Ammonia and GABA-ergic Neurotransmission: Interrelated Factors in the Pathogenesis of Hepatic Encephalopathy

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Although hepatic encephalopathy (HE) has long been recognized as a clinically important manifestation of hepatocellular failure, the precise pathogenesis of this common syndrome remains elusive. Currently, the two factors considered to be most important in the pathogenesis of this syndrome are elevated central nervous system (CNS) levels of ammonia1 and increased γ-aminobutyric acid (GABA)–mediated neurotransmission.2-4 The hypotheses implicating these two factors in the pathogenesis of HE have appeared to be unrelated. Indeed, much research has been directed toward providing evidence that provides support for or detracts from one of these hypotheses, rather than toward determining whether the two hypotheses are interrelated and mutually compatible. To facilitate understanding of the possible interrelationship between ammonia and the enhancement of GABA-ergic neurotransmission in the pathogenesis of HE, brief, current perspectives of the evidence for both of these hypotheses are provided. We then propose a concept that unifies these hypotheses and provides a rationale for treating HE by decreasing brain ammonia concentrations and/or reducing GABA-mediated neurotransmission.

THE AMMONIA HYPOTHESIS

The neurotoxicity of ammonia is well recognized.5,6 The implication of ammonia in the pathogenesis of HE was originally based on the association of increased plasma ammonia levels with impaired mental function in patients with liver failure.7 Although ammonia in plasma readily enters the brain,8 correlations between arterial and venous plasma ammonia levels and the severity of HE are poor,9 and do not necessarily imply a causal relationship.

In an effort to determine the neural mechanisms underlying ammonia-induced neurotoxicity, the effects of ammonia on neuronal electrophysiology have been examined. Ammonia increases the resting membrane potential and inhibits axonal conductance and excitatory postsynaptic potential (EPSP) formation at concentrations of approximately 5 mmol/L.10 At lower concentrations (0.75-1 mmol/L), ammonia inactivates neuronal chloride extrusion pumps, suppresses inhibitory postsynaptic potential (IPSP) formation, and depolarizes neurons.10 These effects of ammonia (at 0.75-1 mmol/L) on single neuron electrophysiology, either in vitro or in vivo, are compatible with the behavioral effects of ammonia observed in both animals and patients with hyperammonemia. Specifically, infusing animals with ammonium salts induces a preconvulsive state, followed by overt seizures.11 Similarly, convulsions commonly occur in the congenital hyperammonemias.6,12 However, these neuroexcitatory actions of ammonia superficially appear to be at variance with a central role for ammonia in the pathogenesis of HE, which is characterized by a global increase in neuronal inhibition, not excitation.2 HE is only rarely associated with seizures in patients with chronic liver disease, and the reported changes in the electroencephalogram and visual evoked responses induced by ammonia are not characteristic of those observed in HE.11,13,14 Although total brain ammonia concentrations may rise above 1.5 mmol/L in stage IV HE (coma),15,16 the suppression of IPSPs and EPSPs may not significantly contribute to the manifestations of HE in its earlier stages (0-III), in which total brain ammonia levels are typically below 1.5 mmol/L.

It is clear that brain ammonia levels increase in liver failure and that ammonia modulates neuronal activity. However, the relationship between the concentrations of ammonia in the various compartments of the CNS, the particular mechanisms by which ammonia alters neurotransmission, and the manifestations of HE in its different stages are not known. Indeed, the mechanisms by which ammonia contributes to the global depression of the CNS in HE remains unclear. Accordingly, an important question is whether ammonia, in concentrations commonly observed in liver failure, can increase neuronal inhibition.

THE GABA-ERGIC NEUROTRANSMISSION HYPOTHESIS

GABA is the principle inhibitory neurotransmitter of the mammalian brain.3 GABA-mediated neurotransmission results from the binding of GABA to its site on the GABA_A receptor chloride (Cl^-) ionophore complex (the GABA_A receptor complex). This binding opens the ion channel and increases Cl^- currents into the neuron. Increased GABA-mediated neurotransmission is associated with impairments of motor function and decreased consciousness.1,2 Two of the cardinal manifestations of HE. The following observations have been cited as support for the hypothesis that increased GABA-mediated neurotransmission contributes to HE: 1) similarities between visual evoked responses in animals with HE and in normal animals rendered encephalopathic by drugs that potentiate GABA-mediated neurotransmission (e.g., pentobarbital, diazepam, muscimol); 3) increased resistance of animals with HE to seizures induced by drugs that decrease GABA-ergic tone (e.g., bicuculline, 3-mercapto propionic acid); and 4) reversal of the behavioral and electrophysiological manifestations of HE by GABA_A receptor complex antagonists (e.g., bicuculline, isopropylcyclophosphoro-thionate, flumazenil).17,21

The search for mechanisms that increase GABA-ergic neurotransmission in liver failure has provided direct and indirect evidence implicating increased levels of natural benzodiazepine receptor agonists as pathogenic factors3,22,23 (Fig. 1). In particular, initial reports of the ability of the benzodiazepine receptor antagonist, flumazenil, to ameliorate HE in an appreciable proportion of patients with cirrhosis or fulminant hepatic failure24,25 have been confirmed in both anecdotal and controlled clinical trials.2,26 Furthermore, increased levels of benzodiazepine receptor agonists have been found in the brains of animal models of fulminant hepatic failure,3 as well as in the brain and body fluids of patients with HE caused by cirrhosis or fulminant hepatic failure.23,27

Contributions of other factors to the activation of GABA-
Enhancement of GABA-Mediated Neurotransmission by Ammonia. At concentrations (0.1-0.5 mmol/L) that have minimal effects on neuronal resting potentials or polarization in vivo, ammonia has recently been shown to facilitate GABA-gated Cl⁻ currents in cultured cortical neurons. These concentrations of ammonia are particularly relevant as they fall within the range of ammonia levels in the cerebrospinal fluid of animal models of hyperammonemia and humans with hepatic failure. The cerebrospinal fluid ammonia levels are most representative of the ammonia concentrations to which the GABA₁ receptors are exposed in the extracellular spaces of the CNS. Ammonia increased the amplitude of the GABA-gated Cl⁻ current to a maximum of about 20% (EC₅₀ = 200 μmol/L), and increased the potency of GABA in gating Cl⁻ channels by 15%. These effects of ammonia were not modulated by the benzodiazepine receptor antagonist, flumazenil, suggesting that the effects were not mediated via the central benzodiazepine receptors on the GABA₁ receptor complex.

The nature of this direct interaction between ammonia and the GABA₁ receptor complex was further investigated using radioligand binding assays. Ammonia (50-500 μmol/L) selectively increased the maximal binding of agonist ligands (e.g., flunitrazepam and muscimol) to the GABA₁ receptor complex by about 30%, but had no effect on the binding of antagonists (e.g., flumazenil, SR 95-531) of the complex. Further increasing ammonia concentrations (750-2,000 μmol/L) returned ligand binding to control levels. Moreover, ammonia and benzodiazepine receptor agonists were found to synergistically enhance the binding of the GABA agonist muscimol to a maximum of 90% above control levels. Thus, ammonia not only directly enhances the ability of GABA to depress neuronal activity, but can further inhibit CNS function by its synergistic interactions with natural benzodiazepine receptor ligands.

Although the mechanisms underlying the dual actions of ammonia on ligand binding to components of the GABA₁ receptor complex are unknown, they resemble those underlying the actions of barbiturates. Ligand binding to the GABA₁ receptor complex is enhanced (to a maximum of 30%) by low concentrations of barbiturates, followed by a reduction in ligand binding to control levels at higher barbiturate concentrations. Together, these biochemical and electrophysiological observations suggest that there is a continuum of neuronal actions of ammonia. Inhibitory neurotransmission would be enhanced by low concentrations of ammonia (150-750 μmol/L), consistent with ammonia contributing to the cognitive and motor deficits observed in the early stages (0-I) of HE. Higher ammonia concentrations (750-2,000 μmol/L) would inactivate Cl⁻ extrusion pumps and suppress IPSP generation, leading to neuronal excitation. The latter phenomenon may contribute to the development of seizures in some patients with HE caused by fulminant hepatic failure, as well as those with congenital hyperammonemias.

CONCLUSIONS: A UNIFYING CONCEPT RELATING TO THE PATHOGENESIS OF HE

The direct actions of moderately elevated concentrations of ammonia can now be added to the list of other, ammonia-independent mechanisms that enhance GABA-ergic neurotransmission in liver failure, and thus contribute to the manifestations of HE (Fig. 1). The evidence discussed above indicates that modest increases in ammonia levels and enhanced GABA-ergic neurotransmission can both play significant roles in the pathogenesis of HE. Consequently, the ammonia hypothesis and the GABA-ergic neurotransmission hypothesis are not mutually exclusive. Indeed, it seems probable that ammonia, in concentrations that commonly occur in patients with acute or chronic liver failure, contributes to the manifestations of HE by directly potentiating inhibitory GABA-ergic neurotransmission and synergistically augmenting the actions of endogenous benzodiazepine receptor agonists. This concept unifies the two hypotheses and serves to explain why some patients with HE may have normal plasma levels of ammonia and why some may not respond to a benzodiazepine receptor antagonist. Moreover, this concept rationalizes treatments for HE that either lower plasma ammonia concentrations (e.g., reduction of colonic sources of ammonia, or administration of sodium benzoate or ornithine) or reduce GABA-ergic tone by ammonia-independent mechanisms (e.g., administration of benzodiazepine receptor antagonists). Most importantly, this unifying concept is entirely consistent with HE being a reversible, metabolic encephalopathy with a multifactorial pathogenesis.
REFERENCES

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