The Pathogenesis of Hepatic Encephalopathy*

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ABSTRACT

Hepatic encephalopathy remains a complex clinicopathological problem. Much is known about the biochemical derangements in liver, blood, and brain. The precise pathogenetic mechanism for central nervous system dysfunction remains to be determined. Ammonia continues to be considered as an important neurotoxin and may act synergistically with other toxic substances. Disturbances of amino acid balance may result in a disproportion of inhibitory and excitatory neurotransmitters in the brain. Alternatively, some amino acids may act as false neurotransmitters. Recent clinical and laboratory data have advanced the hypothesis that gamma-aminobutyric acid (GABA) absorbed from the gut may enter the brain and exert a profound inhibitory effect. Drugs which antagonize the GABA-benzodiazepate receptor may offer symptomatic improvements in hepatic encephalopathy.

Introduction and Clinical Considerations

That the liver could produce profound effects on mental function has been known since antiquity. Chinese and Babylonian manuscripts refer to the liver as the seat of the soul. Hippocrates described dramatic behavioral changes in a patient with hepatitis.48

The clinical course of hepatic encephalopathy (HE) begins with confusion and agitation and ends with deep coma and death. Initially, patients are unable to concentrate, neglect their responsibilities, and show personality alterations characterized by passivity or aggression. The initial manifestations may be more evident to families of the patients than to their physicians.17 Early impairment can be documented by formal neuropsychiatric testing.18 As the encephalopathy progresses, motor abnormalities become evident. There is a characteristic flapping tremor or asterixis, and disturbances of tone and reflexes may be elicited.40 A sweetish or fruity odor on the breath, characterized as fetor hepaticus, may be detected, owing to the exhalation of nitrogenous products, including mercaptans. Eventually, coma ensues associated with respiratory alkalosis, apneustic breathing, decerebrate posturing, and loss of brain stem function.10,40,48

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Electroencephalographic changes are present in HE and show generally good correlation with the degree of clinical impairment. Early on, there is bifrontal slowing of the electrical activity of the brain followed by distinctive 4 to 5 Hz triphasic waves, which carry a poor prognosis. In the terminal stages of coma, a pattern of low amplitude slow waves predominates.\textsuperscript{31,51} More recently, abnormal visual evoked responses have been described in HE. It is possible that visual evoked responses may be useful in detecting preclinical HE, assessing its severity, and distinguishing it from other metabolic encephalopathies.\textsuperscript{59}

Pathology

Pathologic changes have been described in the brains of patients dying with HE and in the brains of experimental animals subjected to ammonia infusion, to hepatotoxic agents, or to porto-caval anastomosis. The characteristic histologic finding in autopsied human brains is the presence of Alzheimer Type II astrocytes. These are large, pale, watery astrocyte nuclei with no identifiable cytoplasm, often occurring in pairs or tetrads, and distributed predominantly in the cerebral cortex and basal ganglia. Frequently, the nuclei have a lobulated or convoluted outline and may contain inconspicuous nucleoli just beneath the nuclear envelope.\textsuperscript{37} Because of their peculiar configuration, it was once suggested that they proliferated by a process of "amitotic division".\textsuperscript{28} This has not been substantiated. The relevance, if any, of these astrocytic changes to the clinical manifestations of HE is uncertain. Nerve cells show no consistent morphologic change.

Under experimental conditions in laboratory animals, pathologic changes are slightly different. Clinical and electrophysiologic findings comparable to human HE have been induced in rats and primates by infusion induced hyperammonemia.\textsuperscript{11,22,54} The perfusion fixed brains of these animals reveal only minimal astrocytic pathology when examined by light microscopy. One possible explanation of these results is that classic Alzheimer II astrocytes are a type of artifact induced by immersion fixation techniques of autopsy practice. Electron microscopic examination of brain tissue from experimental animals, however, does demonstrate astrocytic changes consisting of cytoplasmic swelling and membranous whorls.\textsuperscript{54} Similar ultrastructural findings have been described after portocaval anastomoses in rats.\textsuperscript{58}

Biochemistry and Pathophysiology

The biochemical changes in HE are so multiple and so complex as to defy precise analysis. The exact mechanism or combination of mechanisms responsible for encephalopathy is undetermined. The problem is further complicated by the other medical conditions which may attend hepatic coma such as respiratory alkalosis, fluid and electrolyte imbalance, renal failure, and poor nutrition. This issue has been the subject of extensive scrutiny and has provoked some controversy.\textsuperscript{14}

In general, investigations into the pathogenesis of HE have focused on three major lines of inquiry. The first has focused on the effects of putative neurotoxins of which ammonia has received the most attention.\textsuperscript{46,55} A second approach has examined the complex alterations in amino acid metabolism in liver disease and the role of amino acids as neurotransmitters in the brain.\textsuperscript{14} More recently, it has been proposed that gamma amino butyric acid (GABA), a powerful neuro-inhibitory substance, is responsible for the central nervous system dysfunction in liver disease.\textsuperscript{43}
Ammonia

Of all the substances potentially responsible for HE, ammonia has been the most intensively investigated. The concentration of ammonia is elevated in the blood of patients with HE, and ammonia infusion may cause symptomatic encephalopathy in humans with cirrhosis and in experimental animals.\(^3,4,5\) Ammonia enters the portal circulation from the gut where it is derived from bacterial metabolism and dietary protein.\(^5,7\) Portal-systemic shunting or hepatocellular failure permits ammonia to enter the general circulation and, eventually, the brain.\(^4,6\) Unionized ammonia (about five percent of total blood ammonia at physiologic pH) readily crosses the blood brain barrier.\(^1,7,21\) Ammonia is metabolized in astrocytes by the amination of glutamic acid to form glutamine.\(^7\)

The mechanisms of ammonia toxicity are uncertain. It had been proposed that ammonia interferes with cerebral energy metabolism by removing alpha keto glutarate from the Krebs cycle and diverting it into the glutamine pathway. However, these claims have not been confirmed.\(^5,6,61\) Furthermore, recent work has demonstrated an actual increase in glucose metabolism in brain tissue after experimental portocaval shunting.\(^2,9\) Ammonia may interfere with synaptic transmission by inhibiting Na-K dependent adenosine triphosphatase which maintains the transmembrane ion gradients necessary for neuronal activity.\(^8,46\) The significance of these data is uncertain. It has also been shown that ammonia causes disinhibition in neural circuits by blocking the outward extrusion of chloride ions.\(^10,25,39\)

On a clinical level, ammonia intoxication and hepatic coma cannot be equated.\(^49\) While blood ammonia (especially arterial) is elevated in hepatic coma, there is no close correlation between blood ammonia concentration and the level of encephalopathy.\(^1,6,61\) This may be due to inaccuracy in measurement or to the failure of blood to reflect the concentration of ammonia in the brain.\(^24\) Sherlock suggests that “The raised blood ammonium level in hepatic coma may well be more a nonspecific indicator of disturbed brain metabolism than the toxic causative factor.”\(^48\)

Therapeutic measures in HE have been aimed at reducing the availability of ammonia. These have included the restriction of dietary protein and the use of antimicrobials, especially neomycin and metronidazole, to inhibit ammonia production by bacteria in the gut.\(^6,35\) Lactulose has been used to reduce intestinal transit time and to facilitate the incorporation of ammonia into bacterial protein.\(^38,49\)

Multiple Toxins

Other toxic substances have been implicated in the pathogenesis of HE and may act synergistically in concert with ammonia. Compounds including mercaptans, short chain fatty acids, ketones, phenol, and bile salts are found in increased concentrations in the blood of patients with HE.\(^8,1,7,2,4,6,61\) These putative neurotoxins can produce coma in experimental animals and may act by inhibiting Na-K dependent adenosine triphosphatase or by interfering with mitochondrial electron transport. Poorly characterized “middle molecules” of intermediate molecular weight (500 to 5000) have also been implicated.\(^47\)

Amino Acids

A complex alteration in the composition of blood amino acids characterizes hepatic failure.\(^1,7,57\) Typically, there is an increase in the concentrations of the aromatic amino acids, phenylalanine and
tyrosine, an increase in unbound tryptophan, and a decrease in the concentration of the branched chain amino acids valine, leucine, and isoleucine. It is proposed that some amino acids may act as neurotransmitters or neuromodulators in the brain and that amino acid imbalance may result in altered neural activity and encephalopathy.

The neuroactive amino acids are divided into an excitatory dicarboxylic group including glutamate and aspartate, and an inhibitory monocarboxylic group including glycine, taurine and GABA. Hindfelt et al reported lowered concentrations of neuroexcitatory amino acids in the brain tissue of rats with experimental hepatic failure. Furthermore, a correlation is reported between the depth of hepatic coma in humans and the ratio of branched chain to aromatic amino acid concentrations.

Several complex interrelated mechanisms are suggested to explain the role of amino acids in hepatic coma.

1. Tryptophan is the most "toxic" amino acid and is elevated in the brain, cerebrospinal fluid, and blood of patients in hepatic coma. It can cause neurologic disturbances when ingested in large amounts and is a precursor of serotonin, an inhibitory neurotransmitter.

2. Increased phenylalanine and tyramine can compete with normal substrates for tyrosine hydroxylase and dopamine-beta hydroxylase, respectively, leading to loss of dopamine and nor-adrenalin in the brain. Nor-adrenalin levels fall in rat brains as hepatic coma supervenes. Dopamine and nor-adrenalin are important excitatory neurotransmitters, and loss of these compounds could cause the depressed neural activity characteristic of HE.

3. Glutamate, another neuroexcitatory amino acid, is reduced in blood and brain tissue in hepatic coma. It shares with aromatic amino acids a common carrier at the blood-brain barrier. Elevated aromatic amino acids may compete with glutamate in an exchange transport mechanism resulting in depletion of glutamate from the brain. Elevated ammonia may abet this process by converting glutamine to glutamate. In support of this theory, the best available metabolic marker of HE is cerebrospinal fluid glutamine concentration. Therapeutically, the toxicity of ammonia may be obviated by inhibiting glutamine synthesis with the administration of methionine sulfoxamine, an inhibitor of glutamine synthetase.

4. Octopamine is a neuroactive amino acid derived from tyrosine via the oxidation of tyramine. Octopamine concentrations are elevated in the brain in acute hepatic coma, and there is some correlation between the severity of HE and blood octopamine levels. The "false neurotransmitter" hypothesis proposes that octopamine is taken up and released by neurons which normally store nor-adrenalin and dopamine. This eventually leads to the depletion of "normal" neurotransmitters and the substitution of "false neurotransmitters" which are incapable of appropriate synaptic activity. There is then a relative predominance of inhibitory neurotransmitters and a suppression of neural function. There are several problems with the amino acid "false neurotransmitter" theory, however, and several clinical and experimental data seem to weigh against it:

A) No disturbance of consciousness results when octopamine is instilled directly into the cerebral ventricles, even though there is a striking increase in brain octopamine and a fall in brain dopamine and nor-adrenalin.

B) L-dopa and its agonist, bromocriptine, would be expected to restore dopaminergic activity in HE if the false neurotransmitter theory were true. No
consistent improvement has been shown in clinical trials of these drugs.\textsuperscript{30,33,34,52,53}

(C) No reduction in catecholamine levels are found in postmortem studies of the brains of cirrhotics with encephalopathy. Also, brain octopamine levels were lower when compared to a control group of cirrhotics who were not encephalopathic at the time of death.\textsuperscript{9}

**Gamma Amino Butyric Acid**

Gamma amino butyric acid is the principal inhibitory neurotransmitter in the mammalian brain.\textsuperscript{44} Small amounts of GABA introduced intra-cerebrally will produce a coma-like state in rabbits and an electroencephalographic pattern similar to that noted in patients with deep hepatic coma.\textsuperscript{4,50,51} High concentrations of GABA are reported in the plasma of animals with experimental HE.\textsuperscript{13}

These data have prompted Schafer and Jones to propose that GABA, synthesized by gut bacteria, bypasses the liver in portal-systemic shunting or hepatic failure and crosses the blood brain barrier to attain high concentrations in selected grey matter areas.\textsuperscript{42,43} The GABA then acts at its binding site to cause hyperpolarization of the post-synaptic membrane and inhibition of synaptic transmission.\textsuperscript{44} The GABA receptor is a supramolecular complex which has on its surface sites for the binding of the synergistic ligands GABA, barbiturates, and benzodiazepines.\textsuperscript{17} An increase in the number of GABA binding sites is reported in HE.\textsuperscript{45} This is an example of "up-regulation", a process by which occupancy of a receptor site by an appropriate ligand induces multiplication of the receptors in the brain. Up-regulation of GABA receptors may also account for the increased sensitivity of patients with HE to barbiturates and benzodiazepines.\textsuperscript{16,17}

Support for the GABA hypothesis has come from experimental studies which have demonstrated increased GABA-ergic tone and enhanced sensitivity to GABA agonists in the brains of rabbits with HE.\textsuperscript{2} Furthermore, symptomatic improvement of level of consciousness in patients with HE has been reported after administration of flumazenil, a benzodiazepine-GABA receptor antagonist. These data have led to a qualified acceptance of GABA as a major factor in the pathogenesis of hepatic encephalopathy, although some questions remain unresolved.\textsuperscript{49,61} The relationship of GABA to ammonia, other neurotoxins, and amino acid balance remains to be explored.

**Summary**

In summary, HE remains a complex clinicopathological problem. Much is known about the biochemical derangements in liver, blood, and brain. The precise pathogenetic mechanism for central nervous system dysfunction remains to be determined. Ammonia continues to be considered as an important neurotoxin and may act synergistically with other toxic substances. Disturbances of amino acid balance may result in a disproportion of inhibitory and excitatory neurotransmitters in the brain. Alternatively, some amino acids may act as false neurotransmitters. Recent clinical and laboratory data have advanced the hypothesis that GABA absorbed from the gut may enter the brain and exert a profound inhibitory effect. Drugs which antagonize the GABA-benzodiazepate receptor may offer symptomatic improvement in HE.

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