Biochemical mechanisms of hepatic encephalopathy

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Although there are similarities between the encephalopathy of acute hepatic failure and that due to cirrhosis, it is clear that some of the biochemical abnormalities arise in different ways and the relative roles of the various factors contributing to the production of encephalopathy may differ. In addition, in fulminant hepatic failure, changes in the permeability of the blood–brain barrier may enhance the effects of biochemical abnormalities and this may also be important in the genesis of cerebral oedema, which commonly complicates this form of encephalopathy. When considering encephalopathy complicating cirrhosis, it is important to recognize that the latter can be precipitated by a variety of insults each of which may lead to encephalopathy. Most research has concentrated on the accumulation in the blood of ‘toxic’ substances normally metabolized by the liver. Although no single toxin has yet been identified which alone can be responsible for hepatic encephalopathy, excess ammonia, mercaptans, fatty acids and an abnormal plasma amino acid profile have been implicated in its pathogenesis. Irrespective of the ‘toxin’, three main pathogenetic mechanisms have been proposed to account for its action: (a) disturbed brain energy metabolism, (b) deranged neurotransmitter balance, (c) direct effects on neuronal membranes. These mechanisms are not regarded as mutually exclusive but have their final common effect of interrupting normal neurotransmission.

Hyperammonaemia

Reasons for considering ammonia as an important cerebral toxin in hepatic encephalopathy are that blood and cerebrospinal fluid ammonia concentrations are raised in hepatic coma and glutamine and ketoglutarate, products of cerebral ammonia metabolism, are elevated in the cerebrospinal fluid and correlate with coma grade [1, 2]. Ammonia loading in patients with chronic liver disease or in high dosage in animals will produce coma [3] and coma occurs in children with hyperammonaemia due to congenital defects of the urea cycle [4].

The gastrointestinal tract has long been thought to be the major site of ammonia production [5], as a result of bacterial degradation, especially in the colon, of nitrogenous compounds, chiefly dietary protein or urea which diffuses into the intestinal lumen. However, clinical and experimental studies in germ-free animals suggest that non-bacterial sources of ammonia are also important [6, 7]. In the non-fasting state 40% of ammonia from the gastrointestinal tract is produced by bacteria and 60% from small intestinal digestion of dietary protein, and metabolism of circulating glutamine [8, 9]. Liver, muscle, brain, kidney and erythrocytes also possess mechanisms for ammonia production via the purine nucleotide cycle [10]. Kidney and brain, however, appear to be relatively minor sources. Ammonia production by muscle increases arterial ammonia concentration by 10% in patients with liver disease, whereas in normal subjects increases in blood ammonia occur only after heavy exertion [11, 12].

Ammonia is detoxified to urea and glutamine. The former process occurs principally in the liver, and glutamine synthesis occurs in various organs, including kidney and brain. Most urea is excreted by the kidney but about 25% diffuses into the gut and is subsequently hydrolysed to ammonia and reabsorbed [13]. Although the liver metabolizes
most of the ammonia in the portal blood, in the presence of impaired liver function and/or portal systemic shunting, muscle may become an important homoeostatic organ. One study [14] has shown in normal subjects that 50% of arterial ammonia is metabolized by muscle. However, loss of muscle mass is common in chronic liver disease and may contribute to hyperammonaemia [15]. As a consequence of impaired hepatic clearance, ammonia becomes available for cerebral metabolism. With a pK of 9.1, and being 95% un-ionized at physiological pH, ammonia is free to enter cells. Forty-seven per cent of ammonia in arterial blood is extracted in one passage through the brain [14]. It enters the brain largely by diffusion, and is probably metabolized in a small pool of glutamate that is distinct from a larger tissue glutamate pool located possibly in the astrocytes [16].

Disturbances of cerebral energy metabolism

Early studies in patients with hepatic encephalopathy demonstrated a decrease in cerebral oxygen metabolism and ammonia was thought to be responsible since it was thought to be detoxified via the α-ketoglutarate-glutamate-glutamine pathway. Depletion of α-ketoglutarate in the tricarboxylic acid cycle due to its diversion into the glutamine pathway would result in decreased operation of the cycle, with a fall in high energy phosphates and oxygen consumption [17]. A decrease in cerebral α-ketoglutarate, however, has not been confirmed [18] and recent evidence also suggests that glutamine derives from a pool of glutamate that is rapidly turning over, rather than from α-ketoglutarate [16]. Subsequent studies in dogs in coma after hepatectomy did show a reduction in many of the tricarboxylic acid cycle substrates, including α-ketoglutarate, but this was no different from that observed with simple sedation or anaesthesia [19].

Direct measurement of high energy phosphates (adenosine triphosphate and phosphocreatine) in the central nervous system of animals with ammonia-induced coma has yielded conflicting results [18–25]. Such substances are extremely labile and artifactual decreases due to tissue hypoxia during freezing and subsequent separation of the various parts of the brain for analysis are difficult to avoid. More recent experiments using improved fixation techniques in rats with chronic hyperammonaemia after portacaval shunt operations, showed that after an acute ammonia challenge which induced coma, brain-stem concentrations of high energy phosphates decreased but only after 1 h of coma [26]. Thus changes in cerebral energy metabolism could be the result rather than a cause of coma.

Neurotransmitter imbalance

Inhibition of acetylcholine synthesis has been suggested but never substantiated [27, 28]. Accumulation of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter which is derived from glutamate during cerebral ammonia detoxification, has also been incriminated although measurements of brain concentrations of GABA in rats with ammonia toxicity or after liver damage were normal [24, 29]. Hindfelt et al. [26] have demonstrated decreased cerebral concentrations of the excitatory neurotransmitters glutamate and aspartate, in addition to decreased concentrations of some tricarboxylic acid cycle substrates, in rats challenged with ammonia after a portacaval shunt. Such changes occurred during precoma and were followed by alterations in the malate aspartate shuttle of reducing equivalents from cytoplasm to mitochondria, with subsequent alteration of the NAD/NADH ratio within mitochondria. Such changes could ultimately inhibit oxidative energy coupling as a secondary event. Although criticism has been levelled at the calculation used to derive the NAD/NADH ratio [30], this study would provide a link between the early depletion of neurotransmitters which may be the key abnormality in hepatic coma and the late and possibly secondary findings of decreased cerebral energy metabolism.

Direct effects on neuronal membrane Na⁺,K⁺-dependent ATPase

High concentrations of the enzyme are found in the brain, where it is responsible for the maintenance of transmembrane ion gradients which are necessary for normal neuronal activity [31]. Interference with this system may impair membrane repolarization and cause coma, as well as disturbing the functional integrity of the blood–brain barrier. Ammonia has been shown to inhibit neuronal Na⁺,K⁺-ATPase activity by competing with K⁺ [21]. High concentrations of ammonium ions may partly substitute for intracellular sodium and block the outwardly directed chloride pump, which normally maintains a high transmembrane chloride gradient necessary for repolarization [32]. Studies with brain slices in vitro have also shown that ammonia alters its permeability to ions with inhibition of the frequency of spike potentials [33]. Although often
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only a poor correlation can be demonstrated between blood ammonia concentration and degree of coma [34], in terms of toxicity the intracerebral ammonia concentration is likely to be the more important factor. Intracellular levels of ammonia can be 10–15 times the concentrations in blood because migration of ammonia is influenced by pH, lipophilicity and intracellular metabolism. Furthermore, the synergism between ammonia, fatty acids and mercaptans in the experimental production of coma (see below) could well blunt any relationship between the blood concentrations and coma grade.

Amino acid imbalance in blood and brain

Hepatic failure is associated with a considerable increase in plasma concentrations of aspartate, glutamate, phenylalanine, tyrosine, methionine and free tryptophan. Most attention has been focused on phenylalanine and tyrosine because of the precursor relationship to brain catecholamines, and on tryptophan which is converted into the inhibitory neurotransmitter serotonin.

In chronic encephalopathy there is a two- to four-fold rise in the plasma concentrations of the aromatic amino acids, together with a decrease in the plasma concentrations of the branched-chain amino acids valine, leucine, and isoleucine [35]. A combination of catabolism and impaired hepatocellular function is probably responsible. Raised plasma concentrations of glucagon stimulate muscle catabolism with release of amino acids for gluconeogenesis [36]. However, when hepatic function is poor the uptake and metabolism of aromatic amino acids in the plasma are impaired. In contrast, branched-chain amino acids are preferentially metabolized in muscle and fat and their low levels can be explained by their enhanced uptake as a result of the hyperinsulinism [37]. Levels of the aromatic amino acids are considerably raised in fulminant hepatic failure but concentrations of branched-chain amino acids are normal [38]. This pattern is thought to arise largely from massive necrosis of liver cells with release of amino acids into the circulation, and catabolism probably plays a less important role.

Relation to false neurotransmitter hypothesis

Increased intracerebral concentrations of phenylalanine and tyrosine may result in the formation of 'false neurotransmitter' amines, which are thought to displace true neurotransmitters from synaptosomes. Excess tyrosine is decarboxylated to tyramine, which is then converted by dopamine-β-oxidase into octopamine, and phenylalanine is converted into phenylethanolamine and thence into phenylethanolamine. Levels of octopamine and phenylethanolamine were found to be raised in the brains of animals in acute hepatic coma [39]. In addition, phenylalanine competes with tyrosine for the enzyme tyrosine hydroxylase, and tyramine competes with dopamine for dopamine-β-oxidase, with the result that intracerebral formation of the normal stimulatory transmitters (dopamine and noradrenaline) may be reduced [40].

Concentrations of amino acids in the brain depend on the integrity of the blood–brain barrier and the activity of the carrier systems of amino acids as well as on the plasma levels. The neutral amino acids are transported across the blood–brain barrier by a common carrier [41] and there is usually competition between the aromatic and branched-chain amino acids [42]. Thus low circulating levels of branched-chain amino acids, together with their lower affinity constants for the carrier, might be expected to favour transport into the brain of the aromatic amino acids. Concentrations of branched-chain amino acids in the cerebrospinal fluid have been observed to be normal in animals during hepatic coma, despite low plasma concentrations [43], possibly as a result of increased activity of the carrier system [44].

In acute liver failure, the blood–brain barrier is commonly disrupted and may enhance the cerebral effect of plasma amino acid imbalance. Zaki et al. [45], by means of the Oldendorf technique, have shown that the blood–brain barrier is also disrupted in rats 6 weeks after a portacaval anastomosis. Brain uptake of L-[14C]-glucose, D-[14C]-sucrose, and [14C]insulin, substances which do not normally cross the blood–brain barrier, were all increased, in addition to a selective increase in the uptake of neutral amino acids.

The influx of neutral amino acids may be geared to the slow sustained efflux of glutamine [46], a product of ammonia metabolism, hence providing a link between the ammonia toxicity and the false neurotransmitter hypotheses.

Evidence against the false neurotransmitter theory is that octopamine instilled into the ventricles of rats produced a striking increase in its concentration in the brain and a fall in whole brain dopamine and noradrenaline to one-tenth of normal values, without any disturbance in consciousness [47]. Also, in a study in which brain catecholamines were measured post mortem in cirrhotic patients with encephalopathy, no reduction in dopamine or noradrenaline concentra-
tions was found and octopamine levels were decreased compared with cirrhotic patients who were not encephalopathic at the time of death [48].

**γ-Aminobutyric acid**

Neural inhibition in the mammalian brain is principally mediated by the neurotransmitter GABA. Instillation of less than 1 μmol of GABA into the hippocampal region of the brain induces a coma-like state with characteristic encephalographic features [49]. A 12-fold increase in the plasma concentration of GABA has been reported in animal models of fulminant hepatic failure and this is thought to be due to impaired hepatic metabolism of GABA synthesized by gut bacteria [50, 51]. Associated with the development of encephalopathy was an enhanced permeability of the blood–brain barrier and an increased number of binding sites for GABA on postsynaptic neurons within the brain [52]. Thus gut-derived GABA may contribute to the neural inhibition of hepatic encephalopathy, and in liver failure an increased number of binding sites may enhance the cerebral sensitivity to drugs such as barbiturates and benzodiazepam, which share this effector pathway.

**Role of mercaptans, fatty acids and bile acids**

Bacterial metabolism of methionine in the gut produces methanethiol (methyl mercaptan), which has long been recognized as a cerebral toxin. Blood methanethiol concentration in cirrhotic patients with encephalopathy was found to be significantly higher (975 ± 64 pmol/l) than in non-encephalopathic patients with cirrhosis (636 ± 29 pmol/ml). Furthermore, blood levels correlated with the degree of encephalopathy [53]. Experimental studies in animals have shown that mercaptans induce reversible coma, albeit at higher concentrations (5000 pmol/ml) [54]. Studies using low concentrations of methanethiol (0.03 μmol/l) in vitro have demonstrated inhibition of microsomal Na+,K+-ATPase activity. Serum concentrations of short-chain fatty acids are raised four-to-five-fold in hepatic coma [55] and have been shown to cause coma in animals [56]. Studies in vitro have demonstrated that low concentrations inhibit Na+,K+-ATPase in brain homogenates and microsomes [57–59], probably by competitive inhibition of K⁺, unlike mercaptans which are thought to inhibit the Na⁺-dependent portion of the reaction [60]. No correlation has been found between the serum concentrations of short-chain fatty acids and the clinical course of patients with fulminant hepatic failure [61]. However, at pathological concentrations both mercaptans and free fatty acids interfere with ammonia detoxification in the urea cycle, and in addition act synergistically with ammonia to produce coma at blood concentrations lower than those required for each substance individually [54]. Free fatty acids also displace tryptophan from its binding sites on albumin, thus raising the availability of free tryptophan to the brain, which may enhance cerebral serotonin formation [62]. Methionine may also be metabolized to taurine, which may cause cerebral dysfunction [63].

Free phenol concentrations are increased in the plasma in hepatic encephalopathy complicating fulminant hepatic failure [64]; they are lipophilic compounds derived from phenylalanine and tyrosine. They, too, have been shown to be inhibitors of Na⁺,K⁺-ATPase as well as many other membrane-bound enzymes [65] and on a molar basis are more toxic than ammonia or free fatty acids [66].

Raised concentrations of bile salts have been found in the serum, cerebrospinal fluid and brain of patients in fulminant hepatic failure [67]. Bile acids are toxic in many biochemical systems and in vitro cause uncoupling of oxidative phosphorylation [68, 69]. However, the concentrations of serum bile acids found in fulminant hepatic failure were similar to those found in chronic liver disease without encephalopathy, and indeed lower than concentrations found to inhibit brain respiration in vitro. Protein binding in vitro will reduce toxic effects; in fact, patients with cholestasis seem to suffer only from intractable itching.

**Middle molecules**

Improvement of encephalopathy in patients with fulminant hepatic failure after haemodialysis with polyacrylonitrile membrane as opposed to the cuprophane membrane suggested that substances in the middle molecular weight range (500–5000) may be involved in the pathogenesis of hepatic encephalopathy, since the polyacrylonitrile membrane was more permeable to larger solutes. With Sephadex G15 gel filtration, substances as yet uncharacterized, with molecular weights within this range, have been identified in the sera of patients with fulminant hepatic failure and chronic encephalopathy [70] and also in the plasma and brain of animals with acute hepatic failure [71]. Further studies have shown that the serum of patients with fulminant hepatic failure inhibits leucocyte plasma membrane Na⁺,K⁺-
ATPase and that the main inhibitory components appeared in peaks 3, 4 and 5 (the middle molecular weight range) of the ultrafiltrate [72]. Haemodialysis with the polyacrylonitrile membrane removed the middle molecular weight peaks [72]. A similar inhibitory mechanism in the brain might explain a defect in neurotransmission as well as the development of cerebral oedema.

Conclusions

Current evidence would seem to favour neurotransmitter imbalance or direct effects on neuronal membrane function as the main mechanism of hepatic encephalopathy. Further work in the field of toxic 'middle molecular weight' pathology is multifactorial, as shown by the demonstrated synergistic toxic effects of ammonia, fatty acids, mercaptans and phenols. Furthermore, the effect(s) of the toxic factor(s) are likely to be moderated by endogenous metabolic abnormalities such as hypoxia, hypoglycaemia and acid–base and electrolyte abnormalities.

References


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