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The Pathogenesis and Treatment of Hepatic Encephalopathy: Evidence for the Involvement of Benzodiazepine Receptor Ligands

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I. Introduction

ALTHOUGH the relationship between mental disturbances and liver disease has been described in Hippocratic texts, the mechanisms by which the liver affects normal brain function remain poorly understood. Liver failure is associated with impaired hepatic metabolism, resulting in altered circulating levels of numerous substances including amino acids, ammonia, and mercaptans. Many of these substances have been implicated in the pathogenesis of the changes in neuropsychiatric status that accompany liver failure, for which the term HE† is applied. Moreover, the proposed pathogenic mechanisms have had a significant impact on the development of therapeutic modalities for the management of this syndrome.

Schafer and Jones (1982) proposed that an increase in CNS GABAergic neurotransmission is involved in the pathogenesis of HE. Given our current understanding of the structure and function of GABA-gated chloride channels (Skolnick and Paul, 1988; Skolnick, 1990), an increase in "GABAergic tone" could be effected by several mechanisms. These include changes in one or more of the proteins constituting the GABA/BzR complex or increased concentrations of ligands which bind to this complex. This latter mechanism is consistent with several lines of clinical evidence indicating that humoral factors of gut origin may be precipitants of HE (Sherlock et al., 1954). The original description of high-affinity, saturable, and stereospecific binding sites in the mammalian CNS for Bzs and related compounds (Squires and Braestrup, 1977; Mohler and Okada, 1977) led to speculation that endogenous ligands are present that subserve the physiological function of these receptors. During the past decade a number of structurally diverse substances have been isolated that interact directly with the central BzR, including peptides, proteins, purines, 1,4-Bzs, and β-carbolines (Marangos et al., 1978; Mohler, et al., 1979; Skolnick et al., 1979; Guidotti et al., 1983; Sangameswaran et al., 1986; Pena et al., 1986). Increased concentrations of BzR ligands with agonist (i.e., diazepam-like) properties have recently been identified in the CNS and peripheral tissues of both animal models and patients with HE. These substances, such as exogenously administered 1,4-Bzs, can enhance the potency of endogenous GABA through the BzR, thereby contributing to the manifestations of HE by enhancing the suppression of neuronal activity in those pathways subserved by this transmitter.

The hypothesis that this neuronal mechanism is involved in mediating the manifestations of HE represents the first pathological condition associated with increased levels of "endogenous" BzR ligands. Furthermore, both this hypothesis and the data supporting it constitute a rational basis for developing a new therapeutic modality for the management of HE. In particular, BzR antagonists may represent a significant addition to currently available strategies for the management of this syndrome. BzR antagonists mediate their effects rapidly, their toxicity is minimal, they can be readily administered orally on an outpatient basis, and they are relatively inexpensive.

The objectives of this review are to characterize the syndrome of HE, including its clinical and pathophysiological manifestations, and describe current knowledge of the putative mechanisms contributing to the pathogenesis of this syndrome. This will then be followed by a brief evaluation of the traditional management of HE and an elaboration of the potential role of a new, experimental therapeutic modality involving BzR antagonists in the management of the syndrome.

II. Hepatic Encephalopathy

A. Definition

HE is a complex neuropsychiatric syndrome characterized by a global depression of CNS function, which may progress to impaired consciousness and coma (Adams and Foley, 1953; Davidson and Summerskill, 1956; Read et al., 1967; Gazzard et al., 1986). Acute HE is a complication of acute, subacute, or chronic hepatocellular failure (fig. 1) (Conn and Lieberthal, 1978; Schafer and Jones, 1990; Sherlock, 1989). HE is usually associated with appreciable portal-systemic shunting of blood along with hepatic insufficiency in patients with chronic liver disease. The surgical creation of portal-systemic shunts in such patients may be a major factor in precipitating or exacerbating HE. PSE is a term frequently used for HE associated with chronic liver disease and

† Abbreviations: HE, hepatic encephalopathy; CNS, central nervous system; GABA, γ-aminobutyric acid; PSE, portal-systemic encephalopathy; FHF, fulminant hepatic failure; EEG, electroencephalographic; VER, visual evoked response; CSF, cerebrospinal fluid; Bz, benzodiazepine; BzR, benzodiazepine receptor; GalN, galactosamine; TAA, thioacetamide; ATPase, adenosine triphosphatase; AAA, aromatic amino acid; BCAA, branched-chain amino acid; NE, norepinephrine; DA, dopamine; DMCM, methyl 6,7-dimethoxy-4-ethyl-3-carbomethoxy-β-carboline; P_1, first positive component of the VER; HPLC, high pressure liquid chromatography; DBI, diazepam-binding inhibitor.

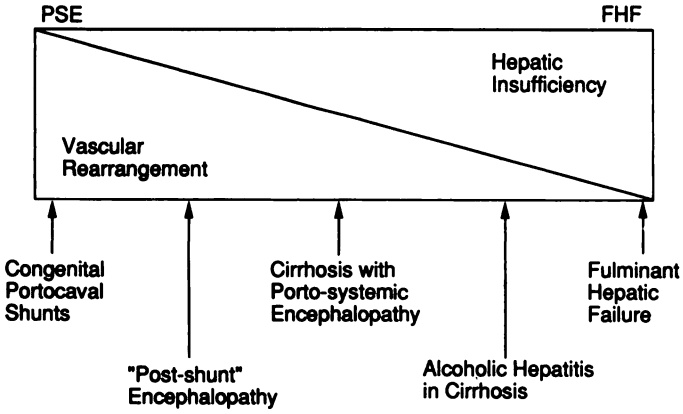

FIG. 1. HE is associated with a spectrum of hepatic insufficiency and vascular rearrangement. FHF is at one end of the spectrum at which hepatic insufficiency predominates. Congenital portacaval shunts may be considered to be at the other end of the spectrum, where vascular rearrangement predominates. Most patients with HE have degrees of hepatic insufficiency and vascular rearrangement that lie between these two extremes.

increased portal-systemic shunting. FHF is the term applied when HE complicates acute hepatocellular failure, and the total duration of liver disease is <8 weeks (Jones and Schafer, 1990). However, HE occurs most frequently as a complication of chronic hepatocellular disease. (Common precipitants of acute hepatocellular failure include viral hepatitis or drug overdose. Alcoholic cirrhosis and chronic viral hepatitis are the most common examples of chronic hepatocellular failure.) When an acute episode of HE occurs in this setting, it is commonly associated with a recognizable precipitating factor. Chronic HE or chronic PSE is a milder, more persistent, and more episodic variant of the syndrome that occurs in patients with chronic liver disease and appreciable portal-systemic shunts. HE is generally considered to be a reversible metabolic encephalopathy, a concept that would exclude changes in neuronal morphology in the development of the syndrome (Conn and Lieberthal, 1978). This distinguishes HE from irreversible neurological deficits and structural lesions of the CNS including hepatocerebral degeneration, cortical thinning, loss of neurons and fibers in the cerebellum and basal ganglia, laminar necrosis, spinal cord demyelination, and astrocytosis (Leigh and Card, 1949; Zieve et al., 1960; Victor et al., 1965; Read et al., 1967; Finlayson and Superville, 1981; Tarter et al., 1986). These lesions are not considered to be essential components of the syndrome of HE but may be distinct manifestations of chronic alcoholism or of chronic hepatocellular disease with chronic liver failure and large portal-systemic shunts. Furthermore, the increased intracranial pressure and cerebral edema that often complicate FHF can be classified separately from HE associated with FHF (Jones and Schafer, 1990).

The term "subclinical" HE has been applied to patients with chronic liver disease who do not display overt behavioral, neurological, or EEG changes but who have abnormal scores in psychomotor tests. These changes are reversible by conventional management of HE (e.g., lactulose, dietary protein restrictions) (Gitlin, 1988; Morgan et al., 1989). Alternatively, the subtle nature of the changes in subclinical HE may reflect the actions of a pathogenic mechanism(s) which is different from those responsible for the more overt manifestations of HE, as opposed to a difference in the degree of the defect.

HE research has been greatly facilitated by studies in which appropriate animal models are used. Unfortunately, there is great confusion in the literature regarding what constitutes an appropriate animal model of HE. This is due, in part, to both the incomplete knowledge of the mechanisms of pathogenesis of the syndrome and the inability to measure some of the more subtle psychomotor manifestations of the syndrome in animals. In a satisfactory model of HE, progressively more advanced neurological deficits with eventual coma should be manifested in the absence of potentially confounding complications of FHF such as hypoglycemia, hypothermia, renal failure, and severe acid-base disturbances. The behavioral manifestations of HE appear to be model dependent, ranging from lethargy and decreased locomotor activity to pronounced agitation and significant decrements in motor function leading to nystagmus, incoordination, and loss of reflexes. The syndrome of FHF in both humans and animal models has features that distinguish it from the syndrome of chronic liver failure with HE and may result from fundamental differences in the mechanism of pathogenesis of HE associated with acute and chronic liver failure. However, as is subsequently described in this review, it appears that certain findings in animals with FHF, such as the partial reversal of encephalopathy with BzR antagonists and the increased brain levels of Bz agonist ligands, also apply to humans with FHF and chronic liver failure.

B. Clinical Manifestations

The features of HE include a broad spectrum of fluctuating psychiatric and neurological abnormalities. Individual abnormalities are nonspecific and may occur in other metabolic encephalopathies, such as uremia, hypercapnia, and hypoglycemia or in sedative overdose.

In the routine clinical assessment of HE, neurological status can be conveniently graded into four stages based on mental state and neuromuscular function (Conn, 1987; Zieve, 1987; Schafer and Jones, 1990) (Table 1). Stage IV (coma) can be further subdivided based on the presence or absence of responsiveness to painful stimuli. The earliest abnormalities in psychiatric and cognitive function associated with HE may not be readily detectable during a conventional clinical assessment but may be apparent to family members and friends (Gazzard et al., 1986). Such early signs include personality changes, childishness, euphoria, irritability, and apathy, reflecting forebrain dysfunction. Slowness and brevity of response leading to dysphasia and perseveration may also be pres-

TABLE 1
Clinical stages of hepatic encephalopathy

Stage	Mental state	Neuromuscular state
I	Mild confusion, euphoria, depression, decreased attention, slowed analytical ability, irritability, sleep inversion	Mild incoordination, impaired handwriting
II	Drowsiness, lethargy, gross deficits in analytical ability, obvious personality changes, inappropriate behavior, intermittent disorientation. EEG abnormalities: high-amplitude, low frequency waves with non-focal changes	Asterixis, ataxia, dysarthria, paratonia, apraxia
III	Somnolent but rousable, unable to perform analytical tasks, disorientation with respect to time and/or place, amnesia, rage, slurred speech	Hyperreflexia, muscle rigidity, fasciculations, abnormal Babinski's sign, seizures (rare)
IV_A	Coma	Oculovestibular responses lost, response to painful stimuli lost
IV_B	Deep coma	Decerebrate postures, no response to painful stimuli

ent. In addition, moderate intellectual deterioration may appear in stage I or subclinical HE. Disturbances in spatial recognition may be manifested as constructional apraxia. Simple psychometric tests are useful in detecting and quantifying subtle defects in mental function in subclinical or stage I HE. Although not specific for HE, these tests are often more sensitive in detecting defects of mental function in early HE than either the EEG or clinical assessments. They include orientation to time, person, and place, recall of current events, the subtraction of serial 7's, handwriting, figure drawing, and the number connection test (Conn, 1977; Rikkers et al., 1978; Gilberstadt et al., 1980). A variety of neuromuscular abnormalities are also associated with HE. Decreased spontaneous movement and fixed stare often accompany the most characteristic neurological abnormality in HE: asterixis (liver flap or flapping tremor) (Adams and Foley, 1953; Leavitt and Tyler, 1964). Asterixis is demonstrated by wrist dorsiflexion when the arm is extended. Within 30 s the hand falls forward, followed by a rapid recovery of the posture. This results from impaired reticular processing of proprioceptive information from the metacarpophalangeal and wrist joints. Asterixis differs from tremor in that it is intermittent, of lower frequency, and bilaterally asynchronous. Other neurological disorders include exaggeration of deep tendon reflexes, muscle rigidity, sustained ankle clonus, fasciculations, ataxic gait, decerebrate postures, and bizarre facial expressions.

Deficits in consciousness are also observed in patients with HE. Hypersomnia is often an early manifestation of the syndrome, which can progress to inversion of sleep rhythm. Delirium and seizures are atypical but may occur during the often rapid evolution of HE due to FHF. Progression from the early stages of HE to coma may occur within hours, regardless of therapy. Nonetheless, at any stage of HE, progression of the syndrome may cease, followed by an amelioration. Coma at first resembles normal sleep but may progress to complete unresponsiveness to painful stimuli. In general, the prognosis for HE due to FHF is more grave than that of a typical episode of HE complicating chronic hepatocellular disease, particularly when the latter is associated with an obvious precipitating factor.

Common precipitants of HE in a patient with cirrhosis include an intestinal nitrogen load in the form of dietary protein or a gastrointestinal hemorrhage from esophageal varices, constipation, urinary, pulmonary, or peritoneal infections (e.g., spontaneous bacterial peritonitis), diarrhea, vomiting, hypoxia, anemia, hypotension, abdominal paracentesis, dehydration, and azotemia (Hoyumpa et al., 1979; Zieve, 1987). Electrolyte and/or acid-base disturbances may also precipitate HE, a common example being hypokalemic metabolic alkalosis due to diuretic therapy (Naranjo et al., 1979). The administration of sedative-hypnotics such as Bzs or barbiturates or analgesics such as morphine which depress CNS function can precipitate or exacerbate HE in patients with poor hepatocellular function (Laidlaw et al., 1961; Branch et al., 1976; Bakti et al., 1987). Moreover, surgical procedures in patients with hepatocellular disease are likely to precipitate HE by exacerbating hepatocellular failure. Hypoglycemia may also compound encephalopathy in FHF (Jones and Schafer, 1990). When HE appears spontaneously (i.e., without obvious precipitants) in patients with decompensated cirrhosis, it is attributed to deteriorating hepatocellular function and the prognosis is usually grave. Although proper management of precipitants is crucial for the clinician, an understanding of the mechanisms by which these factors precipitate HE would provide further insight into the pathogenesis of the syndrome.

C. Neuropathology

1. Anatomy. The presence of alterations in gross CNS anatomy appears dependent on the acute or chronic

nature of the liver failure. The gross anatomy of postmortem brains is apparently normal following acute episodes of HE (Sherlock, 1989). Although approximately 50% of patients with FHF in prolonged, deep coma develop cerebral edema (Gazzard et al., 1975; O'Brien et al., 1987), and have elevated intracranial pressure, these developments are well-recognized complications of FHF in both animal models and humans and can be classified separately from HE per se. In contrast, cerebral atrophy has been reported in computed tomographic scans of cirrhotic patients with chronic recurrent HE (Acker et al., 1982; Zeneroli et al., 1987). It was not clear from these studies whether the brain atrophy resulted solely from the effects of chronic liver disease or from alcohol abuse. Subsequent computed tomographic studies of patients with chronic, non-alcoholic liver disease indicated the presence of cerebral edema and significant cortical atrophy in patients who were not overtly encephalopathic (Tarter et al., 1986; Bernthal et al., 1987). These morphological abnormalities are associated with decreased performance on psychometric tests and correlate with the degree of liver dysfunction but not with blood ammonia levels. Although it appears that liver failure mediates the CNS atrophy, the mechanisms responsible for these changes are unknown. Furthermore, it is not clear that changes in morphology, as opposed to metabolic changes, are directly involved in the pathogenesis of HE. Bernthal and coworkers (1987) assert that variants of HE may not be entirely based on metabolic derangements but on the CNS atrophy observed in patients with chronic liver disease. However, some unknown, chronic metabolic alterations must be involved in the development of subclinical HE. This is indicated by the ability to reverse the psychomotor deficits in subclinical HE with standard therapies (Morgan et al., 1989), despite the continued presence of brain atrophy, and the requirement that some metabolic derangement must be present to cause the initial degeneration of cortical structures observed in these studies.

Histological examination of patients who have died with cirrhosis and portal-systemic shunts often reveals the presence of Alzheimer type II astrocytosis in the cerebral cortex, cerebellum, putamen, and globus pallidus (Norenberg, 1981). In patients with cirrhosis, increases in the density of "peripheral-type" BzRs, which are located primarily on nonneuronal elements (Syapin and Skolnick, 1979; Bender and Hertz, 1985) are also observed (Lavoie et al., 1989). This particular type of astroglial change has been proposed to reflect a toxin-induced (probably ammonia) gliopathy (Norenberg, 1977; Gregorios et al., 1985a,b). However, the relevance of these changes to the neuropsychiatric manifestations of HE is uncertain (Norenberg, 1981). No significant changes in neuron structure in HE have been observed at the level of the light or electron microscope (Conn, 1987; Jones and Gammal, 1988). The lack of significant alterations in neuron morphology, the presence of only minor astrocytic changes in the early stages of HE, and the clinical impression that this syndrome can potentially be completely reversed are compatible with the concept of a metabolic encephalopathy.

2. *Electrophysiology.* The EEG changes associated with this syndrome are not specific for HE. Assessments of CNS electrical activity using the EEG indicate a generalized slowing and an initial suppression of the α rhythm (Laidlaw and Read, 1963). As the syndrome progresses, an unstable, high-voltage α rhythm appears with paroxysmal waves of 5–7 cps, beginning bilaterally in the frontal and temporal regions and spreading posteriorly. Finally, the overall amplitude of the EEG decreases, and bilaterally synchronous 2- to 3-cps waves are found primarily over the frontal lobes. Global electrophysiological recording techniques such as the EEG are useful for monitoring HE (MacGillivray, 1976), particularly when combined with computer-assisted frequency and amplitude analysis (De Groot et al., 1985). In FHF, small decreases in the mean frequency of a continuous EEG recording may precede clinical deterioration (Trewby et al., 1978). In exacerbations of chronic HE, the appearance of triphasic waves is indicative of a poor prognosis, as is a progressive decrease in EEG wave amplitude associated with periods of EEG suppression.

Recording of evoked responses may be more useful than the EEG in assessing the neurophysiological status of HE in animal models and in comparing with other models of encephalopathy. However, evoked response measurements have not been widely applied to humans with HE. Repeated flashes of light (VER), aural pulses (auditory-evoked response), or tactile stimuli (somatosensory-evoked responses) evoke synchronous volleys of excitatory and inhibitory discharges through postsynaptic neuronal networks in subcortical and cortical regions of the visual and/or other sensory-processing areas (Chiappa and Ropper, 1982; Martines et al., 1984; Zeneroli et al., 1984; Dyer et al., 1987; Yang et al., 1985). Changes in some VER components may be attributable to specific neuronal mechanisms, such as alterations in GABAergic tone (Zemon et al., 1980; Bassett et al., 1987). Although abnormal VERs may occur in preclinical (latent) HE and are certainly observed in overt HE (Zeneroli et al., 1984; Casellas et al., 1985), they do not correlate well with the clinical stage of HE in patients (Johansson et al., 1989). This may possibly be attributed to the superficial placement of the electrodes. Furthermore, there is significant inter- and intraindividual variability in the amplitudes and latencies of the VER components (Sandford and Saul, 1988). Thus, the VER may be useful only as a research tool in animal models of HE, in which these variables can be more rigidly controlled (e.g., by the placement of epidural electrodes). Furthermore, although investigators in a recent clinical study of the efficacy of flumazenil in the treatment of patients with

HE associated with acute and chronic liver failure recorded improvements in the somatosensory-evoked response (Grimm et al., 1988b), it is not clear that changes in somatosensory-evoked response waveform parameters can quantitatively assess the severity of HE. In contrast, a recent study (Weissenborn et al., 1990) indicated that measurements of wave latencies in auditory-evoked responses may be a highly appropriate test for the detection of early HE, with a sensitivity equivalent to that of psychometric testing. This may result from the relatively low variability of the amplitude and latency of the P300 component of the auditory-evoked response in normal subjects (Polich, 1986; Sklar and Lynn, 1984) compared to other types of evoked responses.

III. Proposed Mechanisms of Pathogenesis

The basic pathophysiological changes associated with HE involve the accumulation of neuroactive and potentially comagenic substances in the systemic circulation. These compounds are normally absorbed from the gut and metabolized by the liver, but in hepatocellular failure they bypass the diseased liver and enter the systemic circulation (Sherlock, 1989). These putatively toxic substances then cross the blood-brain barrier and accumulate in the brain. Such substances may alter CNS function through a variety of mechanisms, including depression of neuronal electrical activity via neurotransmitter receptors (Schaïer and Jones, 1982), inhibition of electrogenic pumps (Lux, 1971; Ahmed et al., 1984), or inhibition of neuronal oxidative metabolism (Hawkins and Mans, 1989). The shunting of portal venous contents into the systemic circulation is clearly a major mechanism involved in the accumulation of gut-derived neurotoxins in the peripheral circulation in liver failure (Sherlock, 1989; Zieve, 1987; Jones and Gammal, 1988) (fig. 2). Normally, the liver plays a central role in removing potentially neurotoxic substances, which include metabolites produced by enteric bacteria, as well as many drugs and other low molecular weight substances. However, in patients with hepatocellular failure, these compounds could accumulate in plasma as a result of reduced hepatic extraction. In addition, in patients with chronic hepatocellular disease, such substances may also have increased access to the systemic circulation through collateral portal-systemic venous channels which completely bypass the liver parenchyma. This portal collateral circulation occurs most commonly as a consequence of portal hypertension in patients with cirrhosis. The surgical construction of portal-systemic shunts to alleviate portal hypertension may further increase this shunting of blood. Moreover, portal-hepatic venous anastomoses may develop around the regeneration nodules in a cirrhotic liver and act as intrahepatic shunts. Thus, HE is associated with both hepatic insufficiency and vascular rearrangement (fig. 1). In some cases (e.g., FHF) hepatic insufficiency predominates, whereas in others (e.g., congenital portacaval shunts) vascular rearrangement predominates. However, the degree of hepatic insufficiency and vascular shunting lies between these two extremes in most cases of HE.

Alterations in the permeability of the blood-brain barrier have been hypothesized to contribute to the pathogenesis of HE. Increased blood-brain barrier permeability may occur in liver failure as a result of elevated plasma concentrations of substances such as ammonia, methyl octanoate, mercaptans, phenol, or dehydrocholate (Zaki et al., 1983; Spigelman et al., 1983). The accumulation of such substances in the plasma during liver failure could increase blood-brain barrier permeability by acting on capillary enzymes involved in the regulation of cerebral blood flow, altering the function of glial transporter systems, and/or increasing membrane fluidity/patency (Goldstein, 1984). Studies of changes in blood-brain barrier permeability in animal models of HE have produced conflicting results (Horowitz et al., 1983; Ede, et al., 1984; Huet et al., 1984; Lo et al., 1987; Traber et al., 1987; Knudsen et al., 1988; Bassett et al., 1990). This may reflect differences in the validity of the models of HE studied or the techniques used to measure blood-brain barrier permeability. Studies using the Oldendorf intracarotid artery injection technique (Oldendorf, 1981) indicate that the permeability of the blood-brain barrier to a variety of polar molecules, which do not normally cross the blood-brain barrier (including sugars, GABA, and amino acids), is increased in HE (Bassett et al., 1990; Zaki et al., 1983). Although studies in which this experimental approach has been used have been criticized because of the inherent limitations of the technique (such as variable uptake of the reference tracer and susceptibility of data to changes in cerebral blood flow and volume; Fenstermacher et al., 1981), recent studies designed to avoid these problems indicate that blood-brain barrier permeability is nonspecifically increased in models of FHF (Horowitz et al., 1983; Bassett et al., 1990). Thus, the development of HE in FHF may involve an increase in blood-brain barrier permeability leading to an enhanced transfer across the barrier and the accumulation in the CNS of neuroactive metabolites. It should be emphasized that, although data concerning increased permeability of the blood-brain barrier in animal models of FHF are persuasive, it is less certain that a major change in blood-brain permeability occurs in chronic liver failure.

Although there is a consensus of opinion regarding the route by which substances in the portal vein may reach the CNS in liver failure, the identity of the neuroactive substance(s) responsible for the development of HE remains controversial. When one considers both the factors that precipitate HE and the effective modalities of treatment, any neuroactive substance relevant to the pathogenesis of HE should be (*a*) nitrogenous, (*b*) of enteric origin, (*c*) synthesized by gut flora and/or present in the diet, (*d*) found in the portal circulation, (*e*) metabolized

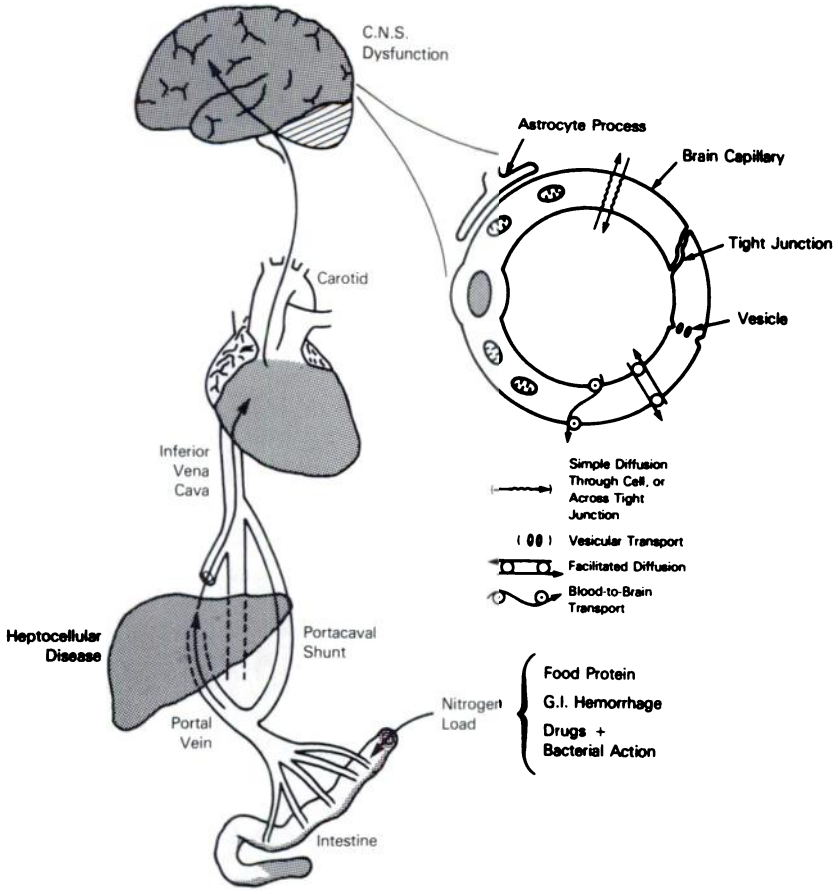

FIG. 2. Traditional concepts of the development of HE or PSE. Nitrogenous substances arising from the gut (e.g., derived from metabolism of dietary protein by enteric bacteria) are absorbed into the portal venous system and delivered to the liver. Under physiological conditions, most of these substances are efficiently extracted and metabolized by the liver. However, with liver disease and impaired hepatocellular function, these substances may be inefficiently extracted by the liver and consequently pass directly into the systemic circulation. Alternatively, they may be shunted through portal-systemic venous collaterals around the liver and into the systemic circulation. After these compounds enter the systemic circulation, they may gain entry to the CNS by a variety of mechanisms as illustrated. If these substances are required by the CNS (e.g., as precursors for neurotransmitters), high-capacity active transport or facilitated diffusion systems may be present. Alternatively, such compounds may diffuse along their concentration gradient across vascular endothelial cells or their intercellular tight junctions to enter the brain. Regardless of the route of entry, the presence of elevated concentrations of these compounds in the peripheral blood plasma would tend to result in their increased uptake into the CNS. If the compounds are neuroactive, this process may lead to the modulation of brain function. Even if these substances are not required for CNS function and are efficiently excluded from the CNS by the normal blood-brain barrier, they may enter the brain through an abnormally permeable blood-brain barrier. An increase in blood-brain barrier permeability to [^{3}H]GABA, [^{14}C]α-amino isobutyric acid, and a vascular permeability tracer (transferrin) has been reported to occur in an animal model of FHF, suggesting that HE due to acute liver failure is associated with a nonspecific increase in the permeability of the barrier. Modified from Sherlock, 1989.

by the normal liver, and (*f*) able to cross the blood-brain barrier in liver failure and affect CNS function (Sherlock 1989). Elevated or otherwise abnormal concentrations of a large number of substances are found in the plasma, CSF, and brains of animal models and patients with HE (Zieve, 1987; table 2). These findings have led to the formulation of several hypotheses of the pathogenesis of HE, none of which has been conclusively validated. However, given the range of metabolic and neuropsychiatric abnormalities observed in liver failure, it is highly likely that the etiology of HE is multifactorial (Hoyumpa et al., 1979; Hoyumpa and Schenker, 1982; Crossley et al., 1983; Schenker and Hoyumpa, 1984; Zieve, 1987). Several factors must be considered when determining the relevance of a particular metabolic abnormality to the pathogenesis of HE: (*a*) Does the abnormality result in HE-like neurological deficits? (*b*) Is there a correlation between the degree of the metabolic abnormality and the severity of HE? (*c*) Is the derangement found in all patients with HE? and (*d*) At what specific stage(s) of HE (or liver failure) is the abnormality manifested?

During the past four decades elevated levels of ammonia and certain amino acids have been widely advocated as playing major roles in the pathogenesis of HE (Zieve, 1987). Other substances, such as mercaptans, free fatty acids, and phenols, have also received extensive consideration (Zieve and Brunner, 1985; Zieve, 1985). Most recently, GABA and BzR agonists have been proposed as prominent factors in the evolution of HE (Schafer and Jones, 1982; Mullen et al., 1988a; Basile and Gammal, 1988). In the following sections, the potential roles of the more intensively investigated substances

TABLE 2
*Metabolites found in abnormal levels in HE**

Category	Blood	CSF	Brain
Electrolytes	↓ Na^+, K^+, Mg^{2+}, Zn^{2+}, Ca^{2+}, PO_4^{2-}		
TCA cycle intermediates	↑ Pyruvate, lactate, α-ketoglutarate, acetone, butylene glycol, citrate, α-ketoglutaramate ↑ acetoacetate	↑ Pyruvate, lactate, α-ketoglutarate	↓ Malate, oxaloacetate, α-ketoglutarate, fumarate
Urea cycle intermediates	↑ Citrulline, ornithine		
Amino acids	↑ Met, Phe, Tyr, Trp (free), Asp, Glu, Lys, Gly, His, Cys ↓ Leu, Ileu, Val, Arg	↑ Asparagine, glutamine	↑ Glutamine
Neurotransmitters, false neurotransmitters, and their metabolites	↑ GABA, homovanillic acid, 5-hydroxyindolacetic acid, octopamine	↑ Glutamate, purines, norepinephrine, dopamine, serotonin, GABA, 5-hydroxyindolacetic acid, tryptophan, normetanephrine, octopamine, phenylethylamine	
Neurotoxins	↑ Mercaptans, ammonia		↑ Mercaptans
Miscellaneous	↑ Phenols, tyramine, straight and branched chain fatty acids		

* These substances have been reported to occur in increased concentrations in the blood, CSF, and/or CNS of patients with HE. Some of these compounds may directly influence neuronal activity through receptor-mediated mechanisms. Other compounds may alter neuronal function indirectly by suppressing oxidative metabolism, altering pH, or disrupting neurotransmitter synthesis or catabolism. Increased levels of these substances in the blood may result in elevated CNS concentrations, particularly in the event of increased blood-brain barrier permeability. Furthermore, whole brain levels of these compounds may not be representative of their concentrations at the synaptic level. None of these substances, either alone or in combination, would affect the binding of the Bz antagonist [^{3}H]Ro 15-1788 to BzR in the cerebral cortex at the concentrations reported to occur in HE.

that have been linked to the pathogenesis of HE are discussed. First, the development of various animal models of the syndrome will be reviewed because they have been invaluable in assessment of the relative importance of specific neural mechanisms in the pathogenesis of HE.

A. Animal Models

Several apparently satisfactory animal models of HE due to FHF have been developed. One model that closely resembles HE due to FHF in humans, particularly with respect to the development of neuromuscular deficits, is the rabbit with GalN-induced FHF (Blitzer et al., 1978; Traber et al., 1986a, 1987; Mullen et al., 1988b). Within 24–48 h after administration of a single dose of GalN (a selective hepatotoxin) (Decker and Keppler, 1972), the rabbit develops a series of readily staged decrements in neuromuscular function (table 3). Initially, the animal is lethargic and shows reduced response to external stimuli. In the more advanced stages, rabbits lose flexor tone and become disoriented and ataxic, and nystagmus is present in about 40% of the animals. These changes in neuromuscular function indicate that, in this model, the syndrome of HE includes a prominent deficit in cerebellar processing. Approximately 6 h after the initiation of these behavioral changes the rabbit becomes comatose. The evolution of the behavioral features in this model of

TABLE 3
*Behavioral characteristics of the rabbit with hepatic encephalopathy due to GalN-induced fulminant hepatic failure**

Stage	Characteristics
I	Sedation, impaired responsiveness to stimuli
II	Mild ataxia, poor head posture
III	Severe ataxia, hindlimb extension, loss of righting reflex, loss of flexor tonus, nystagmus
IV	Light coma, surgical coma

* From Basile and Gammal, 1988.

HE is associated with a progressive, distinctive and reproducible series of changes in the VER (Schafer et al., 1984). Histological examination of GalN-treated rabbits indicates the presence of patchy (rather than massive) necrosis of the hepatocytes (Blitzer et al., 1978), suggesting that hepatocellular failure may be due largely to organelle failure. Other than mild edema, there is no gross CNS pathology in this model (Traber et al., 1986a, 1987). The behavioral, neurochemical, and electrophysiological manifestations of encephalopathy following the administration of GalN to other animals are generally less satisfactory and less specific for HE (Mullen et al., 1988b; Olasmaa et al., 1990). GalN-treated guinea pigs develop an encephalopathy secondary to hypoglycemia, hypothermia, and hypotension (Brachtel and Richter, 1986). Rats display major strain differences in sensitivity

to GalN-induced liver failure (ranging from 0–100% mortality) (Ten Berg et al., 1985), and the degree of encephalopathy is consistently milder than that observed in rabbits (Tabata and Chang, 1980; Mullen et al., 1988b). Indeed, many GalN-treated rats die without developing stage IV encephalopathy.

A second satisfactory model of HE is the rat with TAA-induced FHF (Hilgier et al., 1983; Jones et al., 1987; Zimmermann et al., 1989; Gammal et al., 1990). Although this model does not manifest the dramatic behavioral changes (e.g., nystagmus, profound ataxia) observed in the GalN-treated rabbit, motor activity is generally decreased, the righting reflex is lost by stage III, and distinct and reproducible electrophysiological changes (as measured by VER) occur. An improved version of this model avoids hypothermia and corrects hypoglycemia and renal dysfunction by giving glucose and electrolyte supplements. This model has the advantage that the full evolution of the syndrome of HE takes several days, thereby facilitating objective behavioral studies (Gammal et al., 1990).

Other models of HE due to acute liver failure tend to be less satisfactory (Schafer, 1984). Examples include the induction of liver failure or encephalopathy using acetaminophen, nitrosamines, viruses, and hepatic vascular diversion. In general, these models have a variety of drawbacks, including the lack of hepatospecificity of the toxins or viruses used and the development of hypothermia or seizures (Rzepczynski et al., 1986; Traber et al., 1986b). Thus, the relevance of the encephalopathy induced in some of these models to the human condition must be questioned.

Despite the usefulness of some models of FHF, animal models of HE due to chronic liver failure would also be valuable, because chronic rather than acute liver failure is the most common setting in which HE develops in humans. One model of chronic liver failure is the rat with carbon tetrachloride-induced cirrhosis and ascites (Proctor and Chatamra, 1982). Although suitable for studying some of the manifestations of chronic hepatocellular failure, rats treated with carbon tetrachloride do not develop HE, even after blood gavage or large doses of diuretics (Martin et al., 1989). Similarly, animals with portacaval shunts in the absence of hepatocellular failure (including rats with portacaval shunts and dogs with Eck fistulas) do not reliably develop overt encephalopathy (Bircher, 1979; Miyai et al., 1985; Okamoto et al., 1985; de Boer et al., 1986; Rossle et al., 1986; Thompson et al., 1986), and it is subtle when manifested (Bircher, 1979; Butterworth and Giguere, 1986). Thus, it is necessary to take additional steps to induce overt encephalopathy in animals with portacaval shunts, for example the administration of nitrosamines, blood gavage, partial hepatectomy, feeding of ammonium salts, or hepatic artery ligation (Norenberg, 1977; Baraldi et al., 1984b; Schafer, 1984; Rossle et al., 1986; Rzepczynski et al., 1986; Traber et al., 1986b). There is apparently little involvement of GABAergic neurotransmitter systems in the encephalopathy that is induced in these models (Rzepczynski et al., 1986; Ferenci et al., 1987; Zieve et al., 1987; Roy et al., 1988; Olasmaa et al., 1990). Furthermore, the significance of encephalopathy in an animal with a portacaval shunt is difficult to assess. For example, the prevention of encephalopathy by an appropriate diet in the dog with an Eck fistula is intriguing and currently unexplained (Thompson et al., 1985).

In summary, the syndrome of HE in humans with FHF can be substantially reproduced by treating rabbits with GalN or rats with TAA. Rats with portacaval shunts and dogs with Eck fistulas may be useful models of portal-systemic shunting but are unsatisfactory models of HE because of acute or chronic liver failure. At present, no satisfactory model of HE due to chronic liver failure is available.

B. Ammonia

Ammonia is clearly neurotoxic (Torda, 1953), and hyperammonemia is widely regarded as a major contributor to the pathogenesis of HE. Gabuzda and coworkers (1952) reported that the similarity of the tremor and mental changes observed in patients with cirrhosis given ammonium-containing resins to the neurological manifestations of impending coma suggests a common biochemical basis. (It should be noted that these patients became constipated as a result of the administration of the ammonia-bearing resin. Thus, constipation, rather than ammonia, may have been a significant factor in the precipitation of HE in this study.) The gut is a major site for ammonia production, where it is generated by bacterial degradation of amines, amino acids, and urea (Lockwood et al., 1979; van Leeuwen et al., 1984). Contracting muscle also produces ammonia by purine metabolism (Lowenstein, 1972). Liver failure impairs the conversion of ammonia and glutamine to urea by the Krebs-Hensleit urea cycle. Thus, gut-derived ammonia bypasses the liver, increasing peripheral blood plasma concentrations of ammonia. Under conditions of portal-systemic shunting, skeletal muscle reverses its role as an ammonia generator, becoming an ammonia "trap," by converting ammonia to glutamine, which is then converted to urea (Duda and Handler, 1958).

Ammonia has manifold effects on normal CNS function. Electrophysiological studies of single neurons and isolated synaptic networks and biochemical studies have shown that increasing ammonia concentrations inactivate Cl^- extrusion pumps (Lux, 1971; Inagaki et al., 1987). The resulting elevation of intracellular Cl^- concentration blocks the formation of the hyperpolarizing inhibitory post-synaptic potential, impairing postsynaptic inhibitory processes throughout the brain (cerebral cortex, thalamus, brainstem, and spinal cord) (Lux et al., 1970; Lux, 1971; Llinas and Baker, 1972; Raabe and Gumnit, 1975). This leads to transient increases in ex-

citatory phenomena (Theoret and Bossu, 1985), including seizures at high ammonia concentrations. Further elevations in ammonia levels suppress excitatory postsynaptic potential formation as well, causing presynaptic conduction blocks and an overall depression of neuronal electrical activity (Raabe, 1989). These observations are consistent with the lethargy and EEG slowing induced by the acute administration of ammonium chloride by slow intravenous infusion to normal rabbits (Pappas et al., 1984b; Ferenci, et al., 1984b). However, the changes in the EEG and VER induced by hyperammonemia are not characteristic of those observed in HE (Cohn and Castell, 1966; Pappas et al., 1984b; Jones et al., 1987). The electrophysiological effects of ammonia in normal animals or on preparations of normal neurons are quite different from the electrophysiological changes that occur in animal models of HE or PSE. Cats with portacaval shunts appear to have normal inhibitory postsynaptic potentials, despite chronically elevated plasma ammonia concentrations (Raabe and Onstad, 1985). This observation has been attributed to the development of Cl^- pump tolerance to the persistently increased levels of ammonia (Raabe, 1989), although the change in activity of these pumps has never been confirmed by direct measurement. Despite the apparent tolerance of neurons to ammonia in animals with portacaval shunts, the CNS becomes more sensitive to acute ammonia challenges, which decrease excitatory postsynaptic potential and inhibitory postsynaptic potential amplitudes (Raabe and Onstad, 1985). Thus, in chronic liver failure, hyperammonemia may serve to sensitize the CNS to additional metabolic insults and neurotoxins (Walker and Schenker, 1970).

Hyperammonemia also alters cerebral energy metabolism by a variety of mechanisms, such as suppression of the tricarboxylic acid cycle and the malate/aspartate shuttle (oxidation of pyruvate and α-ketoglutarate) (McKhann and Tower, 1961; Hindfelt et al., 1977; Lai and Cooper, 1986) and stimulation of glycolysis (Lowry and Passonneau, 1966). Hyperammonemia decreases Na^+/K^+ ATPase activity (Schenker et al., 1980) and the global cerebral metabolic rate for O_2 (Mans et al., 1983). These changes may contribute to the cerebral edema and increased intracranial pressure observed in FHF (Voorhies et al., 1983). However, because these changes are also observed in patients with liver failure, but without encephalopathy, their contribution to HE is unclear (Fazekas et al., 1956; Posner and Plum, 1960). Furthermore, changes in the constituents of the tricarboxylic acid cycle are not universally observed in HE or in hyperammonemic states (Shorey et al., 1967; Duffy et al., 1974; Hindfelt et al., 1977). Recent studies have not effectively linked the actions of hyperammonemia on cerebral metabolism to the development of HE. Acute hyperammonemia in normal rats and/or rats with portacaval shunts reportedly increases brain glucose metabolism, particularly in the reticular activating system (Lockwood et al., 1986). This increase in glucose metabolism observed in rats 8 weeks after shunting is paradoxical, because significant decreases in cerebral blood flow and oxygen metabolism are observed at this time as well (Gjedde et al., 1978). Moreover, the issue has been raised whether changes in cerebral energy status are the result rather than the cause of ammonia-induced encephalopathy (Crossley et al., 1983; Hawkins and Mans, 1989). Finally, although chronic hyperammonemia causes a type II astrocytosis in animals similar to that observed in chronic liver failure in humans (Norenberg, 1977, 1981), it has not been established that this complication of hyperammonemia contributes to the pathogenesis of HE.

Clinical evidence suggesting a role for ammonia in HE is based on the observation that ammonia accumulates in liver failure and readily enters the brain (Lockwood et al., 1979). Studies of cirrhotics ingesting ammoniagenic compounds, such as amino acids, urea, and ammonia-releasing resins (Gabuzda et al., 1952), suggested an association between the accumulation of ammonia and encephalopathy. In congenital defects of urea cycle enzymes and valproate poisoning, encephalopathies develop in parallel with hyperammonemia (Shih, 1976; Coulter and Allen, 1980; Flannery et al., 1982; Arn et al., 1990), although the plasma ammonia concentrations that occur in these syndromes are typically much higher than those associated with acute or chronic liver failure. In addition, CSF levels of the ammonia metabolites glutamine and α-ketoglutaramate correlate well with the clinical severity of HE (Hourani et al., 1971; Vergara et al., 1974; Oei et al., 1979). Moreover, therapies that decrease intestinal ammonia absorption are often followed (after several hours) by an amelioration of HE in patients with cirrhosis (Conn and Lieberthal, 1978; Zieve, 1987; Sherlock, 1989).

Nonetheless, there are several findings that are inconsistent with a prominent role of ammonia in the pathogenesis of HE. Plasma ammonia levels do not correlate well with the expression of HE or the severity of encephalopathy (Stahl, 1963). A variety of factors may contribute to this variability, including ammonia production by contracting muscle, differential ammonia tolerance, altered ammonia compartmentation resulting from pH and electrolyte imbalances, and technical problems associated with taking proper blood samples (Conn, 1987). Indeed, plasma ammonia levels may be normal in liver failure, but CNS levels may be elevated [particularly after saturation of the glutamine pool (Ehrlich et al., 1980)]. An additional problem in relating ammonia to HE (particularly when associated with FHF) is that progressive acute ammonia intoxication is characterized by a preconvulsive, lethargic state, seizures, and postictal coma (Torda, 1953; Roberge and Charbonneau, 1969; Pappas et al., 1984b). Although seizures are common in congenital hyperammonemia syndromes (Shih, 1976;

Flannery et al., 1982), they are unusual in acute or chronic HE (Conn and Lieberthal, 1978). The former observation would argue against the proposal that the function of neuronal Cl^- pumps becomes tolerant under conditions of chronic ammonia excess. When ammonium acetate was administered to patients with chronically compromised liver function, EEG alterations typical of HE were not induced (Cohn and Castell, 1966). In addition, in patients with portal-systemic shunts the development of encephalopathy correlated better with the size of the shunt than with plasma ammonia levels (Ohnishi et al., 1985). Finally, hemodialysis of patients in hepatic coma due to acute or chronic liver failure yields inconsistent results. Although significant reductions in plasma ammonia levels are achieved using a variety of dialysis techniques (Opolon, 1980), enhancement of neurological function occurs in <50% of the patients so treated. Indeed, some dialysis techniques appear to hasten neurological deterioration in patients with HE (Opolon, 1980).

Whereas a significant body of information indicates that elevations in the brain and plasma levels of ammonia (and related metabolites) occur in liver failure and that ammonia can modulate neuronal function, evidence that hyperammonemia is solely responsible for the development of HE is lacking. Furthermore, ammonia intoxication does not reproduce the subtle changes in personality and mentation and the sleep inversion that are significant components of HE due to chronic liver failure. Although ammonia should be regarded as one of multiple factors responsible for altered CNS function in liver failure, its relative contribution to the global manifestations of HE remains undefined.

C. Branched-Chain Amino Acids, Aromatic Amino Acids, and "False" Neurotransmitters

In addition to its direct neurotoxic effects, an indirect role for ammonia in the pathogenesis of HE has been proposed in relation to increases in the CNS concentrations of AAAs, which affect the metabolism of certain neurotransmitters (Iber et al., 1957; Fischer and Baldessarini, 1971; Ansley et al., 1978; James et al., 1979; Zieve, 1979; Cascino et al., 1982). These aberrations are believed to result from the following series of events that have been proposed to occur in liver failure (James et al., 1979). Impaired hepatic urea synthesis leads to elevated blood ammonia levels, which stimulate glucagon secretion (Strombeck et al., 1978), and enhance gluconeogenesis from amino acids. The resulting elevation of blood glucose causes hyperinsulinemia which stimulates BCAA uptake by muscle and decreases the plasma BCAA/AAA ratio. Because BCAAs and AAAs share the same carrier system in cerebral endothelial cells, this altered ratio would facilitate AAA transport into the CNS (Fischer and Baldessarini, 1976; Oldendorf and Szabo, 1976). Further enhancement of the rate of AAA transport across the blood-brain barrier in liver failure may be driven by ammonia-induced elevations of brain levels of glutamine (Martinez-Hernandez et al., 1977; Cardelli-Cangiano et al., 1981), which shares the same antiporter system with AAAs. The resulting elevation of CNS AAA levels would enhance false neurotransmitter synthesis, contributing to HE by depleting the levels of biogenic amine neurotransmitters.

This hypothesis is supported by the following evidence. The plasma concentrations of free AAAs (tyrosine, phenylalanine, and tryptophan) and methionine are increased in chronic liver failure. The increase in plasma tryptophan levels results from tryptophan displacement from albumin by nonesterified fatty acids and reduced plasma albumin levels (Curzon et al., 1973; Ono et al., 1974; Sherwin et al., 1974; James et al., 1976). The increase in plasma phenylalanine and tyrosine levels may result from the hyperglucagonemia-induced enhancment of protein catabolism and a decrease in hepatic deamination. In contrast, plasma levels of BCAAs, such as valine, leucine, and isoleucine, are decreased in chronic liver disease, possibly due to hyperinsulemia-induced increases in their catabolism (Munro et al., 1975).

Increased levels of tryptophan, tyrosine, phenylalanine, and other neutral amino acids are observed in the brains of several animal models of HE (Curzon, et al., 1973; Cummings et al., 1976; James et al., 1978; Jonung et al., 1985) and in the CSF of humans with HE (Borg et al., 1982; Riggio et al., 1985). After they enter the brain, however, the precise mechanism by which AAAs alter CNS function is unclear. Some AAAs, particularly tryptophan, have been found to be directly neurotoxic (Smith and Prockop, 1962; Zieve, 1987; Huet et al., 1981), albeit at extremely high doses (2–10 g/kg). AAAs may exhibit indirect neurotoxicity in two ways, either by suppressing catecholamine neurotransmitter synthesis (section III.E.2) (Silk, 1986; Masserano et al., 1989) or by serving as precursors for the synthesis of false neurotransmitters (James et al., 1979). Elevated levels of phenylalanine and tyrosine are associated with increased brain levels of false neurotransmitters such as octopamine and phenylethanolamine in animal models of liver failure (Buxton et al., 1974; Bloch et al., 1978; Record et al., 1976; Baraldi et al., 1983; Hilgier et al., 1985). These false neurotransmitters may deplete catecholamine neurotransmitter stores by displacing NE and DA from synaptic vesicles (allowing them to be rapidly degraded in the cytoplasm) (Saavedra, 1989) and by suppressing their synthesis through the competitive inhibition of tyrosine hydroxylase and dopamine β-hydroxylase activity (Kopin et al., 1969). In addition, false neurotransmitters also act as low-potency catecholamine receptor agonists (Trendelenburg, 1972; U'Prichard et al., 1977). However, it has recently become apparent that compounds such as octopamine and tyramine occur naturally in the mammalian CNS as "trace" amines (Boulton and Juorio, 1982). These substances may interact with spe-

cific receptors (Hauger et al., 1982) and have significant neuromodulatory functions as well as some direct actions on neuronal activity that are independent of noradrenergic, dopaminergic, and serotonergic neurotransmitter systems (Jones, 1983). The nature of the neuromodulatory effects of trace amines (such as octopamine, tyramine, and tryptamine) is complex, because they can potentiate both the excitatory and depressant effects of catecholamine and indoleamine neurotransmitters on neuronal functions (Jones, 1983). Some trace amines (such as phenylethylamine and tyramine) cause an indirect increase in motor activity and stereotypy similar to amphetamines (Mantegazza and Riva, 1963; Stoof et al., 1976; Diamond et al., 1983), although at concentrations higher than normally found in the brain. Although false neurotransmitters may lower CNS NE and DA concentrations, the extent of this depletion, in concert with their intrinsic agonist properties, is not sufficient to induce significant behavioral alterations, let alone the manifestations of HE. Thus, the behavioral and electrophysiological actions of the false neurotransmitters are at variance with the picture of CNS dysfunction observed in HE.

Despite the pronounced decrease in the plasma BCAA/AAA ratio observed in chronic liver failure, this ratio is not a reliable indicator of the onset or severity of HE (Morgan et al., 1978; Record et al., 1976). Indeed, changes in the BCAA/AAA ratio appear to parallel the severity of liver damage rather than the presence or degree of encephalopathy (Sherwin et al., 1974; Morgan et al., 1978; McCullough et al., 1981). Further evidence indicating the lack of significant involvement of BCAAs in mediating HE is suggested by the observations of increased plasma concentrations of all amino acids in FHF (Iber et al., 1957; Record et al., 1976; Rosen et al., 1977). Although there is a report that toxic coma resulting from the long-term infusion (6–7 h) of massive doses of AAAs to dogs was reversed by BCAAs (Rossi-Fanelli et al., 1982), this observation has not been confirmed, and the resulting plasma and CSF concentrations of phenylalanine and tryptophan were much higher than the corresponding concentrations observed in animal models of HE (Zieve, 1987). Although the CNS depression observed in these dogs was postulated to result from the conversion of AAAs to false neurotransmitters, no direct measurements were performed (Rossi-Fanelli et al., 1982). In contrast, octopamine concentrations were decreased in rats with hepatic coma (Dodsworth et al., 1974), and the intracerebroventricular administration of octopamine alone to normal rats was without overt behavioral effect (Zieve and Olsen, 1977). Whereas some studies report a correlation between octopamine levels in urine or plasma and the grade of HE (Fischer and Baldessarini, 1971; Cascino et al., 1982), other investigations of brain octopamine levels in humans found them to be lower in patients with cirrhosis who died with HE than in patients who died without liver disease (Cuilleret et al., 1980).

Finally, therapeutic strategies for the management of HE in patients with chronic liver disease aimed at correcting the abnormal BCAA/AAA ratio by the administration of BCAA supplements have failed to confirm that this approach ameliorates the encephalopathy (Eriksson and Conn, 1989; Alexander et al., 1989; section IV.B). Overall, the data do not support either a direct or indirect (mediated by false neurotransmitters) role for decreased BCAA/AAA ratios in the pathogenesis of HE.

D. Synergistic Neurotoxins

1. Mercaptans. The isolation of methanethiol and dimethyldisulfide from a patient in hepatic coma due to FHF served as the original basis for the association of mercaptans with HE (Challenger and Walshe, 1955). These compounds appear to result from methionine metabolism by gut bacteria (Zieve and Brunner, 1985), because dimethylsulfide concentrations increase in cirrhotics given methionine (Phear et al., 1956; Chen et al., 1970). However, the liver may also contribute to the formation of methanethiol and is apparently not required for the detoxification of methanethiol (Blom et al., 1990). High concentrations of mercaptans reversibly induce coma in animals and suppress Na^+/K^+-ATPase activity (Ahmed et al., 1984; Zieve and Brunner, 1985). However, their concentrations in liver failure are not sufficient to induce overt encephalopathy (Zieve et al., 1984). Indeed, patients with chronic liver failure may have elevated methanethiol concentrations and not be encephalopathic (McClain et al., 1980). Despite their profound anesthetic properties, mercaptans can induce seizures, which are not part of the typical clinical picture of HE (Pappas et al., 1984b; Jones et al., 1987). Similarly, mercaptan administration results in VER abnormalities that bear no resemblance to the evoked potentials observed in HE (Zeneroli et al., 1982b; Pappas et al., 1984b; Jones et al., 1987). Finally, recent improvements in the assays for plasma mercaptans suggest that the original observations of increased levels of mercaptans in liver disease may be erroneous. It has been observed that quantifying mercaptan levels by treating plasma with zinc and acid (Doizaki and Zieve, 1977) leads to the artifactual formation of mercaptans, possibly as a result of methionine degradation (Tangerman et al., 1985). Thus, although plasma methanethiol concentrations were significantly increased in cirrhotic patients (Tangerman et al., 1985), there is not a robust correlation between the plasma and brain levels of mercaptans and the severity of HE in animal models and man (McClain, et al., 1980; Blom et al., 1990).

2. Fatty acids. Short-, medium-, and long-chain fatty acids have a variety of metabolic effects (Zieve, 1985) including the uncoupling of oxidative phosphorylation and inhibition of oxygen consumption and Na^+/K^+-ATPase activity (Hird and Weidemann, 1966; Ahmed

and Thomas, 1971). Short- and medium-chain fatty acids can cause reversible coma (White and Samson, 1956; Zieve, 1985), with a positive correlation between chain length and comagenic potency. However, they appear to have inconsistent influences on CNS electrical activity, with reports of EEG slowing (Muto et al., 1964; Teychenne et al., 1976), spiking, and hypersynchrony (Marcus et al., 1967) or, in the case of evoked responses, no significant effect (Zeneroli et al., 1982b; Pappas et al., 1984b; Jones et al., 1987). Fatty acid-induced changes in the VER do not resemble the VER abnormalities observed in HE (Zeneroli et al., 1982b; Pappas et al., 1984b; Jones et al., 1987). Although increased plasma concentrations of short-, medium-, and long-chain free fatty acids have been observed in patients with chronic liver disease (Muto, 1966; Rabinowitz et al., 1978; Wilcox et al., 1978), there is no consistent evidence indicating that plasma fatty acids are increased in HE or hepatic coma (Lai et al., 1977; Wilcox et al., 1978).

3. Phenols. As noted in section III.C, AAA levels are increased in liver failure. Similarly, the plasma and CSF concentrations of the tyrosine and phenylalanine metabolite phenol are increased in liver failure (Muting and Reikowski, 1965; Faraj et al., 1981; Zieve and Brunner, 1985). Phenols are both neurotoxic and hepatotoxic, depressing the activity of a variety of brain and liver enzymes (including monoamine oxidase, succinic dehydrogenase, lactate dehydrogenase, and proline oxidase) (Zieve and Brunner, 1985), and have been reported to directly induce coma and to augment the comagenic potency of other substances (Windus-Podehl et al., 1983). Although plasma and CSF levels of phenols increase with the severity of the liver disease (Muting and Reikowski, 1965), the concentrations observed in patients with hepatic coma are at least 4 times lower than those that induce coma in normal rats and rabbits (Brunner et al., 1981; Windus-Podehl et al., 1983).

4. Neurotoxin synergism. Although the individual comagenic concentrations of mercaptans, fatty acids, or phenols are much higher than the levels observed in liver failure and do not always correlate with the severity of HE, several reports indicate that the comagenic potency of these substances is dramatically increased when more than one of them are present together (Hirayama, 1971; Huet et al., 1981; Zieve, 1981; Zeneroli et al., 1982b; Windus-Podehl et al., 1983; Zieve et al., 1984; Zieve and Brunner, 1985). Furthermore, each of these classes of compounds has been found to suppress Na^+/K^+-ATPase activity, which may lead to cerebral edema and impaired CNS function (Djuricic et al., 1984). Combinations of ammonia, dimethyldisulfide, and octanoic acid were found to cause VER alterations in rats similar to those seen in rats with HE due to GalN-induced FHF (Zeneroli et al., 1982b). However, these VER findings were not confirmed in two other studies in which the changes in VER induced by specific doses of these "synergistic neurotoxins" in normal rats and rabbits were compared to the VER abnormalities observed in rats with TAA-induced FHF and rabbits with GalN-induced FHF, respectively (Pappas et al., 1984b; Jones et al., 1987). Altering the relative amounts of these neurotoxins either had no measurable effect on behavior, VER, or EEG or resulted in seizures, which are not characteristic of HE. Although the neuropsychiatric manifestations of HE may result from the synergistic interactions of multiple metabolic abnormalities (including agents other than mercaptans, fatty acids, phenols, and ammonia), the relevance of the comagenic potential (the penultimate result of organ system failure) of these synergistic neurotoxins to the spectrum of neurological changes characteristic of HE is unclear.

E. Neurotransmitters

Neurotransmitter systems are obvious targets for investigating the changes in CNS function resulting from liver failure and may potentially have a role in the pathogenesis of HE. The function of these systems can be altered by changes in the rate of neurotransmitter turnover (synthesis, release, and/or catabolism), the presence of agents acting directly at the neurotransmitter receptor, alterations in receptor density and/or affinity, or changes in the efficiency of the transduction systems subserved by these receptors. The following sections will examine the available data implicating amino acid and biogenic amine neurotransmitter systems in the pathogenesis of HE.

1. Serotonin. One of the tenets of the false neurotransmitter hypothesis (section III.C) is that altered levels of biogenic amine neurotransmitters (serotonin, NE, and DA) may contribute to the manifestations of HE. Elevations of serotonin were postulated to be involved in the CNS depression observed in HE and could result from the increased brain levels of the precursor amino acid tryptophan observed in liver failure (Mans et al., 1979). Some, but not all (Tyce et al., 1967), investigations of animal models of HE (Cummings et al., 1976; Mans and Hawkins, 1986; Bengtsson et al., 1989; Yurdaydin et al., 1989) and HE in humans (Jellinger et al., 1978; Riederer et al., 1980; Borg et al., 1982; Bergeron et al., 1989) indicate that the concentrations of serotonin or its metabolite 5-hydroxyindolacetic acid are increased in several brain regions and CSF. However, this increase in serotonin concentration in HE implies neither an increase in serotonin release (DeSimoni et al., 1987) nor a correlation with the severity of HE (Lookingland et al., 1986). It was first reported by Lal and coworkers (1974) that the turnover of serotonin was similar in cirrhotics with and without HE. More recent investigations of serotonin turnover in animal models of PSE, ammonia intoxication, cirrhosis, hepatic ischemia, and hepatectomy have indicated no correlation between alterations in brain serotonin metabolism and neurological status (Bengtsson et al., 1989). Moreover, although tryptophan

excess (Smith and Prockop, 1962) results in drowsiness, disturbances in motor function, and generalized CNS depression, these changes occur at concentrations greater than those observed in liver failure and are not specific for HE.

2. Catecholamines. Another component of the false neurotransmitter hypothesis is that catecholaminergic neurotransmission is reduced in HE. Because the role of trace amines in reducing catecholamine neurotransmission (due to the low potency of the trace amines at catecholamine receptors) in HE is unsubstantiated, the other mechanism by which this may occur is through decreased neurotransmitter concentrations or by reductions in receptor density and/or transduction efficacy.

The levels of NE have been reported to be decreased in the cerebral cortex and hippocampus of animal models of HE (Dodsworth et al., 1974; Herlin et al., 1983; Yurdaydin et al., 1989; Zimmerman et al., 1989). This may be the result of competitive inhibition of tyrosine hydroxylase and dopamine β-hydroxylase activity by phenylalanine and false neurotransmitters (Kopin et al., 1969). However, it is not clear that the depletion of NE in the CNS is causally related to the neuropsychiatric manifestations of HE. When CNS NE levels in normal rats were reduced by approximately 90% by octopamine administration, no significant alterations in behavior were observed (Zieve and Olsen, 1977). Sedation and significant decreases in motor activity are observed in normal rats after a more thorough depletion of NE using α-methyl-*p*-tyrosine or reserpine, but a neurological syndrome resembling HE is not produced. Moreover, brain NE levels are not depleted to this extent in animal models of HE (Dodsworth et al., 1974; Zieve and Olsen, 1977) and were either unchanged or increased in studies of patients who died with HE due to cirrhosis (Cuilleret et al., 1980; Bergeron et al., 1989).

Reports of alterations in DA levels in HE are less controversial. Numerous investigators have reported no changes in DA levels in the whole brain or striatum from animal models of HE (Zieve and Olsen, 1977; Bengtsson et al., 1985; Ferenci et al., 1986; Mans and Hawkins, 1986; Yurdaydin et al., 1989) and in necropsy specimens of substantia nigra, caudate, hypothalamus, thalamus, and frontal cortex from humans with cirrhosis and HE (Cuilleret et al., 1980; Bergeron et al., 1989). One study has presented evidence that DA turnover may be enhanced in patients with liver failure as indicated by increased levels of homovanillic acid in the caudate nucleus and frontal cortex (Bergeron et al., 1989). Furthermore, although the DA receptor density is decreased in some models of liver failure (Baraldi et al., 1983), the functional activity of postsynaptic DA-sensitive adenylate cyclase is normal in rabbits with FHF (Ferenci et al., 1986). The lack of involvement of DA in the pathogenesis of HE is further supported by the absence of consistent effects of the dopaminergic agents L-DOPA or bromocriptine in inducing ameliorations of HE in patients with chronic liver disease (Uribe et al., 1979, 1983; Michel et al., 1980). In summary, these findings strongly suggest that indoleamine and catecholamine neurotransmitters, their amino acid precursors, and trace amines play no significant role in the pathogenesis of HE.

3. Excitatory amino acid neurotransmitters. It has been proposed that the CNS depression present in HE may be explained by a decrease in the activity of excitatory neurotransmitter pathways (Bradford and Ward, 1975). Because glutamate and aspartate are the primary excitatory neurotransmitters in the mammalian CNS, these systems have been the logical foci of studies of the role of impaired excitatory neurotransmitter activity in HE. Observations of elevated CNS glutamine levels in concert with elevated plasma ammonia concentrations led to the hypothesis that glutamate concentrations would be reduced as a result of ammonia-induced suppression of glutaminase (Benjamin, 1982; Kvamme and Lenda, 1982). This hypothesis was supported by reports of decreased brain glutamine synthetase and glutaminase activity in rats with portacaval shunts (Bradford and Ward, 1976; Cooper et al., 1985; Butterworth et al., 1988) and in necropsy samples from cirrhotic humans (Lavoie et al., 1987), as well as the observations of decreased glutamate and aspartate levels in the whole brain and specific brain regions of animals following ammonia treatment (Moroni et al., 1983; Lin and Raabe, 1985), portacaval shunting (Cremer et al., 1975; Butterworth and Giguere, 1986), or hepatectomy (Tyce et al., 1967; Holmin et al., 1983) and in autopsy tissues from patients who died in hepatic coma (Record et al., 1976; Lavoie et al., 1987). Unfortunately, glutamate serves as a metabolic precursor as well as a neurotransmitter, and these studies were unable to distinguish between glutamate concentrations in neurotransmitter and metabolic pools.

Electrophysiological studies of normal hippocampal neurons in vivo and in vitro indicated that acute applications of ammonium salts suppressed neuronal activity without altering axonal conduction or electrical excitability (Theoret and Bossu, 1985; Theoret et al., 1985). These findings were interpreted as indicating that ammonia suppressed the synaptic release of glutamate and/or aspartate in the hippocampus.

In contrast to studies indicating that a decrease in the activity of excitatory amino acid-containing neurons may contribute to HE, other reports indicate either no change or an increase in glutamatergic function in animal models. Evoked glutamate release from brain slices derived from normal rats or those with portacaval shunts is either unaffected or increased by ammonia (Hamberger et al., 1980; Butterworth et al., 1989). Furthermore, glutamate release from the surface of the cerebral cortex in situ was increased by ammonia in normal rats or those with portacaval shunts (Moroni et al., 1983). This in-

crease in neurotransmitter glutamate release may result from ammonia-induced decreases in astrocytic reuptake of glutamate (Butterworth et al., 1989). The hypothesis that excitatory amino acid release is increased in chronic liver failure is consistent with the compensatory down-regulation of receptors binding [^{3}H]glutamate, [^{3}H]kainate, and [^{3}H]aspartate throughout the CNS of rats with portacaval shunts (Butterworth et al., 1989) and rabbits with FHF (Ferenci et al., 1984b). However, ammonia-induced increases in glutamate release from brain slices is only observed using ammonia concentrations that are 2–4 times higher than those observed in HE.

Although a pathogenic mechanism for HE that invokes a reduction in the function of excitatory neurotransmitter systems is attractive, the present data do not clearly substantiate an involvement of this mechanism in HE. Indeed, it is possible that the efficacy of neurotransmitter glutamate pools may be enhanced in chronic liver failure as a result of increased neuronal release or decreased glial uptake. These changes may compensate for the neuronal depression mediated by other mechanisms. This rapidly evolving area of research may yield some interesting new insights in the future.

4. γ-Aminobutyric acid. a. ORGANIZATION AND FUNCTION OF THE γ-AMINOBUTYRIC ACID RECEPTOR COMPLEX. The $GABA_A$ receptor complex is a hetero-oligomeric complex (Olsen and Tobin, 1990) that is a member of a superfamily of ligand-gated ion channels which includes the nicotinic acetylcholine receptor and the glycine receptor (Stevens, 1987; Schofield et al., 1987). The $GABA_A$ complex has been traditionally subdivided on a pharmacological basis into a $GABA_A$ receptor, a BzR, and a chloride ionophore (which is thought to contain sequences that recognize barbiturates and "cage" convulsants such as picrotoxinin). These regions are physically and pharmacologically coupled to form a "supramolecular complex" (Tallman et al., 1980; Skolnick and Paul, 1982), (fig. 3). This complex is currently thought to consist of at least 3 distinct, but highly homologous, protein subunits (termed α, β, and γ). The α subunit has at least six clonal variants (Levitan et al., 1988; Lolait et al., 1989, Khrestchatisky et al., 1989; Shivers et al., 1989; Ymer et al., 1989a; Malherbe et al., 1990; Luddens et al., 1990), the β subunit three (Ymer et al., 1989b), and the γ subunit 2 (Pritchett et al., 1989). An additional subunit appears to be associated with a high-affinity $GABA_A$ receptor complex that lacks Bz-binding capacity and has a unique neuroanatomical distribution (Shivers et al., 1989; Olsen et al., 1990).

The potential for numerous combinations of these subunits is thought to result in the different neuroanatomical distributions, pharmacological properties, and physiological functions of the $GABA_A$ receptor complex. Although homo-oligomeric or α, β hetero-oligomeric complexes of each subunit are capable of interacting to some degree with GABA, Bzs, and barbiturates (Blair et al., 1988; Bureau and Olsen 1988), expression studies in the *Xenopus* oocyte and cultured cell lines indicate that the complete physiological and pharmacological characteristics of the $GABA_A$/BzR complex require at minimum the presence of α, β, and γ subunits (Pritchett et al., 1989). Based on minimum molecular weight estimates and radiation inactivation studies (Nielsen, et al., 1985), the present data indicate that a functional $GABA_A$ complex has a molecular weight between 240 (Mamalaki et al., 1987) and 400 kDa or higher (Ray et al., 1985; King et al., 1985), which suggests that five or more subunits are necessary to form a fully functioning complex. However, the precise number of each subunit and their arrangement is not known (Olsen et al., 1990; Skolnick, 1990).

These subunits aggregate to form an anion-selective channel that is gated (opened) by the binding of GABA to its receptor. Thus, activation of the $GABA_A$ receptor (by GABA or a GABA mimetic) opens the chloride ionophore, increasing neuronal membrane permeability to Cl^- (Hamill et al., 1983). Because the Cl^- resting potential of the neuron is more negative than the resting membrane potential, Cl^- enters the cell effecting a hyperpolarization. Changes in GABAergic tone can alter cortical and subcortical functions to such an extent that consciousness and motor control are impaired (Lloyd et al., 1977; Tamminga et al., 1979; Lloyd, 1980).

The basic function of the BzR is to allosterically modulate GABA receptor gating of the chloride ionophore. Occupation of the BzR by an agonist (e.g., diazepam) increases the frequency of GABA-gated Cl^- channel openings (Study and Barker, 1981) and the affinity of GABA for its receptor. The principal pharmacological (i.e., sedative, myorelaxant, anxiolytic, and anticonvulsant) properties of BzR agonists are mediated through this mechanism (Skolnick and Paul, 1988) (fig. 4). In contrast, "inverse" agonists of the BzR (e.g., DMCM) possess proconvulsant, convulsant, and anxiogenic actions due to their ability to decrease GABA-gated Cl^- conductance (g_{Cl-}) by decreasing the frequency of GABA-gated chloride channel openings (Study and Barker, 1981). Antagonists of the BzR (e.g., Ro 15–1788 and Ro 14–7437) lack intrinsic activity over a wide concentration range. BzR antagonists do not alter the frequency of chloride channel openings but compete with agonists and inverse agonists for binding sites on the BzR. Moreover, in electrophysiological and behavioral assessments of normal animals and humans, BzR antagonists may show moderate activating or inhibitory properties. The depressant effects of the BzR antagonist may result from partial agonist characteristics of the compound, whereas neuronal activation may result either from an intrinsic inverse agonist property or by the displacement of endogenous agonists from the BzR by the antagonist (sections III.E.4.c and IV.B.6.b).

In general, ligands acting at the BzR do not directly

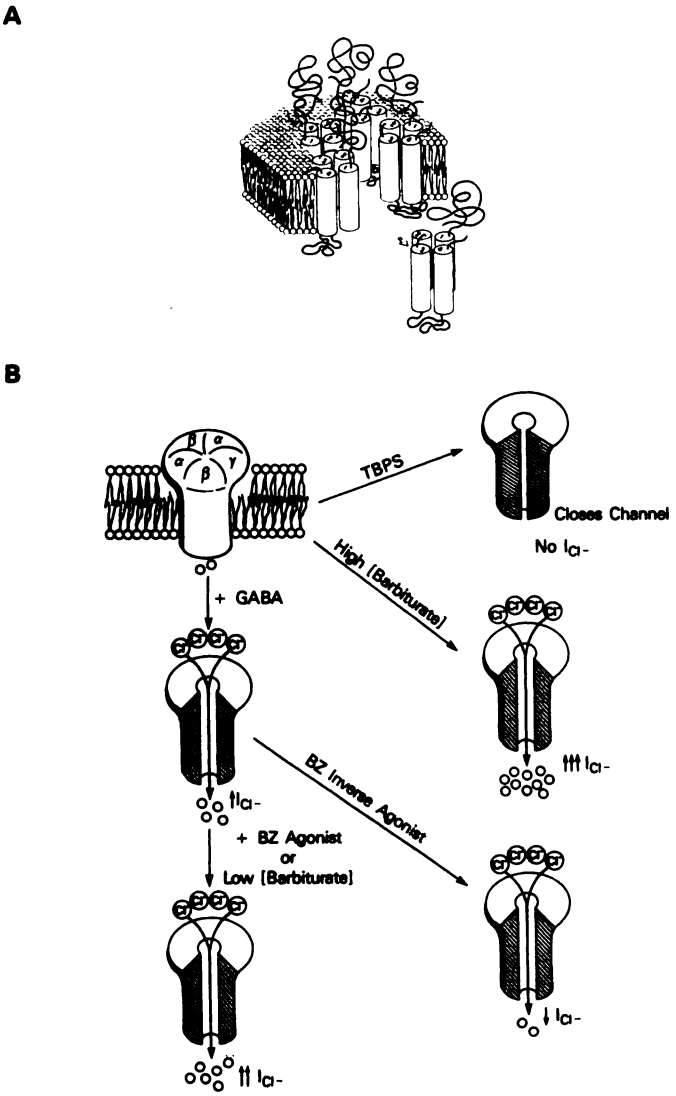

FIG. 3. *A*, model of the GABA/BzR/chloride ionophore supramolecular complex. This ligand-gated ion channel is a hetero-oligomeric complex containing at least five subunits of three different types (α, β, and γ). Although the precise subunit composition, stoichiometry, and number of subunits is presently unknown, a 2α, 2β, 1γ stoichiometry has been proposed. Each subunit contains four membrane-spanning domains which combine with the other subunits to form an anion channel with relatively high selectivity for chloride. This channel contains binding sites for barbiturates, cage convulsants, ethanol, and possibly gaseous anesthetics. *B*, function of the GABA/BzR supramolecular complex. The binding of GABA to its receptor will open the chloride ionophore. Driven by the E_{Cl-}, Cl^- ions flow from the outside of the membrane to the inside of the neuron, increasing the Cl^- current (I_{Cl-}). Addition of BzR agonists increases the frequency of opening of the chloride channel, thereby further increasing the I_{Cl-}. In contrast, BzR inverse agonists reduce the I_{Cl-}. BzR antagonists have no intrinsic effect on chloride channel function. However, they may appear to have an activating effect when they displace a BzR agonist. Barbiturates have two distinctly different actions on the function of the complex. Low concentrations of barbiturates (corresponding to a sedative dose), in the presence of GABA, reduce the ionophore resistance in a fashion similar to that of BzR agonists. However, high concentrations of barbiturates or ethanol (correlating with a hypnotic dose) can directly open the channel, "short-circuiting" the GABA-gating system and greatly increasing the I_{Cl-}. The ensuing hyperpolarization will effectively depress all neuronal activity and may account for the propensity of a barbiturate overdose to be fatal. Finally, cage convulsants such as t-butylbicyclosphosphorothionate (TBPS) completely block the Cl^- ionophore. This greatly increases membrane resistance, simultaneously reducing the I_{Cl-}, and massively depolarizes the neuron. *A*, reprinted with permission from Olsen and Tobin, 1990.

alter chloride ion conductance. However, barbiturates can either enhance GABA-gated g_{Cl-} by increasing the mean open time of the GABA-gated Cl^- channel or, at high concentrations, directly increase g_{Cl-} independently of GABA (Schulz and MacDonald, 1981; Study and Barker, 1981). These properties of barbiturates may account for their anxiolytic, anesthetic, and anticonvulsant effects. Thus, binding sites for both Bzs and barbiturates are intimately associated with the GABA receptor and the Cl^- ionophore and serve to modulate GABAergic tone.

b. ROLE OF THE γ-AMINOBUTYRIC ACID/BENZODIAZEPINE RECEPTOR COMPLEX IN THE PATHOGENESIS OF HEPATIC ENCEPHALOPATHY. Electrophysiological, neurochemical, and behavioral evidence for an involvement of the $GABA_A$ receptor complex in HE has been obtained using several different animal models of HE due to FHF. Initially, functional changes in GABAergic tone were assessed using electrophysiological techniques to record neuronal activity. In animal models, VERs were found to be superior to EEG analysis in terms of their specificity in discriminating between the CNS effects of different pharmacological agents and in their ease of quantitation (MacGillivray, 1976; Zemon et al., 1980; Chiappa and Ropper, 1982; Schafer et al., 1984; Gammal et al., 1990). VERs are derived from the occipital EEG by averaging signals temporally linked to a photic stimulus and consist of a series of positive and negative peaks of electrical activity (Bassett et al., 1987; Schafer et al., 1984) (fig. 5). The development of HE in GalN-treated rabbits was associated with a significant increase in the amplitude of P_1 (Schafer et al., 1984; Jones and Gammal, 1988) (fig. 5). The P_1 amplitude was also significantly increased in normal rabbits rendered encephalopathic by administration of a barbiturate (pentobarbital), a BzR agonist (diazepam), or a GABA mimetic (e.g., muscimol or γ-vinyl-GABA) but not by the gaseous anesthetic ether (Schafer et al., 1979, 1984; Pappas et al., 1984b; Bassett et al., 1987). Furthermore, the amplitude of the P_1 wave in rabbits with HE can be normalized by the administration of a GABA receptor antagonist (bicuculline) or a Cl^- channel blocker (isopropyl-bicyclophosphorothionate). These improvements in VER abnormalities were observed concurrently with ameliorations in the neurological manifestations of HE, including deficits in alertness, muscle tone, response to painful stimuli, and spontaneous motor activity (Bassett et al., 1987). The seizure threshold to bicuculline was also found to be increased in rabbits with HE due to GalN-induced FHF (Bassett et al., 1987) and to 3-mercaptopropionic acid in rats with HE due to TAA-induced FHF (Ferreira et al., 1988).

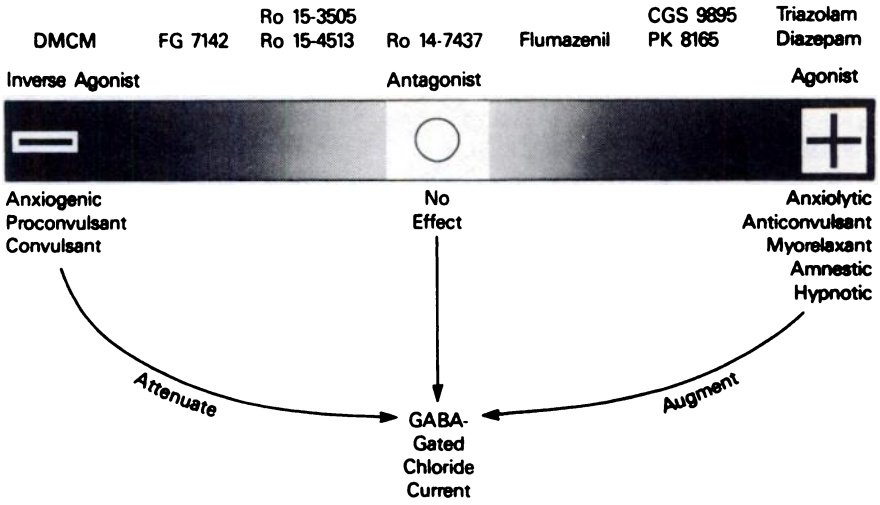

FIG. 4. The spectrum of intrinsic activities of BzR ligands. Agonists such as diazepam increase the potency of GABA, resulting in an increased frequency of opening of GABA-gated chloride channels and a greater degree of neuronal hyperpolarization. BzR antagonists bind with high affinity to the receptor but are devoid of any physiological or pharmacological function. The changes in $GABA_A$ complex function elicited by inverse agonists are opposite to those of the agonists. Inverse agonists decrease the potency of GABA and the frequency of opening of GABA-gated chloride channels. Those compounds that fall between either end of the scale (partial agonists and inverse agonists) do not manifest the full spectrum of pharmacological actions associated with either "classic" 1,4-Bz agonists or inverse agonists.

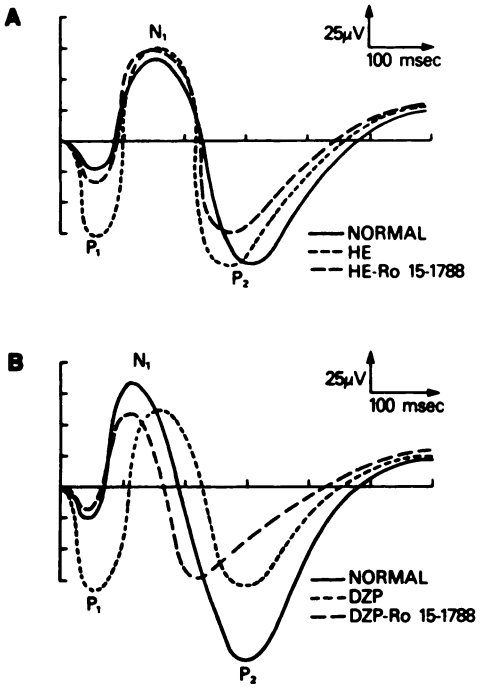

FIG. 5. Abnormalities in the VER of rabbits with HE due to GalN-induced FHF. *A*, composite VERs from normal rabbits and rabbits in HE. Note the pathological increase in the amplitude of the P_1 of the VER from rabbits with HE. The amplitude of this component of the VER reflects the intensity of GABAergic tone in the occipital cortex and adjacent subcortical regions. *B*, a similar change in P_1 amplitude is observed in normal rabbits treated with an encephalopathic dose of diazepam (DZP). The abnormal P_1 amplitudes in HE and in diazepam-induced encephalopathy are normalized by the administration of the BzR antagonist flumazenil (Ro 15–1788). From Bassett et al., 1987.

Because these drugs induce seizures by reducing GABAergic tone, these findings are consistent with the presence of increased GABAergic tone in HE.

Although the ability to reverse the electrophysiological manifestations of HE in rabbits with GalN-induced FHF with bicuculline and isopropyl-bicyclophosphorothionate is consistent with an enhancement of GABAergic tone in HE, it may also be argued that the improvements in the VER reflect a generalized activating (convulsant) property of these compounds. Ideally, it would be most desirable to test in animal models of HE compounds that can incrementally modulate the function of the GABA-gated chloride channel rather than completely open (e.g., high concentrations of pentobarbital) or close (e.g., picrotoxinin) the channel (Havoundjian et al., 1987). Such incremental modulation of GABAergic neurotransmission can be mediated through the BzR. To test the effects of BzR modulation of GABAergic activity in HE, BzR antagonists that lack intrinsic activity over a wide concentration range were examined in rabbit and rat models of FHF.

The BzR antagonist flumazenil (Ro 15–1788, Anexate) reversed the increase in amplitude of the P_1 component of the VER observed in rabbits following an encephalopathogenic dose of diazepam and in GalN-treated rabbits in stage III-IV HE (fig. 5). In addition, both flumazenil and Ro 15–4513 (an antagonist with partial inverse agonist properties structurally related to flumazenil) (Harris et al., 1987) partially reversed the VER abnormalities (i.e., increased the pathologically reduced amplitude of the first negative potential associated with HE in rats with TAA-induced FHF. Ro 15–4513 appeared to be more effective in reversing the electrophysiological manifestations of HE than flumazenil at doses that had no

obvious effect on the VER or behavior of normal animals. These observations are consistent with the first report of an electrophysiological improvement of encephalopathy in GalN-treated rats following the administration of the structurally unrelated BzR antagonist CGS 8216 (Baraldi et al., 1984a).

These VER findings are consistent with an augmentation of GABAergic tone occurring in HE due to FHF. Given the remarkable specificity of these ligands for the BzR, this phenomenon is most likely mediated by the BzR. However, the VER represents an average of the electrical activity of large and poorly defined neuronal populations in the occipital cortex and adjacent subcortical regions, and it could be assumed that agents acting at the GABA/BzR complex might evoke multiple changes in the VER and other indices of EEG status (Myslobodsky, 1987). Thus, a technique with higher resolution of neuronal activity is required to ascribe with confidence the changes in CNS electrophysiological activity observed in HE to alterations in the function of a specific neurotransmitter system. One such complementary technique is to record the changes in electrical activity of single neurons from encephalopathic animals in response to pharmacological agents specific for the $GABA_A$ receptor complex. In view of the pronounced cerebellar deficits seen in GalN-treated rabbits in HE, the spontaneous activity of Purkinje neurons in cerebellar slices maintained in vitro was studied (Basile et al., 1983). This preparation is particularly useful because it has an extensive GABAergic innervation, it eliminates variables associated with changes in the blood-brain barrier and peripheral drug metabolism that would confound recording in situ, and it allows the isolation of neurons in the slice from their synaptic inputs.

The spontaneous activity of Purkinje neurons from rabbits with HE due to FHF, as recorded using the in vitro slice technique, was found to be 3–5 times more sensitive to depression by the GABA mimetic muscimol and by flunitrazepam (a 1,4-Bz structurally and functionally related to diazepam) than neurons from control animals (Basile et al., 1988) (fig. 6). This hypersensitivity to depression appears to be specific for agents acting at the supramolecular complex, because no differences in the sensitivity of these neurons to phenylephrine, a depressant α-adrenergic receptor agonist, were observed (fig. 7). In contrast, the application of each of two different BzR antagonists (flumazenil and Ro 14–7437) significantly increased the spontaneous activity of Purkinje neurons from rabbits with HE at concentrations which either had no effect on or depressed the activity of control neurons (Basile et al., 1988) (fig. 8). Finally, pretreatment of these neurons with the BzR antagonist (Ro 14–7437) eliminated the hypersensitivity of neurons from rabbits with HE to depression by muscimol (fig. 7). These observations are all consistent with an augmentation of GABAergic neurotransmission in HE, but they may be

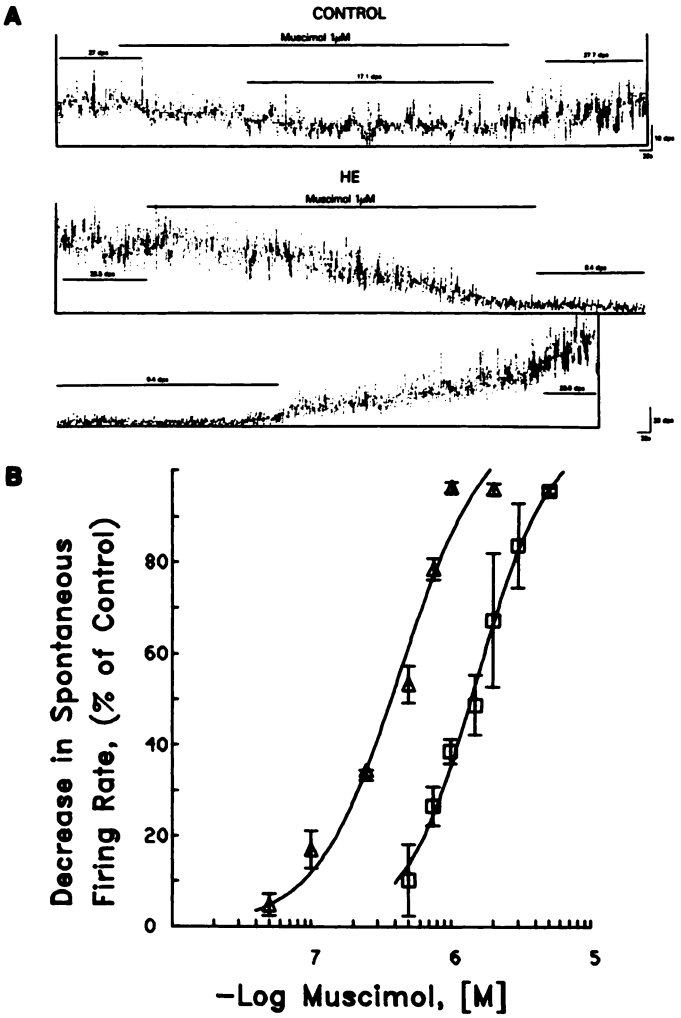

FIG. 6. *A*, rate meter records of Purkinje neuron responses to the GABA mimetic, muscimol. Application of muscimol (1 μM) decreased the spontaneous firing rate of this Purkinje neuron from a control rabbit by 37% (*top*). However, the same concentration of muscimol depressed the firing rate of a neuron from an HE rabbit to a greater degree (99%) and for a longer period (*bottom*). *B*, hypersensitivity of neurons from rabbits with HE to depression by muscimol. The IC_{50} values of muscimol for control and HE neurons are 1.37 and 0.30 μM, respectively. Symbols: △, HE neurons; □, control neurons. From Basile et al., 1988.

explained by several possible mechanisms. One possibility would involve pharmacodynamic changes in the $GABA_A$ receptor complex resulting in an increase in either the density or affinity of the receptors. This possibility is not readily testable using electrophysiological techniques but could explain the hypersensitivity of HE neurons to depression by GABA and BzR agonists. The presence of elevated concentrations of humoral factors (such as GABA or BzR ligands) in the brains of animals with HE may serve as an alternative explanation of the enhanced depression of HE neurons by muscimol and flunitrazepam.

Concomitant with the improvements in VER and single-unit electrophysiology, BzR antagonists also improved some of the behavioral manifestations of HE in

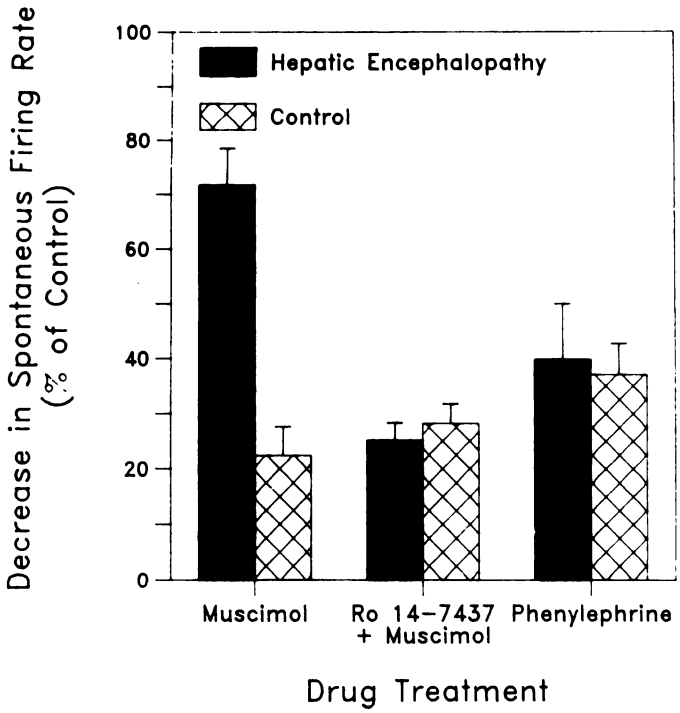

FIG. 7. The differential sensitivity of Purkinje neurons from control rabbits and rabbits with HE to inhibition by muscimol: reversal by Ro 14–7437. Purkinje neurons from rabbits with HE were significantly more sensitive to depression by muscimol (0.75 μM) alone than control rabbit Purkinje neurons (*left*). However, pretreatment of Purkinje neurons from rabbits with HE using a subthreshold concentration of Ro 14–7437 (0.5 μM) reduced the subsequent muscimol-induced depression to levels indistinguishable from control neurons (*center*). *, significantly different from all other groups, $P < 0.05$. Also shown is the specificity of the hypersensitivity of HE neurons to depression by agents acting at the GABA-BzR (*right*). Application of phenylephrine (50 μM) depressed the spontaneous activity of Purkinje neurons from controls and rabbits with HE to a similar extent. This suggests that Purkinje neurons from GalN-treated rabbits are not nonspecifically hypersensitive to the actions of all depressants. From Basile et al., 1988.

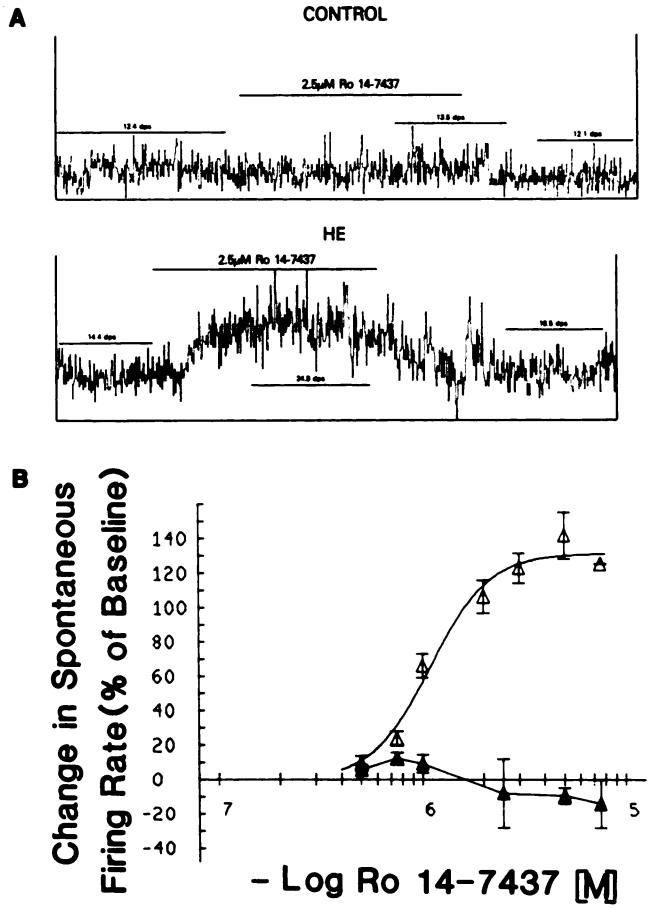

FIG. 8. *A*, rate meter records of Purkinje neuron responses to the BzR antagonist Ro 14–7437. Unlike flumazenil, which showed partial agonist properties in this preparation, Ro 14–7437 (2.5 μM) did not alter the spontaneous firing rate of Purkinje neurons from control rabbits (*top*). In contrast, Ro 14–7437 increased the firing rate of a neuron from a rabbit with HE by 142% (*bottom*). This excitatory effect was reversible, as indicated by the recovery of neuronal firing after the termination of drug application. *B*, dose-response curves indicating the effect of Ro 14–7437 on rabbit Purkinje neuron spontaneous activity. Ro 14–7437 had no effect on control rabbit Purkinje neuron activity over a concentration range of 0.5–7.5 μM but elicited a robust increase (≈120%) in the spontaneous activity of Purkinje neurons from rabbits with HE (IC_{50} = 1.43 μM). Symbols: △, HE neuron; ▲, control neuron. From Basile et al., 1988.

animal models. In contrast to the effects of the chloride channel blockers and GABA receptor antagonists, BzR antagonists, such as flumazenil, have little or no intrinsic activity and do not cause seizures. Flumazenil improved the behavioral indices of HE in the GalN-treated rabbit (Bassett et al., 1987). In addition, the BzR antagonists flumazenil and Ro 15–4513 improved both the subjectively scored behavioral manifestations of HE and the objective scoring of open field (motor) activity in rats with TAA-induced FHF (Gammal et al., 1990) (fig. 9). In this latter study, Ro 15–4513 appeared to be more effective in reversing the behavioral manifestations of HE than Ro 15–1788 at doses that did not affect motor function in normal animals. Furthermore, the effects of Ro 15–4513 on this model do not appear to be related to its partial inverse agonist properties at the BzR, because subconvulsant doses of the inverse agonist DMCM were less effective in improving motor function in these rats. The efficacy of Ro 15–4513 in improving the behavioral manifestations of HE in animal models has been independently confirmed (Bosman et al., 1990; Steindl and Ferenci, 1990). Ameliorations of the behavioral manifestations of HE by other BzR antagonists (e.g., CGS-8216 and Ro 15–3505) were observed in GalN-treated rats (Baraldi et al., 1984a), TAA-treated rats (Steindl and Ferenci, 1990), and rats with hepatic ischemia (Bosman et al., 1990). It should be noted that BzR antagonists are not universally effective in reversing the behavioral effects of HE in animal models of FHF (Steindl and Ferenci, 1990; Bosman et al., 1990), and when they do, the effects are transient. The transient nature of the behavioral improvements in HE induced by BzR antagonists in animal models may reflect their rapid catabolism by plasma esterases (Lister et al., 1984), their partial agonist properties (particularly high doses of flumazenil) (Higgett et al., 1986; Basile et al., 1988), and the severity

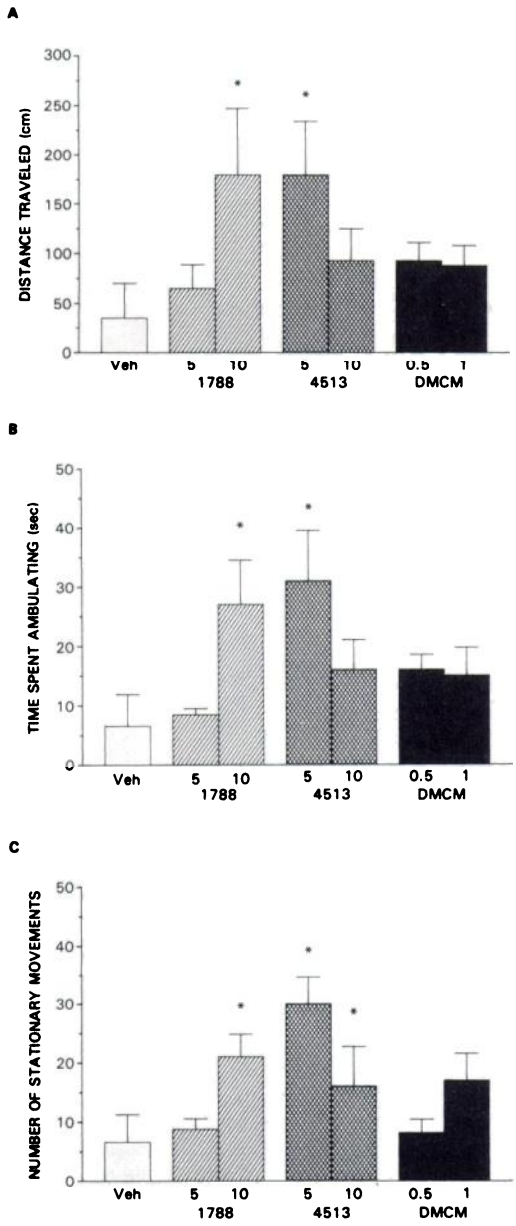

FIG. 9. The effects of BzR ligands on the open field performance of rats with HE due to TAA-induced FHF. Rats in stage III-IV HE exhibited almost total lack of movement in the open field. The administration of 10 mg/kg of flumazenil (1788, hatched) to rats with HE significantly increased the distance traveled (*top*), the time spent ambulating (*center*), and the number of stationary movements (*bottom*) during a 10-min interval compared to the administration of vehicle (Veh) to rats with HE. Similar results were obtained following the administration of 5 mg/kg of Ro 15–4513 (4513, cross-hatched). However, the administration of DMCM did not significantly improve motor performance in these animals. The lack of effect of DMCM and the decrease in motor activity observed following the administration of 10 mg/kg of Ro 15–4513 may reflect the inverse agonist properties of these compounds. *, significantly different from vehicle, $P < 0.05$. Neither flumazenil nor Ro 15–4513 at the doses indicated increased the distance traveled by normal rats during any time interval (<10 min) after injection. From Gammal et al., 1990.

and rapidity of progression of the syndrome of acute liver failure. Furthermore, animal models of liver failure (e.g., the portacaval shunt/hepatic ischemia models), which exhibit either a partial or no response to BzR antagonists (Zieve et al., 1987), do not appear to have an appreciable increase in GABAergic neurotransmission (Olasmaa et al., 1990) and do not appear to be satisfactory models of HE (see section III.A.).

Finally, one must address the conclusion that the efficacy of these BzR ligands in reversing the manifestations of HE depends on their intrinsic activity as inverse agonists (Gammal et al., 1990). The administration of analeptics such as bicuculline, isopropyl-bicyclophosphorothionate, or DMCM may activate the EEG but do not efficaciously reverse the behavioral manifestations of HE. Instead, they decrease the locomotor activity of the animal models and may cause convulsions and prodromal states, which are not noted following the administration of either Ro 15–4513, Ro 15–3505, or Ro 15–1788. The efficacy of Ro 15–4513 and Ro 15–3505 (in comparison to Ro 15–1788) in reversing the electrophysiological and behavioral manifestations of this syndrome in animals may reflect their higher affinity for the BzR (Ro 15–3505), their selective affinity for BzR subtypes in the cerebellum (Ro 15–4513) (Luddens et al., 1990), and/or pharmacokinetic differences rather than their weak inverse agonist properties. Finally, Ro 15–1788 (flumazenil) shows no consistent intrinsic activity, yet it potently ameliorates HE in humans (section IV.B.6). Together, these results should be taken as evidence that the efficacy of BzR ligands in ameliorating HE depends more on their affinity for the BzR and pharmacokinetics than on any intrinsic inverse agonist properties.

Increases in either the density or affinity of receptors in the $GABA_A$ complex or the CNS concentrations of GABA could explain the depression of motor function and the neuronal hypersensitivity to inhibition by GABA and BzR agonists observed in animal models of HE. However, these factors do not adequately account for the behavioral ameliorations of HE and the increase in HE neuron activity observed after the administration of BzR antagonists. The mechanism most consistent with these behavioral and electrophysiological observations is the presence of an increased concentration of substances with Bz-like (i.e., agonist) properties in HE. Thus, the presence of a BzR agonist in HE would enhance the apparent potencies of exogenously administered GABA and BzR agonists, which would account for the hypersensitivity of neurons from HE rabbits to depression by these agents. Furthermore, displacing a BzR agonist in HE with an antagonist could disinhibit neuronal activity, thereby explaining the observed excitatory response (Carlen et al., 1983). However, alterations in the constituent components of the $GABA_A$ receptor complex in HE might offer an alternative explanation of the excitatory responses to BzR antagonists. This possibility would

require a change in either the functional or recognition properties of the BzR so that BzR antagonists would act as inverse agonists (Lawson et al., 1990). If a BzR agonist is involved in the electrophysiological and behavioral manifestations of HE, it would be necessary to apply neurochemical techniques to determine the identity of this substance and to correlate its concentrations with the severity of the encephalopathy.

Measurement of the densities and apparent ligand affinities of the constituent recognition sites on the GABA/BzR complex have been used to address some of these issues. An apparent increase in the density of both low- and high-affinity GABA receptors and of BzRs was reported to be associated with HE in brains from GalN-treated rabbits (Schafer et al., 1983), GalN-treated rats (Baraldi and Zeneroli, 1982; Baraldi et al., 1984a), nitrosamine-treated dogs with portacaval shunts (Baraldi et al., 1984b), and humans with cirrhosis (Ferenci et al., 1988b). This increase in GABA receptor density was manifested during acute liver failure, before the expression of the overt behavioral manifestations of HE, in the GalN-treated rat (Pappas, 1984). One study indicated that, as the severity of the encephalopathy intensified, the density of the high-affinity sites increased moderately, whereas the low-affinity sites increased but then disappeared in severe HE (Baraldi and Zeneroli, 1982). The observations by Baraldi and coworkers (1982, 1984a,b) of increased GABA/BzR density in animals with HE were proposed to reflect a compensatory response to the degeneration of nerve terminals with the progression of liver failure. However, no evidence to support this hypothesis has been reported.

Subsequent investigations using autoradiography and radioligand binding to washed membrane preparations in several animal models of HE (Pappas et al., 1986; Pappas and Gordon, 1986; Ferenci et al., 1987; Maddison et al., 1987a,b; Rossle et al., 1989a; Roy et al., 1988; Zimmermann et al., 1989; Basile et al., 1989, 1990b; Basile, 1990) and in humans with HE (Lal et al., 1987; Ferenci et al., 1988b) failed to confirm that the density of either the GABA or BzRs was increased. There are several potential explanations for these discrepant results. The first possible explanation concerns the relative importance of the $GABA_A$ receptor complex to the development of encephalopathy in the models studied. For example, evidence for the involvement of GABAergic systems in the encephalopathy observed in rats with portacaval shunts (Ferenci et al., 1987; Maddison et al., 1987b; Olasmaa et al., 1990) and dogs (Roy et al., 1988) or murine viral hepatitis (Pappas et al., 1986) is not compelling, so it is not surprising that no changes in receptor density are observed in these models. The second explanation relates to differences in the tissue preparation techniques used. The original studies showing increased GABA receptor densities in HE appear to have uniformly used the detergent Triton X-100 in preparing brain membranes. This procedure has been found to preferentially solubilize BzR from normal animals (Rossle et al., 1989b), thereby decreasing the density of receptors in normal brain preparations relative to those from animals with HE. Furthermore, Speth and coworkers (1979) reported an increase in the density of BzR in brain membranes prepared in distilled water from rats given a large dose of diazepam. A similar change was not observed if these membranes were prepared using buffered media (R. Speth, personal communication). These observations suggest that the occupation of the BzR induces a physicochemical change in the receptor that reduces the efficiency of detergents in solubilizing membrane-bound receptors. Agonist-induced changes in the physicochemical characteristics of the $GABA_A$ complex are also manifested as a slower rate of heat denaturation (McIntyre et al., 1988). Thus, reports of apparent increases in receptor density in brains from animals with HE may reflect not only changes in membrane composition (Pappas et al., 1984a) but also the presence of ligands that alter the physicochemical properties of the receptor.

The inconsistent results of radioligand-binding studies suggest that increased GABAergic tone in HE is not dependent upon changes in the GABA/BzR complex. An alternative mechanism by which GABAergic tone could be enhanced in HE involves increased concentrations of $GABA_A$ receptor agonists (e.g., GABA or GABA-like compounds) in the CNS. Early reports indicated that plasma levels of a GABA-like (as measured using a radioreceptor assay) substance were elevated about 12-fold in rabbits with acute hepatocellular failure several hours before the onset of overt HE (Schafer et al., 1980b,c; Ferenci et al., 1983). GABA levels were also found to be elevated, albeit to a lesser degree, in studies of dogs with Eck fistulas (Thompson et al., 1985) and patients with HE due to acute or chronic liver failure (Borg et al., 1982; Ferenci et al., 1983; Minuk et al., 1985; Singh et al., 1985; Levy et al., 1987). Increases in plasma GABA levels correlate more closely with the severity of HE in FHF than in chronic liver disease in man (Levy and Losowsky, 1989). Nonetheless, plasma GABA levels are not uniformly elevated in animal models of HE (Maddison et al., 1986; Ferenci et al., 1987), and when increased, they do not always correlate with the severity of encephalopathy (Thompson et al., 1985; Loscher et al., 1989). Discrepancies between the amounts of GABA detected using HPLC and radioreceptor-binding techniques in whole plasma from models of HE raised the issue of whether additional GABA-like substances were present in HE (Maddison et al., 1987a,b; Ferenci et al., 1988a). Subsequent investigations indicated that not only is there a significant correlation between plasma GABA concentrations measured by the two techniques (Minuk et al., 1985; Levy et al., 1987) but that GABA and the related inhibitory amino acid taurine make up

most of the GABA-like substances in plasma (Maddison et al., 1990). Although it has been hypothesized that enteric bacteria such as *Escherichia coli* and *Bacillus fragilis* may be a major source of the elevated plasma levels of GABA in HE (Schafer et al., 1980a, 1981a), GABA may also be synthesized in the gut wall (van Berlo et al., 1987).

Do elevated plasma GABA levels imply increased brain concentrations of GABA? If brain GABA concentrations are elevated, then what is the relevance of whole brain GABA levels to the compartmentation and release of GABA? The results of investigations of these issues are mixed and dependent upon the animal model used. Although studies in some models suggest that blood-brain barrier permeability and brain GABA uptake are unchanged in hepatic failure (Zeneroli et al., 1982a; Knudsen et al., 1988; Lo et al., 1987; Traber et al., 1987; Roy et al., 1988), other studies indicate that there is a nonspecific increase in blood-brain barrier permeability in acute liver failure (Livingstone et al., 1977; James et al., 1978; Horowitz et al., 1983), allowing increased plasma to brain transfer of GABA (Bassett et al., 1990). The concentration of GABA associated with neurotransmitter pools may be elevated in HE, not only due to increased transfer from plasma but also through alterations in synthesis or catabolism of GABA within the CNS. For example, mechanisms that would potentially increase extracellular GABA levels in HE include decreased catabolism of GABA due to inhibition of GABA-transaminase activity [as reported in GalN-treated rabbits (Ferenci et al., 1984a), increased GABA release from glial cells (Albrecht and Rafalowska, 1987), or activation of arginine conversion to GABA (as observed in TAA-treated rats; Albrecht et al., 1990)]. In contrast, whole brain GABA levels (which may not reflect GABA levels in the neurotransmitter pool) and glutamic acid decarboxylase activity were unchanged or reduced in portacaval shunt models (Zeneroli et al., 1982a; Diaz-Munoz and Tapia, 1988; Roy et al., 1988; Zimmermann et al., 1989) and in humans who died with HE (Lavoie et al., 1987). Although the preponderance of evidence suggests that extracellular GABA concentrations in the CNS are increased in HE, further studies are clearly required. Two additional issues need to be addressed before increased GABA levels can be linked to the pathogenesis of HE. The first issue is whether GABA (and taurine) levels are significantly elevated in neurotransmitter pools or the synaptic cleft in liver failure. The second issue focuses on the level to which extracellular brain GABA levels must be increased to contribute to the manifestations of HE. Large increases in whole brain GABA levels may not be relevant to HE if they are not relegated to the neurotransmitter pool and/or if glial GABA uptake systems are functioning normally. However, if glial function is compromised (e.g., by ammonia, octanoic acid, or phenol) (Norenberg, 1989) or a synergist is present (e.g., a BzR agonist), small increases in GABA concentrations at the $GABA_A$ receptor may contribute significantly to the development of HE.

Taken together, interdisciplinary studies of the effects of BzR-specific agents provide strong evidence for a functional increase in GABAergic tone in animal models of HE due to FHF. Although increases in GABA levels or GABA receptor density/affinity could account for the observed electrophysiological hypersensitivity to GABA and BzR agonists in HE due to FHF, these changes alone cannot adequately explain the improvements in the behavioral and electrophysiological manifestations of HE observed following the administration of BzR antagonists. To account for the observed effects of BzR antagonists, it is necessary to postulate that an increase in the CNS concentration of some substance that acts as a BzR agonist occurs in HE.

c. EVIDENCE FOR AN INVOLVEMENT OF BENZODIAZEPINE RECEPTOR LIGANDS IN THE PATHOGENESIS OF HEPATIC ENCEPHALOPATHY. During the past decade, a number of substances have been identified in the CNS that possess measurable affinities for the BzR. These include purines (e.g., inosine and hypoxanthine) (Skolnick et al., 1978; Asano and Spector, 1979), nicotinamide (Mohler et al., 1979), 3-carbobutoxy-β-carboline (Pena et al., 1986), a family of 1,4-Bzs (e.g., diazepam, N-desmethyldiazepam, deschlorodiazepam, lorazepam, and oxazepam) (Sangameswaran et al., 1986; Wildmann et al., 1986; Wildmann and Ranalder, 1988), and proteins/peptides (DBI-octadecaneuropeptide) (Guidotti et al., 1983; Marquardt et al., 1986). Although some of these compounds may be synthesized de novo, recent evidence suggests that N-desmethyldiazepam and diazepam may arise from dietary sources (Medina et al., 1988; Wildmann, 1988; Wildmann et al., 1988).

To date, none of these substances have been conclusively demonstrated to be an endogenous ligand (i.e., physiologically relevant) for the BzR. Nonetheless, electrophysiological and behavioral studies in three animal models of HE (Baraldi et al., 1984a; Bassett et al., 1987; Basile et al., 1988; Gammal et al., 1990) indicate that increased concentrations and/or availability of a BzR agonist are present in HE. As noted in section IV.H.2, numerous studies indicate that BzR antagonists do not alter neuronal electrical activity, responsiveness to GABA, or behavior in normal animals over a wide dose range. Thus, the ability of these antagonists to reversibly increase neuronal activity in HE is best explained by the displacement of an agonist from the BzR.

Several lines of more direct evidence support the presence of elevated concentrations of BzR ligands in HE. The binding of either [^{3}H]flumazenil or [^{3}H]flunitrazepam to BzR in several brain areas, including the cerebral cortex and cerebellar cortex, was determined using autoradiographic techniques. The levels of radioligand binding to the BzR were found to be significantly reduced

in unwashed sections from GalN-treated rabbits with HE relative to controls (fig. 10) (Basile et al., 1990b). The binding to the BzR in HE sections could be normalized by washing for 20 min before incubation with the radioligands. These results suggest that a BzR ligand reversibly occupied these receptors in the brains of HE rabbits. Furthermore, this ligand appears to have the pharmacological characteristics of an agonist, because [^{3}H]flunitrazepam binding could be further reduced by incubating the sections in the presence of muscimol and NaCl [a positive "GABA shift," (Mohler and Richards, 1981; Braestrup et al., 1982; Skolnick et al., 1982)]. No changes in the binding of [^{3}H]muscimol or [^{3}H]PK-11195 to the GABA or the peripheral-type BzRs, respectively, were observed in these tissue sections.

Similar studies were performed using well-washed brain membrane preparations from the GalN-treated rabbit (Basile, 1990) and the TAA-treated rat with HE (Basile et al., 1989). There were no significant differences between the animal models of HE and their respective controls in either receptor densities or ligand affinities to the constituent components of the $GABA_A$ receptor complex (i.e., $GABA_A$ receptors, BzR, and chloride ionophore) in well-washed preparations of cerebral cortex and cerebellum. In addition, there was no difference in the ability of NaCl or GABA to enhance the E_{max} or EC_{50} of [^{3}H]flunitrazepam binding to the cortex or cerebellum from animals with HE. These observations are consistent with the results of previous studies that indicated that the characteristics of ligand binding to the GABA/BzR

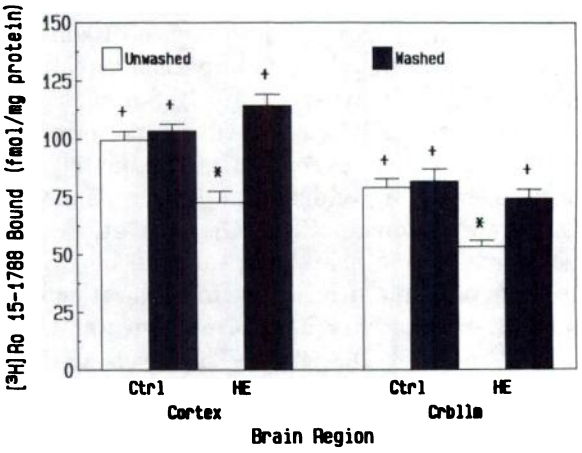

FIG. 10. Evidence for the presence of a reversible BzR ligand in the brain of rabbits with HE due to GalN-induced FHF. The levels of [^{3}H] Ro 15–1788 (flumazenil) binding to the cerebral cortex (*left*) and cerebellum (*right*) from control (Ctrl) rabbits and rabbits with HE were determined using autoradiographic techniques. Adjacent brain sections were either washed (solid bars) for 30 min before incubation with radioligand or incubated without prewashing (open bars). [^{3}H]Ro 15–1788 binding to unwashed cerebral cortex and cerebellum from rabbits with HE was significantly lower than that to control sections. However, in the sections from HE rabbits that were prewashed, [^{3}H] Ro 15–1788 binding is not significantly different from controls. This indicates that a reversible BzR ligand is present in HE sections. *, significantly different, $P < 0.05$ from groups labeled +. From Basile et al., 1990b.

complex are unaltered in both animal models of HE (Maddison et al., 1987a,b; Rossle et al., 1989a; Zimmermann et al., 1989) and in humans with HE (Lal et al., 1987).

Overall, these findings are consistent with the presence of a reversible BzR ligand in HE. This hypothesis is supported by the seminal observations of Mullen et al. (1986, 1989) that CSF from GalN-treated rabbits with HE inhibited the binding of [^{3}H]3-ethyl-β-carboline-3-carboxylate and [^{3}H]Ro 15–1788 to normal rabbit cerebral cortical membranes to a significantly greater extent than control CSF. Subsequent investigations in which crude brain extracts from GalN-treated rabbits and TAA-treated rats with HE were used demonstrated the presence of elevated levels of a substance(s) that reversibly and competitively inhibited [^{3}H]Ro15–1788 binding to the BzR (Basile et al., 1989). Extracts made from several peripheral organs (including liver, heart, plasma, and kidney) from animals with HE also contain higher levels of this inhibitory activity than equivalent volumes of extracts from control animal organs. The potency of these extracts in inhibiting [^{3}H]flunitrazepam or [^{3}H] flumazenil binding was enhanced in the presence of GABA, indicating that the BzR ligand present in HE has agonist properties. This finding reinforces the results from the autoradiographic studies (Basile et al., 1990b). The inhibitory factors in these extracts were resistant to boiling and treatment with proteolytic enzymes (including pronase, protease K, and trypsin).

Based on the ability of these HE brain extracts to inhibit the binding of [^{3}H]flumazenil and [^{3}H]flunitrazepam, both highly specific radioligands for the BzR, as well as the results of applying radioimmunoassays specific for 1,4-Bzs to body fluids from animals and humans with HE (Mullen et al., 1989; Olasmaa et al., 1989, 1990), it was proposed that the BzR ligands in the tissue extracts from animal models and humans with HE were 1,4-Bzs. This hypothesis is particularly attractive in that it not only provides a mechanism for increased GABAergic tone in HE but is independent of changes in blood-brain barrier permeability which may be present in liver failure, because Bzs readily cross the intact blood-brain barrier. However, additional purification and isolation of these compounds were clearly required to confirm their structural identity. Subsequent purification of the whole brain extracts from GalN-treated rabbits and TAA-treated rats using reverse-phase HPLC techniques revealed the presence of four to eight peaks that inhibited [^{3}H]flumazenil binding (fig. 11). The profile of this activity from each HE extract varied with respect to the number of peaks, their retention times, and the activity in each peak. However, the overall inhibitory activity of extracts from animals with HE was consistently higher than that from controls. Furthermore, some of these peaks of activity had retention times similar to those of known 1,4-Bzs, including diazepam and N-desmethyldi-

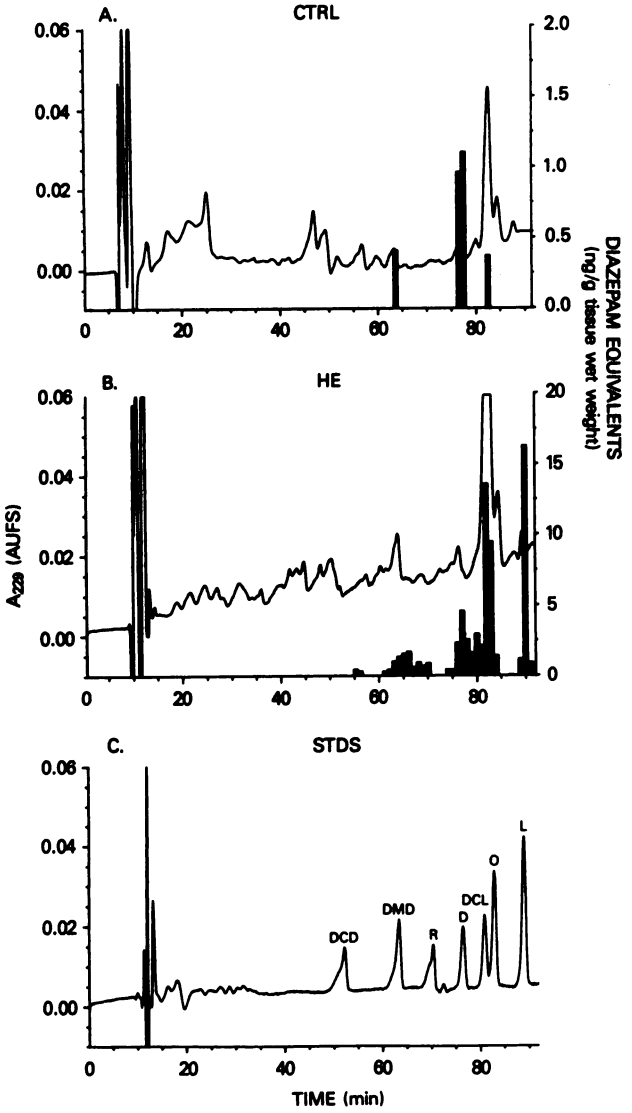

FIG. 11. BzR ligands are elevated in the brains of rats with HE. Representative reverse-phase chromatograms of extracts from ≈20 g of brain from control (CTRL) (*A*) and rats with HE due to TAA-induced FHF (*B*). *C*, elution profile of Bz standards (STDS). The Bz standards used include deschlorodiazepam (DCD), N-desmethyldiazepam (DMD), Ro 7–1986 (R), diazepam (D), deschlorolorazepam (DCL), oxazepam (O), and lorazepam (L). Solid bars overlaying the chromatograms in A and B, the inhibition of [^{3}H]Ro 15–1788 binding to BzR in diazepam equivalents (in ng/g of tissue wet weight) per fraction (right ordinate). Note the presence of five major peaks of radiometric activity in the brain extracts from HE rats, which have retention times similar to those of N-desmethyldiazepam, diazepam, deschlorolorazepam, oxazepam, and lorazepam. The total inhibitory activity in the HE rat brain extract was ≈10-fold greater than in control rat brains. The presence of N-desmethyldiazepam and diazepam in HE rat brain extracts was confirmed by mass spectroscopy in fractions taken at 61–66 and 74–78 min, respectively. From Basile et al., 1990c.

azepam. Subsequent mass spectroscopic analysis established that two of these peaks contained diazepam and N-desmethyldiazepam (Basile et al., 1990c; Basile, 1990). The concentrations of diazepam and N-desmethyldiazepam together constitute between 17 and 55% of the total activity in each extract of HE brain, depending upon the animal model of HE studied. Presently, the identities of the substances responsible for the remaining inhibitory activity are not established.

These findings are supported by other studies that indicate the presence of diazepam, N-desmethyldiazepam, and other as yet unidentified BzR ligands in extracts of brain and serum from TAA- and GalN-treated rats and GalN-treated rabbits (Mullen et al., 1986, 1989; Olasmaa et al., 1990). In these studies several different polyclonal antibodies to 1,4-Bzs were used to quantify the levels of inhibitory activity in crude and HPLC-purified extracts. The rank order of the amount of BzR ligand activity in the animal models used in these studies indicates that the TAA-treated rat has the highest brain levels of BzR ligands, followed by the GalN-treated rabbit and the GalN-treated rat. Consistent with its classification as an unsatisfactory model of HE, no significant elevation of BzR ligand concentrations was present in the rat with a portacaval shunt. These results are in temporal agreement with the development of encephalopathy (the TAA-treated rat being the slowest), the quality of the encephalopathy (the TAA-treated rat and GalN-treated rabbit being the most profound), and the relative contribution of GABAergic systems to the development of the encephalopathy (lowest in the rat with a portacaval shunt).

If an increase in BzR ligands is relevant to the development of HE in humans, it is necessary to show that elevated levels of BzR ligands are also present in the human condition. The increased sensitivity of cirrhotics to triazolam, which is not readily attributable to impaired drug elimination, suggested the presence of substances in humans with chronic liver failure that increase brain sensitivity to Bzs (Bakti et al., 1987). Samples of CSF from patients with HE due to acute and chronic liver failure were reported to contain approximately 3-fold greater concentrations of diazepam-like immunoreactivity compared to control CSF (Olasmaa et al., 1989). Furthermore, in another study samples of CSF and plasma from patients with HE due to cirrhosis contained levels of Bz-like activity 5- to 7-fold greater than in controls (Mullen et al., 1990). The levels of Bz-like immunoreactivity in the cirrhotic patients were on the order of 54 ng of diazepam equivalents/ml serum and 195 ng of oxazepam equivalents/ml of CSF. When quantitative radioreceptor assays were used, Bz-like activity in serum from cirrhotic patients was 911 ± 385 ng (mean ± SEM) of diazepam equivalents/ml (Mullen et al., 1990). Plasma Bz-like activity appeared to correlate with the severity of the encephalopathy, with significant increases in activity seen only in stages III-IV HE. Finally, a recent investigation of brain samples from patients who died with HE due to FHF indicated the presence of several peaks of Bz-like activity (fig. 12) (Basile et al., 1990a). Two of these peaks were positively identified as diazepam and N-desmethyldiazepam based on HPLC

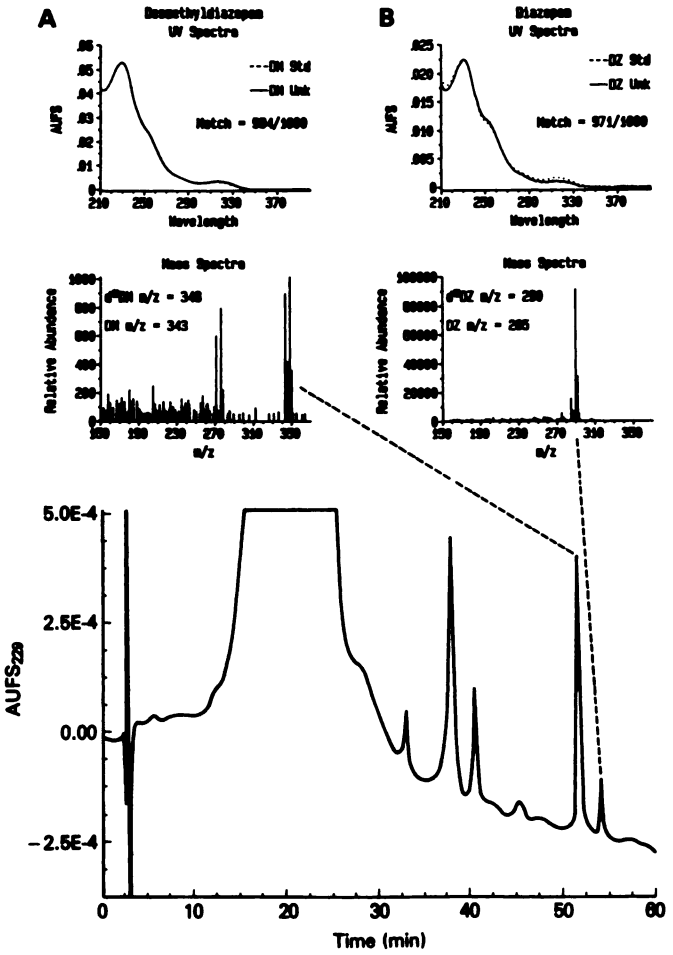

FIG. 12. Diazepam (DZ) and N-desmethyldiazepam (DM) are present in the brains of humans with HE. *Bottom*, representative reverse-phase chromatogram of a brain extract (2 g) from a patient who died of HE secondary to FHF. This patient was not given Bzs during hospital treatment. The two peaks of ultraviolet (UV) absorption have retention times of 52 and 54 min which are similar to the retention times for N-desmethyldiazepam and diazepam standards, respectively. Aliquots of these fractions significantly inhibited [^{3}H]flumazenil binding to BzR in rat brain preparations in vitro. Scanning ultraviolet analysis of these peaks indicated an absorption profile consistent with that of the N-desmethyldiazepam (*top, A*) and diazepam (*top, B*) standards. Derivatizing the peaks and subjecting them to gas chromatography/negative chemical ionization mass spectroscopy yielded mass spectra consistent with the presence of the trimethylsilyl derivative of N-desmethyldiazepam (*bottom, A*) and of diazepam (*bottom, B*). Mass spectral peaks at 348 and 274 (*A*) and 285 (*B*) m/z represent the presence of trimethylsilyl-N-desmethyldiazepam-d_5, N-desmethyldiazepam-d_5, and diazepam-d_5, standards added after HPLC and before derivatization.

retention times as well as both ultraviolet and mass spectral analysis (Basile et al., 1990a). The overall Bz-like activity in these brain samples was significantly higher than in controls (380 ± 100 versus 80 ± 29 ng of diazepam equivalents/g of brain) (fig. 12), as were diazepam and N-desmethyldiazepam levels (diazepam, 110 ± 42 versus 54 ± 33 ng/g; N-desmethyldiazepam, 130 ± 59 versus 28 ± 16 ng/g). The overall level of Bz activity in these patients with HE due to FHF was lower than the level reported in the serum from cirrhotic patients with HE (Mullen et al., 1990). This difference may be related to both the time course of development and duration of the encephalopathy. Further analysis of the distribution of total Bz activity and N-desmethyldiazepam in the patients with HE due to FHF revealed that they were not a homogeneous population (Basile et al., 1990a). The majority of patients (≈60%) had elevated brain levels of total Bz activity and N-desmethyldiazepam concentrations, whereas the remainder had brain levels that were not significantly different from those of controls. Thus, the responsiveness of patients to treatment with flumazenil may be determined by this differential expression of BzR ligands in patients with HE due to FHF.

1,4-Bzs are not the only BzR ligands that are reportedly increased in patients with HE. The levels of DBI are elevated in the CSF of patients with chronic HE (Rothstein et al., 1989). However, given the pharmacological properties of DBI, this observation is difficult to interpret. DBI is a low-affinity BzR ligand that may produce inverse agonist actions in some paradigms. Rothstein and coworkers have suggested that the increase in DBI levels is a compensatory response to the elevation of BzR agonists observed in HE. However, a recent report demonstrates that DBI has the same amino acid sequence and subcellular and tissue localization as acyl-coenzyme A ester-binding protein (Knudsen and Nielsen, 1990). Together, these findings make it difficult to evaluate a potential role for DBI in the pathogenesis of HE.

The origin of the elevated levels of 1,4-Bzs present in HE is unknown. The presence of N-desmethyldiazepam and diazepam has been demonstrated in a variety of mammalian tissues, including brain, kidney, liver, spleen, and plasma (Sangameswaran et al., 1986; Wildmann et al., 1986; DeBlas et al., 1987; Medina et al., 1988; Wildmann and Ranalder, 1988; Unseld et al., 1989, 1990). These compounds were found in human brains preserved prior to the commercial availability of 1,4-Bzs (Sangameswaran et al., 1986; Unseld et al., 1990). However, there is only indirect evidence for the de novo synthesis of these 1,4-Bzs by mammalian cells, specifically by NG108–15 cells (a transformed neuroblastoma/glioma cell line) cultured in Bz-free, serum-free medium (DeBlas et al., 1987). Thus, it appears unlikely that 1,4-Bzs are synthesized de novo in mammalian tissues.

Alternatively, diazepam and N-desmethyldiazepam could be produced by prokaryotes in the gut. It is known that a Bz nucleus can be synthesized by both *Penicillium* and *Aspergillus* fungi by the condensation of anthranilic acid (a tryptophan derivative) with phenylalanine and methionine (fig. 13*A*) (Luckner, 1984). Furthermore, the inhibition of the methionine-induced potentiation of HE by antibiotics (Phear et al., 1956) is circumstantial evidence for the gut flora as a source of Bzs. However, these compounds do not possess a 5-phenyl C ring. Furthermore, although aromatic chlorinations catalyzed by chloroperoxidase are known to occur in biological systems

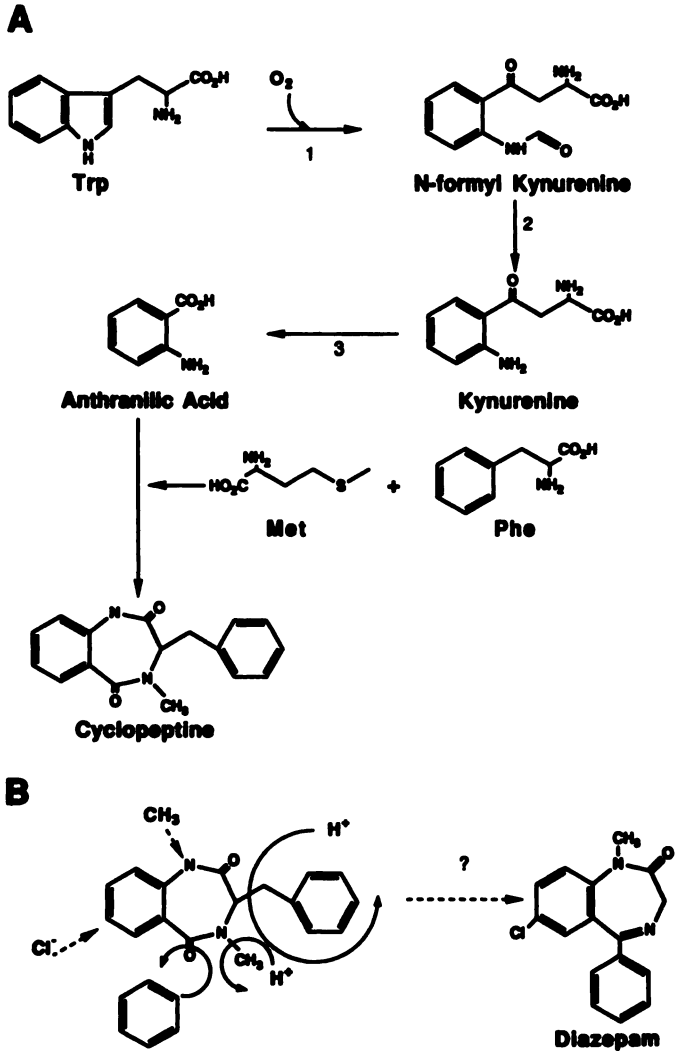

FIG. 13. Biosynthesis of Bz nuclei. *A*, a possible pathway for the biosynthesis of the Bz cyclopeptine, found in *Penicillium cyclopium*. Anthranillic acid is formed in mammals from the amino acid tryptophan (Trp) in the first three steps using 2,3-dioxygenase-pyrrolase (1), formamidase (2), and kynurenidase (3). Cyclopeptine is then synthesized by the cyclopeptine synthase complex, which activates anthranilic acid and *l*-phenylalanine (Phe), forms peptide bonds between them, and uses *l*-methionine (Met) as a methyl donor. However, several additional modifications of cyclopeptine must occur to create a 1,4-substituted Bz like diazepam (*B*). These include the addition of the C ring, removal of the 3-phenyl group, N-demethylation, and halogenation of the A ring. Although the 3-phenyl group may be removed by substituting glycine for Phe, and chlorination of the A ring via halogen peroxidases are possible, the other modifications of cyclopeptine to yield diazepam are hypothetical.

(Neidleman, 1975), there is no evidence that a 7-chlorination of the Bz A ring occurs in such systems (fig. 13*B*). The discovery of such pathways will be required before the source of the 1,4-Bzs observed in HE can be confidently ascribed to biosynthesis by enteric flora.

A final possibility is that these compounds may be dietary in origin, being ingested either as preformed Bz or as Bz precursors which are subsequently modified in the gut or after absorption. Several studies have indicated that a family of 1,4-Bzs (including diazepam and N-desmethyldiazepam) are present in foodstuffs, such as wheat and potatoes (Wildmann et al., 1988), milk (Medina et al., 1988), soy beans, rice, and mushrooms (Unseld and Klotz, 1989). Furthermore, Bz levels increased 5- to 8-fold during germination of wheat and potatoes, suggesting that these plants may biosynthesize Bzs for use as growth regulators (Wildmann, 1988).

Regardless of their origin, a focal issue is whether the elevated concentrations of BzR ligands in the CNS observed in liver failure are capable of sufficiently enhancing GABAergic tone to result in the expression of the neuropsychiatric manifestations of HE. After the administration of 1.3 mg/kg of diazepam (a behaviorally active but nonhypnotic dose) to normal rats, the whole brain concentrations of N-desmethyldiazepam and diazepam are on the order of 200–300 ng/g (Mennini and Garattini, 1983). These levels are 5–10 times higher than the whole brain Bz concentrations in rats with TAA-induced FHF (fig. 14). Diazepam and N-desmethyldiazepam levels in cerebral cortex from patients with HE due to FHF (≈100 ng) are somewhat closer to the reported plasma levels of these compounds after the administration of 5–10 mg of diazepam to normal humans (Klotz et al., 1980). These findings suggest that the whole brain levels of 1,4-Bzs and Bz-like compounds in HE are below those induced by encephalopathogenic doses of diazepam. Nonetheless, these levels may be sufficient to cause the subtle derangements of psychomotor function observed in subclinical HE in humans. Furthermore, the autoradiographic investigations of radioligand binding to BzRs in brain sections from rabbits with HE indicated that between 20 and 40% of the receptors were occupied by BzR ligands (Basile et al., 1990b). This level of BzR occupation is sufficient to mediate the anticonvulsant [e.g., blockade of audiogenic and metrazol-induced seizures (Paul et al., 1979; Petersen et al., 1984)], and anxiolytic [(four-plate, chlordiazepoxide discrimination and conflict tests (Lippa et al., 1979; Petersen et al., 1984)] effects, and some of the motor impairment [exploratory motility, muscle relaxation (Klockgether et al., 1985; Petersen et al., 1986)] associated with BzR agonists like diazepam. Furthermore, this autoradiographic study indicated that a significant regional heterogeneity in the distribution of BzR agonists exists in the brains of rabbits with GalN-induced HE, implying that local concentrations of BzR agonists may be much higher than whole brain concentrations (Basile et al., 1990b). Thus, the regionally high levels of BzR ligands found in the cerebral cortex and cerebellum may be sufficient to cause the ataxia and motor impairment observed in the GalN-treated rabbit. Moreover, the ability of BzR antagonists to substantially correct both the electrophysiological and behavioral manifestations of HE in several animal models (Bassett et al., 1987; Basile et al., 1988; Gammal et al., 1990) and patients with acute or chronic liver failure (Scollo-Lavizzari and Steinmann 1985; Grimm et al., 1988a,b; Ban-

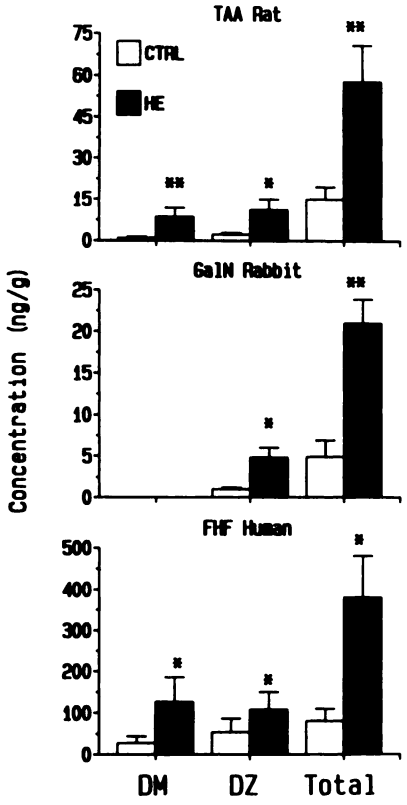

FIG. 14. Summary of the levels of N-desmethyldiazepam (DM), diazepam (DZ), and total BzR ligand activity in the brains of rats (*top*), rabbits (*center*), and humans (*bottom*) with FHF. Concentrations are in absolute ng/g of tissue wet weight for N-desmethyldiazepam and diazepam and in ng/g of diazepam equivalents for total BzR ligand activity. Levels were quantified by radiometric analysis of fractions collected by isocratic (TAA-treated rat) and gradient (both models and human) HPLC. Overall levels of BzR ligand activity are elevated approximately 4-fold in both animal models and patients with HE compared to controls. However, the absolute levels of activity are 5 times greater in humans with FHF (380 ng) than in the rat with FHF (71 ng) and 18-fold greater than the rabbit with FHF (21 ng/g). Variability in the levels of the 1,4-Bzs are observed in rat, rabbit, and human syndromes. Both N-desmethyldiazepam and diazepam are present in the rat model and humans with FHF but only diazepam was positively identified in the rabbit model. Bz levels in HE were significantly greater than those in control (animal models: *, $P < 0.05$; **, $P < 0.01$, Student's t test; humans: *, $P < 0.05$, Mann-Whitney U test).

sky et al., 1985, 1989; Ferenci et al., 1990; Ferenci and Grimm, 1989) indicate that the BzR ligand concentrations are sufficiently elevated to produce some of the behavioral manifestations of HE. The encephalopathic effects of BzR agonists may be further enhanced by other metabolic changes that may occur in liver failure, such as increased GABA concentrations in brain extracellular fluid (Bassett et al., 1990).

In summary, BzR agonists, regardless of their source, should be extracted and metabolized by a normal liver (Klotz et al., 1980). Severe hepatocellular disease would allow an increase in the levels of free BzR ligands in peripheral blood plasma by reducing the efficacy of the detoxification mechanism, by decreasing the synthesis of the plasma albumin that sequesters Bzs, and/or by increased portal-systemic shunting of blood. The subsequent elevation in the CNS levels of BzR agonists would augment GABAergic tone, thereby contributing to the development of HE.

IV. Therapeutic Modalities for the Management of Hepatic Encephalopathy

A. General Principles

The primary objective of treatment for HE is to normalize the patient's mental status. Several principles are relevant to achieving this goal. First, any precipitating factors should be identified and either removed or corrected. Second, in accordance with traditional concepts of the pathogenesis of PSE (Sherlock et al., 1954), standard maneuvers are instituted to minimize the absorption of nitrogenous substances from the intestinal tract. Third, any complications of the liver disease that tend to exacerbate HE are treated. Fourth, as HE abates when adequate hepatocellular function is restored, any appropriate measures are taken to improve hepatocellular function. Fifth, if possible, increased portal-systemic shunting of blood is reduced. Finally, it may be possible to administer specific therapies that correct pathophysiological neural mechanisms that contribute directly to the pathogenesis of HE.

Routine management of the patient with acute HE includes maintenance of fluid and electrolyte balance, plasma oncotic pressure, and renal function. Glucose, which reduces protein catabolism, is provided as a source of energy.

1. Precipitating factors. Withdrawal or correction of any potential precipitating factors is a routine part of management of all patients with HE. However, amelioration of HE following such action is likely to be more substantial if the patient has cirrhosis rather than FHF (Conn and Lieberthal, 1978; Sherlock, 1989). Measures taken in this context include correction of constipation, acid-base disturbances, diarrhea, vomiting, hypoxia, anemia, hypotension, hemorrhage, dehydration, azotemia, and hemodynamic disturbances, treatment of infections (e.g., pneumonia and spontaneous peritonitis), and discontinuing diuretic therapy. The volume of an abdominal paracentesis should be limited to that needed for diagnostic purposes only. Enteric intake of nitrogenous substances (protein, amino acids) is reduced (chronic HE) or stopped altogether (acute HE) (Hoyumpa et al., 1979). Hypoglycemia may compound the encephalopathy of FHF and must be detected early and treated vigorously with intravenous dextrose.

All sedative-hypnotic drugs are discontinued. The importance of this rule has been reemphasized because a substantial role of the GABA/BzR complex has been defined in the pathogenesis of HE. Appropriate drug antagonists (e.g., naloxone and flumazenil) should be given, if available. Despite difficult nursing problems posed by a restless, agitated, or even delirious patient with HE (usually FHF), the temptation to give sedative-

hypnotic drugs should be resisted. The rare occurrence of seizures in the context of HE (usually FHF) and their self-limiting nature is not an indication for barbiturate or Bz anticonvulsant therapy.

2. Reduction of absorption of nitrogenous substances. a. ACUTE HEPATIC ENCEPHALOPATHY. Ingestion of protein, amino acids, and other nitrogenous substances into the gastrointestinal tract is stopped. The bowel is evacuated by administering enemas and cathartics. There is a general consensus that to suppress the enteric bacterial flora and hence reduce biotransformation of nitrogenous substances in the intestine, a broad spectrum, poorly absorbed antibiotic should be administered orally. The most widely used antibiotic is neomycin (Dawson et al., 1957). Lactulose (Conn, 1988) or a disaccharide with similar properties (e.g., lactitol) (Morgan and Hawley, 1987; Heredia et al., 1987) given orally is also a standard treatment. During lactulose therapy, it is necessary to carefully monitor electrolyte and volume status to avoid hypernatremia. Lactulose may also be administered by enema. As recovery occurs, dietary protein intake is gradually increased.

b. CHRONIC HEPATIC ENCEPHALOPATHY. Protein intolerance is an indication to reduce dietary intake of protein (Eriksson and Conn, 1989) and it is conventional to administer lactulose (Conn, 1988) or lactitol (Uribe et al., 1987; Morgan and Hawley, 1987; Heredia et al., 1987; in doses sufficient to produce two or three bowel actions daily. Protein intake should only be reduced to an extent necessary to suppress the features of chronic PSE and should not be reduced below 40 g daily for long periods, because negative nitrogen balance would be induced (Conn and Lieberthal, 1978; Sherlock, 1989). Neomycin may be given if lactulose is poorly tolerated. Lactulose and neomycin appear to be equally efficacious in the treatment of chronic PSE (Conn, 1977). However, long-term neomycin therapy may cause ototoxicity and nephrotoxicity (Conn and Lieberthal, 1978; Sherlock, 1989). Metronidazole is more effective than neomycin in suppressing *Bacteroides* and other anaerobes and is as effective as neomycin in chronic PSE (Morgan et al., 1982). However, metronidazole should not be administered on a long-term basis because of dose-related CNS toxicity. Whether lactulose and neomycin have additive or synergistic effects on chronic PSE is uncertain (Weber et al., 1989).

3. Complications of liver disease. Management of HE includes the treatment of complications of acute or chronic liver disease that tend to compound or exacerbate HE, such as renal failure, hemorrhage, and hypoglycemia.

4. Improvement of hepatocellular function. Although HE represents an advanced stage of evolution of either acute or chronic hepatocellular failure, administration of standard therapies to reverse or arrest the process(es) responsible for the liver disease may have a beneficial effect on HE by reducing deterioration of hepatocellular function or by improving it. In some instances, specific measures can be taken to protect the liver from continuing hepatocellular injury. For example, administration of N-acetylcysteine and methionine can decrease hepatocellular injury following an overdose of acetaminophen (Read et al., 1986), and corticosteroids may decrease hepatocellular necrosis due to reactivation of hepatitis B in an hepatitis B surface antigen carrier following withdrawal of immunosuppressive drugs (Hoofnagle et al., 1982). Stimulation of hepatic regeneration is a desirable therapeutic goal for patients with FHF. This approach is being studied experimentally and is currently not a component of standard management for HE (Ohkawa et al., 1985; Pappas et al., 1985).

As expected, when the failing liver is successfully replaced by a normally functioning liver (orthotopic liver transplantation), complete and sustained resolution of HE occurs. Liver transplantation is becoming an increasingly important therapeutic option for patients with decompensated chronic hepatocellular failure and HE (or recent HE) or FHF (Maddrey and van Thiel, 1988).

HE may also be improved by providing artificial liver support. This approach is only relevant in selected patients with FHF and potentially reversible liver disease to permit sufficient time for hepatic regeneration to occur or in patients with FHF or decompensated chronic liver disease in preparation for liver transplantation (Jones and Schafer, 1990). Currently, no methods of providing artificial hepatic support are of proven clinical value in the management of patients with HE.

5. Reduction of portal-systemic shunting. In patients with intractable chronic PSE associated with appreciable portal-systemic shunting of blood (spontaneous or surgically induced) and only modestly impaired hepatocellular function, it may be possible to achieve a dramatic and sustained amelioration of the encephalopathy by reversing portal venous blood flow from hepatofugal to hepatopedal. This result can sometimes be achieved by occluding shunts (Bismuth et al., 1983; Potts et al., 1984; Uflacker et al., 1987; Clarke et al., 1989).

B. Therapeutic Modalities

In the following sections, the results of clinical trials of several therapeutic regimens for the management of HE will be reviewed. Many of these clinical trials suffer from a number of problems (Pomier-Layrargues et al., 1989a). These include the subjective nature of the methods and criteria of clinical staging used. Even when the criteria are generally agreed upon, the staging of encephalopathy is subject to significant interobserver variability, lack of reproducibility, nonspecificity of many criteria for HE (as in the case of EEG and psychometric tests), the need for patient collaboration (which is difficult with delirious or comatose patients), and a lack of correlation of some criteria with the severity of encephalopathy (as in sensory-evoked responses). Furthermore,

because of the ethical concerns regarding withholding adequate therapy from patients with HE, many of these studies have crossover designs and lack placebo controls. Thus it is difficult to determine the efficacy of the therapies in their own right, instead of in comparison to "benchmark" standard therapies. This is particularly true in cases of acute HE due to FHF. Finally, a major confounding factor in all of these studies is the tendency for HE to undergo spontaneous remission in many patients, particularly those with chronic liver disease. Three double-blind, placebo-controlled studies (Michel et al., 1980, 1985; Wahren et al., 1983) have shown that in 33–48% of cirrhotic patients with acute HE who do not die within 48 h HE will spontaneously remit. Thus, although effects attributable to removing precipitating factors may be apparent, it is often difficult to determine whether even "standard" therapies, particularly those that are administered for days to weeks (e.g., lactulose, neomycin), are truly efficacious treatments.

1. Diet. Dietary protein intake plays the most significant role of the major nutrients in precipitating HE. Animal models of liver failure and humans with compromised liver function will develop clinical signs of encephalopathy when an excessive protein load, primarily meat (Hahn et al., 1893; Uribe, 1989) is administered. Cessation of protein intake ameliorates the symptoms of HE. Unfortunately, chronic restriction of dietary protein intake in patients with advanced liver disease often exacerbates the depletion of muscle and liver protein reserves, hampering liver regeneration in patients suffering from protein-calorie malnutrition and inducing a negative nitrogen balance (Yanez et al., 1986). Thus, an important goal in managing HE is to maintain nitrogen balance without precipitating or exacerbating HE.

Quantity and quality of dietary protein initially appeared to be the most important factors in determining protein tolerance by patients with liver disease. A positive nitrogen balance can be achieved with 0.8–1 g/kg of protein/day, titrated to avoid precipitation of HE (e.g., frequent small meals). The quality of dietary protein and its role in precipitating HE is a matter of some controversy. The apparent hierarchy of encephalopathogenic potential for dietary protein is meat > dairy > vegetable protein (Greenberger et al., 1977). This effect is proposed to reflect (*a*) the lower methionine and AAA content of vegetables, (*b*) their higher fiber content and cathartic action, and (*c*) their ability to acidify the colon and increase fecal nitrogen content (Uribe, 1989). Several clinical studies comparing the therapeutic benefits of a vegetable protein diet relative to meat protein have been performed (Uribe et al., 1982, 1985; Uribe, 1989). These studies found no significant difference in mental state, EEG, or arterial blood ammonia content between patients with chronic liver failure treated with meat protein, neomycin, and laxatives versus vegetable protein (Uribe et al., 1985). Interestingly, these parameters were not significantly improved in the group fed meat protein plus standard therapy relative to basal values, suggesting that the standard therapy of neomycin plus laxatives may be ineffective as well. Thus, the absolute amount of protein in the diet appears to be of more importance in the management of chronic PSE than the relative proportions of different types of protein (Shaw et al., 1983; DeBruijn et al., 1983; Keshavarzian et al., 1984; Uribe et al., 1982, 1985). Furthermore, the results of subsequent studies suggest that the quality of vegetable or dairy protein may matter less than the cathartic action of accompanying dietary fiber (Uribe, 1989) or lactose (in lactase-deficient patients) (Fenton et al., 1966). This catharsis may improve psychometric test scores, increase fecal nitrogen content, and enhance glucose/protein tolerance in patients with chronic liver disease, without significant reductions in blood ammonia levels.

2. Antibiotics. One of the general goals in the treatment of HE is to reduce the absorption of nitrogenous substances from the gut. Although neomycin was the most widely used antibiotic because of its poor absorption from the gut (Atterbury et al., 1978), the same effect may be achieved by the administration of any of several broad spectrum antibiotics, such as paromomycin (Stormont et al., 1958), chlortetracycline (Summerskill et al., 1957), vancomycin (Tarao et al., 1985), or metronidazole (Morgan et al., 1982).

The initial rationale for the use of antibiotics to treat HE was to reduce ammonia absorption by eliminating bacterial urease and proteases responsible for ammonia formation in the gut (Fisher and Faloon, 1957; MacBeth et al., 1965). Although some studies show that blood ammonia levels decrease after antibiotic therapy (Pirotte et al., 1974; Dawson et al., 1957), antibiotics may be efficacious in treating HE by reducing the absorption of other nitrogenous toxins of bacterial origin. This is supported not only by the lack of correlation between blood ammonia levels and the stage of HE but also by investigations demonstrating that antibiotics may ameliorate HE while having no effect on blood ammonia (Phear et al., 1956; Morgan et al., 1982).

Initial studies of the efficacy of neomycin in treating HE were not performed under double-blind and controlled conditions (Dawson et al., 1957; Fisher and Faloon, 1957; Najarian et al., 1959). Nonetheless, the results were sufficiently striking that neomycin administration became the standard treatment for HE. Subsequent clinical studies were designed to include neomycin as the therapy of comparison. These double-blind studies of patients with both acute and chronic HE indicated that neomycin was as effective as other antibiotics (e.g., metronidazole, Morgan et al., 1982) or cathartics such as oral lactulose (Conn et al., 1977; Atterbury et al., 1978; Orlandi et al., 1981) or lactulose enemas (Uribe et al., 1981).

Because antibiotics are no more efficacious than lac-

tulose in the treatment of HE and carry an increased risk of toxicity, they are no longer the primary treatment for HE due to chronic liver failure. Neomycin can cause significant oto-, neuro-, or nephrotoxicity (Berk and Chalmers, 1970) and malabsorption (Jacobsen et al., 1960). Chronic antibiotic therapy can also predispose to pseudomembranous colitis (Bolton, 1979). However, in refractory patients, neomycin is sometimes administered along with lactulose (Weber, 1989).

3. Lactulose and related carbohydrates. Lactulose is a synthetic disaccharide composed of galactose and fructose. It (or the related disaccharide, lactitol) is presently a standard therapy for HE. Lactulose is not absorbed or metabolized in the upper gastrointestinal tract where enterocytes lack the relevant disaccharidases. However, it is degraded by anaerobic bacteria in the lower gut to several organic acids, primarily lactate (Hoffman et al., 1964; Vince et al., 1978; Lieberthal, 1988).

It was first proposed by Ingelfinger (1964) that a compound like lactulose may reduce the absorption of nitrogenous compounds by promoting the growth of urease-negative lactobacilli in the colon. Several subsequent clinical studies have failed to confirm this mode of action (Bircher et al., 1971; Vince et al., 1973). Furthermore, although lactulose lowers blood ammonia concentrations in patients with severe liver disease (Conn et al., 1977), this is not a result of ammonia ion trapping in the acidified colonic contents (Agostini et al., 1972; Weber, 1989; Lebek and Luginbuhl, 1988). The present hypothesis for the mechanism of lactulose action involves a combination of cathartic and metabolic actions. Lactate formation from lactulose decreases the pH of the colonic environment. In combination with the osmotic action of the parent compound, lactulose has a laxative effect, thereby reducing the time available for toxin absorption from the gut. In addition, the presence of metabolizable carbohydrates such as lactulose in the colon promotes increasing ammonia nitrogen incorporation into bacterial protein, with a resultant decrease in urea and ammonia production (Vince, 1973, 1978). This inference is supported by reports of significant lactulose-induced increases in fecal nitrogen excretion, located primarily in the fecal bacteria fraction (Stephen and Cummings, 1980; Weber et al., 1987).

The first clinical trial of lactulose efficacy was performed by Bircher and coworkers (1966). Although it was a small and open study which only partially characterized the clinical manifestations of HE, it established that lactulose was more efficacious than the glucose placebo in reversing the mental aberrations of HE. Subsequently, a large number of clinical trials of lactulose have been conducted, most often comparing the efficacy of lactulose therapy to the previous standard therapy, neomycin. In a recent paper (Orlandi et al., 1988), the results of 30 of these trials, of which 16 were controlled and three were double blind, were reviewed. There was a great deal of variation among investigations, including the criteria for patient inclusion, the dose and duration of lactulose administration, the nature of the control group, and the type of ancillary treatments. Overall, lactulose treatment appeared to reduce the severity of the neuropsychiatric manifestations of HE in 25–100% of patients tested. Of the double-blind trials, the studies by Conn and coworkers (Conn et al., 1977; Atterbury et al., 1978) used semiquantitative assessments of mental state (a PSE index) and found that lactulose was equal to or somewhat more effective than neomycin in reducing the severity of HE. It should be noted that at least 36 h of treatment are required prior to the first statistically significant improvements in the PSE index used, with maximal effect achieved by 4 days of continual administration (Morgan and Hawley, 1987).

Not only did mental status improve more rapidly after lactulose treatment than neomycin but lactulose therapy was associated with a better risk to benefit ratio, which was confirmed by a much larger double-blind, randomized trial (Orlandi et al., 1981). Although hemoconcentration (Conn, 1988) and lethal hypernatremia have been reported in association with lactulose administration, these cases are relatively rare (Nelson et al., 1983). In the majority of cases, no serious toxicity results from lactulose administration for a period of up to 3 weeks. However, up to 20% of patients report discomfort from nausea, flatulence, and cramping, which usually resolves spontaneously in a few days without discontinuing lactulose. Although patient compliance with lactulose may be problematic because of its intense sweetness and the unpredictable timing of catharsis, the related compound lactitol tends to be better tolerated. It is slightly less sweet, as efficacious, and faster acting than lactulose (Morgan and Hawley, 1987).

Although lactulose is significantly less toxic than antibiotics such as neomycin, the designs of the published clinical trials do not resolve the issue of whether lactulose therapy would be significantly more beneficial than newer therapies directed toward the end organ. This issue must be considered in light of the rate of spontaneous reversal of HE during the course of lactulose administration, the effects of correcting precipitating factors alone (dietary protein, gastrointestinal hemorrhage), and the effects of nonspecific catharsis in reversing HE independently of any specific effects attributable to lactulose.

4. Branched-chain amino acids. Based on the BCAA/false neurotransmitter hypothesis of the pathogenesis of HE (James et al., 1979), it was proposed that the administration of BCAAs would correct the decrease in plasma BCAA/AAA observed in liver failure, thereby reversing the syndrome of HE. It appears that the evidence supporting this underlying hypothesis for the pathogenesis of HE is unconvincing (section III.C). Thus, even without reviewing the results of clinical trials of BCAA therapy, the rationale for the use of these substances in the

treatment of HE is questionable. Nonetheless, during the past 9 years, a number of trials examined the efficacy of BCAA or BCAA-enriched amino acid preparations administered intravenously or orally or of the α-keto analogs of BCAA given orally in the treatment of HE associated with acute or chronic liver failure.

The results of seven major trials, including 3 double-blind studies, examining the efficacy of BCAA therapy for acute HE complicating chronic liver failure have been published (Rossi-Fanelli et al., 1981; Wahren et al., 1983; Cerra et al., 1985; Michel et al., 1985; Fiaccadori et al., 1985; Strauss et al., 1986; Vilstrup et al., 1990). Three studies were placebo controlled (comparing the efficacy of BCAA therapy to glucose or conventional amino acid administration) (Wahren et al., 1983; Michel et al., 1985; Vilstrup et al., 1990). Wahren and coworkers (1983) reported that, in cirrhotic patients with HE, BCAA administration had no effect on mental status compared to placebo (48 versus 56% improvement, respectively), whereas the mortality rate was actually higher in patients treated with BCAA (40 versus 20%, BCAA versus placebo). In the other two placebo-controlled studies, BCAA therapy had no significant effect on either mortality rate or improvement of mental status in patients with acute HE. The remaining, unblinded studies attempted to determine whether BCAA therapy was as efficacious as conventional (e.g., lactulose or neomycin) therapy. In all of these studies, BCAA treatment was as efficacious as the standard therapy in restoring mental status. However, in one study (Rossi-Fanelli et al., 1981), the patients included in the BCAA groups had definite precipitating factors present (such as gastrointestinal hemorrhage or infection). Treatment of these precipitants alone could have been as efficacious as BCAA therapy. In other studies, correction of an abnormal nitrogen balance could have contributed to a positive effect (Michel et al., 1985).

The efficacy of BCAA therapy in the management of chronic HE or chronic PSE is not clearly established. Of six double-blind, crossover studies in which the efficacy of BCAA therapy was compared to casein, carbohydrates, or amino acid diets (e.g., Hepatic Aid), BCAA administration was not superior to control treatments in normalizing HE in five (Schafer et al., 1981b; Eriksson et al., 1982; McGhee et al., 1983; Horst et al., 1984; Mendenhall et al., 1985; Egberts et al., 1985).

The overall results of these studies indicate that, although this therapeutic approach effectively corrects abnormal levels of plasma amino acids, it does not consistently ameliorate the manifestations of acute, chronic, or subclinical HE. These trials have led to discussions of issues that relate to the appropriateness of their design, including the nature of the primary caloric source, documentation of side effects, and duration of follow-up (Ferenci, 1986; Alexander et al., 1989; Eriksson and Conn, 1989). Indeed, the similarity in rates of improvement of mental status and mortality between patients receiving standard therapy (lactulose, neomycin) and placebo controls even raises questions about the efficacy of conventional therapies (Eriksson and Conn, 1989). Irrespective of any possible effect on HE, BCAA administration may improve the nutritional status of patients with chronic liver disease. In patients in whom the minimal daily requirements of dietary protein induce recurrent HE, oral supplements of BCAA may induce a similar degree of positive nitrogen balance as an equivalent amount of dietary protein without inducing HE as frequently (Horst et al., 1984). Several recent reviews of these studies, including one rigorous meta-analysis (Naylor et al., 1989), conclude that the results do not favor the use of BCAA solutions in the management of cirrhotic patients with HE (Ferenci, 1986; Alexander et al., 1989; Eriksson and Conn, 1989).

5. Miscellaneous therapies. In patients with chronic liver disease, beneficial effects on HE of sodium benzoate and sodium phenylacetate (Mendenhall et al., 1986) and of zinc supplementation have been reported (Reding et al., 1984). Rational explanations for these findings are not readily apparent, and these agents have no established role in the management of HE.

As indicated in section III.E.2, dopaminergic agents such as L-DOPA and bromocriptine do not appear to be efficacious in the treatment of HE (Uribe et al., 1979, 1983; Michel et al., 1980).

6. Benzodiazepine receptor antagonists. a. FLUMAZENIL PHARMACOKINETICS. The imidazobenzodiazepine flumazenil (Ro 15–1788, Anexate) is a selective, high-affinity, competitive antagonist of the BzR. Flumazenil is rapidly and almost completely absorbed when administered orally (absorption $t_{1/2}$ = 18 min) with peak plasma concentrations observed after 20–90 min (Roncari et al., 1986). The amount of binding to plasma proteins is relatively low [free fraction, 54–64% (Klotz et al., 1984)]. Following intravenous administration, flumazenil is rapidly [≈24 min (Klotz et al., 1984)] and extensively distributed throughout the body (Vd_{SS} = 0.63 ± 0.18 liters/kg, mean ± SD), indicating considerable tissue uptake. The plasma clearance is rapid in normal humans (Cl_p = 16.3 ± 2.6 ml/min/kg, mean ± SD) as is the plasma $t_{1/2}$ (45.7 ± 8.5 min) (Pomier-Layrargues et al., 1989b). However, the Cl_p decreases to 4–7 ml/min/kg and the $t_{1/2}$ increases to 75–142 min in patients with cirrhosis (Pomier-Layrargues et al., 1989b). Following initial distribution according to cerebral blood flow, flumazenil is taken up by gray matter structures in the brain, with the highest specific retention found in the cerebral cortex and moderate levels in the cerebellum, thalamus, and basal ganglia (Shinotoh et al., 1986). Cerebral levels of flumazenil decrease with a half-life of 25–38 min (Samson et al., 1985).

The bioavailability of flumazenil through the oral route is low (16%) because of extensive first-pass hepatic metabolism (Roncari et al., 1986). Hepatic elimination

is rapid ($t_{1/2\beta}$ = 0.9 ± 0.2 h, mean ± SD) as is the rate of plasma clearance [Cl_p = 41.5 ± 13 liters/h, mean ± SD (Klotz et al., 1984)]. Flumazenil is extensively metabolized (>99.8%), primarily by N-demethylation and ester hydrolysis to three inactive metabolites (Zell and Timm, 1986) which are excreted primarily in the urine [90–95% (Roncari et al., 1986)]. Flumazenil and its metabolites are completely eliminated within 48–72 h. Because of its rapid onset of action (<1 min) and short elimination half-life, flumazenil is usually administered intravenously.

b. PHARMACOLOGICAL PROPERTIES OF FLUMAZENIL. Numerous in vitro and in vivo binding studies indicate that flumazenil competitively displaces BzR ligands and interacts with binding sites that have the same density and distribution as those that bind [^{3}H]clonazepam (Hunkeler et al., 1981; Polc et al., 1981; Mohler and Richards, 1981, 1983). The binding of [^{3}H]flumazenil is unaffected by the presence of GABA, pentobarbital, SQ 20009, and Cl^-, conditions that enhance agonist binding to the BzR (Mohler and Richards, 1981). Consistent with the preponderance of evidence indicating that BzR antagonists have no intrinsic actions, flumazenil blocks all of the specific behavioral and physiological effects of BzR agonists, including their anxiolytic, sedative, hypnotic, and muscle relaxant properties (Hunkeler et al., 1981; Mohler and Richards, 1981; Krespan et al., 1984).

Despite the evidence indicating that its primary action is that of an antagonist, flumazenil does display intrinsic, dose-dependent effects. In animals, low doses have a mild anxiogenic effect and increase neuronal activity (File et al., 1982a,b; Skerrit and MacDonald, 1983; King et al., 1984; Vicini, et al., 1986). However, at higher concentrations agonist effects predominate, including mild anxiolytic and anticonvulsant properties (Lloyd et al., 1981; File et al., 1982b; Nutt et al., 1982) and depression of neuronal activity (Polc et al., 1981; Skerrit and MacDonald, 1983; Basile et al., 1988).

Analogous effects are seen when flumazenil is administered to normal humans. Relatively low doses (1–5 mg, i.v., or 30 mg, p.o.) were found to have central activating properties, including anxiogenesis and autonomic arousal (Emrich et al., 1984; Darragh et al., 1983; Schopf et al., 1984; Higgitt et al., 1986), sleep disturbances (Gaillard and Blois, 1983; Ziegler et al., 1986), and increased neuronal electrical activity (Schopf et al., 1984; Higgitt et al., 1986). At higher doses (2.5 mg, i.v.; 100–400 mg, p.o.) flumazenil displays more agonist properties, acting as an anticonvulsant (Scollo-Lavizzari, 1984), causing motor impairment and suppressing neuronal electrical activity (Higgittt et al., 1986).

Unlike GABA receptor antagonists, BzR inverse agonists, and chloride channel blockers, flumazenil is not a convulsant and does not appear to have intrinsic neuronal activating properties. Thus, the anxiogenic and CNS-activating responses reported in scattered animal and human studies may represent the displacement of endogenous ligands with agonist properties from BzRs. In the majority of paradigms, flumazenil has been found to lack significant intrinsic activity of any type (Darragh et al., 1981; Emrich and Lund, 1983; Emrich et al., 1984; Lupolover et al., 1984), and to be free from any significant side effects, with doses of up to 600 mg, p.o., or 100 mg, i.v., being well tolerated (Darragh et al., 1981; Higgitt et al., 1986). In summary, although flumazenil has been found to elicit a spectrum of subtle centrally mediated effects, its primary actions are consistent with those of a very weak partial agonist.

c. POTENTIAL CLINICAL USE OF FLUMAZENIL. The therapeutic use of flumazenil is presently restricted to Europe (where it is approved for clinical use) and to relatively special clinical situations involving the reversal of the sedative and hypnotic actions of BzR agonists. Specifically, flumazenil can rapidly (<5 min) reverse the sedative and amnestic effects of Bz agonists administered for diagnostic procedures [e.g., endoscopy, 0.6–1 mg, i.v. (Kirkegaard et al., 1986; Holloway and Logan, 1988), surgical premedication (Nilsson et al., 1988), or anesthesia, 10 mg, i.v. (Alon et al., 1985)]. Generally, no adverse effects were noted, other than occasional reports of anxiety (Lauven et al., 1986; Ricou et al., 1986) or some resedation related to its short duration of action (Lauven et al., 1986; Klotz and Kanto, 1988). Flumazenil is also effective in the treatment of Bz overdose and in reversing the Bz component of multidrug overdoses. Numerous anecdotal (Hofer and Scollo-Lavizzari, 1985) and double-blind, placebo-controlled studies (Lheureux and Askenasi, 1986; O'Sullivan and Wade, 1987; Rouzioux et al., 1988) have been performed, indicating that low doses of flumazenil (1 mg, iv) significantly improve the coma rating within 5 min in patients who had taken Bz alone or mixed drug overdosages. In one case, 5 mg of flumazenil, i.v., immediately aroused a patient who ingested 10 g of diazepam (Klotz and Kanto, 1988). Finally, flumazenil is effective in terminating the sedative actions of Bz as a prelude to weaning patients from mechanical ventilators (Kleinberger et al., 1985). These studies clearly indicate that flumazenil is clinically efficacious in reversing all of the neurological effects of BzR agonists. Given the data from basic science studies of animal models and humans with HE indicating that BzR agonists are involved in the pathogenesis of HE, it is logical to assume that flumazenil may be efficacious in ameliorating some of the manifestations of HE in man.

Two anecdotal observations appeared simultaneously in 1985 reporting the effects of flumazenil in the treatment of patients with HE (Bansky et al., 1985; Scollo-Lavizzari and Steinmann, 1985) (table 4). A young female drug addict with FHF due to hepatitis B was treated with flumazenil (Scollo-Lavizzari and Steinmann, 1985). She was in stage IV HE, motionless, and unresponsive to painful stimuli and had an EEG showing flat periods

TABLE 4
*Flumazenil: efficacy in clinical trials**

Investigators	No. of cases	No. of episodes	Clinical setting	No. of responders	No. of positive episodes
Bansky et al., 1989	14	14	CI	10	10
Ferenci and Grimm, 1989	1	2	PSE	1	2
Pidoux et al., 1989	7	7	CI	6	6
van der Rijt et al., 1989	17	17		5	5†
Klotz and Walker, 1989	3	3	CI	1	1‡
Grimm et al., 1988a	17	20	FHF, CI	10	12§
Burke et al., 1988	1	2	CI	1	2
James, 1988‖	2	2	FHF	2	2
Grimm et al., 1988b	6	6	CI	6	6
Grimm et al., 1987	5	5	FHF, CI	4	4
Sutherland and Minerk, 1988	1	1	FHF	0	0¶
Meier and Gyr, 1988	3	5	PSE, CI	3	5
Bansky et al., 1985	4	4	CI	2	2#
Scollo-Lavizzari and Steinmann, 1985	1	1	FHF	1	1
Total	82	89		52 (63%)	58 (65%)

* The majority of the observations were made in uncontrolled, open investigations. CI, cirrhosis.

† This study was a double blind, crossover design with placebo and Bz screen. Of those patients in the controlled study, three of eight responded to flumazenil. An additional nine patients tested positive for Bz and were excluded from the controlled study. Two of these patients responded to flumazenil.

‡ This study was of randomized, double-blind, crossover design with placebo. The patients were screened for the presence of Bz. The one patient who showed a response to flumazenil screened positive for Bz.

§ This study screened 11 patients for the presence of Bz, but the limit of sensitivity of the screen was approximately 200 ng/ml of urine.

‖ O.L.F. James, University of Newcastle-Upon-Tyne, personal communication.

¶ This patient, in a late stage of HE, may have had cerebral edema.

Two additional patients in this study displayed a slight response to flumazenil.

with intermittent triphasic slow wave complexes. The intravenous infusion of 0.5 mg of flumazenil improved the EEG and behavioral manifestations of HE, such that the patient opened her eyes, reacted to verbal commands and painful stimuli, and spontaneously moved. This improvement lasted 1 h and could be sustained with additional flumazenil treatments. The other study included a patient with alcoholic cirrhosis in stage IV HE with continuous 1- to 2-Hz triphasic wave EEG activity. Forty seconds after intravenous flumazenil (0.3 mg), her encephalopathy had improved to stage II, with behavioral arousal and responsiveness to painful and verbal stimuli. This clinical improvement was accompanied by an increase in EEG frequency to 4- to 5-Hz theta rhythm (fig. 15). This state lasted approximately 1 h and could be regained by additional flumazenil administrations. Anecdotal observations of improvements in EEG pattern, somatosensory-evoked potentials, and indices of consciousness continue to be observed in patients with HE who were not otherwise exposed to exogenous synthetic Bzs (Bansky et al., 1985, 1989; Grimm et al., 1987, 1988a,b; Burke et al., 1988; Meier and Gyr, 1988; Ferenci et al., 1990; Pidoux et al., 1989). In two of the larger studies (Grimm et al., 1988a; Bansky et al., 1989), 19 of 31 (61%) patients with HE responded to flumazenil administration, with an average improvement of 1.4 ± 0.1 HE stage units, or 2.8 ± 0.38 units on the Glasgow coma scale (mean ± SEM). Compared to conventional

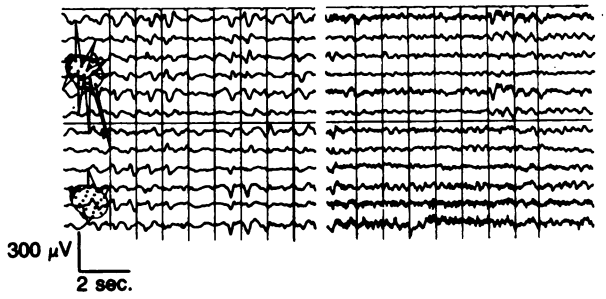

FIG. 15. Amelioration of HE in a 58-year-old woman with cirrhosis following the administration of the BzR antagonist flumazenil. *Left*, before treatment, when the patient was in stage IV HE, the EEG showed continuous 1- to 2- Hz triphasic wave activity; *right*, 40 s after the intravenous administration of 0.3 mg of flumazenil, the patient's degree of HE had improved to stage II, and the EEG showed 4- to 5-Hz theta background activity. Reprinted with permission from Bansky et al., 1985.

treatments for HE, the rate of response to flumazenil was rapid, with the time from injection to first responses ranging from 28 s to 30 min. The beneficial effects of an intravenous dose of flumazenil were evident for 0.58–4 h. There has been considerable variability in the doses of flumazenil and methods of its administration. As little as 0.2 mg has been given by intravenous bolus, and as much as 15 mg has been infused intravenously over 525 min.

Flumazenil has also been shown to be effective in the long-term treatment of chronic PSE on an outpatient basis (Ferenci et al., 1990) (fig. 16). This patient had an

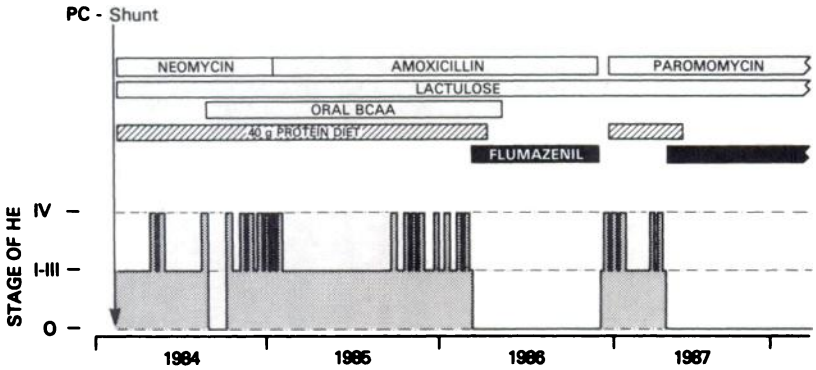

FIG. 16. Remissions of chronic intractable PSE in a 42-year-old woman associated with the oral administration of flumazenil. Degrees of encephalopathy are depicted as 0 (clinically normal mental function), stages I-III, and stage IV (coma). A two-thirds hepatectomy and end-to-side portacaval shunt were followed by the development of chronic incapacitating encephalopathy with episodes of coma. Treatment with oral broad spectrum antibiotics, lactulose, and dietary protein restriction did not appreciably ameliorate the encephalopathy. Addition of oral BCAA therapy was associated with a transient improvement in the encephalopathy. In contrast, flumazenil administration (25 mg, twice/day) was associated with a complete and sustained remission of the encephalopathy which included a normalization of tolerance to dietary protein. Discontinuing flumazenil therapy precipitated a recurrence of the encephalopathy; reinstituting it was followed by a further complete and sustained remission. Reprinted with permission from Ferenci et al., 1990.

end-to-side portacaval anastomosis constructed at the time of a partial (two-thirds) hepatectomy for the removal of a chronic inflammatory tumor. Three weeks after surgery the patient became encephalopathic. The encephalopathy was not controlled by conventional treatment with a low-protein diet, neomycin, and lactulose. During a period of several months, the patient had multiple episodes of coma in between frequent periods of stage I-III HE (fig. 16). During an episode of hepatic coma, the patient regained consciousness 30 s after the administration of 1 mg, i.v., flumazenil and remained conscious for 2 h. Subsequently, she lapsed back into HE. Oral flumazenil (25 mg, twice/day) was then initiated, and a sustained reversal of the encephalopathy was achieved despite normalization of dietary protein intake. Flumazenil treatment was stopped after 9 months as part of the assessment of a fever of unknown origin. Two days later, the patient became comatose and remained encephalopathic with episodes of coma until flumazenil administration was reinstated. Although this study was a single case report, the inference that flumazenil was effective in reversing the manifestations of chronic portal systemic encephalopathy is strongly supported by the refractory nature of the encephalopathy to long periods of conventional therapy and, by contrast, the sustained reversal of encephalopathy through long periods of flumazenil administration. This study suggests that flumazenil may be a safe and efficacious therapy for the long-term, outpatient treatment of patients with chronic PSE.

In summary of the reported studies (Grimm et al., 1988b; Bansky et al., 1989), flumazenil administration has been associated with an amelioration of encephalopathy in 69% of the patients (total number = 55) and 76% of the episodes of HE studied. Approximately 88% of those patients who did not respond to flumazenil were in stage IV HE, 60% had increased intracranial pressure, and 60% subsequently died within 3 days (Ferenci and Grimm, 1989). The degree of response was independent of the stage and etiology of HE when flumazenil was administered. Patients with HE due to FHF or acute HE associated with chronic liver disease (cirrhosis) showed similar rates of response (72 and 69%, respectively). It seems likely that the responsiveness of patients to flumazenil may be determined by the levels of BzR ligands. Brain levels of BzR ligands were significantly elevated in 7 of 12 (58%) patients who died of FHF and who had not received Bzs (Basile et al., 1990a). Thus, measuring the levels of BzR ligands in patients with HE may be a useful predictive test for responsiveness to flumazenil therapy. Indeed, in two of three studies in which flumazenil was reported to be ineffective in the management of HE (Klotz and Walker, 1989; van der Rijt et al., 1989), patients were intentionally screened for lack of Bz-like activity in their blood. In the other study (a single case) in which flumazenil was ineffective (Sutherland and Minuk, 1988), the patient with FHF appeared to have cerebral edema and hence would not have been expected to respond to flumazenil. Indeed, the lack of responsiveness of a patient in HE to flumazenil may indicate a poor prognosis (Schafer, 1987; Bansky et al., 1989). Clearly, flumazenil would not influence any underlying liver disease. Consequently, BzR antagonists would not be expected to influence the survival rate in FHF or cirrhosis without other therapeutic interventions. Nevertheless, flumazenil may greatly facilitate the management of HE in patients with acute or chronic liver failure.

Currently, there are five potential clinical applications of flumazenil and other BzR antagonists in the management of HE. First, these agents can be used to reverse the effects of any exogenously administered Bz. Second, patients' responses to BzR antagonists may be of value

in the differential diagnosis of encephalopathies. Third, these agents may permit patients with potentially reversible encephalopathy (and hence with a better prognosis) to be identified. Fourth, these drugs may be given to patients, perhaps by intravenous infusion, in an attempt to optimize brain function as a part of supportive therapy in FHF or in preparation for liver transplantation. Finally, BzR antagonists may be of value when given orally in the management of chronic PSE.

V. Conclusions

It is clear that the mechanisms responsible for the pathogenesis of HE are unresolved. Because of the multiplicity of metabolic abnormalities that accompany liver failure, it has been difficult to discriminate between those dysfunctions that are causally related to HE and those that are epiphenomena. Nonetheless, it is likely that multiple factors contribute to the pathogenesis of HE. Although changes in the plasma concentrations of amino acids, free fatty acids, and other cytotoxic metabolites such as phenol and mercaptans are often observed in liver failure, the evidence conclusively linking these changes to the pathogenesis of HE is unconvincing. The preponderance of evidence points to aberrations in the function of two major systems, ammonia metabolism and GABAergic neurotransmission, as the primary contributors to the pathogenesis of encephalopathies associated with liver failure, including HE. Increasing ammonia levels transiently disinhibit CNS neuronal activity and then depress it. Furthermore, increased ammonia concentrations may result in elevated intracranial pressure and cerebral edema, direct contributors to death due to FHF. There is significant evidence for concurrent increases in CNS GABAergic tone in HE, which may be summarized as follows: (*a*) Gross neurophysiological and single neuron electrophysiological studies in animal models of HE indicate that GABAergic tone is increased because of BzR-mediated potentiation of GABA potency. (*b*) Biochemical studies of patients with HE and animal models indicate that GABA and BzR agonist levels are increased in the brain and body fluids. (*c*) BzR antagonists are effective in ameliorating the electrophysiological and behavioral manifestations of HE.

Thus, the increase in GABAergic tone observed in HE may mask the potential for increased neuronal activity observed at low concentrations of ammonia. Higher concentrations of ammonia would depress CNS activity, an effect enhanced by increased GABAergic activity. This mechanism is also novel because it indicates for the first time a pathogenic mechanism that involves BzR ligands. Further insights into the normal role of the BzR in the operation of the GABAergic neurotransmitter system, its regulation of neuronal activity and higher mental processes, and the influence of diet on BzR function may be provided by further investigations of the pathogenesis of HE.

The results of studies using animal models and patients with HE indicate that the involvement of the GABAergic neurotransmitter system may have a significant impact on the development of rational modalities for the treatment of HE. Indeed, some of the conventional treatments, originally designed to reduce plasma ammonia levels by reducing gut ammonia production/absorption, may coincidentally reduce GABAergic tone by mechanisms independent of their effects on ammonia absorption. Thus, lactulose/neomycin may reduce the prokaryotic synthesis and absorption of BzR agonists. Modifications of diet can have a 3-fold action in suppressing the development of HE by eliminating direct sources of Bzs, by reducing the concentrations of precursors of Bzs and other nitrogenous substances (primarily in the form of amino acids, such as tryptophan, phenylalanine, and methionine), and by altering gut flora as a result of the laxative effects of high fiber.

It is clear, however, that BzR antagonists can play a useful role in correcting the abnormalities of BzR function observed in HE. BzR antagonist therapy in the management of HE has distinct advantages, including:

1. Rapid action: The BzR antagonist flumazenil elicits a response in patients with HE over a time frame of seconds to minutes. This rate of response is considerably faster than the response to conventional therapy (days), and the doses effective in ameliorating HE are consistent with those recommended for diazepam overdose.

2. Duration of action: The mean duration of action when administered intravenously to patients with HE is between 1 and 2 h (consistent with the $t_{1/2\beta}$ of flumazenil in humans with liver failure). These effects can be sustained with repeated administration, and flumazenil is orally active.

3. Therapeutic index: Flumazenil has a very high therapeutic index. Except for some anxiogenic properties, no significant adverse reactions have been observed.

4. Outpatient management: Flumazenil-induced suppression of enhanced GABAergic activity may be associated with increased protein tolerance in patients with chronic liver disease. The resulting increase in dietary protein is likely to optimize liver function and improve nutritional status.

5. Index of prognosis: A favorable neurological response to BzR antagonists probably indicates that the encephalopathy is uncomplicated, and potentially reversible, thereby providing an index of prognosis. Furthermore, the use of diagnostic tests (e.g., radioimmunoassy or radioreceptor assay for Bzs) for the presence of endogenous BzR ligands in patients with liver failure should indicate whether BzR antagonist therapy is likely to be effective. It is unlikely that these agents would be effective in ameliorating HE complicated by increased intracranial pressure, cerebral edema, hypoxia, or hypoglycemia or in the agonal stages of liver failure.

Because the manifestations of HE may spontaneously reverse within 2–3 days and lactulose/neomycin therapy

takes 1–2 days to induce a minimal amelioration of HE, it is unclear whether standard therapy induces a better outcome than flumazenil. Flumazenil rapidly induces a one- to two-grade improvement in the level of HE, suggesting that it would be useful for patients with early stages of HE and particularly for the outpatient management of chronic PSE. Furthermore, HE may be useful in inducing a more rapid and complete amelioration of the later stages of HE in combination with other therapies.

In summary, multiple factors appear to contribute to the pathogenesis of HE. For neuroscientists, it manifests a condition whereby GABAergic neurotransmission is enhanced by the presence of naturally occurring BzR ligands, suggesting a normal function for the BzR. Consistent with the involvement of the BzR in HE, anecdotal clinical observations indicate that BzR antagonists may be of value in managing the syndrome. Clearly, double-blind controlled studies comparing BzR antagonists to placebo and conventional therapies in patients with HE are necessary. However, currently available data suggest that BzR antagonists are likely to be an important adjunct to conventional therapies for HE.

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