Review Article

THE PATHOGENESIS OF HEPATIC ENCEPHALOPATHY

(Received 23 May, 1997)

C. Yurdaydın M.D.*

* *Professor, Sub-department of Gastroenterology, Department of Internal Medicine, Faculty of Medicine, Ankara University, Ankara, Turkey.*

A careful reader of manuscripts on the pathogenesis of hepatic encephalopathy (HE) will notice that most of them state in their introduction that its pathogenesis is "poorly understood". This may indeed be the case but progress has been made, and this review will try to provide some new developments in the field as well as recapitulate the classic knowledge on the pathogenesis of HE.

The modern pathophysiological concept of HE comes from the landmark paper by Dame Sheila in the 50's (1). Accordingly, under normal conditions 'neuroactive' nitrogenous substances derived from the intestine would be efficiently extracted and metabolized by the liver whereas in liver failure, these substances would tend to bypass the liver, as a consequence of impaired extraction and/or portal-systemic collateral venous channels, and accumulate in peripheral blood plasma (Fig. 1). However, credit must be given to William Shakespeare (1564-1616) who in the "Twelfth Night" quite nicely describes the same concept in the following words by one of the characters in the play: " I am a great eater of beef, and I believe that this harms to my wit."

The classical hypotheses of the pathogenesis of HE are listed below:

1. Ammonia Hypothesis
2. Synergistic Neurotoxin Hypothesis and Serotonin
3. False Neurotransmitter Hypothesis
4. Gamma aminobutyric acid (GABA) Hypothesis
5. A Hypothesis implicating Glutamate

1. Ammonia Hypothesis:

Ammonia very much follows the route which Dame Sheila had suggested (1). It is a gut derived neurotoxin. It is metabolized and detoxified by the liver. In serious liver disease with portacaval shunting it escapes degradation in the liver to urea and glutamine, and accumulates in the systemic

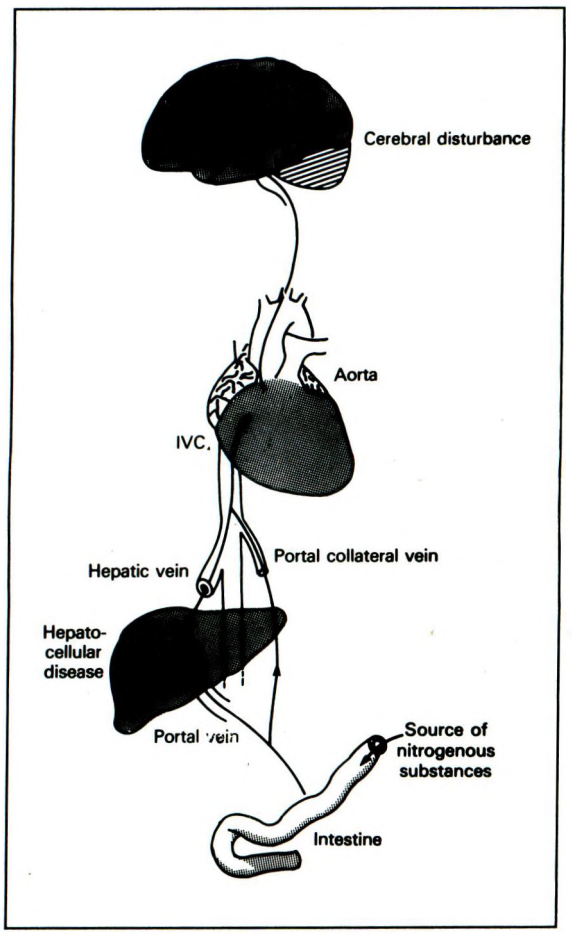

Fig.1: Diagrammatic representation of the traditional concept of the development of hepatic encephalopathy in a patient with advanced liver disease. Under normal physiological conditions nitrogenous substances arising from the gut reach the liver through the portal venous system and are detoxified. In a patient with serious liver disease nitrogenous substances may be inefficiently extracted by the liver. In addition, these substances may bypass the liver through portosystemic collaterals. Consequently, toxic nitrogenous substances accumulate in the systemic circulation and may enter the central nervous system where they may modulate brain function.

circulation. As a non-polar substance, it readily crosses the blood-brain barrier and enters the central nervous system.

What is the effect of ammonia at the neuronal level? Ammonia impairs postsynaptic neural inhibition (2). At the inhibitory postsynaptic neuron, normally gamma aminobutyric acid (GABA) would lead to the opening of the chloride channel (Fig. 2). Since there is a gradient between extracellular and intracellular Cl^-, the extracellular Cl^- concentration being higher, Cl^- influx into the postsynaptic neuron occurs, intracellular negative charges increase and hyperpolarisation is produced, the basis of GABAergic inhibitory neurotransmission. When GABA's action on the postsynaptic receptor is terminated chloride channels close. Excess Cl^- in the postsynaptic neuron is removed towards the extracellular space through a transport system. Ammonia inactivates the Cl^- extrusion. As a consequence, the opening of Cl^- channels by GABA no longer causes an influx of Cl^- into the neuron hence impairing postsynaptic neural inhibition (2).

Ammonia also affects excitatory neurotransmission by blocking the conduction of action potentials into the presynaptic terminals. However, excitatory neurotransmission may only be affected by ammonia intoxication when central nervous system tissue levels of ammonia exceed those necessary to affect postsynaptic inhibition (2).

What makes the ammonia hypothesis attractive? Ammonia, as mentioned above, accumulates in liver failure and its uptake by the brain is increased. It causes encephalopathy (3). Therapies leading to reduced intestinal absorption of ammonia ameliorate HE (3). Ammonia also contributes to the accumulation in the brain of other substances implicated in the pathogenesis of HE such as tryptophan (4) and aromatic amino acids (5).

However, ammonia is unlikely to be the only answer for a couple of reasons, some of which are listed below. Plasma ammonia levels correlate poorly with the stages of HE (6). Experimental hyperammonemia is characterized by a preconvulsive state and coma

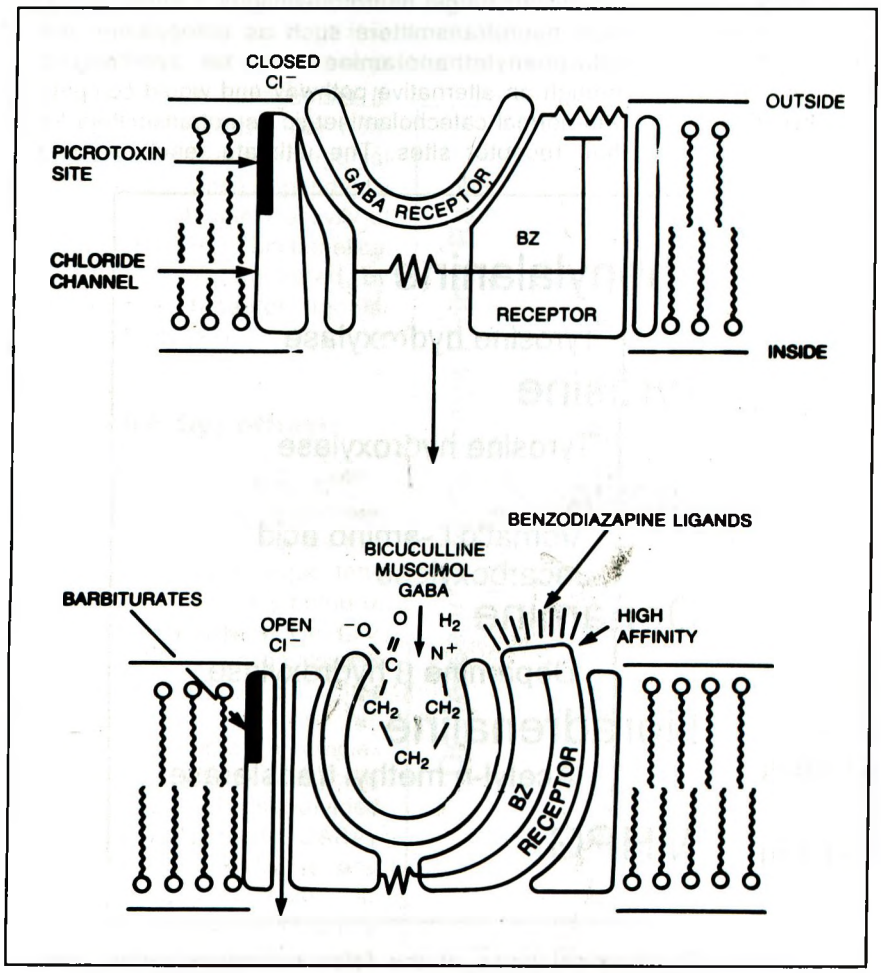

Fig.2:

Diagrammatic representation of the $GABA_A$ / benzodiazepine receptor/chloride ionophore complex of a postsynaptic neuron. Receptors are depicted for GABA, picrotoxin (the barbiturate binding site) and benzodiazepines. (A) The receptor complex in the unactivated state with the Cl^- channel closed. (B) The receptor complex in the activated state with the Cl^- channel open. Activation is induced by GABA or GABA agonists binding to GABA receptors or barbiturates or benzodiazepine agonists interacting respectively with their specific receptors in the presence of GABA with resultant opening of the chloride channel. Cl^- moves from the synaptic cleft across the cell membrane to the cytoplasm and leads to transformation of the cell membrane from a depolarized state to a hyperpolarized state with resultant mediation of inhibitory neurotransmission (From Paul SM, et al: Biol Psychiatr 1981; 16:213-229).

occurs only after seizure activity. Seizures are common in the congenital hyperammonemia syndromes but are unusual in HE (6).

Very recent data suggest that ammonia at concentrations that have minimal effects on neuronal resting potentials or polarization in vivo and that commonly occur in patients with HE, contributes to the manifestations of HE by directly potentiating inhibitory GABAergic neurotransmission and synergistically augmenting the actions of endogenous benzodiazepine receptor agonists (7-9).

2. Synergistic Neurotoxin Hypothesis

This hypothesis implicates methionine derived mercaptans, such as methanethiol and dimethyl disulfide, phenolic compounds and fatty acids in conjunction with ammonia in the pathogenesis of HE. Mercaptans are derived from methionine by enteric bacterial metabolism and phenolic compounds arise through the catabolism of aromatic amino acids such as tyrosine and phenylalanine. It was postulated that the combination of these substances at subencephalopathic levels could induce coma as a consequence of their synergistic effects on the central nervous system. For a detailed description of this hypothesis the reader is referred to a review on this subject by a leading proponent of this hypothesis (10).

More recent studies have questioned the role of mercaptans using more refined methodology (11, 12). Visual evoked potential changes obtained with synergistic neurotoxins were also found to be not consistent with those obtained in HE (13, 14).

3. False Neurotransmitter Hypothesis and Serotonin

This hypothesis is based on the accumulation during HE of aromatic amino acids in the brain. In liver failure, the ratio of branched chain amino acids (BCAA) to those of aromatic amino acids (AAA) decreases. BCAA's and AAA's compete for a common transport carrier at the blood brain barrier. The decreased BCAA/AAA ratio together with the increased efflux of glutamine from the brain as a result of increased cerebral ammonia metabolism are thought to be responsible for the accumulation of AAA's such as phenylalanine, tyrosine and tryptophan (5). Thyrosine at high concentrations may inhibit tyrosine hydroxylase, the key enzyme for the synthesis of dopamine and noradrenaline (Fig. 3) with resultant decrease of the synthesis of catecholaminergic neurotransmitters. Consequently, false neurotransmitters such as octopamine and beta-phenylethanolamine may be synthesized through an alternative pathway and would compete with normal catecholaminergic neurotransmitters for their receptor sites. The ultimate result of such

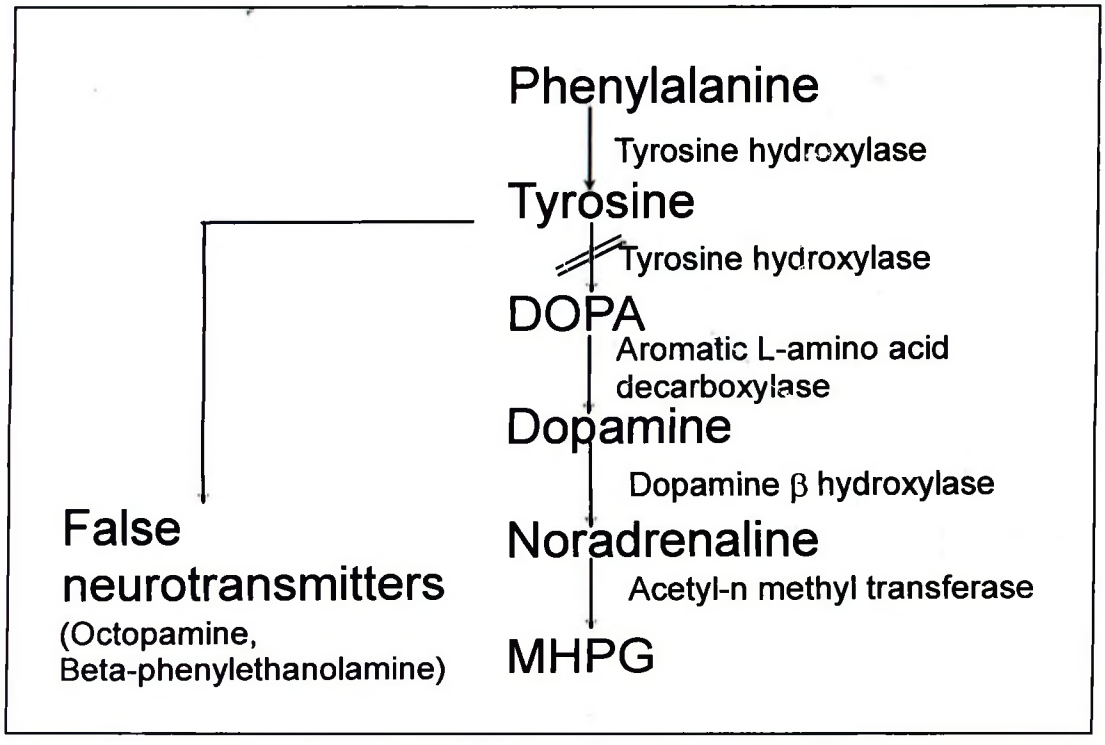

Fig.3: Schematic representation of the biochemical basis of the false neurotransmitter hypothesis MHPG (3- Methoxy - 4- Hydroxi Phenyl/Glycol)

changes was postulated to be decreased noradrenergic and dopaminergic neurotransmission contributing to the neural inhibition in HE (15).

Although theoretically an attractive hypothesis it remains unproven. For example, intraventricular administration of octopamine caused no obvious change in consciousness (16). Furthermore, therapies of HE based on the false neurotransmitter hypothesis such as the use of branched chain amino acids (17), L-dopa and bromocriptine (18-20) have not convincingly shown a beneficial effect.

One of the best studies neurotransmitters in the pathogenesis of HE is serotonin. It is synthesized from the aromatic amino acids trytophan in two steps. It has been consistently shown that HE is associated with increased brain levels of the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) and normal or slightly increased serotonin concentrations (21). 5-Hydroxytrytophan is also increased suggesting increased serotonin turnover in HE (22). However, enhanced serotonin metabolism may be without changes in serotonin release into the synaptic cleft (23, 24). Furthermore, in studies using in vivo brain dialysis methodology, serotonin levels in extracellular space were found not to be increased (25). A simple approach to circumvent these problems could be the use of serotonin antagonists. In a preliminary study, the non-selective serotonin antagonist methysergide dose dependently increased ambulatory activity of rats with HE (26). This benefical effect may be mediated by serotonin1A receptor agonist ligand activity (27).

4. GABA hypothesis

The GABA hypothesis of the pathogenesis of HE, developed in the early 80's, suggests that an increased GABAergic tone contributes to at least some of the manifestations of HE (28-30). The GABA/benzodiazepine receptor (BZR)/chloride ionophore complex is an oligomeric glycoprotein complex that has been pharmacologically and biochemically subdivided into three components: $GABA_A$ receptors, central BZRs and chloride ionophores. These units are allosterically linked to form a "supramolecular" complex (Fig. 2). Binding of GABA to the $GABA_A$ receptor increases neuronal membrane permeability to Cl^- by opening the Cl^- ionophore. Cl^- entering the neuron cause membrane hyperpolarization. This prevents neuronal membrane depolarization in response to other synaptic events and hence causes neural inhibition. This phenomenon is the basis of GABAergic inhibitory neurotransmission. BZR agonists (e.g. diazepam) increase the frequency of GABA gated Cl^- channel openings.

Theoretically, an increased GABAergic tone could be due to non-humoral factors such as changes in the status of the chloride ionophore or the GABA/BZR or to humoral factors (30). Available evidence is against an involvement of non-humoral factors but humoral factors could play a role. Two types of humoral factors, GABA receptor and/or BZR agonists, may be involved (29,31, 32).

There is in vitro and in vivo evidence supporting the GABA hypothesis. For example, Basile et al (33) have shown that the spontaneous activity of Purkinje neurons from rabbits with HE was 3-5 times more sensitive to depression by the GABA mimetic muscimol and the benzodiazepine agonist flunitrazepam than neurons from control animals (Fig. 4). The visual evoked response changes in animal

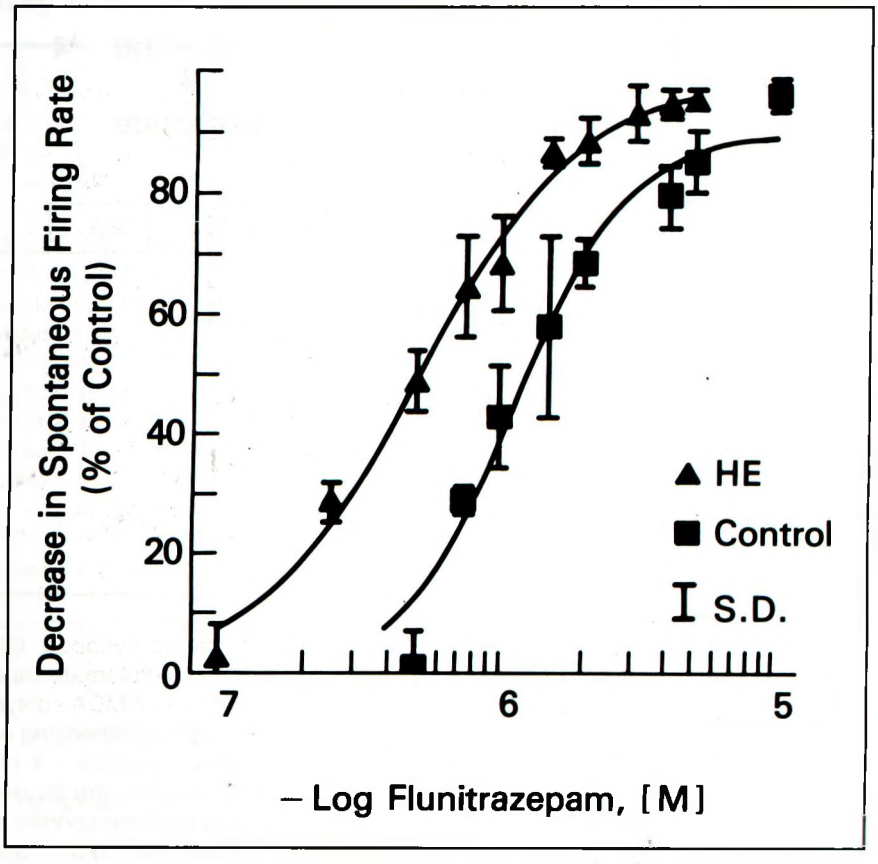

Fig.4: Hypersensitivity of Purkinje neurons from rabbits with HE compared to normal rabbits to depression by flunitrazepam

models of HE are similar to those associated with encephalopaties induced by drugs which increase GABAergic tone (34). BZR ligands are increased in animal models of HE and human HE and correlate with the stages of HE (35, 36). Another support for the GABA hypothesis comes from human studies with the BZR antagonist where flumazenil was partly beneficial (37-39).

What are the problems with the GABA hypothesis? First, evidence for increased BZR ligand levels have not been found in all studies (40, 41). Brain levels of benzodiazepines may not be sufficient to account for manifestations of HE. Origin of BZR ligands is still not known although indirect evidence for the synthesis of BZR ligands in the brain from BZR ligand precursors has been provided (42). Finally, despite intensive research efforts most of the BZR ligands are still unknown.

5. A HYPOTHESIS IMPLICATING GLUTAMATE:

There is a recent renewed interest to the potential role of the glutamatergic neurotransmitter system in the pathogenesis of HE. Glutamate is the principle excitatory neurotransmitter. It is synthesized in the presynaptic neuron from glutamate , stored in presynaptic vesicles and then released into the synaptic cleft (Fig. 5). Glutamate may then act on postsynaptic receptors or reuptake into the perineuronal astrocyte occurs. Here glutamate is transformed to glutamine. Hence, glutamate serves as a metabolic precursor as well as a neurotransmitter. While glutamate present in the neuronal compartment can be biologically active, glutamate in the glial compartment is not. Based on neurochemical studies in which decreased glutamate levels were found, it was suggested that an

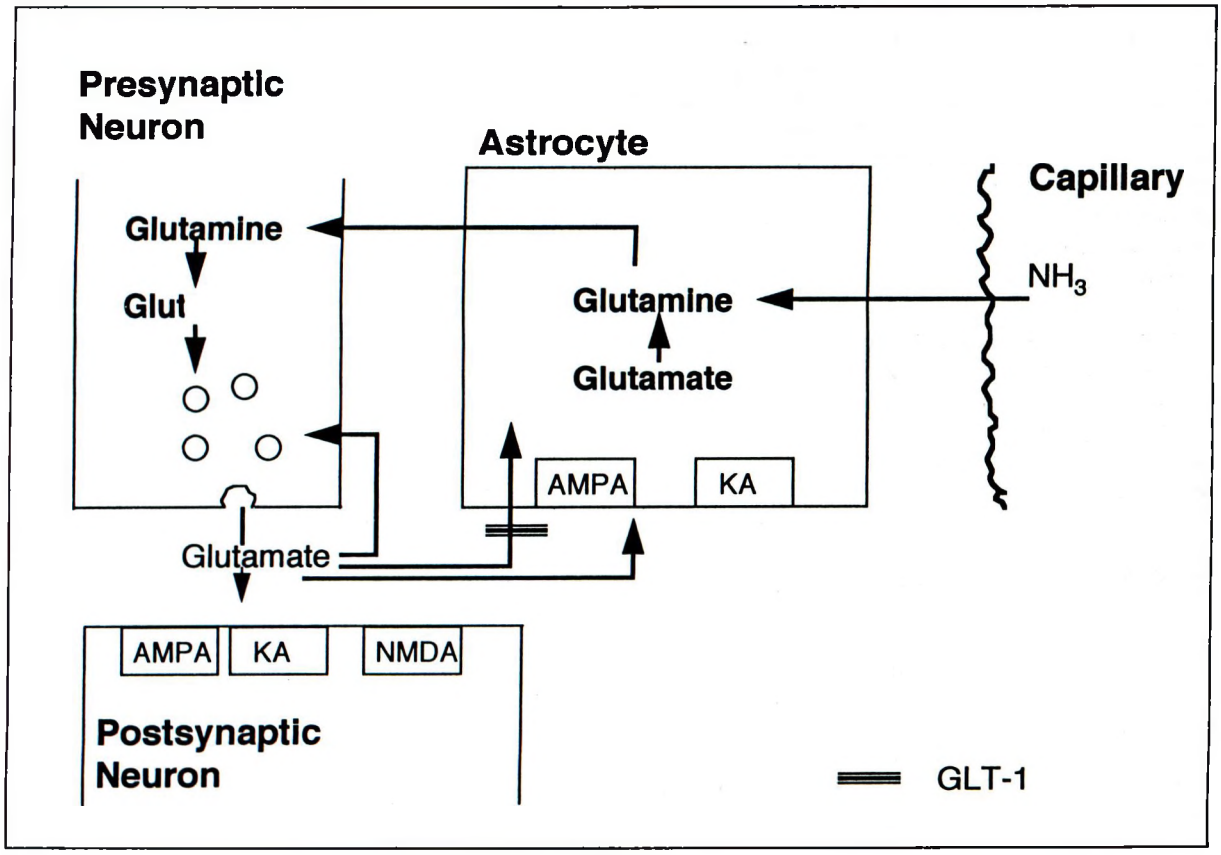

Fig.5: Diagrammatic illustration of the glutamatergic synapse. Glutamate released from the presynaptic neuron binds to postsynaptic or astrocytic glutamate receptors. Glutamate receptors are subdivided into N-Methyl-D-Aspartate (NMDA) or non-NMDA subtypes according to their affinity for NMDA. The non-NMDA receptors are further classified depending upon their affinities to kainate and AMPA. (Alpha - amino - 3 hydroxy - 5 methylisoxazole - 4 propionic acid) While NMDA receptors are located only neuronally, non-NMDA receptors are located on neuronal as well as on astrocytic membranes. Glutamate in the synaptic pool is transported to the presynaptic neuron or astrocyte by specific transporters of which the astrocytic glutamate transporter GLT-1 is well characterized (see text).

imbalance between excitatory and inhibitory neurotransmission may be responsible for the neural inhibition of HE (43) . However, those measurements were not able to differentiate between the biologically active and inactive glutamate.

Recent studies using in vivo brain dialysis may provide more insight. Using this methodology increased extracellular glutamate levels were found in experimental ischemic liver failure (44-46). These increased levels may be secondary to reduction in gene expression of the recently cloned and sequenced astrocytic glutamate transporter GLT-1 (47). Hence, increased glutamatergic neurotransmission may contribute to the pathogenesis of HE in acute liver failure. How this can be reconciled with the neural inhibition of HE accompanying cirrhosis is unanswered.

NEWER HYPOTHESES OF HEPATIC ENCEPHALOPATHY

1. Hepatic Encephalopathy and the Opioid System
2. Hepatic Encephalopathy and Melatonin
3. Hepatic Encephalopathy and Manganese
4. Hepatic Encephalopathy and Osmolytes

1. Hepatic Encephalopathy and the Opioid System:

Patients with liver cirrhosis are hypersensitive to neuroinhibitory effects of morphine (48). Increased levels of opioid receptor ligands have been reported in acute and chronic liver disease (49, 50). Opioid peptides interact with a wide variety of neurotransmitters (51) most of which are implicated in the pathogenesis of HE (52). This background information justifies to study a possible contribution of the opioid system to the manifestations of HE. Recent studies support such a contribution. Indeed, in a rat model of HE, changes of opioid ligands, especially ß-endorphin in the hypothalamo-pituitary-adrenal axis and met-enkephalin in the striatum, have been shown to occur in HE (53). The latter is associated with down regulation of the density of delta opioid receptors, and may contribute to the motor inactivity of HE. This suggestion is supported by the opioid receptor antagonist naloxone-induced ameliorations of HE in this rat model of fulminant hepatic failure (53). Elevated met-enkephalin levels have also been observed in plasma and cerebrospinal fluid of patients with HE (54).

2. Hepatic Encephalopathy and Melatonin:

Frequent complaints of patients with cirrhosis and subclinical hepatic encephalopathy are sleep disturbances, i.e., alterations of the sleep/wake cycle or the inability to sleep during the night. The timing of sleep is a circadian function. This circadian function is regulated by a circadian pacemaker located in the suprachiasmatic nucleus of the anterior hypothalamus, the "biological clock" (55). The hormone melatonin secreted from the pineal gland is considered to be the output signal of the "biological clock" (56). It was suggested that the sleep disturbances of cirrhotics may be secondary to an abnormality in the circadian clock.

A marked alteration of the rhythm of plasma melatonin was found in patients with cirrhosis and subclinical hepatic encephalopathy (57) supporting the hypothesis that an alteration of the circadian rhythmicity is responsible for the alteration of the sleep/wake cycle seen in cirrhosis. Neurochemical changes initiating HE may be responsible for the alterations of the circadian rhythm or alternatively a decreased hepatic clearance of melatonin with resultant high endogenous melatonin levels may by itself alter the circadian system (57). Recent data suggest the latter (58, 59). Furthermore, sleep disturbances may be a consequence of the anxiety and depression of chronic disease and not related to changes in the circadian rhythm (60).

3. Hepatic Encephalopathy and Manganese:

Hyperintense globus pallidi on T1-weighted magnetic resonance imaging is a reproducible finding observed in the majority of cirrhotic patients (Fig. 6) (61-66).

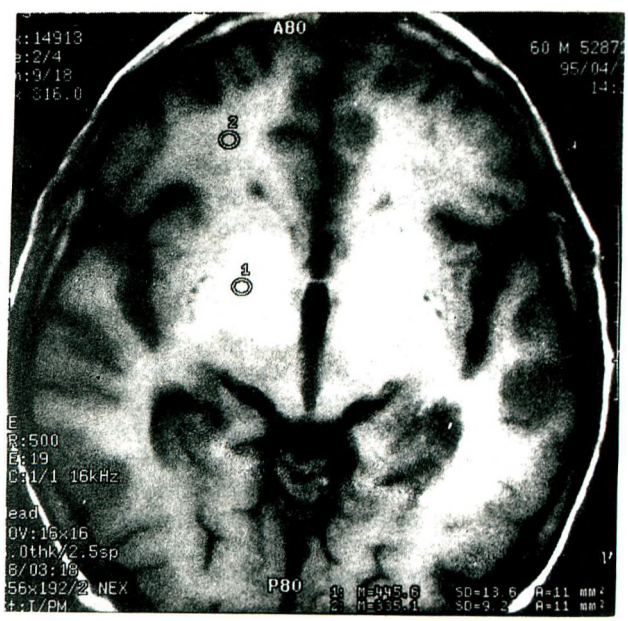

Fig.6: Representative nuclear magnetic resonance image of a patient with decompansated cirrhosis showing hyperintense signals in the globus pallidi

Substantial evidence suggests that manganese deposition is the cause of pallidal MRI signal hyperintensity (67, 68). Manganese is absorbed from the gut into the portal vein and is eliminated by the liver via biliary excretion (69). This explains why manganese accumulates in liver disease. Manganese toxicity has been studied in miners exposed to manganese dust and experimentally in primates and is known to cause extrapyramidal symptoms and basal ganglia lesions (70, 71). Intensity of pallidal signal hyperintensity was correlated with severity of liver disease, grade of encephalopathy, and plasma ammonia level. These studies revealed inconsistent results (63-65). However, a significant correlation was found between pallidal signal hyperintensity and the degree of portosystemic shunting (61) and the extrapyramidal symptoms (66). Hence, manganese may be responsible for the extrapyramidal symptoms of HE. Furthermore, since manganese intoxication in nonhuman primates results in Alzheimer type II astrocytosis (72) similar to what is observed in human HE manganese may contribute to the astrocytic changes of HE.

4. Hepatic Encephalopathy and Osmolytes

Osmolytes are osmotically active substances. If a cell is exposed to a hyperosmotic medium, osmolytes accumulate in the cell to preserve cell volume homeostasis (73). Myo-inositol and glutamine are important osmolytes in astrocytes. In recent years, proton magnetic resonance spectroscopy has been widely used for semiquantitative assessment of brain metabolites. An almost universal finding was a decrease of myo-inositol and an increase in glutamine signal in patients with HE (74). The increase in glutamine, most likely due to intraastrocytic accumulation of glutamine as a result of hyperammonemia, may have been compensated with the decrease of myo-inositol. It has been suggested that changes in the hydration state, especially cell swelling may contribute to the pathophysiology of HE, through effects of astrocyte swelling on glial nerve communications (74). Interestingly, in patients with fulminant hepatic failure the glutamine increase was not found to be associated with a compensatory myo-inositol decrease (75) which may suggest that organic osmolytes may contribute to the brain edema of acute liver failure. It has to be remembered that these studies do not cover all osmolytes in the body. In addition, the duration of the anisosmotic condition also plays a role in the accumulation of osmolytes (76).

CONCLUSION

The precise mechanism(s) of the pathogenesis of hepatic encephalopathy is still not known. Among the many factors suggested to contribute to the manifestations of HE ammonia and GABA are probably the most important ones. Hence the possible cooperative action of these two key factors to the neurobiology of HE is an interesting new venue which has to be further investigated. Nevertheless, there is enough reason to believe that other neurotransmitters such as serotonin, glutamate, opioid peptides and other substances may contribute to the broad spectrum symptomatology of HE, and that HE is a syndrome of multifactorial pathogenesis.

REFERENCES

1. *Sherlock S, Summerskill WHJ, White LP, Phear EA. Portal-systemic encephalopathy: neurological complications of liver disease. Lancet 1954; 2:453-457*
2. *Raabe WA. Neurophysiology of ammonia intoxication. In: Butterworth RF, Pomier Layrargues G, eds. Hepatic encephalopathy: Pathophysiology and Treatment. New Jersey: Humana Press, 1989: 49-78*
3. *Butterworth RF. Pathogenesis and treatment of portal-systemic encephalopathy: an update. Dig Dis Sci 1992; 37:321-327*
4. *Grippon P, Le Poncin-Lafitte M, Boschat M, et al. Evidence for the role of ammonia in the intracerebral transfer and metabolism of tryptophan. Hepatology 1986; 6:682-686*
5. *James JH, Zipparo V, Jepsson B. Hyperammonemia, plasma amino acid imbalance and blood brain amino acid transport: a unified theory of portal systemic encephalopathy. Lancet 1979; 2;772-775*
6. *Yurdaydın C, Jones EA. Hepatic encephalopathy. In: Gitnick, G. ed. Principle and Practice of Gastroenterology and Hepatology, 2nd edition. Elsevier Science Publishing Co., Inc. 1994: 985-995*
7. *Takahashi K, Kameda H, Kataoka M, et al. Ammonia potentiates GABAA response in dissociated rat cortical neurons. Neurosci Lett 1993; 151:51-54*
8. *Ha J-H, Basile AS. Modulation of ligand binding to components of the GABAA receptor complex by ammonia: implications for the pathogenesis of hyperammonemic syndromes. Brain Res 1996; 720:35-44*
9. *Basile AS, Jones EA. Ammonia and GABA-ergic neurotransmission: interrelated factors in the pathogenesis of hepatic encephalopathy. Hepatology 1997; 25:1303-1305*

10. Zieve L. Pathogenesis of hepatic encephalopathy. Metab Brain Dis 1987; 2:147-165
11. Blom HJ, Chamuleau RAFM, Rothuizen J, et al. Methanethiol metabolism and its role in the pathogenesis of hepatic encephalopathy in rats and dogs. Hepatology 1990; 11:682-689
12. Blom HJ, Ferenci P, Grimm G, et al. The role of methanethiol in the pathogenesis of hepatic encephalopathy. Hepatology 1991; 13:376-379
13. Pappas SC, Ferenci P, Schafer DF, Jones EA. Visual evoked potentials in a rabbit model of hepatic encephalopathy: II. Comparison of hyperammonemic encephalopathy, postictal coma and coma induced by synergistic neurotoxins. Gastroenterology 1984; 86:546-551
14. Jones DB, Mullen KD, Roessle M, et al. Hepatic encephalopathy: application of visual evoked responses to test hypotheses of its pathogenesis in rats. J Hepatol 1987; 4:118-126
15. Fischer JE, Baldessarini RJ. False neurotransmitters and hepatic failure. Lancet 1971; 2:75-80
16. Zieve L, Olsen RL. Can hepatic coma be caused by a reduction of brain noradrenalin or dopamin? Gut 1977; 18:688-691
17. Eriksson LS. The cons of BCAA in patients with cirrhosis and acute hepatic encephalopathy. In: Capocaccia L, Merli M, Riggio O (Eds). Advances in hepatic encephalopathy and metabolic nitrogen exchange. Boca Raton, FL: CRC Press 1995:543-548
18. Michel H, Solere M, Grainer P. Treatment of cirrhotic hepatic encephalopathy with l-dopa: a controlled trial. Gastroenterology 1980; 79:207-211
19. Morgan M, Jakobovits AW, James M, et al. Succesful use of bromocriptine in the treatment of hepatic encephalopathy. Gastroenterology 1980; 78:663-670
20. Uribe M, Farca A, MarquezMA, et al. Treatment of chronic portal systemic encephalopathy with bromocriptine. Gastroenterology 1979; 76:1347-1351
21. Yurdaydın C, Hörtnagl H, Steindl P, et al. Increased serotoninergic and noradrenegic activity in hepatic encephalopathy in rats with thioacetamide-induced acute liver failure. Hepatology 1990; 12:695-700
22. Bugge M, Bengtsson F, Nobin A, Jeppsson B, Herlin P. The turnover of brain monoamines after total hepatectomy in rats infused with branched chain amino acids. World J Surg 1987; 11:810-7
23. DeSimoni MG, Sokola A, Fodritto F, Dal Toso G, Algeri S. Functional meaning of tryptophan-induced increase of 5-HT metabolism as clarified by in vivo voltametry. Brain Res 1987; 411:819-24
24. Lookingland KJ, Shannon NJ, Chapin DS, Moore KE. Exogenous tryptophan increases synthesis, storage, and intraneuronal metabolism of 5-hydroxytryptamine in the rat hypothalamus. J Neurochem 1986; 47:205-12
25. Bergquist PBF, Vogels BAPM, Bosman DK, et al. Neocortical dialysate monoamines of rats after acute, subacute, and chronic liver shunt. J Neurochem 1995; 64:1238-1244
26. Yurdaydın C, Herneth AM, Püspök A, Steindl P, Singer EA, Ferenci P. Modulation of hepatic encephalopathy in rats with thioacetamide-induced acute liver failure by serotonin antagonists. Eur J Gastroenterol Hepatol 1996, 8:667-671
27. Herneth AM, Steindl P, Ferenci P. Central acting serotonin (5-HT) ligands which ameliorate hepatic encephalopathy require partial 5-HT1A agonistic properties (Abstract). Hepatology 1994; 20:108A
28. Schafer DF, Jones EA. Hepatic encephalopathy and the g-aminobutyric acid neurotransmitter system. Lancet 1982; 1:18-20
29. Basile AS, Jones EA, Skolnick P. The pathogenesis and treatment of hepatic encephalopathy: evidence for the involvement of benzodiazepine receptor ligands. Pharm Rev 1991; 43:27-71
30. Jones EA, Yurdaydın C, Basile AS. The GABA hypothesis-state of the art. Adv Exp Biol Med 1994; 368:89-101
31. Albrecht J, Rafalowska U. Enhanced potassium-stimulated gamma-aminobutyric acid release by astrocytes derived from rats with early hepatic encephalopathy. J Neurochem 1987; 49:9-11
32. Wysmyk U, Oza SS, Saransaari P, Albrecht J. Enhanced GABArelease in cerebral cortical slices from rats with thioacetamide-induced hepatic encephalopathy. Neurochem Res 1992; 17:1187-1190
33. Basile AS, Gammal SH, Mullen KD, et al. Differential responsiveness of cerebellar Purkinje neurons to GABA and benzodiazepine receptor ligands in an animal model of hepatic encephalopathy. J Neurosci 1988; 8:2414-2421
34. Schafer DF, Pappas SC, Brody LE, et al. Visual evoked potentials in a rabbit model of hepatic encephalopathy: I. Sequential changes and comparisons with drug-induced comas. Gastroenterology 1984; 86:540-545
35. Yurdaydın C, Gu Z-Q, Nowak G, et al. Benzodiazepine receptor ligands are elevated in an animal model of hepatic encephalopathy: relationship between brain concentration and severity of encephalopathy. J Pharmacol Exp Ther 1993; 265:565-571
36. Basile AS, Harrison PM, Hughes RD, et al. Relationship between plasma benzodiazepine

receptor ligand concentration and severity of hepatic encephalopathy. Hepatology 1994; 19:112-121
37. Grimm G, Ferenci P, Katzenschlager R, et al. Improvement in hepatic encephalopathy treated with flumazenil. Lancet 1988; 2:1392-1394
38. Bansky G, Meier PJ, Riederer E, et al. Effects of the benzodiazepine receptor antagonist flumazenil in hepatic encephalopathy. Gastroenterology 1989; 97:744-750
39. Pomier-Layrargues G, Giguere J-F, Lavoie J, et al. Flumazenil in cirrhotic patients in hepatic coma: a randomized double-blind placebo-controlled crossover trial. Hepatology 1994; 19:32-37
40. Püspök A, Hernerh AM, Steindl P, ferenci P. Hepatic encephalopathy in rats with thioacetamide-induced acute liver failure is not mediated by endogenous benzodiazepines. Gastroenterology 1993; 105:851-857
41. Widler P, Fisch HU, Schoch P, et al. Increased benzodiazepine-like activity is neither necessary nor sufficient to explain acute hepatic encephalopathy in the thioacetamide-treated rat. Hepatology 1993; 18:1459-1464
42. Yurdaydın C, Walsh TJ, Engler HD, et al. Gut bacteria provide precursors of benzodiazepine receptor ligands in a rat model of hepatic encephalopathy. Brain Res 1995; 679:42-48
43. Schenker S, Brady CE. Pathogenesis of hepatic encephalopathy. In: Conn HO, Bircher J, eds. Hepatic encephalopathy: Management with lactulose and related carbohydrates. Medi-Ed Press, East Lansing, Michigan: Medi-ed Press, 1988:15-30
44. Bosman DK, Neutz NEP, Maas MAW, et al. Amino acid release from cerebral cortex in experimental acute liver failure, studied by in vivo microdialysis. J Neurochem 1992; 59:591-599
45. de Knegt RJ, Schalm SW, van der Rijt CCD, et al. Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute liver failure: an experimental study based on in vivo brain dialysis. J Hepatol 1994; 20:19-26
46. Michalak A, Rose C, Butterwoth J, Butterworth RF. Neuroactive amino acids and glutamate (NMDA) receptors in frontal cortex of rats with experimental acute liver failure. Hepatology 1996; 24:908-913
47. Knecht K, Michalak A, Rose C, Butterworth RF. Decreased glutamate transporter (GLT-1) gene expression in brain in acute liver failure. Hepatology 1996; 24:248A
48. Laidlaw J, Read AE, Sherloch S. Morphine tolerance in hepatic cirrhosis. Gastroenterology 1961; 40:389-396
49. Thornton JR, Losowsky MS. Opioid peptides and primary biliary cirrhosis. Br Med J 1988; 297:1501-1504
50. Thornton JR, Losowsky MS. Methionine enkephalin is increased in plasma in acute liver disease and is present in bile and urine. J Hepatol 1989; 8:53-59
51. Cooper JR, Bloom FE, Roth RH. Neuroactive peptides. In: The biochemical basis of neuropharmacology, 6th ed. New York, Oxford: Oxford University Press, 1991:381-427,
52. Yurdaydın C, Jones EA: Hepatic encephalopathy. In: Principles and Practice of Gastroenterology and Hepatology, 2nd edition, G. Gitnick, ed., Elsevier Science Publishing Co., Inc. 1994:985-995
53. Yurdaydın C, Li Y, Ha J-H, Jones EA, Rothman R, Basile AS. Brain and plasma levels of opioid peptides are altered in rats with thioacetamide-induced fulminant hepatic failure: Implications for the treatment of hepatic encephalopathy with opioid antagonists. J Pharmacol Exp Ther 1995; 273:185-192
54. Yurdaydin C, Karavelioglu D, Yasa H, Onaran O. Opioid peptides in human hepatic encephalopathy. Hepatology 1996; 24:454A
55. Moore RY. Organization and function of a central nervous system circadian oscillator: The suprachiasmatic nucleus. Fed Proc 1983; 42:2783-2789
56. Rosenthal NE. Plasma melatonin as a measure of the human clock. J Clin Endocrinol Metab 1991; 73:225-226
57. Steindl PE, Finn B, Bendok B, Rothke S, Zee PC, Blei AT. Chronic liver disease and the circadian "clock": Disruption of the diurnal rhythm of plasma melatonin in cirrhosis. Ann Intern Med 1995; 123:274-277
58. Cordoba J, Steindl P, Cabrera J, Blei AT. Melatonin and sleep disturbances in cirrhosis (Abstract). Hepatology 1996; 24:451A
59. Steindl PE, Schwaiger B, Marktl W, Gangl A, Ferenci P. Evidence for impaired hepatic catabolism of melatonin in patients with liver cirrhosis (Abstract). J Hepatol 1997; 26:104
60. Cabrera J, Cordoba J, Lataif L, Blei AT. High prevalance of insomnia in compensated cirrhosis: A controlled survey (Abstract). Hepatology 1996; 24:452A
61. Inoue E, Hori S, Naruni Y, Fujita M, Kuriyama K, Kadfota T, Kuroda C. Portal-systemic encephalopathy: presence of basal ganglia lesions with high signal intensity on MR images. Radiology 1991; 179:551-555
62. Krieger S, Jauss M, Jansen O, Theilmann L, Geissler M, Krieger D. Neuropsychiatric profile and hyperintense globus pallidus on T1-weighted magnetic resonance images in liver cirrhosis. Gastroenterology 1996; 111:147-155
63. Pujol A, Pujol J, Graus F, et al. Hyperintense globus pallidus on T1-weighted MRI in cirrhotic patients is associated with severity of liver failure. Neurology 1993; 43:65-69

64. Kulisevsky J, Pupol J, Balazo J, et al. Pallidal hyperintensity on magnetic resonance imaging in cirrhotic patients: Clinical correlations. Hepatology 1992; 16:1382-1388
65. Thuluvath PJ, Edwin D, Yue NC, et al. Increased signals seen in globus pallidus in T1-weighted magnetic resonance imaging in cirrhotics are not suggestive of chronic hepatic encephalopathy. Hepatology 1995; 21:440-442
66. Spahr L, Butterworth RF, Fontaine S, et al. Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. Hepatology 1996; 24:1116-1120
67. Pomier-Layrargues G, Spahr L, Butterworth RF. Increased manganese concentrations in pallidum of cirrhotic patients (letter). Lancet 1995; 345:735
68. Krieger D, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H. Manganese and chronic hepatic encephalopathy. Lancet 1995; 346:270-274
69. Papavasiliou PS, Miller ST, Cotzias GC. Role of liver in regulating distribution and excretion of manganese. Am J Physiol 1966; 211:211-216
70. Yamada M, Ohno S, Okayasu I, Okeda R, Hatakeyama S, Watanabe H, Ushio K, et al. Chronic manganese poisoning: a neuropathological study with determination of manganese distribution of the brain. Acta Neuropathol (Berl) 1986; 70:273-278
71. Shinotoh H, Snow BJ, Hewitt KA, Pate BD, Doudet D, Nugent R, Perl DP, et al. MRI and PET studies of manganese intoxicated monkeys. Neurology 1995; 45:1199-1204
72. Pentschew A, Ebner F, Kovatch R. Experimental manganese encephalopathy in monkeys: a preliminary report. J Neuropathol Exp Neurol 1963; 22:488-499
73. Häussinger D, Lang F, Gerok W. Regulation of cell function by the cellular hydration state. Am J Physiol 1994; 267:E343-E355
74. Häussinger D, Laubenberger J, von Dahl S. et al. Proton magnetic resonance spectroscopy on human brain myo-inositol in hypo-osmolarity and hepatic encephalopathy. Gastroenterology 1994; 107:1475-1480
75. McConnell JR, Antonson DL, Ong CS, et al. Proton spectroscopy of brain glutamine in acute liver failure. Hepatology 1995; 22:69-74
76. Córdoba J, Gottstein J, Blei AT. Glutamine, myo-inositol, and organic brain osmolytes after portacaval anastomosis in the rat: implications for ammonia-induced brain edema. Hepatology 1996; 24:919-923