

## The GABA Hypothesis of the Pathogenesis of Hepatic Encephalopathy: Current Status

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Gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter of the mammalian brain, can induce coma. Outside the central nervous system it is synthesized by gut bacteria and catabolized largely in the liver. GABA and its agonists, as well as benzodiazepines and barbiturates, induce neural inhibition as a consequence of their interaction with specific binding sites for each of these classes of neuroactive substances on the GABA receptor complex of postsynaptic neurons. In a rabbit model of acute liver failure: (i) the pattern of postsynaptic neuronal activity in hepatic coma, as assessed by visual evoked potentials, is identical to that associated with coma induced by drugs which activate the GABA neurotransmitter system (benzodiazepines, barbiturates, and GABA agonists); (ii) the levels of GABA-like activity in peripheral blood plasma increase appreciably before the onset of hepatic encephalopathy, due at least in part to impaired hepatic extraction of gut-derived GABA from portal venous blood; (iii) the blood-brain barrier becomes abnormally permeable to an isomer of GABA,  $\alpha$ -aminoisobutyric acid, before the onset of hepatic encephalopathy; and (iv) hepatic coma is associated with an increase in the density of receptors for GABA and benzodiazepines in the brain. These findings are the bases of the following hypotheses: (i) when the liver fails, gut-derived GABA in plasma crosses an abnormally permeable blood-brain barrier and by mediating neural inhibition contributes to hepatic encephalopathy; (ii) an increased number of GABA receptors in the brain found in liver failure increases the sensitivity of the brain to GABA-ergic neural inhibition; and (iii) an increased number of drug binding sites mediates the increased sensitivity to benzodiazepines and barbiturates observed in liver failure by permitting increased drug effect.

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### INTRODUCTION

Hepatocellular failure, whether acute or chronic, is characterized by jaundice, fluid retention, a hemorrhagic diathesis, and hepatic encephalopathy [1]. Of these four complications of liver disease the pathogenesis of hepatic encephalopathy is least well understood. It has not even been established whether hepatic encephalopathy occurs as a result of failure of the liver to synthesize a substance that is necessary for the maintenance of normal brain function or failure of the liver to metabolize adequately neuroactive substances that are capable of inducing encephalopathy.

301

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However, two observations tend to favor the second of these possibilities. The first is the clinical finding that the administration of therapies which reduce the production of nitrogenous substances by gut bacteria is often followed by an amelioration of hepatic encephalopathy in patients with cirrhosis [1,2]. The second is the result of carefully conducted cross-circulation experiments between normal and liverless rats in which the electroencephalogram of the liverless rats was monitored. Brain function of liverless rats was found to improve more rapidly when their aortic blood was infused into the portal vein (systemic-portal cross-circulation) rather than the jugular vein (systemic-systemic cross-circulation) of normal rats [3].

As a consequence of the impaired hepatic metabolism of many substances in liver failure a large number of metabolites accumulate in plasma. Some of these metabolites are neuroactive [2,4-8]. However, it is not apparent which, if any, of the neuroactive metabolites that accumulate in liver failure plays a central role in the pathogenesis of hepatic encephalopathy. Clearly if a metabolite in plasma contributes to the mediation of hepatic encephalopathy it must gain access to the brain by crossing the blood-brain barrier. Many studies, in which attempts have been made to draw inferences concerning the pathogenesis of hepatic encephalopathy, have involved deriving correlations between levels of putatively neuroactive metabolites in plasma, cerebrospinal fluid, or brain, and the degree of severity of hepatic encephalopathy [2,4-8]. When such a correlation is found there are three possible explanations: (i) the increased level of the metabolite is causally related to hepatic encephalopathy; (ii) the increased level of the metabolite occurs as a consequence of hepatic encephalopathy; and (iii) some other pathophysiologic disturbance, perhaps liver failure per se, causes both hepatic encephalopathy and the increased level of the metabolite. Furthermore, it is important to emphasize that because of the regional and subcellular compartmentalization of substances within the brain [9] mean changes in the concentration of a metabolite in the brain may not be relevant to the pathogenesis of hepatic encephalopathy. However, changes in the concentration of a metabolite at a critical site within the brain, such as synaptic clefts, could well be of critical importance [10]. A major deficiency of most hypotheses of the pathogenesis of hepatic encephalopathy is that the presence of putative neurotoxins cannot be correlated with specific neural mechanisms [7,8]. It seems unlikely that major advances in elucidating the pathogenesis of hepatic encephalopathy will come from studies that rely solely on measuring the levels of metabolites. Important clues as to which compounds are important in the pathogenesis of hepatic encephalopathy may, however, come from studies designed to throw light on which neurophysiologic mechanisms could conceivably mediate the syndrome [10].

Four hypotheses of the pathogenesis of hepatic encephalopathy have received most attention. These are that the syndrome is mediated by (i) ammonia-induced neurotoxicity [2,7,8,11]; (ii) synergistic neurotoxic interactions between ammonia, mercaptans, and fatty acids [7,8]; (iii) false neurotransmitter-induced neural inhibition [12-14]; and (iv) activation of the gamma-aminobutyric acid (GABA) inhibitory neurotransmitter system [10,15]. Some of the observations on which the first three of these hypotheses are based and some of the main deficiencies of these three hypotheses have recently been discussed elsewhere [16,17]. This review article concentrates on the last and most recent of the four hypotheses, the GABA hypothesis. After briefly reviewing some important aspects of GABA metabolism and stating the GABA hypothesis, the interpretation and potential significance of experimental findings which have been cited in support of the GABA hypothesis are discussed. To

conclude, some current perspectives and speculations relating to the GABA hypothesis and other hypotheses of the pathogenesis of hepatic encephalopathy are given.

### GABA METABOLISM

GABA is the principal inhibitory neurotransmitter of the mammalian brain. It is synthesized in presynaptic neurons by decarboxylation of glutamate (Fig. 1). The total brain content of GABA is 200–1,000 times greater than that of the biogenic amine neurotransmitters. GABA is stored in cytoplasmic vesicles within presynaptic neurons. Presumably GABA at these intracellular sites exerts no biological effect. When released from presynaptic storage sites it enters the synaptic cleft and binds to receptors on postsynaptic neurons. The interaction between GABA and its receptors results in increased chloride ion conductance across the postsynaptic neural membrane, hyperpolarization, and the generation of an inhibitory postsynaptic potential. GABA is quickly eliminated from the synaptic cleft by efficient mechanisms of re-uptake into astrocytes and neurons and is either reincorporated into storage vesicles or catabolized. GABA transaminase is the enzyme which mediates the biotransformation of GABA to succinic semialdehyde, which is a precursor of succinic acid [18–20] (Fig. 1).

The GABA receptor on postsynaptic neurons consists of a supramolecular complex which possesses sites on its external surface for the interactive but noncompetitive binding of at least three classes of synergistic ligands—GABA and its agonists, benzodiazepines, and barbiturates. The sedative-hypnotic effects of benzodiazepines and barbiturates are mediated by the GABA neurotransmitter system. The binding of a benzodiazepine or a barbiturate to its binding site on the GABA receptor complex potentiates the response to GABA by augmenting GABA-induced chloride ion conductance across the membrane [21–24]. Benzodiazepines increase the frequency of chloride channel opening whereas barbiturates increase the average time that chloride channels are open [25].

### THE GABA HYPOTHESIS

The GABA hypothesis relating to the pathogenesis of hepatic encephalopathy consists of three components: (i) when the liver fails, gut-derived GABA in plasma crosses an abnormally permeable blood-brain barrier and by mediating neural inhibition contributes to hepatic encephalopathy; (ii) an increased number of GABA receptors in the brain found in liver failure increases the sensitivity of the brain to GABA-ergic neural inhibition; and (iii) an increased number of drug binding sites mediates the increased sensitivity to benzodiazepines and barbiturates observed in liver failure by permitting increased drug effect [10,15].

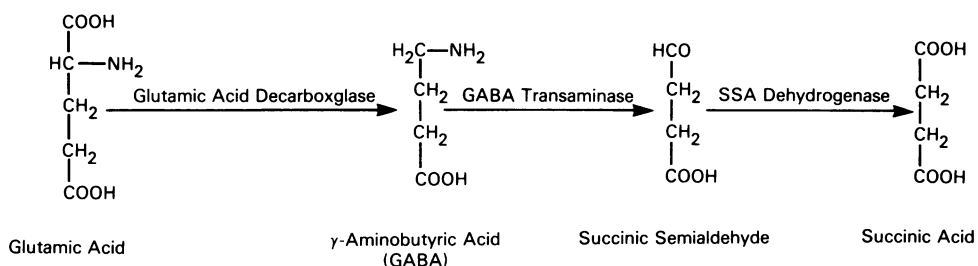


FIG. 1. The synthesis and catabolism of  $\gamma$ -aminobutyric acid (GABA).

## THE POTENTIAL VALIDITY OF FINDINGS CITED AS SUPPORTING THE GABA HYPOTHESIS

### *Data Obtained Using Animal Models of Hepatic Encephalopathy*

Most of the experimental findings that are cited in support of the GABA hypothesis have been generated using a rabbit model of galactosamine-induced fulminant hepatic failure [26]. This model has been well characterized. Galactosamine is a selective hepatotoxin. It induces acute liver failure, followed by hepatic encephalopathy, hepatic coma, and death. The model fulfills the requirements of an appropriate animal model of hepatic encephalopathy [27] in that the encephalopathy is reversible (e.g., by cross-circulation with a normal rabbit [Schafer DF: unpublished observations]), the model is reproducible, death is from liver failure, the animal is sufficiently large for most experimental purposes, and the model is not hazardous to laboratory personnel. This model is regarded as one of the most satisfactory animal models of acute hepatic encephalopathy [28]. The issue arises over whether findings supporting the GABA hypothesis using this model are specific for this model or would also be found in other models of acute and chronic hepatic encephalopathy. Indeed, another pertinent issue is whether findings supporting the GABA hypothesis are specific for the syndrome of hepatic encephalopathy or also apply to other metabolic encephalopathies.

### *The Effect of GABA on Consciousness*

A crucial property of a substance of importance in the pathogenesis of hepatic encephalopathy would be that it can induce encephalopathy and coma. GABA appears to fulfill this requirement. It is not only a potent inhibitor of single neurons [29], but, in a potentially important study conducted by Smialowski [30], it was shown that GABA can probably induce coma as well. Within ten seconds of the instillation of less than 1  $\mu$ mole of GABA into the hippocampal region of conscious rabbits the animals "became quiet; the spontaneous locomotor activity declined and the animals mostly lay on the cage floor." These behavioral changes were associated with the development of spreading delta-wave electroencephalographic activity similar to that reported in man [31] and rabbits [26] with hepatic encephalopathy. Furthermore, when large doses of GABA were administered intravenously to normal rabbits coma was induced [Ferenci P, Pappas SC: unpublished observations]. This observation presumably indicates that when the plasma content of GABA is increased to sufficiently high levels, the absolute quantities of GABA that traverse an intact blood-brain barrier in this nonphysiologic situation are large enough to induce a degree of activation of the GABA neurotransmitter system that is sufficient to result in clinically overt encephalopathy. The issue whether in liver failure GABA itself in the quantities available for distribution to synaptic clefts could mediate encephalopathy is crucial to the feasibility of the GABA hypothesis. Accordingly, further studies of the ability of this inhibitory neurotransmitter to induce changes in mental status would be of considerable interest and potential importance.

### *GABA-ergic Neurotransmission in Liver Failure*

If activation of GABA-ergic neural mechanisms plays an important role in the mediation of hepatic encephalopathy, then it would be expected that the pattern of neuronal activity in the brain in this syndrome would be similar to that associated with direct drug-induced activation of the GABA neurotransmitter system. The methods currently available for studying changes in the patterns of neuronal activity associ-

ated with different types of encephalopathy are limited. Recordings of the electroencephalogram seem to be too nonspecific and too difficult to quantitate to be useful in this context [32]. However, the recording of visual evoked potentials (VEPs) appears to be more promising [33]. Indeed, measurements of VEPs in experimental animals have been shown to be a useful objective measurement of neuronal events in encephalopathies, and it has been suggested that this technique may prove to be particularly useful in assessing the role of altered neurotransmission in the pathogenesis of experimental hepatic encephalopathy [34,35].

When visual afferent stimuli are applied in the form of flashes of light, the central nervous system responds by generating synchronous volleys of discharges through neuronal networks [36,37]. This response constitutes the VEP. It can be monitored via chronically implanted epidural electrodes. In practice, a computer of average transients is used to derive the VEP from the electroencephalogram by averaging responses which are time-locked to the flashes of light and simultaneously removing other noncoherent electroencephalographic activity [35].

The results of studies of VEPs in an animal model of fulminant hepatic failure are entirely consistent with hepatic encephalopathy being associated with activation of GABA-ergic neural mechanisms [35,38]. The pattern of the VEP trace in the animal model exhibited highly distinctive and reproducible abnormalities (Fig. 2) which are

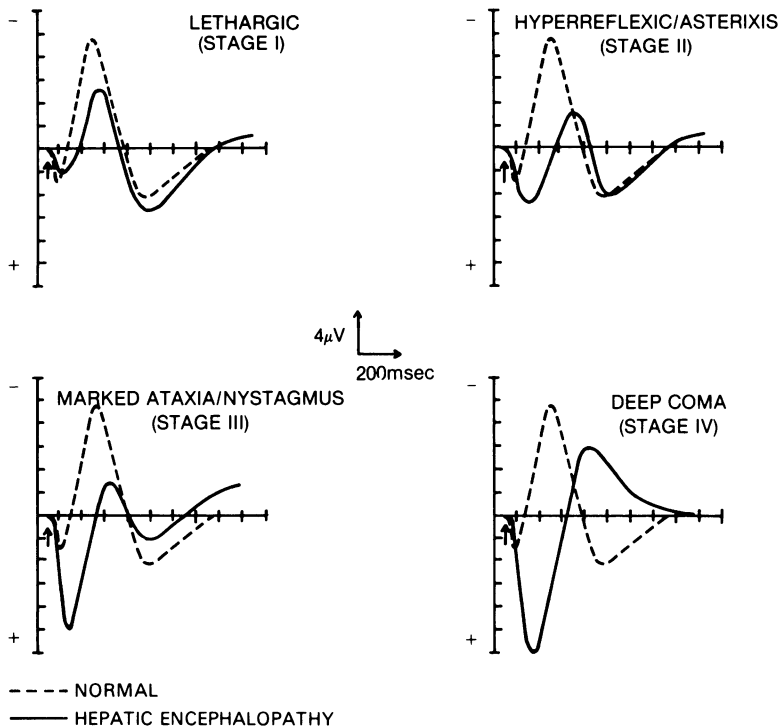


FIG. 2. The highly reproducible sequence of changes in the visual evoked potential waveform as a rabbit succumbs to galactosamine-induced fulminant hepatic failure. Visual evoked potentials associated with four different clinical stages of hepatic encephalopathy are depicted. The arrows indicate the time of the light flash stimuli. The waveform in stage IV hepatic encephalopathy (hepatic coma) is identical to that found in coma induced by a benzodiazepine (diazepam), a barbiturate (pentobarbital), or a GABA agonist (muscimol). (Reproduced from [35] with permission of *Gastroenterology*).

identical to those exhibited by animals with coma that was not precipitated by liver failure per se, but by direct drug-induced activation of the GABA neurotransmitter system. The particular drugs used in these studies were the benzodiazepine, diazepam; the barbiturate, pentobarbital; and the potent GABA agonist, muscimol [35]. In contrast, the patterns of the VEP trace in animals with coma or encephalopathy induced by a variety of neurotoxic agents (ether, ammonia, dimethyldisulfide, octanoic acid, a mixture of phenylalanine and tryptophan, and a mixture of subcoma doses of ammonia, dimethyldisulfide, and octanoic acid), which do not act preferentially on the GABA system, also exhibited highly distinctive and reproducible abnormalities, but these abnormal patterns were fundamentally different from the pattern of the VEP trace in hepatic coma [35,38–40].

There are potential problems with the interpretation of studies of VEPs. There is still debate regarding the precise origin of the VEP trace and correspondingly what inferences can justifiably be drawn from changes in the pattern of VEPs [41]. VEPs are summated measurements of the electrical activity in nervous tissue in response to a stimulus. They probably “reflect excitatory postsynaptic potentials, inhibitory postsynaptic potentials or some mixture of both” [37]. However, the observation that VEP waveforms in different neurologic states are identical does not constitute unequivocal proof that the different syndromes share common neural mechanisms. Phenomena related to the generation and measurement of VEPs may influence the results of VEP studies [42]. Nevertheless, the overall patterns of VEPs may be helpful in determining whether similar or dissimilar postsynaptic neural events are associated with particular neurologic states. Thus, from the findings in the VEP studies it would be reasonable to assume that the neurologic mechanisms which mediate hepatic encephalopathy more closely resemble the neurologic mechanisms which mediate drug-induced activation of the GABA neurotransmitter system [35], than those associated with encephalopathies induced by a variety of other agents whose effects on the central nervous system do not primarily involve activation of the GABA neurotransmitter system [35,38–40].

The observation that the abnormal pattern of the VEP in experimental animals with post-ictal coma is identical to that of animals in hepatic encephalopathy due to fulminant hepatic failure [38], adds further support to the notion that hepatic encephalopathy is associated with activation of the GABA neurotransmitter system, since it has recently been found that activation of the GABA neurotransmitter system appears to play a major role in the pathogenesis of post-ictal coma [43]. The potential validity of inferences regarding which neural mechanisms mediate specific neurologic syndromes from VEP data should become clearer when adequate data correlating changes in VEPs with changes in cerebral physiology are available [41].

#### *A GABA Agonist in Plasma in Liver Failure*

At least three decades ago it became apparent that neuroactive nitrogenous products of gut bacteria were likely to play an important role in the pathogenesis of hepatic encephalopathy. It was assumed that such substances are absorbed and are normally extracted from portal venous blood and metabolized by the liver. It was further assumed that in liver failure appreciable amounts of these substances gain access to the systemic circulation and ultimately the brain as a result of passage through portal-systemic venous collateral channels and/or impaired hepatic extraction [44]. As an alternative to hepatic encephalopathy, the term portal-systemic encephalopathy was introduced to embrace these concepts, and this term has withstood

the test of time. When considering the GABA hypothesis in relation to portal-systemic encephalopathy, it is germane to consider whether in liver failure a substance is present in plasma which can bind to GABA receptors and whether intestinal bacteria are a major source of such a substance.

The sensitive radioreceptor assay for GABA developed by Enna and Snyder [45] depends on the principle that when  $^3\text{H}$ -GABA is incubated with synaptic membranes the amount of  $^3\text{H}$ -GABA that specifically binds to the membranes is inversely proportional to the total amount of unlabeled GABA in the incubation medium. In other words, this assay measures the concentration of GABA and/or other substances which can bind to GABA receptors. Application of this assay has revealed that the concentration of GABA-like activity in portal venous plasma is about twice that in aortic plasma in normal rabbits [46], an observation which points to the gut as a source of GABA. Furthermore, *E. coli* and *B. fragilis* isolated from human feces, when cultured anaerobically, produce abundant quantities of GABA-like activity [46]. These findings are consistent with the enteric bacterial flora being a source of an appreciable amount of the GABA found in peripheral blood. Further application of the radioreceptor assay has shown that peripheral blood plasma levels of GABA-like activity increase by an order of magnitude in an animal model of acute liver failure before the onset of clinically overt hepatic encephalopathy [47]. In addition, plasma levels of GABA-like activity tend to be increased several-fold in patients with decompensated acute and chronic hepatocellular disease, particularly if hepatic encephalopathy has developed [48]. Plasma levels of GABA-like activity do not increase in rabbits in uremic coma [49], indicating that the increase in plasma levels of GABA-like activity associated with hepatic encephalopathy is not a nonspecific finding common to metabolic encephalopathies in general. The correlation between plasma levels of GABA-like activity and the stage of hepatic encephalopathy in patients is not close [48]. However, this negative finding does not necessarily detract from the GABA hypothesis since data on the prevailing permeability of the blood-brain barrier in the patients studied are not available. Plasma levels of GABA-like activity have been shown to increase several-fold in cirrhotic patients following hemorrhages into the gastrointestinal tract (Fig. 3) [48], an observation that suggests that blood in the gut acts as a substrate for GABA synthesis by intestinal bacteria.

The specificity of the radioreceptor assay for GABA is not absolute. However, it should be emphasized that very few physiologically occurring substances are known to interfere with this radioreceptor assay. Two that have been cited are homocarnosine and  $\beta$ -alanine, substances with affinities for GABA receptors that are, respectively, 50 and 200 times less than that of GABA itself [45,48]. Furthermore, in rabbits in acute liver failure there is complete concordance between the ion-exchange chromatography profiles of the GABA-like activity in plasma and  $^3\text{H}$ -GABA [10,15]. Thus, it seems probable that the GABA-like activity produced by intestinal bacteria and detected in plasma in increased amounts in liver failure is due to GABA itself. However, this inference has not been proved by applying another more specific assay for GABA. Attempts to separate GABA from other amino acids that are present in very high concentrations in plasma in liver failure using high-performance liquid chromatography and gas chromatography/mass spectroscopy have been unsuccessful [48]. The technical problems currently preventing the definitive resolution of this issue will almost certainly be overcome in due course.

The liver probably plays an important role in extracting gut-derived GABA from portal venous blood. In the rabbit more than 80 percent of the total body activity of

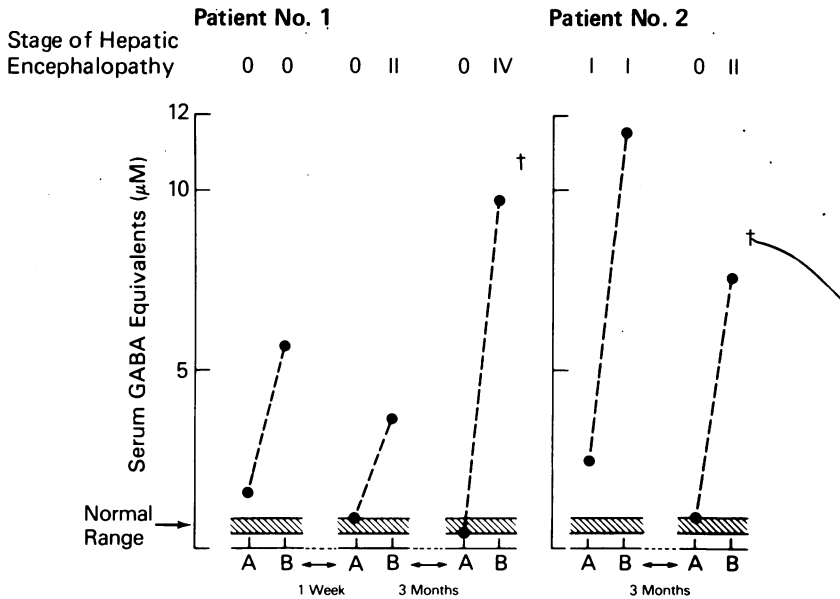


FIG. 3. The effect of gastrointestinal hemorrhages on serum levels of GABA-like activity in two patients with cirrhosis. A = before bleeding; B = 12-16 hours after bleeding; † = died. (Reproduced from [48] with permission of *The Lancet*).

GABA transaminase, the enzyme responsible for GABA catabolism, has been found to be present in the liver [50]. Accordingly, reduced hepatic catabolism of gut-derived GABA may contribute to the elevated plasma levels of GABA-like activity found in liver failure. Both nonmodel-dependent [47] and model-dependent [51] analyses of data on the *in vivo* kinetics and catabolism of  $^3\text{H}$ -GABA in rabbits have confirmed that hepatic extraction of GABA is reduced in acute liver failure. However, both analyses also indicated that the magnitude of the decrease in hepatic clearance was not sufficient to account for the magnitude of the increase in plasma GABA-like activity observed in acute liver failure. Thus, studies of GABA metabolism *in vivo* not only provide support for the notion that gut-derived GABA contributes to the increased plasma levels of GABA-like activity in liver failure, but they also suggest that in liver failure there is an increase in the delivery rate of GABA into the circulation. The mechanism of the latter phenomenon is unknown; possibilities include increased absorption of GABA from the gut and increased brain-to-plasma transfer of GABA across the blood-brain barrier.

Thus, from the available experimental findings it can be concluded that in liver failure there is an increased ability of plasma to displace  $^3\text{H}$ -GABA from GABA receptors on postsynaptic membranes and that a major source of this plasma GABA-like activity is the enteric bacterial flora. It is assumed, but it has not been proved, that the GABA-like material in plasma in liver failure is not a GABA antagonist but a GABA agonist.



*Permeability of the Blood-Brain Barrier to GABA in Liver Failure*

Clearly if gut-derived GABA contributes to the neural inhibition of hepatic encephalopathy, it is necessary for GABA in plasma to gain access to the brain by crossing the blood-brain barrier. This barrier consists of a continuous layer of capillary endothelial cells which are almost devoid of vesicles and are joined together by tight intercellular junctions (Fig. 4). Normally the transcapillary movement of most solutes and water is greatly hindered by this barrier [52]. GABA moves across the normal intact blood-brain barrier slowly by what appears to be a nonspecific process [53]. A potential problem with trying to quantitate the blood-to-brain transfer of GABA directly is the rapid metabolism of GABA both within and outside the central nervous system [18–20,24,47]. An isomer of GABA,  $\alpha$ -aminoisobutyric acid (AIB) [ $(\text{CH}_3)_2\cdot\text{C}\cdot\text{NH}_2\cdot\text{COOH}$ ] is free from this disadvantage.  $^{14}\text{C}$ -labeled AIB has several properties which make it a most attractive tracer to study the permeability of the blood-brain barrier [54]: (i) it is not metabolized in mammals so that the distribution of the label is not complicated by metabolism; (ii) it crosses the intact blood-brain barrier relatively slowly; (iii) it is rapidly taken up by and trapped in brain cells at the point where it crosses the blood-brain barrier, thereby minimizing backflow of label across the blood-brain barrier and diffusive and convective flow of label through the extracellular space of the brain; (iv) it is rapidly cleared from plasma; and (v) its transfer across the blood-brain barrier is not mediated by a specific amino acid carrier system but is a function of its lipid solubility and diffusivity. Because of the last of these properties an increase in the transfer of AIB across the blood-brain barrier implies a nonspecific increase in the permeability of the blood-brain barrier.

The transfer of  $^{14}\text{C}$ -AIB across the blood-brain barrier has been studied in rabbits in acute liver failure. The methods used involved computerized imaging techniques combined with quantitative autoradiography of sections of the brain to produce

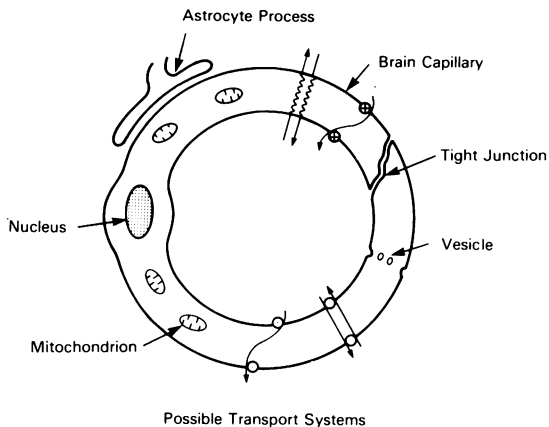


FIG. 4. Diagrammatic representation of the blood-brain barrier. A cerebral capillary with a tight intercellular junction is depicted in close relationship to an astrocyte process. Possible mechanisms for the blood-to-brain and brain-to-blood transport of solutes across this barrier are indicated.

color-referenced representations of the blood-to-brain transfer of  $^{14}\text{C}$ -AIB. In normal rabbits transfer of  $^{14}\text{C}$ -AIB across the barrier was minimal. However, with the onset of acute liver failure there was an increase in the permeability of the blood-brain barrier to  $^{14}\text{C}$ -AIB in certain gray matter areas of the brain. This change in permeability occurred several hours before the onset of clinically overt hepatic encephalopathy [54]. This finding implies a nonspecific increase in the permeability of the blood-brain barrier in acute liver failure. This inference is also supported by the finding that hepatectomy in rats is followed by an increase in the permeability of the blood-brain barrier to L-glucose, D-sucrose, inulin, and trypan blue dye [55]. Such a nonspecific increase in the permeability of the blood-brain barrier would presumably facilitate increased transfer of GABA from plasma to the extracellular fluid of the brain in liver failure before the onset of hepatic encephalopathy.

The mechanism of the increased permeability of the blood-brain barrier in acute liver failure could be an increase in transcapillary vesicular transport, as suggested by the ultrastructural observation of a marked increase in the number of vesicles within cerebral capillaries of rats with surgically created portacaval shunts [56]. Alternatively, the mechanism could be the opening of a few intercellular tight junctions of cerebral capillaries, a phenomenon which may be difficult to demonstrate ultrastructurally.

#### *The Status of the GABA Neurotransmitter System in Liver Failure*

If enhanced GABA-ergic neurotransmission plays an important role in the mediation of hepatic encephalopathy, changes in the GABA neurotransmitter system in the brain might be expected in liver failure. To assess the functional status of the GABA neurotransmitter system in liver failure, measurements have been made of the specific binding of  $^3\text{H}$ -GABA and  $^3\text{H}$ -flunitrazepam to postsynaptic neural membranes prepared from the brains of rabbits. Scatchard plot analysis of the GABA binding data suggested the presence of two independent classes of receptors with different (high and low) affinities. The development of hepatic coma due to acute hepatic failure was associated with no changes in the affinities but with an increase of about twofold in the densities of both GABA receptors [57,58] (Fig. 5). Scatchard plots of the benzodiazepine binding data indicated a single class of binding sites. The development of hepatic coma was associated with no change in the affinity but with an increase of about 40 percent in the number of these binding sites [58,59] (Fig. 6). These findings suggest that binding sites for GABA and benzodiazepines on the GABA receptor complex are regulated as a single operational unit. In an analogous study in rats, in which there also appeared to be two classes of receptors for GABA, mild hepatic encephalopathy was also associated with an increase in the density of both classes of receptors, but more severe hepatic encephalopathy was associated with a loss of high affinity receptors [60]. Similar changes in GABA receptors to those found in this study in rats have also been found in mild and severe hepatic encephalopathy in a dog model of chronic liver failure [61] and in patients who had died with cirrhosis and hepatic encephalopathy [62]. Not surprisingly the human autopsy data are not as clear-cut as the animal data, but nevertheless appear to show that the pattern of changes in GABA receptors associated with hepatic encephalopathy does not occur in patients dying from a variety of nonhepatic causes [62].

Changes in neurotransmitter receptors in hepatic encephalopathy are not limited to the GABA neurotransmitter system. In rabbits with acute liver failure, hepatic coma is associated with an increase in the density of receptors for another inhibitory

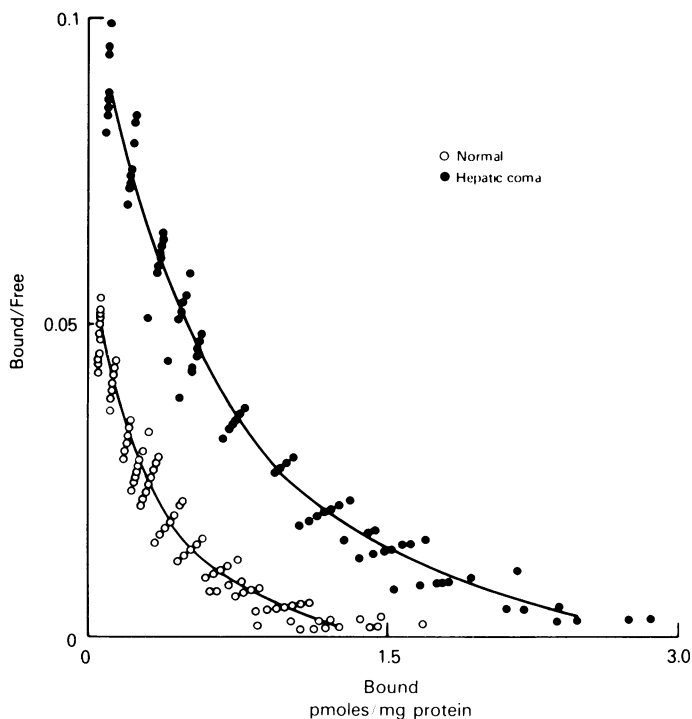


FIG. 5. Scatchard plots of data on the binding of GABA to postsynaptic membranes from the brains of eight normal rabbits and five rabbits in hepatic coma. The continuous lines are the respective (computer-derived) isotherms generated from each of the two groups of data points. The curvilinear plots are compatible with the existence of two independent binding sites for GABA which have different affinities. The finding that the value for the intercept of the hepatic coma isotherm with the abscissa is greater than the corresponding value for the control isotherm indicates that the density of GABA receptors is increased in hepatic coma. (Reproduced from [58] with permission of the *Journal of Laboratory and Clinical Medicine*).

amino acid neurotransmitter, glycine; a decrease in the density of receptors for three excitatory amino acid neurotransmitters, glutamate (the principal excitatory neurotransmitter of the mammalian brain), aspartate, and kainic acid; and no appreciable change in the densities of receptors for certain nonamino acid neurotransmitters, specifically dopamine, opiates ( $\mu$ -opiate), enkephalins ( $\delta$ -opiate), and acetylcholine [63,64]. The specificity of this pattern of changes in neurotransmitter

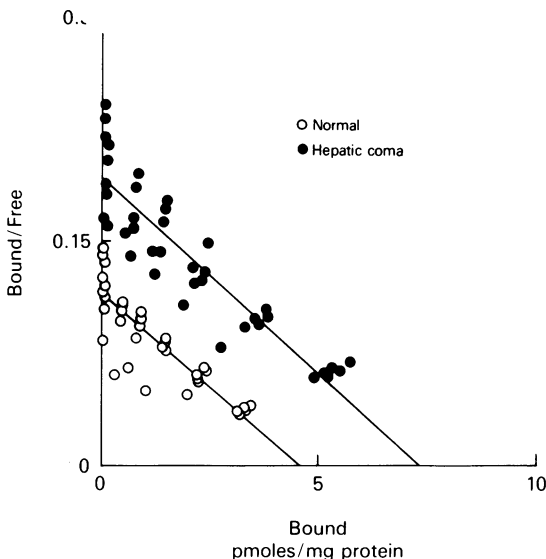


FIG. 6. Scatchard plots of data on the binding of flunitrazepam to neural membranes from the brains of six normal rabbits and six rabbits in hepatic coma due to galactosamine-induced fulminant hepatic failure. The continuous lines are the respective (computer-derived) binding isotherms generated from each group of data points. The linear plots are compatible with the existence of a single class of binding sites for benzodiazepines. The finding that the value for the intercept of the hepatic coma isotherm with the abscissa is greater than the corresponding value for the control isotherm indicates that the density of binding sites for benzodiazepines is increased in hepatic coma. (Reproduced from [58] with permission of the *Journal of Laboratory and Clinical Medicine*).

receptors for the syndrome of hepatic encephalopathy has not been established. However, the observation in rabbits that uremic coma is not associated with any appreciable changes in the densities of GABA [58] or glutamate [Ferenci P: unpublished observations] receptors indicates that the changes in the densities of these receptors found in hepatic encephalopathy are not common to metabolic encephalopathies in general.

The mechanisms underlying the observed changes in neurotransmitter receptors in hepatic encephalopathy are unknown. One possibility is that the changes are secondary to alterations in the composition of neural membranes. The cholesterol and phospholipid content of synaptic membranes isolated from the cerebral cortex of rabbits in hepatic coma due to acute liver failure has been found to be increased without any associated changes in the relative composition of phospholipid subclasses or membrane protein content [65]. It is not clear whether such changes in the composition of neural membranes reflect altered lipoprotein metabolism in neurons or changes in the composition of lipoproteins in brain extracellular fluid. It is conceivable that the increase in the lipid-to-protein ratio of synaptic membranes in hepatic coma is associated with changes in the functions of membrane receptors. The interpretation of the observed changes in neurotransmitter receptors in hepatic encephalopathy is hindered by the paucity of knowledge concerning the mechanisms which normally regulate neurotransmitter receptors and whether these mechanisms are operational in liver failure. The changes in GABA receptors in a rat model of hepatic coma have been attributed to "denervation supersensitivity" [60]. As denervation would be associated with a loss of presynaptic buttons which are rich in glutamate decarboxylase, the observation that brain glutamate decarboxylase levels are not reduced in an animal model of hepatic coma provides no support for this possibility [66].

The most important issue relating to the changes in neurotransmitter receptors in liver failure is their behavioral implications. Unfortunately, it is not possible to extrapolate from the available *in vitro* data on the binding of neurotransmitters to their receptors in hepatic encephalopathy to *in vivo* events with any degree of confidence. One possibility is that a neurotransmitter system becomes supersensitive to its neurotransmitter when the concentration of that neurotransmitter in synaptic clefts is decreased [67]. However, the most attractive potential implication of the neurotransmitter binding data in hepatic encephalopathy, and one that is entirely consistent with the GABA hypothesis, would be that the sensitivity of the brain to neurotransmitters varies directly with their density [63,64]. Thus, it is possible that in liver failure there is increased sensitivity to inhibitory amino acid neurotransmitters, decreased sensitivity to excitatory amino acid neurotransmitters, and unaltered sensitivity to other neurotransmitters which modulate neural function. Such changes in the sensitivity of the brain to neurotransmitters afford a feasible pathophysiologic basis for the neural inhibition which characterizes the syndrome of hepatic encephalopathy. As GABA is the principal inhibitory neurotransmitter of the mammalian brain [18-20], an important component of this potential interpretation of the neurotransmitter binding data in hepatic encephalopathy is the assumption that an increase in the number of receptors for GABA in liver failure increases the sensitivity of the brain to GABA-ergic neural inhibition by permitting more interaction between GABA and its receptors [58]. Increased GABA-ergic neural inhibition in liver failure could be mediated by GABA itself or by an as-yet-unidentified GABA agonist.

## CONCLUDING PERSPECTIVES AND SPECULATIONS

As with other hypotheses of the pathogenesis of hepatic encephalopathy, the GABA hypothesis has not been definitively validated. In contrast to other hypotheses such as the ammonia [2,7,8,11], the synergistic neurotoxins [7,8], and the false neurotransmitter [12-14] hypotheses, in which the neural mechanisms responsible for inducing encephalopathy have not been precisely defined, an attractive feature of the GABA hypothesis is that the neural mechanisms responsible for mediating neural inhibition when the GABA neurotransmitter system is stimulated are well characterized [18-24,29]. Furthermore, some recent experimental data provide no support for the ammonia, the synergistic neurotoxins, and the false neurotransmitter hypotheses. For example, it has not been possible to reproduce the abnormal pattern of VEPs that is associated with an animal model of hepatic coma by infusing ammonium chloride or by administering simultaneously subcoma doses of ammonium chloride, a mercaptan, and a fatty acid [38]. Also, it has not been possible to reproduce the changes in receptors for GABA and glutamate that occur in an animal model of hepatic coma by infusing ammonium chloride [58,63,68]. Furthermore, while the false neurotransmitter hypothesis assumes that decreased dopaminergic neurotransmission occurs in hepatic coma, the finding of no significant changes in the molecular components of the postsynaptic dopamine receptor in an animal model of hepatic coma [69], suggests that there is no appreciable change in dopaminergic neurotransmission in hepatic coma. In one recent study the pattern of VEPs in a rat model of hepatic coma was reported to be similar to that in rats with coma induced by administering a mixture of subcoma doses of ammonia, a mercaptan, and a fatty acid [40]. However, in this study it is not completely clear whether the animals in coma following the administration of the three neurotoxins had not had an episode of seizures immediately preceding the onset of coma. Consequently the possibility exists that the coma in these animals may not have been due to the direct interactions of the three neurotoxins on the brain but rather to post-ictal coma. Thus, the findings in this study are not necessarily incompatible with the GABA hypothesis. Indeed, experimental data incompatible with this hypothesis have yet to be generated.

Clearly further studies are necessary to determine definitively what role, if any, the GABA neurotransmitter system plays in the pathogenesis of hepatic encephalopathy. In particular, the applicability of this hypothesis to human hepatic encephalopathy is currently unknown. Furthermore, it is not known whether the GABA hypothesis applies to other metabolic encephalopathies. There is a need to study GABA metabolism and the GABA neurotransmitter system in models of hepatic coma other than galactosamine-induced fulminant hepatic failure, including models of chronic liver failure. It would be highly desirable, using an appropriate model of hepatic encephalopathy, to measure the concentration of GABA in synaptic clefts, to assess the effects of blocking GABA-ergic neurotransmission, and to make direct measurements of GABA-ergic neurotransmission. Such data should provide further insights into the pathogenesis of hepatic encephalopathy.

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