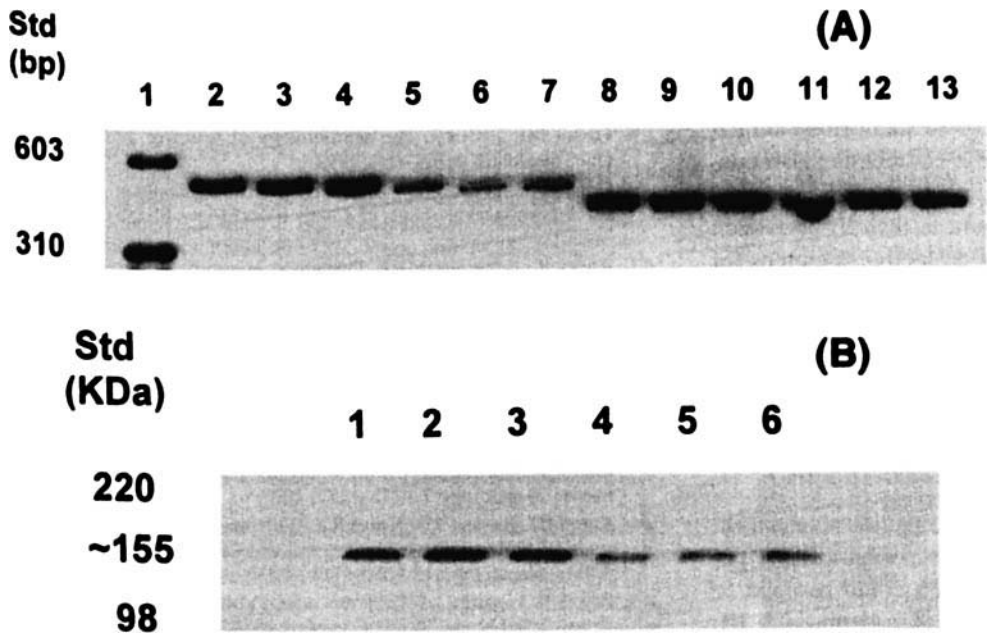

Figure 5. NOS in portacaval-shunted rat brain. (A) nNOS gene expression in the cerebellum of rats that underwent portacaval anastomosis (PCA) (Lanes 2–4) and sham-operated (Lanes 5–7) rats. Lane 1 shows molecular-weight markers (bp). Expression of GAPDH that was used as an internal standard is shown for PCA (Lanes 8–10) and sham-operated (Lanes 11–13) rats. (B) Western blot analysis of cytosol protein from the cerebellum of PCA (Lanes 1–3) and sham-operated (Lanes 4–6) rats. [Reproduced from Ref. 138, with permission.]

glutamine synthetase) are impaired in activity, leading to hyperammonemia. Both sodium benzoate and phenylacetate have been used to treat PSE with some success (149). Benzoate forms hippurate with glycine, and phenylacetate reacts with glutamine to produce phenylacetylglutamine thereby leading to the removal of waste nitrogen into the urine. Over the last decade, a series of randomized, controlled trials have been performed with L-ornithine-L-aspartate, all of which showed some beneficial qualities including a lowering of blood ammonia and improving neuropsychiatric test scores in patients with PSE (150, 151). A recent study showed that L-ornithine-L-aspartate was able to lower brain ammonia levels in experimental chronic HE (152). Another agent with therapeutic potential is L-carnitine, an agent with a demonstrated potential to prevent acute ammonia toxicity. In the portacaval-shunted rat, administration of L-carnitine was shown to have a protective effect in ammonia-precipitated coma (153, 154), an effect that appears to be centrally mediated involving improved mitochondrial respiration. Hypocarnitinemia is a characteristic feature of primary carnitine deficiency that interestingly is also observed in many cirrhotic patients (155). Furthermore, animals with portacaval-shunts also show decreased levels of L-carnitine (156). These findings suggest that this agent may have an important role in the treatment of PSE. At present, however, further studies are necessary to elucidate its mechanism of action as well as its true potential for the treatment of HE.

As described above, modifications of several neurotransmitter systems have been identified in HE resulting from acute or chronic liver failure. In some cases, these neurotransmitter changes are related to an action of ammonia whereas in other cases (for example, the endogenous opioid system), there is no obvious link to ammonia toxicity. Neurotransmitter systems are open to pharmacologic manipulation using appropriate agonists and antagonists, and preliminary data from animal experiments suggest that such pharmacologic manipulations could be of benefit in the treatment of HE. For example, administration of serotonin antagonists such as methysergide and metitepine resulted in significant improvement in neurological status in an animal model of FHF (157). Although these compounds also possess efficacy at dopaminergic and catecholaminergic receptor sites (158), which may contribute to their effects, such agents may be useful in developing protective strategies for use in HE. The administration of antagonists of the glutamate (NMDA) receptor have been shown to protect animals against the lethal effects of acute hyperammonemia (159) and improve neurological status in experimental FHF (160, 161). Follow-up studies of these interesting leads are now required to assess their effectiveness in the prevention and treatment of HE in humans.

Manganese deposition in the globus pallidus is the most likely explanation for the MR signal hyperintensity in patients with chronic liver disease, which could respond to chelation therapy. Finally a subgroup of patients with HE continue to benefit from treatment with the benzodiazepine antagonist flumazenil (Ro-15 1788) by mechanisms that remain to be established. More recent findings of altered densities of receptors for endogenous opioids in brain in acute and chronic liver disease may represent an alternative mechanism to explain the neuropsychiatric signs characteristic of HE. Further elucidation of the multiple neurotransmitter systems involved and the pathogenesis of the spectrum of neurological and neuropsychiatric symptoms characteristic of HE should provide rational therapeutic strategies for the prevention and treatment of this serious complication of acute and chronic liver failure.

The authors are grateful to Dominique D. Roy for her help in the preparation of this manuscript.

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