

NEUROTRANSMITTERS IN HEPATIC ENCEPHALOPATHY

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Key words: hepatic encephalopathy, neurotransmitters, histamine, histidine

Abstract. The pathogenesis of hepatic encephalopathy (HE), a neuropsychiatric disorder resulting from liver failure, is still a matter of debate. Recently attention has been focused on brain neurotransmitters. The accumulating evidence indicates that the imbalanced metabolism and turnover as well as the altered functions of neurotransmitters (dopamine, noradrenaline, serotonin, GABA) and false neurotransmitters (phenylethanolamine, octopamine, synephrine) may be of high importance in the pathomechanism of HE. Our data permit adding histamine (HA) to the multifactorial pathogenesis of HE. The enhanced rate of histidine influx into the brain following portocaval anastomosis and a dramatic increase in the HA content in the hypothalamus may suggest the involvement of HA in the symptomatology of hepatic encephalopathy.

INTRODUCTION

Chronic liver disease is frequently accompanied by a neuropsychiatric disorder — hepatic encephalopathy (HE). Clinically, the early stages of HE are manifested by restlessness, irritability, altered mental and intellectual functions (personality, judgment) and neuromuscular disorders (incoordination, spasticity, asterixis). The mild symptoms eventually progress to drowsiness, confusion and finally pass to a coma. Hepatic

encephalopathy is commonly attributed to the inability of the diseased liver to remove the excess of gut generated cerebrotoxic substances, which then enter the systemic circulation, some of them reaching the brain. In fact, the location of the liver in relation to the splanchnic circulation, which drains the vascular beds of the spleen, the pancreas, the stomach, the large and small intestines and the mesentery, makes the liver the main barrier against the influx to the systemic circulation of noxious products which may be ingested or generated in the gastrointestinal tract. Ammonia (3, 4), unestrified fatty acids (9), false neurotransmitters (12) and GABA (11, 47) have been incriminated in the pathogenesis of HE. On the other hand, the absence or shortage of some protective substances normally formed in the healthy liver and important for correct brain functioning (glucose, albumin) cannot be entirely ruled out (18, 45).

The fundamental differences exist in the pathogenesis of the fulminant hepatic failure (FHF) and encephalopathy complicating chronic liver failure. The removal of the liver in laboratory animals, which resembles FHF progressing to a coma in patients, is associated with marked changes in the brain. In hepatectomized rats an increased permeability to trypan blue, L-glucose, D-galactose and inulin, the substances which do not normally cross the blood-brain-barrier (BBB), was demonstrated (26), indicating the loss of BBB function. The comatose rats were found to have cerebral edema, swollen astrocytes and perivascular astrocyte foot processes (26). Thus, it is possible that after hepatectomy the breakdown of the BBB is a factor determining the development of cerebral edema. It is of interest to note that encephalopathy in FHF is often associated with cerebral edema.

A severe impairment of the liver by high doses of hepatotoxin D-galactosamine in rabbits (another animal model of FHF) has been found to increase the brain uptake of nonmetabolisable amino acid, namely α -aminoisobutyric acid, which suggests that in this case there occur non-specific changes in the blood-brain-barrier transport (19).

In chronic liver disease encephalopathy is potentially reversible, which is consistent with the subtle, not significant, structural changes in the brain. The principal morphological feature is protoplasmic astrocytosis (Alzheimer type II astrocyte), characterized by cytoplasmic hypertrophy with marked proliferation of mitochondria and endoplasmic reticulum, generally associated with cells exhibiting increased metabolic activity (34, 35).

BLOOD-BRAIN BARRIER PERMEABILITY

Recent evidence indicates that the changes in the BBB may play an important role in the mechanism of HE. Endothelial cells of cerebral capillaries have tight junctions between the plasmolemma of adjacent cells. They form thereby a continuous barrier between the blood and the brain. Lipid soluble compounds move across the BBB by dissolving in the lipoproteins of the cell membrane. The flux of hydrophylic compounds occurs via distinct, specialized carrier systems located in the luminal and abluminal membranes of the endothelial cells. These systems show specificity for groups of substrates such as hexoses, monocarboxylic acid, neutral basic and acidic amino acids, nucleosides and purine bases. They differ in their properties and kinetics (37). By controlling nutrient availability in the CNS the blood-brain barrier plays a significant role in the regulation of many pathways of the brain metabolism.

BLOOD-BRAIN BARRIER IN LIVER DISEASE

The available data show that liver failure may produce marked changes in the properties of transport mechanisms through the BBB. Their magnitude depends on the severity of the disease. Although the ultrastructural image of increased vascular transport of horseradish peroxidase has been obtained in rats with portocaval anastomosis (26), the model commonly used because of its resemblance in many respects to chronic liver failure in patients, the brain uptake of small molecules, ^{22}Na (44), ^{24}Na (24) and $^{51}\text{Cr-EDTA}$ (44), ^{14}C sucrose (24) as well as lipid soluble octanoate (7) was found to be unchanged, whereas the uptake of monocarboxylic acids: acetate, butyrate and pyruvate (45) transported by the carrier of ketone bodies and the transport of 3-hydroxybutyrate (16) were reduced. These experiments indicate both the preservation of physical integrity in the BBB and diminution in the amount of the carrier of ketone bodies. The glucose carrier system does not seem to be affected; the decreased influx of glucose to the brain was in direct proportion to the lowering of plasma glucose concentrations (16, 29, 45).

AMINO ACIDS CARRIER SYSTEMS

The concentration of amino acids in the brain is closely related to the integrity of the BBB, the activity of carrier systems, and the plasma levels of amino acids. There is growing evidence suggesting that after portocaval anastomosis the change in the passage of amino acids into

the brain is mainly due to both the altered capacity of various carrier systems and the competition effects; the latter are of primary importance in the transport of neutral amino acids (37). As has been mentioned above, there are three independent carrier systems for amino acids: neutral, basic and acidic (39). During hepatic encephalopathy these carriers are not equally affected.

The transport of acidic amino acids. Glutamate and aspartate, precursors of neuroexcitatory and inhibitory transmitters, glutamine, GABA and aspartic acid, is mediated by a transport system of a high affinity and very low capacity. This carrier is supposed to mediate rather the egress of excitatory neurotransmitters from the brain than the rate of unidirectional influx of glutamate into the brain (37). In rats with portocaval shunts the plasma levels of glutamate do not change significantly (28) whereas the brain levels have been found to be somewhat lower (5, 17). On the other hand the brain levels of glutamine are more than doubled as compared with sham operated rats (4, 5, 17, 30). Similar changes in brain glutamate and glutamine levels have been obtained in cirrhotic patients who died in a hepatic coma (5). All these changes suggest indeed that the transport system for acidic amino acids is not significantly affected by hepatic insufficiency. The increase in cerebral glutamine most probably results from both the increased synthesis and the ammonia-induced decrease of glutamine degradation (product inhibition) (5).

For lysine (28) and other basic amino acids, i.e., arginine (20) and citrulline (49), transported by the same carrier, the BBB after portocaval anastomosis in rats and dogs becomes almost impermeable. As the rates of transport of the basic amino acids into the brain are very similar to the rates of protein synthesis, it has been suggested that the availability of basic amino acids may be a limiting factor of the rate of protein synthesis (16).

The most pronounced changes have been found during HE in the transport of neutral amino acids into the brain. The BBB neutral amino acid transport system is analogous to the L-system (leucine preferring) and mediates the equilibrative bidirectional movement of amino acids through cell membranes, i.e., it does not concentrate amino acids within the cell. This system carries neutral amino acids at similar rates and can be described by the Michaelis-Menten kinetics (38, 39). Since K_m values for neutral amino acids are similar (38) and are close to the values of their plasma concentrations, the rate of entry into the brain of any particular amino acid is influenced by the presence of other amino acids.

The most striking abnormality of plasma amino acid patterns in patients and animals with HE is that the level of large, branched-chain

amino acids (VAL, LEU, ILE) is decreased (20, 28, 36, 41) or unchanged (30, 49) whereas that of aromatic and sulphur-containing amino acids: PHE, TYR, free TRY, HIS, MET is highly increased (13, 20, 28, 30, 36, 49). It has been suggested that the decrease of the branched-chain amino acids results from sustained hyperinsulinemia, which stimulates their uptake in the muscles and fat (32), where these glycolytic amino acids are preferentially metabolized. It is worth noting that some data contradict this hypothesis (30). The elevated plasma level of aromatic amino acids is thought to arise mainly from necrotic liver; the catabolism of a striated voluntary muscle may also contribute to a certain extent (41).

NEUTRAL AMINO ACIDS IN THE BRAIN

The abnormal plasma distribution of neutral amino acids during HE may lead directly to the imbalance of amino acid content in the brain. In fact, in rats with portocaval anastomosis the brain levels of PHE, TYR, HIS and MET were found to be markedly increased, whereas those of VAL, LEU, ILE were unchanged (20, 28). Also in shunted dogs neutral amino acids show this characteristic pattern both in the plasma and in the cerebrospinal fluid (CSF); the aromatic amino acids were increased while the branched chain amino acids were almost unchanged (49). The fact that the brain levels of aromatic amino acids were much higher than could be predicted from their plasma levels and that the branched-chain amino acids were almost unchanged while their plasma levels were reduced leads to the conclusion that the blood-brain transport of neutral amino acids was selectively increased after portocaval anastomosis (20).

The resulting imbalance of neutral amino acids in the plasma and in the brain has been attributed to the development of hepatic encephalopathy (20, 22). In dogs with portocaval anastomosis the infusion of solutions rich in branched-chain amino acids and poor in aromatic amino acids resulted in the normalization of the amino acid profile, with a reduction of aromatic amino acids and their metabolites in the cerebrospinal fluid. These changes were associated with an amelioration of the hepatic coma (49). As a consequence of these investigations the infusions of solutions containing high concentrations of branched-chain amino acids have been included in the treatment of acute and chronic liver diseases. However, the results have not always been advantageous.

BRAIN NEUROTRANSMITTERS

The change in the brain levels of neurotransmitter amines during HE is a consequence of several factors, the most significant of them being the availability of the precursors, penetration into the brain of neurotransmitters produced elsewhere, whose passage is normally limited by the BBB, and the access to the cerebral tissue, in excessive amounts, of the so-called false neurotransmitters: octopamine, phenylethanolamine, tyramine, synephrine, not cleared by the diseased liver. In physiological conditions brain neurotransmitters, namely serotonin (5HT), dopamine (DA), noradrenaline (NA), adrenaline (A), and histamine (HA), are formed locally in the cerebral tissue and do not easily cross the BBB. Their synthesis is controlled by the brain concentration of the precursor amino acids, e.g., free TRY, PHE, TYR, and HIS. Under normal conditions the concentrations of these amino acids are below the level sufficient to saturate particular enzymes. Therefore enhanced availability of any of them due to selectively increased permeability of the BBB caused by liver damage may create favourable conditions for the rise of the synthesis and subsequent elevation of the amine level in the brain. On the other hand, changes in the BBB permeability may facilitate also a direct inflow of neuromediators deriving from peripheral sources.

FALSE NEUROTRANSMITTERS

The most striking change found in patients with hepatic insufficiency and in animals with portocaval anastomosis is a great increase in the levels of phenylethanolamine (6, 12), tyramine, octopamine and synephrine (2, 6, 12, 21, 43). These amines may be formed within and outside CNS, especially in the gut, where the intestinal bacteria play an important role. In a healthy subject the amines are largely oxidized in the liver and cleared from the portal blood. During HE the increase of these compounds in the serum by 50-150%, in the cerebrospinal fluid by 200-300% and in the brain by 150-230% was observed (2, 12, 21). These findings serve as the basis for the false neurotransmitter hypothesis proposed by Fischer and Baldessarini (12), explaining some of the neurological and cardiovascular complications of HF by the accumulation of the trace amines which may replace the proper mediators in functioning. However, the causal relationship of the above mentioned events is not very obvious because even an increase as high as 20,000 fold in brain octopamine which resulted in a reduction of NA and DA in the brain by 90% did not affect the behaviour (alertness and activity) of normal rats (52).

CATECHOLAMINES

Increased intracerebral concentrations of PHE and TYR (20, 28), the precursors of catecholamines, during HE might result in enhanced formation of DA, NA and A. But this is not the case. The dopamine level in the brain was found to be unaffected by portocaval shunting in the rats (9, 16) and that of NA was slightly higher, namely by 22% (16). In only one report concerning L-dopa, a direct precursor of dopamine, there was a 50% decrease of the dopa concentration in the CSF from patients with hepatic coma grade II-III, together with a 6- and 5-fold increase in DA and NA levels, respectively (2). All these findings are inconsistent with the 3-4-fold rise in the brain concentrations of PHE and TYR following liver injury. The most plausible explanation may be that the excess of both the amino acids PHE and TYR undergoes further transformations in the brain, not on the main physiological routes leading to the catecholamines but on the false neurotransmitter pathways, where PHE is converted to phenylethylamine and then to phenylethanolamine and TYR to tyramine, octopamine and synephrine. This can be supported by the fact that octopamine given intraventricularly into rats produced a deep fall of brain DA and NA (52).

SEROTONIN

Serotonin (5HT) has been implicated as a factor contributing to the pathogenesis of HE. Although during HE the brain concentrations of 5HT were found to be moderately increased (1, 9, 16), unchanged (31, 51) or even decreased (10), the levels of 5-hydroxyindoleacetic acid, the oxidative metabolite of 5HT, were constantly higher than in the controls, indicating an increased turnover of 5HT in the liver disease (1, 8, 9, 10, 16, 31, 51). However, the altered tryptophan metabolism in shunted rats, which is at least partly responsible for the behavioral changes involving hypoactivity and diminished response to stimuli, is by itself insufficient to explain the central effects of liver disease in man (51). It is worth mentioning that 5HT has been claimed to act as a "false neurotransmitter" displacing the catecholamines from their sites (1).

HISTAMINE

Recently in our laboratory in the series of experiments on Wistar rats subjected to portocaval anastomosis the involvement of histamine in the pathogenesis of HE has been proved. The levels of HA in the brain were found to be highly increased. The most dramatic change was

found in the hypothalamus, where in the 3rd week following the operation, the peak values were as high as 25.9 nmoles/g. At the same time the serum HA levels remained within the normal range. Histamine concentrations in the hypothalamus tend to decrease with time, but even 6 weeks after portocaval anastomosis they were still 4-fold the normal ones (Table I).

TABLE I
Histamine parameters in rat hypothalamus. The effect of portocaval anastomosis

Parameter	Exp. group	Days after portocaval anastomosis			
		10	20	30	40
HISTAMINE	C	2.87±0.24	2.91±0.39	2.58±0.17	2.88±0.17
nmoles/g	PCA	6.76±0.78*	25.90±2.02*	19.40±1.10*	14.40±2.42*
HISTIDINE	C	148±22	189±14	148±20	176±12
nmoles/g	PCA	327±54*	741±110*	458±32*	528±26*
HDC	C	0.38±0.04	0.39±0.03	0.35±0.02	0.45±0.02
nmole/min/g protein	PCA	0.41±0.03	0.37±0.01	0.39±0.04	0.42±0.03
HMT	C	18.7±2.6	19.8±4.5	19.8±3.1	16.1±0.5
nmole/min/g protein	PCA	18.8±1.0	20.7±4.0	15.2±2.3	18.0±3.5

C, control; PCA, portocaval anastomosis; * $p < 0.01$. The levels of histamine and histidine and the activity of histidine decarboxylase (HDC) and N-methyltransferase (HMT) were estimated by standard isotopic procedures as described by Taylor and Snyder (50).

Our data are consistent with the sole published report concerning histamine in relation to HE in man. It indicates 3- to 4.5-fold elevation of HA levels in CSF. The HE patients displayed also somewhat higher plasma histamine concentrations (30% to 80% above the controls) (2). The increase in hypothalamic HA was not accompanied by any change in the activities of the main two histamine metabolizing enzymes, namely histidine decarboxylase and histamine N-methyltransferase (Table I). The most likely explanation of the phenomenon of unaltered histamine synthesizing enzyme activity is that histidine decarboxylase, being the rate-limiting enzyme for HA synthesis, is normally not saturated. Therefore an increase in the amount of histidine (20, 28) available to the enzyme should be reflected in extensive HA synthesis, especially in the hypothalamus, where the rate of HA formation is greatest and its concentrations higher than in other brain structures (14, 48). Histamine was found to induce an increased transcapillary influx of horse-

radish peroxidase, sucrose, α -aminobutyric acid and albumin (15) as well as an increased number of pinocytotic vesicles in isolated cerebral microvessels (23). The increase in the number of pinocytotic vessels within cerebral endothelial cells was observed after portocaval anastomosis (25). It remains to be checked whether this coincidence is fortuitous or the HA extensively formed after portocaval anastomosis is a factor leading to the modification of the BBB to amino acids.

γ -AMINO BUTYRIC ACID

Schafer and Jones have introduced the concept of the possible role of γ -aminobutyric acid (GABA), the most ubiquitous inhibitory neurotransmitter in the vertebrate CNS in the pathogenesis of HE. In a model of galactosamine-induced FHF they showed previously that the gut-derived GABA is not metabolized normally by the damaged liver and may pass into the circulation (46). On this basis it was conjectured that owing to the impaired BBB this GABA, normally impermeable, would flood into the brain, and exert its function (47). An increased efficiency of the GABA system has also been suggested (11, 47). Some shortcomings of the hypothesis have been noted and the hypothesis has been severely criticized (5, 24, 27, 33, 40, 42). The GABA theory in its present state changes very little in the pathophysiological scenery of HE (33).

CONCLUSIONS

This review deals with the probable role of neurotransmitters, thought to be crucial for the symptomatology of HE. However, this is only one side of the problem. Hepatic encephalopathy cannot be attributed solely to any single abnormality, such as BBB permeability, the capacity and activity of BBB transport systems, the balance of amino acids, normal and false neurotransmitters. These are only single instruments playing their parts in the multifactorial pathogenesis of HE. It remains to be found which of them, if any, is the leading one in any particular case.

This investigation was supported by Project CPBP 06.03 of the Polish Academy of Sciences.

ABBREVIATIONS

BBB blood-brain barrier
CNS central nervous system
CSF cerebrospinal fluid
FHF fulminant hepatic failure

HE hepatic encephalopathy

PCA portocaval anastomosis.

Amino acids and amines

A adrenaline

DA dopamine

GABA γ -aminobutyric acid

HA histamine

HIS histidine

ILE isoleucine

LEU leucine

MET methionine

NA noradrenaline

PHE phenylalanine

TRY tryptophan

TYR tyrosine

VAL valine

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