MINIREVIEW

Current Theories on the Pathogenesis of Hepatic Encephalopathy (43770)

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Abstract. **Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of both acute and chronic liver disease. Several hypotheses have emerged following the development of appropriate animal models of HE and following studies using post-mortem brain tissue from HE patients. It was originally suggested that primary energy failure was responsible for HE; however, there is now mounting evidence that the pathogenetic defect involves neurotransmission failure. Specific neurotransmitter systems implicated in the pathogenesis of portal-systemic encephalopathy (PSE) include the excitatory amino acid glutamate as well as neuroactive and/or neurotoxic biogenic amine metabolites. Although it has been proposed that alterations in the γ-aminobutyric acid (GABA) system may play a pathogenic role in HE associated with both chronic and acute liver failure, there is now overwhelming evidence to the contrary. On the other hand, there is evidence to suggest that a subgroup of patients with HE have increased blood and CSF concentrations of substances that bind to GABA-related benzodiazepine receptors in brain. Alterations of both the glutamatergic and serotoninergic neurotransmitter systems in PSE likely result from the metabolic consequences of chronic exposure of brain to toxic levels of ammonia. In addition to its effects on glutamatergic and serotoninergic systems during chronic liver disease, ammonia has been intimately associated with the brain edema invariably observed in acute liver failure. It is evident that, regardless of the type of liver failure, effective reductions of ammonia levels remains the strategy of choice in the prevention of encephalopathy. The further elucidation of neurotransmitter alterations in HE could result in novel "downstream" neuropharmacologic approaches to its prevention and treatment.**

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Liver failure is frequently accompanied by a neuropsychiatric syndrome generally referred to as hepatic encephalopathy (HE). Depending upon the duration and the degree of hepatic dysfunction, HE may present as one of two major types. The most common form of HE, portal-systemic encephalopathy (PSE), accompanies the development of portal-systemic shunting that arises either following portacaval anastomosis surgery during chronic liver disease or spontaneously following portal hypertension most often due to cirrhosis. Neurologically, PSE develops slowly: Symptoms, including abnormalities of sleep patterns, shortened attention span, and muscular incoordination, are followed by lethargy and ataxia, progressing to stupor and coma. Repeated episodes of PSE precipitated by events such as gastrointestinal bleeding, constipation, and sedative drugs are common. Neuropathologically, PSE is character-

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ized by astrocytic changes known as Alzheimer Type II astrocytosis. The second major type of HE is associated with acute or fulminant hepatic failure and is a clinical syndrome of rapid onset resulting from severe inflammatory and/or necrotic liver disease. The neurological disorder progresses from altered mental status to coma, generally within hours or days. Death frequently results from brain herniation which is a direct consequence of massive brain edema and the accompanying increase in intracranial pressure.

Significant advances in our understanding of the pathogenesis of HE and the subsequent emergence of several hypotheses can be attributed to the development of animal models for both forms of HE. However, a great deal of confusion has resulted from the widespread practice of extrapolating data obtained from animal models of acute liver failure to explain, in part, PSE (and vice versa). A second major approach to the study of the pathogenesis of HE has made use of brain tissue from patients who died with the disorder. In common with many other neurometabolic and neurodegenerative disorders, a great deal of useful information has been forthcoming from such approaches. Such studies, however, require rigorous matching of patient material to that of controls for the effects of age, sex, postmortem delay time, and agonal status. Thirdly, recent data derived from studies using noninvasive techniques such as positron emission tomography (PET) and nuclear magnetic resonance (NMR) have added to the armamentarium of experimental approaches to our understanding of the pathogenesis of HE.

Neurotransmission Failure Rather Than Decreased Brain Energy Metabolism is the Cause of HE

The cerebral metabolic rates for both oxygen (CMR_{O_2}) and glucose ($CMR_{glucose}$) are reduced in PSE. Such reductions parallel the onset of overt clinical symptoms and are directly proportional to worsening of neurological status. Decreased brain glucose utilization in 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 fuel [glucose] consumption). Studies in experimental PSE demonstrate that severe neurological impairment precedes decreases in brain levels of high-energy phosphates (1) 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 brain. Similar conclusions can be drawn from recent ^{1}H-NMR and ^{31}P-NMR spectroscopic studies. These have shown that, in experimental animal models of fulminant hepatic failure, high-energy phosphates are not significantly altered in the brains of severely encephalopathic animals (2, 3).

It is understandable, therefore, that current theories on the pathogenesis of HE continue to focus on the origin of neurotransmitter abnormalities which could be responsible for the neural inhibition characteristic of this syndrome. Pathophysiologic mechanisms involving altered neurotransmitter systems which have been proposed to explain HE include the following:

A. Ammonia neurotoxicity.
   i. Direct effects on inhibitory neurotransmission.
   ii. Direct effects on excitatory neurotransmission.
   iii. Altered neuron-astrocyte interactions resulting in a defect in glutamatergic synaptic regulation.
   iv. Role of ammonia in the pathogenesis of brain edema in acute liver failure.
B. Effects of neuroactive and/or neurotoxic biogenic monoamine metabolites.
C. Increased "GABAergic tone" resulting from increased transport of blood-borne GABA or the presence of endogenous benzodiazepines that modulate GABAergic neurotransmission.

The Ammonia Hypothesis Revisited

Evidence for an association between HE and ammonia dates back over a century to the work by Eck which described the effects of portacaval anastomosis in dogs. Portacaval anastomosis involves the surgical shunting of the hepatic portal circulation directly to the inferior vena cava. This animal model represents the best model developed to date in the search for a better understanding of the effects of a by-pass of the normal hepatic detoxification processes and the resultant PSE observed in humans. 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 "meat intoxication" in these animals (4). In 1952, Gabuzda and colleagues 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 (5).

Arterial blood ammonia levels are frequently (but not always) elevated in patients with PSE. A recent report described the results of PET studies using $^{13}NH_3$ to investigate brain ammonia metabolism in cirrhotic patients with mild PSE (6). A significant increase in the cerebral metabolic rate for ammonia (CMR_{NH_3}) was observed in PSE patients. Furthermore, this increase was associated with an increase in the apparent permeability-surface area product, a

measure of blood-brain barrier permeability (Table I). This apparent ease with which ammonia moves into brain from blood in patients with liver disease could account for the hypersensitivity of cirrhotic patients to ammoniagenic conditions (e.g., high protein diet, gastrointestinal bleeding, constipation) as well as for the presence of brain dysfunction in some patients with near-normal arterial ammonia levels. Due to its inherent labile nature, ammonia cannot be measured accurately in postmortem human brain samples. However, using appropriate sacrifice-fixation techniques, brain ammonia levels have consistently been found to be in the 1–5 m*M* range in the brains of animals at coma stages of HE resulting from fulminant hepatic failure or in experimental PSE (Table II).

An important factor in HE is the absence of a cerebral urea cycle, which renders the brain almost entirely dependent upon glutamine synthesis as a means of ammonia removal. Glutamine concentrations in autopsied brain samples from cirrhotic patients or from patients with fulminant hepatic failure dying in hepatic coma are increased 2- to 5-fold, suggesting previous exposure to high levels of ammonia (12). In addition, CSF glutamine concentrations correlate well (better than any other biochemical parameter studied to date) with the severity of neurological impairment in patients with HE (13). The enzyme responsible for ammonia detoxification by brain (e.g., glutamine synthetase) has an almost exclusively astrocytic localization (14). Not surprisingly, given the integral role played by this cell, the neurological picture in chronic liver failure is one of astrocytic, rather than of neuronal, dysfunction and damage. Histopathological evaluation of brain tissue from patients with PSE reveals Alzheimer Type II astrocytosis. In a study of 50 cirrhotic patients, the degree of astrocytosis was directly related to the severity and duration of PSE (15). Alzheimer Type II astrocytosis has also been observed in brain in other chronic hyperammonemic states resulting either from congenital defects of urea cycle enzymes or following urease infusions (16). These findings serve to reinforce the notion of a pathophysiologic link between chronic hyperammonemia and Alzheimer Type II astrocytosis. The following list, by no means an exhaustive one, indicates the diversity of evidence which, in addition to the evidence derived from neuropathologic studies, suggests that astrocytes are functionally compromised during the course of chronic liver disease: (i) morphometric studies using rats having undergone portacaval anastomosis, and subsequently fed ammonium salts or resins to precipitate severe encephalopathy, demonstrate increased astrocytic number and size, as well as evidence of astrocytic mitochondrial proliferation and swelling (17, 18); (ii) [^{3}H]PK-11195 binding sites (the so-called peripheral-type benzodiazepine receptors), localized on the astrocytic mitochondrial membrane, are increased in density in autopsied brain tissue from cirrhotic patients who died in hepatic coma (19), in the brains of portacaval-shunted rats (20) and in the brains of mice with chronic hyperammonemia resulting from congenital urea cycle enzyme deficiency (21); (iii) the activity of the astrocytic marker enzyme glutamine synthetase is decreased in the brains of patients who died in hepatic coma (22) and in the brains of portacaval-shunted rats (10); (iv) primary cultures of astrocytes exposed to levels of ammonia equivalent to those observed in the brains of animals in hepatic coma show decreased capacity for uptake of K^+ and glutamate (23); (v) chronic liver disease induces astrocytes to express significantly less glial fibrillary acidic protein (24); and (vi) a recent report described hyperintensity of globus pallidus on magnetic resonance imaging (MRI) investigations in patients with PSE (25). Pallidal hyperintensity was significantly correlated with the severity of neurological impairment and with blood ammonia concentrations (Fig. 1). Subsequent neuropathological examination revealed Alzheimer Type II astrocytosis suggesting that the degree of pallidal hyperintensity resulted from the persistent exposure of brain to high levels of ammonia in these patients.

Table I. Ammonia Metabolism in Patients with PSE

	Controls (5)	PSE patients (5)
Arterial NH_4^+ (μmol/l)	30 ± 7	62 ± 20**
aPS product (ml/g/min)	0.13 ± 0.03	0.22 ± 0.07*
CMR_{NH_3} (μmol/100g/min)	0.35 ± 0.15	0.91 ± 0.36**

Note. CMR_{NH_3}: cerebral metabolic rate for ammonia; aPS: apparent permeability-surface area product. Values represent mean ± SEM; number of patients in parentheses; values significantly different from controls indicated by $^*P < 0.05$, $^{**}P < 0.005$. (From Ref. 6, with permission.)

Ammonia exerts a deleterious effect on central nervous system (CNS) function by both direct and indirect mechanisms. Millimolar concentrations of ammonium ion, equivalent to those observed in brain in hepatic coma (Table II) affect both inhibitory and excitatory neurotransmission. For a more complete discussion of the mechanisms implicated in the electrophysiological effects of NH_4^+, the reader is referred to a recent review of this topic (26).

NH_4^+ Impairs Inhibitory Postsynaptic Potentials (IPSPs). *In vivo* studies reveal that NH_4^+ impairs postsynaptic inhibition in cerebral cortex, thalamus, brainstem, and spinal cord (26). Available data indicates that NH_4^+ interferes with the outward pumping of Cl^- (27). Consequently, the inhibitory neurotransmitter is incapable of opening the Cl^- channel thus abolishing inhibitory postsynaptic potential (IPSP) activity. Electroencephalogram (EEG) changes

Table II. Ammonia Concentrations in Rat Brain at Coma Stage of Encephalopathy in Acute Liver Failure and in PSE: Literature Data

Type of HE	Brain ammonia concentration (m*M*)	Reference
Acute liver failure		
PCA/hepatic artery ligation	3.9 (Whole brain)	7
PCA/hepatic artery ligation	5.4 (Cerebral cortex)	8
Galactosamine	2.8 (Whole brain)	9
PSE		
PCA/NH_4^+ salts	3.4 (Cerebral hemisphere)	1
PCA/NH_4^+ salts	4.8 (Cerebellum)	1
PCA/NH_4^+ salts	3.8 (Brainstem)	1
PCA/NH_4^+ salts	4.5 (Cerebral cortex)	10
PCA/NH_4^+ salts	3.2 (Brainstem)	10
PCA/NH_4^+ salts	3.0 (Whole brain)	11

parallel these alterations. Brain concentrations of ammonia required to affect IPSPs in cerebral cortex are of the order of 1 m*M*, well within the range of brain NH_4^+ concentrations encountered in early experimental PSE (10). Interestingly, following portacaval shunting, postsynaptic inhibition in cerebral cortex develops increased sensitivity to an acute ammonia load (28), a situation having clinical parallels; patients who undergo surgical portacaval anastomosis for the treatment of portal hypertension are particularly susceptible to small increases of ammoniagenic substances derived either from the diet or from gastrointestinal bleeding.

NH_4^+ Ions Suppress Excitatory Neurotransmission. Synaptic transmission from Schaffer collaterals to CA1 pyramidal cells in hippocampus is reversibly depressed by 1 m*M* NH_4^+ (29). In addition, it was demonstrated that the firing of CA1 pyramidal [glutamatergic] cells evoked by iontophoretically applied glutamate is inhibited by 2 or 5 m*M* NH_4^+, reinforcing the suggestion that NH_4^+ ions decrease excitatory synaptic transmission by a postsynaptic action. That NH_4^+ often depolarizes neurons, perhaps through a direct reduction of K^+ concentrations, without changing membrane resistance may help elucidate the effect of NH_4^+ on excitatory neurotransmission (27, 29). Two additional mechanisms have been suggested to be responsible for the depression of excitatory transmission by NH_4^+ ions. NH_4^+ ions may (i) reduce the release of glutamate by inhibiting its synthesis from glutamine (30) and (ii) prevent action potentials by invading presynaptic terminals (31).

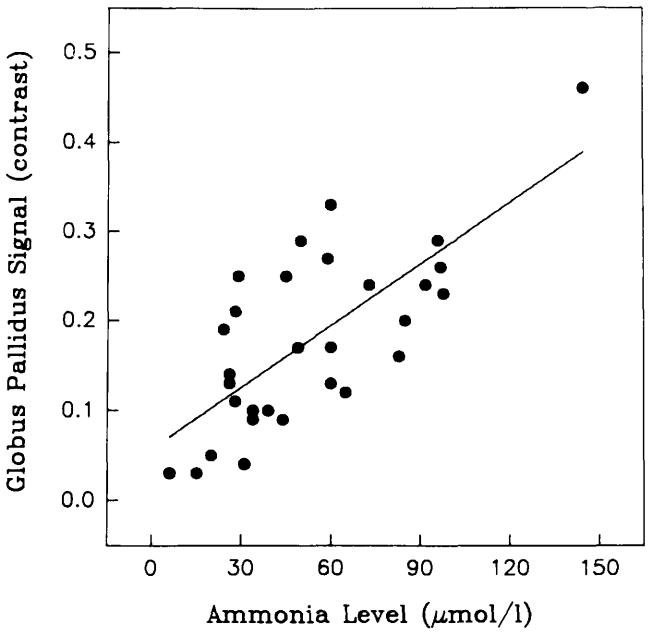

Figure 1. Plot of globus pallidus signal measurements (on T1-weighted magnetic resonance imaging) against plasma levels of ammonia ($r = 0.73$; $F = 31.22$; $P < 0.0000$) (from Ref. 25, with permission).

Chronic Hyperammonemia Results in Impaired Neuronal-Astrocytic Metabolic Interactions and, Consequently, in Glutamatergic Synaptic Dysfunction. The key steps in neurotransmitter glutamate synthesis and in glutamatergic synaptic regulation are shown in Figure 2. Thus, glutamate released from presynaptic nerve terminals is inactivated by uptake mainly into perineuronal astrocytes where it rapidly transformed into glutamine. Glutamine may then be recycled to the presynaptic nerve terminal as the immediate precursor of releasable glutamate. There is a growing body of evidence to suggest that, as a result of the loss of astrocytic integrity in chronic liver disease, glutamatergic synaptic regulation is impaired. Such evidence comes from studies of glutamate uptake and release, as well as from studies of binding sites for postsynaptic glutamate receptor ligands in the brains of humans and animals with experimental HE. For a recent review of glutamatergic function in acute and chronic hyperammonemia see Raghavendra Rao *et al.* (32).

Glutamate uptake. Schmidt *et al.* (33) recently described a dose-dependent inhibition of uptake of

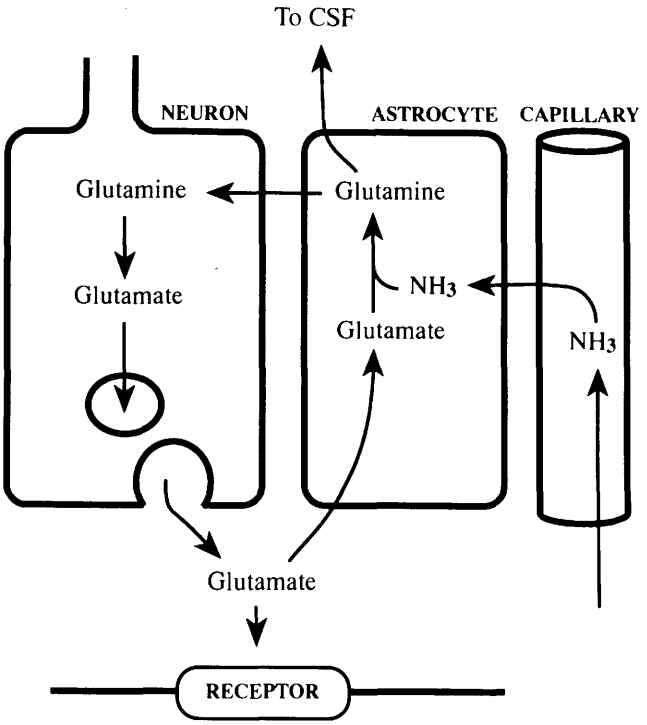

Figure 2. Key steps in glutamatergic synaptic regulation and removal of ammonia by brain. In the neuron: glutamate is synthesized from its precursor glutamine, stored in synaptic vesicles, and ultimately released via a Ca^{++}-dependent mechanism. Once released, glutamate can act upon any of the glutamate receptors (NMDA, non-NMDA, or metabotropic) found in the synaptic cleft. In the astrocyte: glutamate is taken up and converted to glutamine, using NH_3, by glutamine synthetase.

D-aspartate (a nonmetabolizable analog transported by the L-glutamate uptake system) into rat hippocampal slices by blood extracts from patients with varying degrees of severity of PSE. The relative potency of inhibition correlated with the blood ammonia content in these patients. Earlier studies independently described capacity of high-affinity uptake of glutamate into nerve ending preparations following their exposure to 5 m*M* ammonia (34) or into cultured astrocytes exposed to 2 m*M* ammonia for 4 days (23).

Glutamate release. Electrically-stimulated release of glutamate from superfused hippocampal slices taken from portacaval-shunted rats is significantly increased (35). Similarly, the *in vivo* release of glutamate by brain using either the "cortical cup technique" or microdialysis has also been shown to be increased in these animals (36, 37). In view of the findings of NH_4^+-induced decreases in neuronal and astrocytic uptake of glutamate, it is probable that the observations of increased "release" of glutamate represent increased "overflow" rather than release *per se*, i.e., increased extracellular glutamate resulting from decreased uptake of the neurotransmitter into metabolically compromised perineuronal astrocytes.

^{3}H-Glutamate binding sites. Consistent with the observed increase in extracellular glutamate in PSE, several studies have demonstrated a loss of densities of glutamate binding sites in the brains of animals with experimental PSE. Of the three subtypes of excitatory amino acid receptors found in brain, separated as a function of agonist and antagonist affinities (i.e., [i] the N-methyl-D-aspartate [NMDA] receptor, [ii] the non-NMDA receptor [previously the AMPA and kainate receptors] and [iii] a metabotropic receptor [see 38 for review]), the NMDA receptor is the most interesting in the present context given its high affinity for glutamate and the allosteric glycine binding site which modulates its function. Indeed, NMDA-sensitive glutamate binding site density is decreased in rat brain following portacaval anastomosis (39) or following acute exposure to ammonia (40). In addition, it is via the NMDA receptor that quinolinic acid, a metabolite of tryptophan whose concentrations are altered in PSE (41; see below), is thought to induce most of its cellular-directed excitotoxic effects. It has been suggested that the loss of NMDA-sensitive glutamate binding sites could result from sustained exposure of these sites to increased synaptic concentrations of endogenous ligand and a subsequent "down-regulation" (39). Data consistent with this interpretation include the findings of (i) increased extracellular glutamate in the brains of portacaval-shunted rats (see above) as well as (ii) the observation that brain concentrations of quinolinic acid are increased in the brains of these animals (42).

Evidence for glutamatergic synaptic dysfunction in fulminant hepatic failure is less compelling than for PSE. Although brain ammonia levels are increased in fulminant hepatic failure (Table II) as are levels of the ammonia detoxification product, glutamine (43), astrocytes do not undergo Alzheimer Type II alterations. Nevertheless, Ferenci *et al.* (44) reported decreased densities of glutamate receptors in the brains of animals with galactosamine-induced acute liver failure. Ammonia's role in the pathogenesis of HE in fulminant hepatic failure may lie in the direct neurotoxic effects of NH_4^+ ions on inhibitory and excitatory neurotransmission.

NH_4^+-Induced Swelling; Role in Brain Edema in Acute Liver Failure. Recent studies suggest that increased brain ammonia concentrations may be causally related to the phenomenon of cerebral edema in acute liver failure. Increased brain water has been described in dogs with urease-induced hyperammonemia (45) and in rats following ammonium acetate infusions (46). Treatment of isolated cerebral cortical slices with ammonia in concentrations equivalent to those reported in brain in experimental acute liver failure results in significant swelling and in concomitant reductions of inulin space (47). Cerebral edema in both experimental and human acute liver failure is mainly cytotoxic (rather than vasogenic) in nature with astrocytic swelling being consistently reported (48, 49). It is

interesting to note that swelling of cerebral cortical astrocytes is observed following ammonia infusions to primates (50) and exposure of primary cultures of astrocytes to millimolar concentrations of ammonia also results in significant swelling (51).

Recent evidence suggests that ammonia-induced swelling is mediated via a metabolite of ammonia rather than ammonia *per se*. As outlined in an earlier section of this review, ammonia removal in brain depends on glutamine synthesis via the astrocytic enzyme glutamine synthetase. Recent studies delineated a significant correlation between the rise in brain glutamine and brain water concentrations in normal rats receiving an ammonia infusion (45, 46). It was suggested that the ammonia-induced increase in brain water content was mediated by the osmotic effects of increased glutamine in these animals (46). In favor of this possibility, treatment of hyperammonemic animals with methionine sulfoximine, an inhibitor of glutamine synthesis in brain, prevented both the increases of glutamine and water content of the brains of these animals (46). More recently, however, a dose-dependent reduction of glutamine levels was indeed observed with increasing doses of methionine sulfoximine, although brain water content did not decrease further at maximal doses (52), suggesting that brain edema in hyperammonemia does not appear to be solely due to increased glutamine accumulation. Ammonia infusion in rats after portacaval anastomosis results in brain edema of a sufficient magnitude to raise intracranial pressure (53). Under normal physiological conditions, glutamine transport participates in the regulation of water movement in the brain (54). Studies in postmortem brain tissue from patients who died in acute liver failure reveal significantly increased glutamine concentrations (43). Brain glutamine concentrations are increased 6-fold in experimental ischemic liver failure (55) in parallel with increasing brain water content of brain tissue in these animals. Taken together, these findings afford a consistent body of evidence supporting a major role of ammonia via one or more of its metabolites in the pathogenesis of brain edema in acute liver failure.

Monoamine Neurotransmitters in Hepatic Encephalopathy

In 1971, Fischer and Baldessarini (56) proposed the false or "weak" neurotransmitter hypothesis for HE. The false neurotransmitter hypothesis stated that HE could result from the decarboxylation, in the large intestine, of various amino acids leading to the production of false transmitters such as β-phenylethylamine, tyramine, and octopamine (56). It went on to suggest a preference for the synthesis of these false transmitters and a subsequent replacement of the true transmitter (i.e., dopamine and noradrenaline by phenylethylamine, tyramine, or octopamine). This hypothesis was later expanded upon by Sourkes (57) to include neuroactive metabolites of tryptophan such as tryptamine and β-carbolines. The original hypothesis gained support from both the laboratory and the clinical settings. A limited number of patients with HE appeared to improve following administration of L-Dopa (58, 59) which is the immediate precursor for dopamine and noradrenaline and can not be metabolized to form false transmitters (Fig. 3). The accumulation of catecholamine-like false transmitters in serum and urine of patients with PSE correlated roughly with the grade of encephalopathy (58, 60). Levels of octopamine were also significantly increased in rat brain following coma induced by hepatic devascularization and in PCS rats fed high aromatic amino acid content diets (58, 61). These increased levels of brain false transmitters in hepatic devascularized rats were later shown to precede similar increases in serum thus suggesting a cerebral rather than purely intestinal origin of the transmitter (62, 63). In addition, brain levels of norepinephrine are significantly reduced in these same rats (62). The catecholamine aspect of this hypothesis came under fire, however, following the observation that intraventricular administration of octopamine to rats significantly increased the brain concentration (upwards of 20,000-fold) of this transmitter without inducing any adverse behavioral effects (64). In addition, almost total depletion of rat brain dopamine and norepinephrine (down to approximately 10% of control) following octopamine administration failed to alter the behavior in these animals. Finally, the limited number of clinical trials (using L-Dopa or bromocriptine) and their equivocal results (see below) argue against a direct intervention by catecholamine-like false transmitters in HE.

An alternative hypothesis (to that of the false neu-

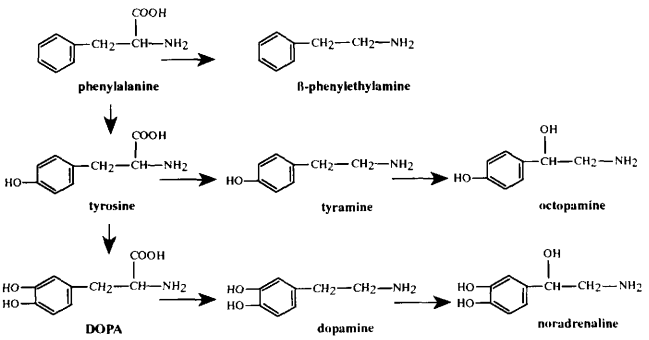

Figure 3. Catecholamine and related false neurotransmitter metabolism. Phenylalanine can be decarboxylated to give β-phenylethylamine or ring-hydroxylated to give tyrosine. In turn, tyrosine can be decarboxylated to give the false neurotransmitters tyramine and ultimately octopamine, which are thought to displace the true neurotransmitters dopamine and noradrenaline, formed following ring-hydroxylation of tyrosine.

rotransmitter) involving the synthesis of neuroactive/neurotoxic metabolites of tryptophan is presently emerging. A massive increase in brain tryptophan concentration, reflecting an abundance of free (i.e., non-albumin bound) plasma tryptophan, has consistently been reported in human and experimental PSE (57). Increased availability of the amino acid precursor during chronic liver failure may promote the synthesis of alternate neuroactive tryptophan metabolites, in addition to the biogenic amine serotonin, including neuroactive intermediates in the kynurenine pathway and tryptamine (see below). It was demonstrated, for example, that hepatic dysfunction altered CNS concentrations of both tryptamine in humans (57, 65, 66) and quinolinic acid in both human and experimental animals (41, 42). Although much of the focus in the study of HE shifted in the early 1980s to the GABA system and its potential as a causative agent in HE (discussed below), there is recent data which suggest that the amine systems, and in particular the metabolites of tryptophan, may play specific and important roles in the pathogenesis of the neuropsychiatric symptoms of HE.

Neuroactive Tryptophan Metabolites. *Serotonin (5-hydroxytryptamine; 5-HT).* A number of observations led to the suggestion that the serotonin system may be implicated in the etiology of HE and hepatic coma. Administration of large doses of tryptophan to Eck-fistula dogs results in a neuropsychiatric syndrome resembling PSE (67). Furthermore, CSF and brain concentrations of tryptophan are increased in patients in hepatic coma (68, 69). There is evidence to suggest that such changes are linked to ammonia accumulation in brain. For example, both portacaval shunting as well as hyperammonemic syndromes resulting from urease infusions (without liver dysfunction) result in increased brain tryptophan uptake by brain (70, 71). Furthermore, this enhanced uptake of tryptophan in chronic hyperammonemia is inhibited following inhibition of glutamine synthetase by methionine sulfoximine (72) suggesting a pathophysiologic link between hyperammonemia, glutamine synthesis in brain and blood-brain barrier uptake of tryptophan. Since tryptophan hydroxylation is the rate limiting step in 5-HT synthesis and since, under normal physiological conditions, the enzyme is not saturated, increased levels of brain tryptophan during liver failure should result in increased brain 5-HT synthesis. Following portacaval anastomosis in the rat, 5-HT turnover is increased in brain whether assessed by measurement of the ratio 5-HT/5-HIAA (5-hydroxyindole acetic acid; the predominant metabolite of 5-HT) (73) or using a decarboxylase inhibition assay (74). Interestingly, the brain regions affected are those associated with the control of levels of arousal and it is known that disruptions of sleep patterns and neuropsychiatric problems form part of the spectrum of clinical signs of early PSE in humans (75). Evidence for altered 5-HT turnover has also been provided in studies of autopsied brain tissue from patients who died with PSE (76). In studies using the portacaval-shunted rat, increased 5-HT turnover was apparent early during the development of PSE (73). It has been suggested that the observed changes in 5-HT turnover could relate to the altered sleep patterns and diurnal rhythms observed early in PSE in both man and experimental animals with chronic liver disease (73, 77). It seems unlikely, however, that increased brain 5-HT turnover is solely responsible for the decreased levels of consciousness observed in severe PSE, as CSF concentrations of 5-HIAA do not correlate with the presence of coma in patients with cirrhosis (68). In addition, changes in brain 5-HT turnover do not correlate with the degree of neurological impairment in portacaval-shunted rats administered ammonium salts to precipitate coma (73), and treatment of patients with liver disease and minimal (subclinical) encephalopathy with a 5-HT reuptake inhibitor failed to result in significant worsening of neurological symptoms (78). On the other hand, a clinical trial aimed at lowering portal hypertension in patients with chronic liver disease with the serotonin 5-HT_2 receptor antagonist ketanserin resulted in the precipitation of PSE in a significant number of patients (79). These findings prompted the investigation of [^{3}H]-ketanserin binding sites in autopsied brain tissue from PSE patients; these studies revealed significant increases in the density of these sites in frontal cortex (80). Furthermore, activity of the 5-HT degradative enzyme MAO_A were found to be significantly increased in the same material (81). These findings are consistent with previous reports of increased concentrations of the 5-HT metabolite 5-HIAA and, together with the findings of increased 5-HT_2 (ketanserin) binding sites (80) and the precipitation of PSE by 5-HT_2 antagonists, suggest that severe PSE may be the result of a synaptic deficit of 5-HT rather than increased 5-HT turnover as had previously been suggested (Fig. 4). Further studies are clearly required in order to resolve this issue.

Tryptamine. Increased uptake of brain tryptophan in hepatic failure may also result in increased synthesis of other neuroactive amines such as tryptamine (Fig. 5). Tryptamine metabolism is much more responsive than is 5-HT metabolism to tryptophan loading (65). In addition, given the relative proportions of CSF indole acetic acid (IAA), the predominant tryptamine metabolite, and 5-HIAA levels in humans and rats, it would appear that tryptamine metabolism is more important in human brain than rat brain (82). Using CSF IAA concentrations as an index of central tryptamine turnover, it was observed that patients with hepatic coma had significantly higher brain trypt-

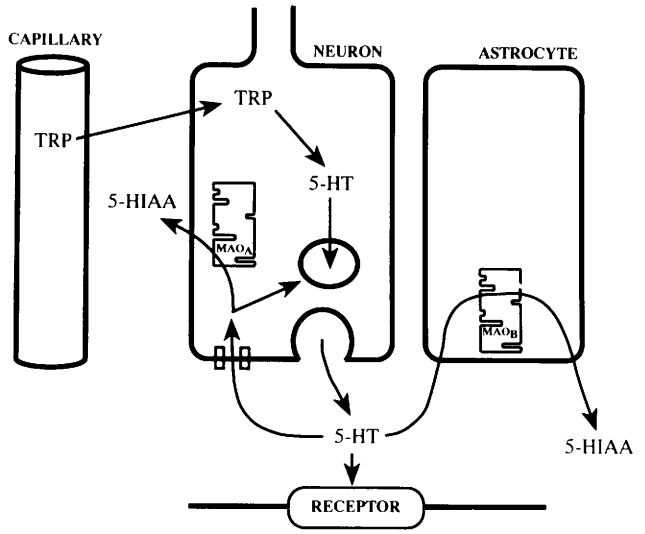

Figure 4. Key steps in serotoninergic synaptic regulation. In the neuron: serotonin (5-HT) is synthesized from its precursor tryptophan (TRP), stored in synaptic vesicles and ultimately released via a Ca^{++}-dependent mechanism. Once released 5-HT can act upon any of the 5-HT receptors found in the synaptic cleft (n.b.: although 5-HT receptors are only shown to be postsynaptic in this diagram, presynaptic receptors have been demonstrated but are left out to simplify the diagram). 5-HT can be taken up by the nerve terminal where it can be stored in synaptic vesicles and recycled, or it can be degraded by the mitochondrial enzyme monoamine oxidase-A (MAO_A) to give 5-hydroxyindoleacetic acid (5-HIAA). In the astrocyte: 5-HT is taken up and converted to 5-HIAA by MAO_B.

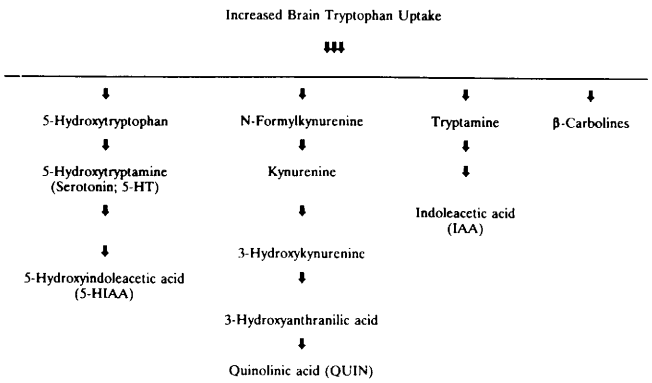

Figure 5. Tryptophan metabolism in brain. Synthetic pathways initiated by different enzymes can lead to the synthesis of serotonin, kynurenines, quinolinic acid, tryptamine and β-carbolines following the uptake of tryptophan into brain. Alterations in all of these pathways, except for the β-carboline pathway, have been demonstrated in HE.

amine turnover when compared with cirrhotic patients not in coma (66). Furthermore, the grade of coma was directly proportional to CSF IAA levels in these patients. Wiltfang *et al.* (83) recently observed a similar trend towards an increase in plasma IAA levels in patients with early PSE. However, the wide scattering of individual values rendered the finding statistically nonsignificant. Consistent with the suggested increase in tryptamine turnover reported by Young and Lal (66), decreases in [^{3}H]-tryptamine binding site density in postmortem brain tissue from patients who died in hepatic coma were recently reported (84; Fig. 6). Whether the observed changes in binding site density are the consequence of region-specific increases in the availability of the amino acid precursor tryptophan or in region-specific increases in brain uptake of tryptamine resulting, for example, from decreased hepatic degradation of the amine in these patients, awaits further studies.

Other metabolites. In addition to being the precursor for neuroactive monoamines such as 5-HT and tryptamine, tryptophan may be transformed in brain into other neuroactive and/or neurotoxic metabolites (Fig. 5). One example involves the kynurenine pathway leading to synthesis of quinolinic acid. Increased concentrations of quinolinic acid have been described in the CSF of patients with PSE (41) and in the brains of portacaval-shunted rats (42). More recently, a preliminary report described increased levels of 3-hydroxykynurenine in postmortem brain samples of patients with PSE (85). Alternatively, the accumulation of these substances could result from decreased quinolinic acid degradation; the enzyme responsible for quinolinic acid metabolism, quinolinic acid phosphoribosyl transferase (QPRT), is reportedly localized in astrocytes (86). Loss of astrocytic integrity and function in PSE and concomitant reductions in activity of QPRT could thus offer an alternate explanation for increased brain levels of quinolinic acid in PSE. As previously mentioned, an increase in the brain levels of these putative endogenous ligands for the NMDA

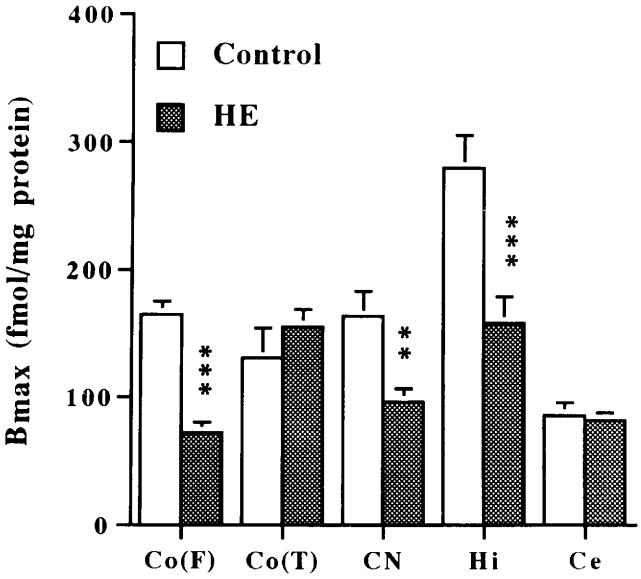

Figure 6. Regional brain [^{3}H]-tryptamine binding site densities, determined by Scatchard analysis of saturation experiments, in autopsied tissue from controls (open bars) and from cirrhotic patients with hepatic encephalopathy (hatched bars). Co(F): frontal cortex; Co(T): temporal cortex; CN: caudate nucleus; Hi: hippocampus; Ce: cerebellum. $^{**}P < 0.01$; $^{***}P < 0.001$. (From Ref. 84, with permission.)

receptor offers an alternative explanation for the selective loss of NMDA binding sites in the brains of animals with experimental PSE (39).

Dopamine. Altered metabolism and/or function of dopamine has been implicated in the etiology of several neurological disorders including Parkinson's and Huntington's disease, neurodegenerative disorders characterized by impaired motor and/or cognitive function. In addition to cognitive and affective disorders, neuromuscular abnormalities such as tremor, muscular incoordination, slurred speech, clonus, and rigidity form an integral part of the HE syndrome (87).

The dopamine system was initially implicated in the clinical symptomatology of PSE because of the beneficial therapeutic effects of both L-Dopa (the immediate precursor to dopamine) and the dopamine agonist bromocriptine (88, 89), although negative results with both agents were also reported (90, 91). Thus the therapeutic efficacy of dopaminergic compounds in the treatment of HE remains contentious. Patients who survive an episode of hepatic coma occasionally experience residual neurologic abnormalities such as choreatic twitching of the limbs, which may worsen with repeated episodes of coma. The basal ganglia (adjacent areas in the brain having a high dopamine content) have recently been investigated extensively, since these structures play a role in facilitating cortically-initiated movement as well as controlling unintended movements under normal conditions. In addition to their involvement in motor function, the basal ganglia also integrate aspects of attention, wakefulness, and sensory processing, all of which are affected in PSE (92). An increase in the levels of the dopamine metabolites homovanillic acid (HVA) and 3-methoxytyramine (3-MT) have been reported in several regions from autopsied brain tissue from cirrhotic patients who died in hepatic coma (78). These findings may reflect increased synthesis and metabolism of dopamine resulting from the increased concentrations of the amino acid precursors phenylalanine and/or tyrosine commonly observed in HE (73, 93). Furthermore, densities of postsynaptic dopamine D_2 receptors have recently been shown to be selectively decreased in the globus pallidus from these patients (94; Fig. 7). In addition to the documented involvement of the dopamine system and the basal ganglia in movement and affective disorders, other evidence supports a function for the basal ganglia (and therefore indirectly implicates the dopamine system) in PSE. PET (6) and single photon emission computed tomography (SPECT) (95) techniques both describe increased blood flow and altered function in basal ganglia which correlates positively with neuropsychological impairment in patients with chronic liver disease. Although there is evidence of structural changes in the basal ganglia of patients with chronic liver disease (96, 97), the recent report of

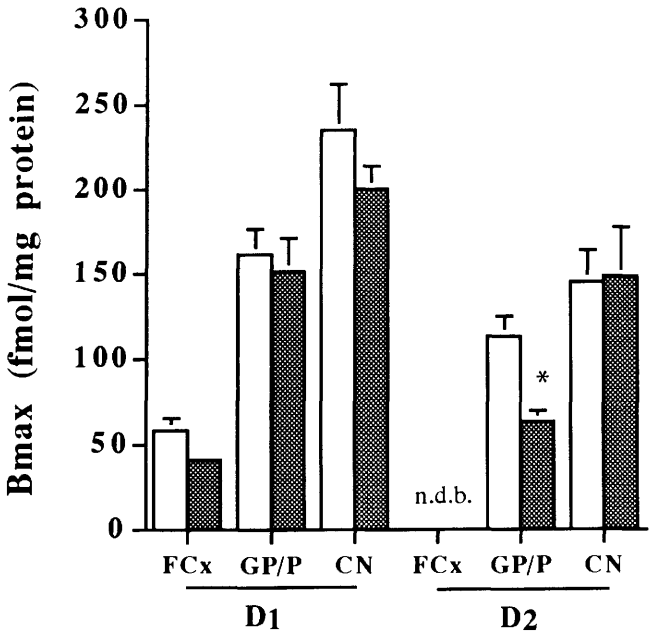

Figure 7. Regional dopamine D_1 and D_2 receptor densities, determined by Scatchard analysis of saturation experiments, in autopsied brain tissue from controls (open bars) and from cirrhotic patients with hepatic encephalopathy (hatched bars). [^{3}H]-SCH 23390 was used for determination of D_1 receptor parameters and [^{3}H]-spiperone was used for determination of D_2 receptor parameters. FCx: frontal cortex; GP/P: globus pallidus/putamen; CN: caudate nucleus; $^*P < 0.001$; n.d.b.: no detectable binding. (From Ref. 94, with permission.)

complete reversibility of MRI hyperintensity in the basal ganglia of cirrhotic patients following liver transplantation (98) argues for the likelihood of a functional rather than a structural origin for the abnormal results associated with these structures in PSE. This functional abnormality most likely involves the dopamine system.

The number of investigations into the function of biogenic amines and related compounds in the pathogenesis of HE has begun to increase following a decade-long hiatus, and the above discussion indicates that recent data from numerous laboratories suggest an interesting and important role for these compounds.

The GABA Theory of HE

In 1982, it was proposed that gut-derived GABA, by virtue of its lack of removal by the liver and its entry into brain via a permeable blood-brain barrier, might contribute to the neural inhibition characteristic of HE (99). Evidence in favor of this hypothesis was derived almost exclusively from studies in the rat and rabbit models of fulminant hepatic failure derived by treatment of animals with the hepatotoxin galactosamine (100, 101). Studies in human and experimental PSE yielded negative results. Thus, although serum GABA levels were reportedly increased in patients with HE due to acute or chronic hepatocellular failure (102), no differences in plasma GABA levels were ob-

served between encephalopathic and nonencephalopathic dogs with portacaval anastomoses (103). Subsequent studies likewise were unable to confirm any evidence of a positive correlation between either plasma or CSF concentrations of GABA and clinical or EEG grading of encephalopathy in patients with chronic liver disease (41, 104). Although a study in 1984 found evidence of alterations of GABA-related enzymes and of GABA binding sites in the brains of dogs with experimental PSE (101), other studies in experimental PSE in rats (105) or dogs (103, 106) did not confirm these findings. In fact, in a systematic study of the GABA system in cirrhotic and portacaval-shunted dogs, with or without PSE, no evidence of significant alterations of either brain GABA uptake, GABA content, GABA-related enzymes, or GABA binding sites was observed (106). Finally, a lack of any observed changes in GABA concentrations (12), GABA-related enzymes (22), or GABA binding sites (107) in postmortem brain tissue from cirrhotic patients who died in hepatic coma confirmed the negative results from most animal studies. Taken together, this considerable body of evidence suggests that PSE does not result from alterations of brain GABA function.

As more studies in different animal models and in human tissue were performed, the evidence for a major pathogenic role for altered brain GABA function in HE resulting from fulminant hepatic failure became, likewise, unconvincing. The initial findings of alterations of GABA-related enzymes and binding sites observed in experimental galactosamine-induced liver failure were not confirmed by other investigators working either with these same experimental models (108) or with other preparations. For example, rats with thioacetamide-induced liver failure and HE had unaltered brain GABA receptor binding affinities and densities (109) and HE following portal vein ligation with or without common bile duct ligation did not lead to increased brain GABA (110). Binding of GABA to synaptic membranes of rats with ischemic liver failure was similarly found to be unaltered (111) and in a study of amino acid changes in autopsied brain tissue from patients with fulminant hepatic failure who died in hepatic coma, no significant increases of GABA were observed (43). Thus, although acute liver failure may result in increased serum levels of GABA (102), this does not appear to result in increased brain GABA nor in alterations of brain GABA function.

"Endogenous" Benzodiazepine-Receptor Ligands Have Been Identified in HE

Benzodiazepines exert their effects on CNS function by interacting with binding sites on the GABA-benzodiazepine-chloride ionophore receptor complex. As outlined in the previous section of this review, in the 1980s, two reports demonstrated increased densities of GABA and benzodiazepine binding sites in the brains of animals with galactosamine-induced fulminant hepatic failure (100, 101). Administration of a benzodiazepine receptor antagonist to such animals resulted in improved neurological status (101). Based upon these observations, the therapeutic usefulness of benzodiazepine receptor antagonists was assessed in human HE. In 1985, two communications reported favorable results using Ro15–1788 (flumazenil) in the treatment of three patients with severe HE (112, 113). It was proposed by Scollo-Lavizzari and Steinman (112) that the reversal of HE by flumazenil was mediated by its blocking action on increased numbers of benzodiazepine receptors and thus preventing the action of an "endogenous benzodiazepine" in the brain.

Following up on these initial reports, benzodiazepine receptor antagonists were found to induce a transient decrease in the clinical severity of HE caused by galactosamine-induced acute liver failure in the rabbit (114). Other studies demonstrated that cerebellar neurons from rabbits with galactosamine-induced HE were hypersensitive to GABA and benzodiazepine receptor agonists (115). On the other hand, HE resulting from hepatic artery ligation following portacaval anastomosis was not reversed by the administration of benzodiazepine receptor antagonists even when these compounds were administered in doses sufficient to reverse diazepam-induced coma (116). Similar negative findings were reported following attempts to ameliorate neurological status in rabbits with ischemic liver failure (117). In one study, administration of high doses of flumazenil resulted in a small improvement in neurological status in rats with thioacetamide-induced liver failure (118). However, a subsequent study using flumazenil in the same animal model was unable to replicate these findings (119).

Increased uptake of ^{11}C-flumazenil measured by PET in four patients with PSE was described (120). Although these findings were initially interpreted as being the consequence of increased binding to benzodiazepine receptors in the brains of these patients, direct studies of brain tissue from cirrhotic patients who died in HE revealed normal densities and affinities of benzodiazepine binding sites (10). Furthermore, a subsequent study showed markedly altered pharmacokinetics of flumazenil in cirrhotic patients (121) suggesting that the PET findings resulted from increased amounts of free flumazenil made available for brain uptake by decreased systemic clearance of the drug.

Attempts have been made to isolate and characterize the "endogenous benzodiazepine" receptor binding substances in HE. Serum and CSF from rabbits with galactosamine-induced fulminant hepatic failure displace ^{3}H-benzodiazepines from cerebral cortical membrane preparations (122). Similar findings were observed by two groups of investigators in brain

extracts from thioacetamide-treated rats with HE (123, 124). However, it is unlikely that these substances contributed to the neural inhibition in these animals, since treatment with benzodiazepine antagonists led to no significant improvement of neurological status in these animals (119). Elevated brain concentrations of benzodiazepines have been reported in autopsied brain tissue from 5/11 patients with fulminant hepatic failure from acetaminophen overdose (125). Levels of diazepam were increased beyond the normal range in 2/11 of these patients (Fig. 8). Whether or not the presence of benzodiazepines in this material was the result of prior exposure to pharmaceutical benzodiazepines could not be definitively established.

Portacaval anastomosis does not lead to increased brain levels of endogenous benzodiazepines (124). On the other hand, increased brain content of such substances has been reported in autopsied brain tissue from patients with PSE (124). However, to date, the identities of many of these benzodiazepine-like substances have not been completely characterized. The CSF of patients with PSE contains several benzodiazepine-displacing substances, some of which have been shown to possess the requisite agonist properties consistent with their role in facilitation of GABAergic neurotransmission. The nature and relative concentrations of endogenous benzodiazepines found in the CSF and brains of humans with PSE are different from those encountered in animal models of fulminant hepatic failure (124). For example, although autopsied brain tissue from patients who died in fulminant hepatic failure was shown by gas chromatography-mass spectrometry to contain increased amounts of diazepam and N,N-desmethyldiazepam (125) no evidence of either of these substances could be found using similar techniques in autopsied brain tissue from PSE patients (124). It should be noted that CSF and brain levels of total (agonist plus others) benzodiazepines encountered in human and experimental PSE are several-fold lower than those reported in PSE sedation (126). Results of a controlled clinical trial of the benzodiazepine receptor antagonist flumazenil revealed significant amelioration of neurologic function in 40% of patients, none of whom had measurable levels of diazepam in blood (127).

In addition to benzodiazepine-like molecules, certain endogenous neuropeptides with high affinity for GABA-related benzodiazepine receptors have also been found in increased amounts in human and experimental HE. In one study, the neuropeptide diazepam binding inhibitor (DBI) was found to be elevated in the CSF of some patients with hepatic encephalopathy (Fig. 9). Clinical stages correlated well with CSF DBI levels and patients with liver disease but without HE had DBI levels within the normal range (128). However, DBI is a benzodiazepine receptor ligand with

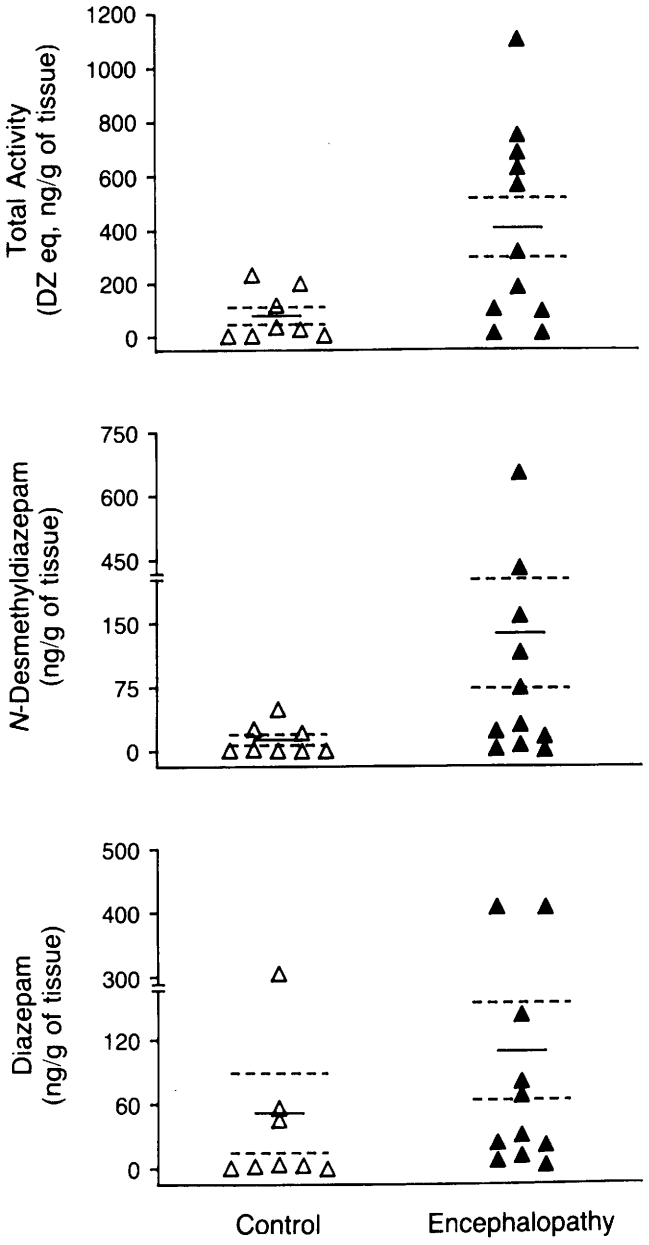

Figure 8. Distribution of levels of total benzodiazepine-receptor-ligand activity as well as N-desmethyldiazepam and diazepam concentrations in autopsied brain tissue from eight control patients (open triangles) and 11 patients who died in acute liver failure (solid triangles). Each solid line with a pair of dashed lines represents the mean ± SEM. The concentrations of benzodiazepine-receptor ligands were determined by quantitative radiometric analysis. Total activity is expressed in diazepam equivalents (DZ eq). (From Ref. 125, with permission.)

negative allosteric modulatory effects on GABAergic neurotransmission (i.e., DBI would be expected to result in decreased facilitation of GABAergic transmission and increased arousal).

It should be borne in mind that DBI as well as its fragmentation product octadecaneuropeptide also bind to the "peripheral-type" benzodiazepine receptor localized on astrocytic mitochondrial membranes (see above). Increased levels of DBI in CSF of patients

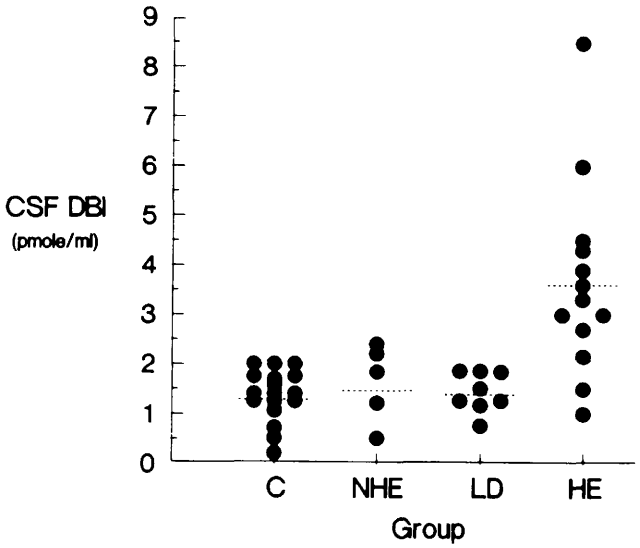

Figure 9. Cerebrospinal fluid (CSF) levels of diazepam binding inhibitor (DBI) in control patients (C), in patients with nonhepatic encephalopathy (NHE), in patients with liver disease without mental staus change (LD), and in patients with hepatic encephalopathy (HE). Dotted line represents the mean for each group. (From Ref. 128, with permission.)

with HE could be a consequence of astrocytic changes in the brains of these patients.

Other Toxins and Synergistic Mechanisms in HE

While the majority of evidence available at the present time implicates ammonia-related neurotoxic mechanisms in the pathogenesis of HE, it is likely that other toxic substances, normally unable by themselves to cause coma, act synergistically with ammonia to "tip the balance" towards neural inhibition in brain. Possible toxic substances include mercaptans, phenols, and fatty acids. Synergism between ammonia and these toxins has been demonstrated experimentally (129). Indeed, administration of fatty acids potentiates the effect of ammonia. These fatty acids are produced by intestinal bacteria (130), absorbed and transported into the portal system (131), and metabolized by the liver (132). The reported plasma levels of fatty acids in cirrhotic patients without manifestations of HE are equivocal (133, 134, 135). In contrast, plasma levels are significantly elevated in HE, whether the patients are cirrhotic or suffering from fulminant hepatic failure (136). The lack of any correlation between fatty acid levels and degree of HE, however, argues against these compounds alone playing a primary role in HE (137), but does not exclude potential synergistic effects with other compounds. Similarly, although endogenous benzodiazepine-like compounds described in blood and CSF of patients with hepatic failure may not be, by themselves, sufficient for HE, they also have the potential to act synergistically with other mechanisms to increase overall brain inhibition.

Summary

The last decade has witnessed the appearance of several new theories of the pathogenesis of HE. In the case of PSE, there is an overwhelming body of data to support the notion that exposure of brain to ammonia results in neuronal dysfunction mediated either by (i) direct effects of ammonium ions on neurotransmission, (ii) glutamatergic regulation resulting from modifications of astrocytic function, or (iii) increased synthesis of neuroactive and/or neurotoxic metabolites of tryptophan. There is no convincing evidence that GABA-mediated neurotransmission *per se* is altered in PSE. Controlled clinical trials with benzodiazepine receptor antagonists have so far yielded beneficial results in only a minority of cases and it is unclear whether the beneficial effects are due to the action of the antagonist on "endogenous" or pharmaceutical benzodiazepines previously ingested by these patients. In fulminant hepatic failure, there is evidence to suggest that rapid increases of brain ammonia and subsequent glutamine synthesis could be implicated in the pathogenesis of brain edema in this condition. Although benzodiazepines have been found in brain in fulminant hepatic failure, their contribution to the pathogenesis of HE is unclear. It is possible that these substances as well as others such as mercaptans and short chain fatty acids could act synergistically with ammonia and other known toxins to produce the overall neural inhibition characteristic of HE in liver failure.

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