

PATHOGENESIS OF HEPATIC ENCEPHALOPATHY - A ROLE FOR THE BENZODIAZEPINE RECEPTOR ?

PATOGÊNESE DA ENCEFALOPATIA HEPÁTICA - UM PAPEL PARA OS RECEPTORES DE BENZODIAZEPÍNICOS ?

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ABSTRACT: Hepatic encephalopathy (HE) is a multifactorial syndrome in which the function of the central nervous system is impaired due to the metabolic consequences of liver disease. The two main components of liver pathology which lead to HE are the decrease in the number of functioning hepatocytes and the vascular rearrangement causing blood from the portal vein to bypass the liver. The symptoms of HE range from mild cognitive impairment to deep coma. Some degree of neuronal loss may be found in HE patients as a consequence of chronic cirrhosis and, in advanced HE, of brain edema; however, most of the HE syndrome is reversible with compensation of the liver disease. The pathogenesis of HE is not fully understood and is likely to be multifactorial. The initial theories implicated accumulation of neurotoxins leading to an impairment of neuronal function. With better understanding of the physiology of neuroreceptors, abnormalities in several neurotransmission systems have been put forward as potential causes of HE, such as a reported increase in GABAergic neurotransmission. There is evidence that this enhancement is related to an increase in the potentiation of GABAergic action by ligands to the benzodiazepine receptor (BZR), which are known to be increased in liver disease. With this evidence in mind, therapy with the benzodiazepine antagonist flumazenil has been attempted in HE, yielding clinical benefit in a variable percentage of patients in recent studies. However, there is still a lack of evidence to support a causal relationship between increased levels of benzodiazepine agonist ligands and HE symptoms. It is feasible to think that this relationship exists in some but not all HE patients.

UNITERMS: Hepatic Encephalopathy. Benzodiazepines. Receptores, GABA-A. Flumazenil.

1. INTRODUCTION

The relationship between psychiatric symptoms and liver impairment has been described in Hippocratic texts. However, the mechanisms through which liver disease impairs brain function are still poorly

understood. The neuropsychiatric syndrome resulting from liver impairment is generically known as hepatic encephalopathy (HE). This syndrome can be classified into different degrees or stages of severity, which range from mild cognitive impairment, detectable only by specific neuropsychological tests, to coma (Table I).

<i>Stage</i>	<i>Mental state</i>	<i>Motor alterations</i>
Pre-clinical	Slowed analytical ability, increase in p300 latency	No change
I	Mild confusion, impaired attention, sleep inversion, mood changes (euphoria, depression, irritability)	Tremor, incoordination, problems in handwriting
II	Amnesia for past events, drowsiness, lethargy, inappropriate behaviour, intermittent disorientation. EEG shows high-amplitude, low-frequency waves	Hypoactive reflexes, asterixis, ataxia, apraxia, dysarthria, paratonia
III	Inability to perform analytical tasks, disorientation, slurred speech, rage	Rigidity, hyperactive reflexes, nistagmus, Babinski's sign
IV	Coma	No response to painful stimuli, dilated pupils, opisthotonus

Liver impairment can also induce irreversible neurological alterations, including astrocytosis, cortical thinning, neuronal loss in the cerebellum and basal ganglia, laminar necrosis, spinal cord demyelination and brain edema and compression^(1/6). However, the term “hepatic encephalopathy” is generally used to describe reversible neurological impairment, and therefore does not include cases with severe degrees of any of the complications mentioned earlier⁽⁷⁾.

The main theories on the genesis of HE are based upon evidence of the accumulation of toxic substances. With increasing degrees of liver damage, many metabolic pathways become obstructed, resulting in the accumulation of numerous substances which may present neurotoxic properties. The HE syndrome appears to be the result of the concomitant action of these different substances. However, it is important to try to distinguish which toxins are responsible for the symptoms in different stages of HE. This distinction is crucial for the design of new and more effective therapeutic approaches. The clinical treatments available for chronic HE so far are palliative; only liver transplantation tackles the real cause of chronic HE, which ultimately consists of a lack of functioning hepatocytes. In acute episodes of chronic HE or primary acute HE episodes (such as those produced by acetaminophen overdose) it is important to prevent the progression of HE symptoms and to reverse the

existing brain impairments as quickly as possible. The emergency status of acute HE episodes is partly due to the fact that the symptoms frequently progress to coma states, which are sometimes accompanied by different degrees of brain edema and neuronal damage. So far, only one drug has been shown to reverse HE related coma in at least a subset of patients: the benzodiazepine antagonist flumazenil.

The rationale for using flumazenil in HE was built up from evidence suggesting the involvement of the GABA-benzodiazepine receptor complex in this syndrome. The first findings suggesting that an increase in GABAergic transmission was involved in HE came from the liver diseases section in the NIH laboratories⁽⁸⁾. After identifying this increase in GABAergic transmission, the NIH group and other research centers began to search for explanations of this change. From the beginning, two hypothesis were proposed: changes in GABA neurotransmission could be explained by alterations of either the structure or the level of ligands to the GABA receptor and its related modulatory sites (see section on the benzodiazepine receptor). The latter mechanism is consistent with the evidence indicating that humoral changes precipitated by substances originated in the gut may contribute to HE⁽⁹⁾. This article aims to review recent evidence on the pathogenesis of HE, focusing on the role of benzodiazepines and of the benzodiazepine receptor (BZR) in this process.

2. HEPATIC ENCEPHALOPATHY

HE is a multifactorial syndrome characterized by depression of the central nervous system. The symptoms are progressive and, in more severe cases, impaired consciousness and coma may occur⁽¹⁰⁾. HE appears to be a result of both vascular and parenchymal mechanisms. The vascular causes include a series of pathological conditions in which toxic metabolites accumulate in the systemic circulation due to vascular bypass of the liver. Congenital portocaval shunts are an example of “pure” vascular disease. The parenchymal causes of HE include the conditions in which the blood supply to the liver is normal but the population of active hepatocytes is decreased. An example of ‘pure’ parenchymal HE is fulminant hepatic failure due to acetaminophen overdose. Most patients with HE have some degree of both parenchymal insufficiency and vascular rearrangement, lying between the pure vascular and pure parenchymal extremes.

HE usually presents itself as a complication of chronic hepatocellular disease, which is a common consequence of alcoholic cirrhosis and chronic hepatitis. Precipitants of acute hepatocellular failure include acute viral hepatitis and drug overdose⁽¹¹⁾. Common precipitants of HE in a patient with cirrhosis include an intestinal nitrogen load in the form of increased protein intake (meat, eggs and other protein-rich foods), constipation and gastrointestinal bleeding (usually from esophageal varices). Other precipitants include infections, diarrhea, vomiting, hypoxia, anemia, hypotension, hypoglycemia, dehydration, electrolyte and/or acid-base disturbances and surgical procedures. The administration of sedative drugs such as benzodiazepines, barbiturates and morphine can also precipitate HE. When such precipitant factors trigger an HE episode, therapy is usually directed to the treatment of these precipitants. However, HE may present as a chronic condition, without a clear precipitant factor. Chronic HE is a milder, more persistent variant of the syndrome, occurring in patients with parenchymal liver disease and some degree of portal-systemic shunting.

2.1. Symptomatology

The earliest symptoms of HE are usually not detectable by conventional clinical assessment but may be apparent to family members or friends⁽¹⁰⁾. These symptoms include personality changes reflecting forebrain dysfunction, such as childishness,

euphoria, irritability and apathy. A general decrease in the speed of verbal and motor responses may also occur, leading to a reduction of spontaneous movements, fixed stare, dysphasia and perseveration in later stages. Another common manifestation in early stages is hypersomnia, which may progress to an inversion of sleep rhythm. Intellectual deterioration may also appear, and may be detected by psychological tests of orientation to time, space and self; episodic memory; subtraction of serial seven’s; handwriting and drawing; trail-making and spatial recognition^(12/14).

In more advanced cases, physical examination signs of HE may emerge. A very typical sign of HE is asterixis (liver flap or liver tremor). Asterixis is a result of impaired reticular processing of proprioceptive information from the metacarpophalangeal and wrist joints. It is elicited by wrist dorsiflexion when the arm is extended: the hand falls forward within 30 seconds, with subsequent recovery of posture. Asterixis differs from tremor because it is intermittent, of lower frequency and bilaterally asynchronous^(15,16). Other signs which may develop in HE include hyperreflexia, muscular rigidity and ankle clonus, fasciculations, ataxic gait, decerebrate postures, bizarre facial expressions, Babinski’s sign, seizures and coma.

Patients with chronic liver disease who do not present with overt symptoms of HE but show some degree of impairment in psychological tests are grouped into the category of subclinical HE⁽¹¹⁾. Subclinical HE is best detected using neuropsychological tests^(17,18,19). More recently, auditory evoked potentials have been proposed as a useful tool to detect HE in its early stages^(20,21). Conventional treatment of liver failure (lactulose, dietary restrictions) may reverse the cognitive impairments of subclinical HE^(22,23,24) and the intravenous injection of flumazenil can improve reaction time⁽²⁵⁾ and performance in auditory evoked potentials testing⁽²⁶⁾, although these results have been contested in later studies⁽²⁷⁾. Chronic alcoholism *per se* also appears to induce deficits in these tests, an effect which might also be related to benzodiazepine activity^(28,29).

2.2 Neuropathology

Cerebral atrophy has been reported in brain scans of cirrhotic patients with chronic recurrent HE^(30,31). Some of these changes might be attributable to the direct effects of alcohol; however, studies with nonalcoholic chronic liver patients without overt HE also indicate the presence of cerebral edema and

cortical atrophy^(32,33). These morphological changes correlate with scores in both neuropsychological and liver function tests. The findings from these studies strongly suggest that liver impairment may lead to cortical atrophy. However, the exact mechanisms through which liver dysfunction produces neuronal loss are still unknown. These morphological changes are possibly involved in the genesis of some HE symptoms which are not reversible by clinical treatment or liver transplantation^(33,34). However, neuronal loss certainly cannot account for all symptoms of the syndrome: most deficits on cognitive performance are reversible with liver transplantation⁽³⁵⁾ and subclinical HE can be reversed with clinical treatment⁽²³⁾. It is likely that the metabolic changes which produce HE induce some degree of neurotoxicity, which may eventually cause neuronal loss in the long run.

The histological examination of the brain of chronic cirrhotic patients frequently shows Alzheimer type II astrocytosis in the cortex, putamen, globus pallidus and cerebellum⁽³⁶⁾. Another finding in these patients is an increase of peripheral-type BZR^(37,38,39). As the peripheral BZR is associated with non-neuronal brain constituents, the increase in peripheral BZR is thought to be a consequence of glial proliferation. No structural changes in individual neurons have been found in HE using electron microscope techniques⁽⁴⁰⁾, but alterations in retinal glia cells, including vacuolization and mitochondrial swelling, have been recently described⁽⁴¹⁾.

2.3. Pathogenesis

The main etiological factor in HE seems to be the accumulation of neurotoxic substances in the systemic circulation⁽⁴²⁾. In normal circumstances, these neurotoxic compounds are absorbed from the gut and cleared from the systemic circulation by the liver. When liver function is seriously impaired, these compounds bypass the liver, gain direct access to the circulation and, once they cross the blood-brain barrier, may accumulate in the central nervous system. There is evidence that toxins can impair neuronal function in HE through several distinct mechanisms, including depression of neuronal electric activity by occupation of brain neuroreceptors⁽⁸⁾; inhibition of ionic pumps^(43,44) and inhibition of neuronal oxidative metabolism⁽⁴⁵⁾. Most of the neurotoxic substances accumulated in HE are thought to be metabolites produced by enteric bacteria, a belief reinforced by the effectiveness of non-absorbable antibiotics in the treatment of chronic HE⁽⁴⁶⁾. Elevated concentrations of several

potentially toxic substances have been reported in HE. These findings led to the postulation of several competing theories about the genesis of HE symptoms, none of which have been conclusively proved or disproved so far. The main hypothesis are summarized in Table II. Given the wide range of metabolic abnormalities and different symptoms in HE it is very likely that the etiology of the syndrome is multifactorial^(47,48).

2.3.1. Increased ammonia levels

The hypothesis that increased ammonia levels resulting from impaired hepatic clearance might result in HE is the oldest theory postulating metabolic changes as the cause of HE. Ammonia is clearly neurotoxic and evidence gathered in the early fifties suggested that HE could result in part from increased levels of this substance^(49,50). The gastrointestinal tract is a major source of ammonia, where it is generated by the degradation of amines, amino acids and urea by enteric bacteria^(51,52). In liver failure, the ammonia produced in the gut bypasses the liver, and higher amounts of it reach the systemic circulation. Liver impairment also reduces the conversion of ammonia and glutamine into urea, resulting in further accumulation of ammonia in the blood.

Several studies have shown that increased levels of ammonia deactivate the ionic pump that actively transports chloride out of the cell^(43,53), leading to a decrease in post-synaptic inhibition. These changes have been demonstrated in the cerebral cortex, thalamus, brainstem and spinal cord in HE^(43,54,55,56). The resulting increase in neuronal excitation may lead to hyperreflexia and seizures⁽⁵⁷⁾. Further elevations of ammonia levels impair the formation of a post-synaptic potential, producing pre-synaptic blockade and overall depression of the central nervous system⁽⁵⁸⁾. Hyperammonemia also induces several detrimental metabolic changes in the Na⁺/K⁺-ATPase activity, the tricarboxylic acid cycle, the malate/aspartate shuttle and the glycolysis pathway^(59/63).

Treatment with oral L-ornithine-L-aspartate, which improves ammonia detoxification, has recently been shown to significantly enhance cognition and scores on the Portosystemic Encephalopathy (PSE) Index⁽⁶⁴⁾, a clinical measure of severity in patients with chronic HE⁽⁴⁰⁾. A correlation between chronic hepatic encephalopathy and gastric colonization by urease-producing *Helicobacter pylori* has also been shown, and eradication of this microorganism appears to improve symptomatology in colonized patients⁽⁶⁵⁾. On the other hand, the finding that plasmatic levels of

Table II - Hypotheses for the pathogenesis of hepatic encephalopathy		
<i>Hypothesis</i>	<i>Evidence in favour of</i>	<i>Evidence against</i>
Ammonia	<ul style="list-style-type: none"> - Clearly neurotoxic. - Abundantly produced by gut bacteria. - Accumulation promoted by liver failure. - Treatment with L-ornithine-L-aspartate improves ammonia detoxification and HE symptoms. - Correlation between H. pylori infection and HE. - Induces upregulation of BZRs and GABAergic enhancement. 	<ul style="list-style-type: none"> - Plasma levels do not correlate with the severity of HE. - Removal by hemodialysis does not improve HE symptoms. - Intoxication not known to produce personality or sleep pattern changes.
Aromatic amino acids	<ul style="list-style-type: none"> - Increased levels found in HE patients. - Levels correlate with HE severity. - May cause synthesis of false neurotransmitter and alter catecholaminergic transmission. 	<ul style="list-style-type: none"> - Studies correlating levels of false neurotransmitters and HE are controversial.
Mercaptans	<ul style="list-style-type: none"> - Clearly neurotoxic. - Produced by gut bacteria. 	<ul style="list-style-type: none"> - Levels do not correlate with HE severity. - Controversies over detection methods.
Short and medium-chain fatty acids	<ul style="list-style-type: none"> - Known to cause reversible coma. - Increased levels found in patients with chronic liver disease. 	<ul style="list-style-type: none"> - No conclusive evidence of increased levels in HE.
Phenols	<ul style="list-style-type: none"> - Neurotoxic and hepatotoxic. - Increased levels found in HE. 	<ul style="list-style-type: none"> - Levels found in HE are at least 4 times lower than those shown to be neurotoxic in animals.
Permeability alterations in the blood-brain barrier	<ul style="list-style-type: none"> - May be increased by toxins (ammonia, mercaptans, phenols) - Evidence for permeability increases in fulminant hepatic failure. 	<ul style="list-style-type: none"> - Little evidence of change in chronic liver disease - More likely to be a potentiating factor for other toxins in HE.
Change in brain monoamines	<ul style="list-style-type: none"> - Increased levels of serotonin found in HE models. - Decreased levels of norepinephrine found in HE models. 	<ul style="list-style-type: none"> - No correlation between neurotransmitter alterations and neurological symptoms. - Similar serotonin turnover in cirrhotics with and without HE.
Enhanced GABAergic transmission (involvement of endogenous benzodiazepines)	<ul style="list-style-type: none"> - Evidence of increase in circulating BZR ligands in animal models and patients with HE. - Increase in the brain levels of benzodiazepines and in receptor density and binding found in both animal models and human patients. - Positive correlation between HE symptoms and BZR ligand levels. - Treatment with flumazenil shows efficacy in a variable percentage of patients. - Evidence for involvement of non-benzodiazepine ligands to the BZR may explain detection controversies. 	<ul style="list-style-type: none"> - Increased levels of benzodiazepines found in a minority of patients. - Levels seem to correlate more strongly with the severity of liver disease than with that of HE. - Treatment with flumazenil shows efficacy in a minority of patients. - Possibility of a non-specific effect of flumazenil cannot be ruled out.

ammonia do not correlate with the severity of HE puts the importance of ammonia in HE etiology in doubt⁽⁶⁶⁾. Another evidence against a central role of ammonia in HE is that hemodialysis, despite significantly reducing ammonia levels, yields improvements in less than 50 % of HE patients and may actually worsen neurological symptoms in some cases⁽⁶⁷⁾. Another argument against the ammonia hypothesis is that ammonia intoxication does not produce the changes in personality or sleep pattern commonly found in the early stages of HE. However, ammonia also induces changes in neurotransmitters such as GABA and can enhance benzodiazepine activity through upregulation of BZRs^(68,69,70), which could account for these alterations.

2.3.2. Changes in amino acid metabolism

It has been proposed that changes in amino acid metabolism may also contribute to the HE syndrome. There is evidence that the levels of aromatic amino acids (tyrosine, phenylalanine and tryptophan) are increased in patients with HE and correlate with the severity of neurological impairment and with ammonia levels in animal models of liver failure⁽⁷¹⁾. Some aromatic amino acids, particularly tryptophan, may be directly neurotoxic^(48,72,73). Moreover, they can also impair the synthesis of catecholamines^(74,75) and serve as precursors to the synthesis of false neurotransmitters such as octopamine and phenylethanolamine^(11,76). These false neurotransmitters can displace dopamine and noradrenaline from synaptic vesicles, increasing their degradation rate⁽⁷⁷⁾. Apart from depleting the pool of monoamines, false neurotransmitters can also suppress their synthesis by inhibiting the activity of tyrosine hydroxylase and dopamine β -hydroxylase⁽⁷⁸⁾ and act as partial agonists at the catecholamine receptors^(79,80). Some studies have suggested a positive correlation between octopamine levels in urine and plasma and the severity of HE^(81,82). However, other studies came to directly opposite conclusions: the level of octopamine in the brain of patients who died with HE was found to be lower than that in patients who died without liver disease⁽⁸³⁾. This leads us to believe that, although abnormalities in the amino acid metabolism may happen in HE, their role in the genesis of the syndrome is not clear.

2.3.3. Other neurotoxins

Substances such as mercaptans, fatty acids and phenols have also been implicated in HE. They can be neurotoxic at certain levels and are potentially

accumulated in HE. There is no consistent evidence, however, that any of these substances alone is capable of producing the HE syndrome. Still, their neurotoxic action may be mutually potentiated by the coexistence of more than one toxin at the same time⁽⁸⁴⁾. This might be a consequence of the ability of all of these substances to reduce the activity of the Na^+/K^+ ATPase, which may lead to brain edema and dysfunction⁽⁸⁵⁾.

Mercaptans such as methanethiol and dimethyldisulfide were first isolated from a HE patient in 1955⁽⁸⁶⁾. These compounds are apparently formed as a result of gut bacteria metabolism⁽⁸⁴⁾, and can induce coma and convulsions by several mechanisms, including suppression of Na^+/K^+ ATPase activity⁽⁸⁴⁾. Their role in HE started to be questioned after the finding that the method initially used to isolate mercaptans could also synthesize them⁽⁸⁷⁾. Moreover, mercaptan concentration also failed to correlate with the degree of HE⁽⁸⁸⁾.

Short and medium chain fatty acids can also cause reversible coma^(84,89). Since increased levels of free fatty acids have been described in patients with chronic liver disease⁽⁹⁰⁾, these compounds too have been implicated in HE. However, there is no conclusive evidence of increased levels of fatty acids in patients who develop HE⁽⁹¹⁾.

Finally, phenols are metabolites of tyrosine and phenylalanine which have been shown to be increased in HE, probably as a consequence of the increase in these aromatic amino acids^(84,92), as discussed above. These substances are both neurotoxic and hepatotoxic and may induce coma or potentiate the ability of other compounds to do so⁽⁹³⁾. However, the fact that the levels of phenols detected in HE are at least 4 times lower than the levels found to be neurotoxic in animal models weakens this hypothesis^(93,94).

2.3.4. Permeability of the blood-brain barrier

Apart from abnormal levels of toxic substances, changes in the blood-brain barrier (BBB) have also been put forward as a possible contributing factor in HE. Some substances that accumulate in liver failure, such as ammonia, methyl octanoate, mercaptans, phenol or dehydrocholate^(95,96) may increase the permeability of the BBB through several different mechanisms. These mechanisms include a deleterious action on enzymes involved in the regulation of transcapilar blood flow, changes in the function of glial transporter systems and an increase in membrane fluidity⁽⁹⁷⁾. There is evidence that the permeability of

the BBB is increased for polar molecules (such as amino-acids) in HE⁽⁹⁸⁾; on the other hand, other studies suggest that the increase in BBB permeability is nonspecific, including both polar and apolar substances^(98,99). It should be emphasized that the findings suggesting increased permeability of the BBB are more convincing regarding cases of fulminant hepatic failure, as there is little evidence of BBB changes in patients with chronic liver disease. Still, it is possible that some degree of increased permeability is present in these patients, which could theoretically potentiate the action of toxins in HE.

2.3.5. Changes in neurotransmission

Finally, changes in several neurotransmitter systems have been implicated in HE. Because of the impairment in amino acid metabolism induced by liver failure, the most investigated systems have been the amino acid and biogenic amine systems. Elevations in the levels of serotonin (5-HT) in the brain were postulated to contribute to the manifestations of HE⁽¹⁰⁰⁾. However, 5-HT turnover has been reported to be similar in cirrhotics with and without HE⁽¹⁰¹⁾. Moreover, no correlation between alterations in 5-HT brain metabolism and neurological status has been demonstrated⁽¹⁰²⁾. On the other hand, the levels of norepinephrine (NE) have been reported to be decreased in animal models of HE⁽¹⁰³⁾. However, it is not clear that the depletion of NE in the brain is causally related to the symptoms of HE⁽¹¹⁾. A decrease in the excitatory activity of glutamate and aspartate has also been put forward as a contributing factor to HE symptoms⁽¹⁰⁴⁾. To this date, however, no firm evidence supporting this hypothesis was reported. Moreover, evidence of a compensatory increase in aspartate neurotransmission has been found in HE⁽¹¹⁾. More recently, the GABAergic system has been implicated in HE. Evidence for this hypothesis will be discussed in the next section.

3. HEPATIC ENCEPHALOPATHY AND THE BENZODIAZEPINE RECEPTOR

3.1. Introduction

The involvement of the GABA_A receptor in HE was first hypothesized by Schafer & Jones⁽⁸⁾. The rationale behind this supposition was that the general depression of the central nervous system found in HE was compatible with a hyperactivity of the GABAergic system. Evidence obtained from animal models of HE

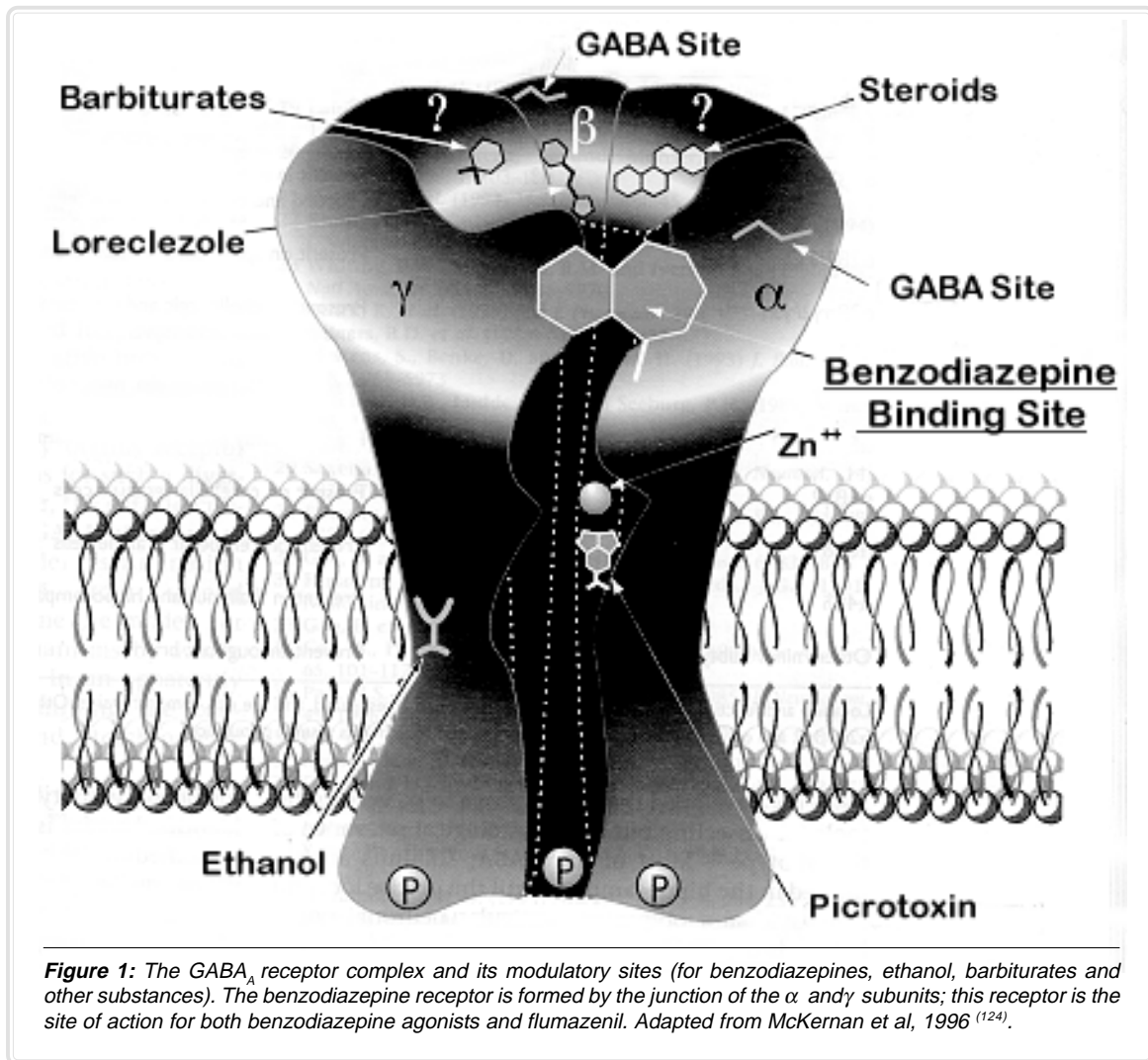
supported this theory, as different groups found that GABA agonists such as barbiturates, benzodiazepines and muscimol potentiated HE symptoms^(105,106), while the administration of the GABA antagonist bicuculline was found to improve neurophysiological changes and behavioural symptoms of HE⁽¹⁰⁶⁾.

The initial studies implicating GABAergic neurotransmission in HE did not provide a specific mechanism for this change. An increase in GABAergic neurotransmission could result from several factors, including higher GABA concentrations, increased GABA receptor density, increased receptor affinity or a combination of these factors. These possibilities have been extensively investigated, but no firm conclusion has been reached so far. Initially, increased receptor density was observed in animal models of HE^(107,108). Other studies failed to confirm these findings, reporting no changes in receptor density or affinity^(109,110,111). An increase in blood concentrations of GABA was also found in HE^(112,113,114) but there is no evidence that it would cross the blood-brain barrier in sufficient amounts to produce a significant effect^(98,115).

3.2. The benzodiazepine receptor and hepatic encephalopathy

Another possible mechanism of enhancement of GABAergic neurotransmission relates to changes in the benzodiazepine receptor (BZR) or in its ligands. The BZR is part of the GABA_A receptor complex (Figure 1) and potentiates GABAergic inhibition when it is activated. An increase in the density of BZRs has been reported in animal models^(108,116,117) and in HE patients⁽¹¹⁸⁾. These changes were contested by subsequent studies reporting no change in these receptors in HE, both in animal models⁽¹⁰⁹⁾ and in humans^(119,120,121). Changes in the levels of ligands to the BZR have also been reported in HE^(122,123). This finding led to the hypothesis that the cause for increased GABAergic tone in HE might be an increase in the level of benzodiazepine-like ligands.

The possibility of having benzodiazepine-like substances circulating and producing some of the symptoms of HE prompted a great deal of research. Initially, these substances were isolated from body fluids of patients with HE and identified as benzodiazepines and benzodiazepine agonists. Upon these findings, treatment with the benzodiazepine antagonist flumazenil was attempted in HE patients and improvements were found in some cases. Larger studies were carried out trying to correlate the level of circulating



benzodiazepines and the severity of liver disease and HE. A positive correlation was found, and currently the debate is on whether this correlation implicates in a causal relationship⁽¹²⁵⁾. However, the implication of benzodiazepines in HE has also been criticized. The grounds for criticism stem from studies reporting that increased levels of benzodiazepines are neither required nor sufficient to induce HE⁽¹²⁶⁾. Moreover, benzodiazepine ligands seem to accumulate in severe liver disease independently of the presence of HE, and their levels are similar to those commonly found in exogenous benzodiazepine consumers⁽¹²⁷⁾. Double-blind, placebo-controlled studies with flumazenil have been carried out to elucidate the role of BZR ligands in HE^(128/131), but the answers are still unclear. Data in favour and against the involvement of “endogenous benzodiazepines” in HE will be summarized below.

3.2.1. Pre-clinical evidence

Increases in the levels of endogenous ligands to the BZR have been demonstrated in a wide range of experiments in animal models and humans. When HE was induced in animals, the brain concentrations of benzodiazepines were shown to be elevated⁽¹²³⁾. As these animals had never been exposed to exogenous benzodiazepines, this increase appears to be due to a process inherent to HE. More recent experiments show that gut bacteria such as *Acinetobacter Iwoffii* may provide precursors of benzodiazepine ligands⁽¹³²⁾. Benzodiazepine concentrations have been shown to be increased in the cerebrospinal fluid^(133,134,135) and in post-mortem brain samples⁽¹³⁶⁾ of patients with HE. BZR binding in the brain of patients with recurrent HE has also been shown to be increased in positron emission tomography (PET) studies⁽¹³⁷⁾. However, one

must consider that liver cirrhosis *per se* can also induce changes in BZR binding⁽¹³⁸⁾.

However, despite the evidence for an increase in BZR activity in the brain, elevated plasmatic levels of benzodiazepines are found in a minority of HE patients^(128,131), even in those who respond to flumazenil (see next section). Moreover, eventual increases can frequently be traced to consumption of commercial benzodiazepines⁽¹²⁸⁾. This prompted a search for non-benzodiazepine ligands to the BZR, such as those isolated in the neurological syndrome known as idiopathic recurrent stupor^(139,140) and later identified as endozepine-2 and -4⁽¹⁴¹⁾. An experiment with 25 patients in different HE stages⁽¹⁴²⁾ showed that most of the benzodiazepine activity in these patients was due to chemically unidentified benzodiazepine agonists, and that total benzodiazepine-like activity correlated more strongly with the clinical stage of HE than did the levels of conventional benzodiazepines (diazepam and n-desmethyldiazepam).

The increase in benzodiazepine-like compounds has been subsequently reported in patients with both acute hepatic failure⁽¹⁴³⁾ and chronic cirrhosis⁽¹²⁶⁾. However, this increase has been inconsistent, suggesting that the occurrence of HE is also dependent on several other factors. A study in which HE was induced in 17 rats found a significant increase of “endogenous benzodiazepines” in only one animal⁽¹²⁵⁾, suggesting that increased BZR ligand activity might be due to increased residence time of exogenous benzodiazepines in the body and that this increase would be neither necessary nor sufficient to induce HE⁽¹²⁵⁾. However the methodology employed in the experiment did not include the extraction and purification protocols applied by most of the authors in the field⁽¹⁴⁴⁾.

More solid criticism is provided by a recent study by AVALLONE et al.⁽¹²⁷⁾ comparing the levels of benzodiazepine-like ligands in cirrhotic patients with and without overt HE. Although these levels were shown to be significantly higher in patients with overt HE as opposed to control cirrhotics, they correlated more clearly with the degree of liver impairment according to the Child-Pugh classification than with the stage of HE itself. Therefore, the increased levels of “endogenous benzodiazepines” in HE might be a mere consequence of more severe liver disease with no causal relationship to the syndrome, a hypothesis reinforced by the fact that these levels are similar to those found in commercial benzodiazepine users without any evidence of neurological impairment⁽¹²⁷⁾.

Increased levels of “endogenous benzodiazepines” might also be related to a decrease in renal function⁽¹⁴⁵⁾, which is a common complication of severe liver disease. Despite these remarks, however, the clinical evidence showing the efficacy of flumazenil in the treatment of HE suggests that endogenous benzodiazepines are involved in the pathogenesis of this process in at least some patients, as will be discussed below.

3.2.2. Clinical evidence - a role for flumazenil ?

The first report implicating “endogenous benzodiazepines” in HE was an anecdotal report of improvement in hepatic coma in a patient after a 1 minute injection of 0.5 mg of flumazenil by SCOLLO-LAVIZZARI & STEIMAN⁽¹⁴⁶⁾. In this case report, the patient improved dramatically: she opened her eyes, reacted to verbal commands and moved spontaneously and reacting to pain. This improvement lasted for approximately one hour, with the patient relapsing into coma afterwards. Since flumazenil does not appear to present intrinsic inverse agonist activity in HE - that is, it cannot alter GABAergic transmission on its own, although partial agonism has been reported in animal models⁽¹⁴⁷⁾ - the reversal of hepatic coma was interpreted as a possible antagonism of endogenous benzodiazepine-like ligands. Several case reports and uncontrolled trials showing positive results followed this initial description. A review of these initial studies⁽¹¹⁾ showed that 69% of the patients improved after flumazenil. The clinical response had the same pattern referred in the case reports, with the improvement being quick and short-lived^(148/156).

In most of these studies, however, intake of synthetic benzodiazepines by the patients could not be ruled out with certainty. Therefore, it is possible that the response to flumazenil injection was just a consequence of the temporary displacement of synthetic benzodiazepines from the BZR. Moreover, in some of the studies, the effect of flumazenil was reported hours after the injection, which is not consistent with the short half-life of the drug. These inconsistencies brought on the need of well-controlled clinical trials to shed more light on the real efficacy and mechanism of action of flumazenil.

The first randomized clinical trial reported in the literature was a double-blind, placebo-controlled, crossover study by POMIER-LAYRARGUES et al.⁽¹²⁸⁾, which showed improvement in 6 of 13 comatose (grade IV HE) patients (46%) treated with intravenous injection of flumazenil, against none of 11 in

the placebo group. However, commercial benzodiazepines were detected in the blood of two responders and two nonresponders - therefore, in two out of the six patients which showed improvement after flumazenil, the clinical effect might have been related to antagonism of the previously used medication.

In a later trial by GROENEWEG et al.⁽¹²⁹⁾ using alterations in the electroencephalogram (EEG) as a measure of clinical improvement in 32 patients with an HE grade of I-III, improvements after flumazenil administration were limited to 29% of cases, as opposed to 13% after placebo. The EEG improvements correlated with the patients' clinical response in 3 out of 5 cases. Another trial analysing a similar population of non-comatose patients, but using the clinical PSE score as a measure of improvement was undertaken by GYR et al.⁽¹³⁰⁾. The overall efficacy of flumazenil remained at 25% (7 out of 28 patients vs. none of 21 in the placebo group) - however, the improvement was greater (43%) in patients with a stage III PSE score, as opposed to only 14% of patients with PSE stage II.

By far, however, the largest clinical trial of flumazenil was the randomized, double-blind, multicenter, placebo-controlled cross-over study recently published by BARBARO et al.⁽¹³¹⁾, comprising 527 patients analysed in the course of 5 years. In this study, flumazenil brought improvement in the neurological score of 17.5% of HE grade III patients and 14.7% of grade IVa patients, while improvements in EEG tracings were seen in 27.8% of grade III patients and 21.5% of grade IVa patients. However, if only the results of the initial treatment (before the groups were crossed over) are considered, these numbers rise to 26.5% (grade III) and 23.3% (grade IVa) for the neurological score and 38.6% (grade III) and 33.8% (grade IVa) for EEG improvements, suggesting that administration of flumazenil early in the course of the illness warrants better results.

Although they failed to recognize it in their analysis, Barbaro et al.'s data provides evidence that patients with alcoholic cirrhosis respond to flumazenil much more frequently than patients with posthepatic cirrhosis (22.3% as opposed to 11.3%)⁽¹⁵⁷⁾. This is in agreement with data showing increased BZR binding in these patients⁽²⁹⁾ and might be useful to identify patients who might respond to flumazenil. This is very relevant because, although it appears clear that flumazenil is beneficial for a subset of patients with HE, the opinions on whether its large-scale use is warranted by this somewhat limited efficacy are still conflict-

ing^(129,131), given the high cost of the therapy and other factors. It is important, therefore, that one can identify consistent indicators of a positive response to flumazenil to be able to direct therapy to these patients.

Another potential application of flumazenil is in the long-term treatment of chronic HE when administered orally⁽¹⁵⁸⁾. In an interesting case reported by FERENCI et al.⁽¹⁵⁹⁾, a patient with refractory HE (12 episodes of coma in 2 years) after extensive liver resection had all signs of encephalopathy cleared for 14 months after twice a day oral administration of flumazenil was instituted. To our knowledge, this kind of approach has been repeated only once in a subsequent case report⁽¹⁶⁰⁾. Still, when one considers that the benzodiazepine antagonist also shows effect in reversing cognitive deficits in alcoholic cirrhotics⁽²⁸⁾, the possibility of long-term treatment seems attractive. Specific studies should be carried out to determine its real usefulness in chronic HE.

4. CONCLUSION

When the efficacy of flumazenil in the clinical trials mentioned above is considered, it appears that BZR ligands should be involved in at least some cases of HE. Still, we cannot rule out that the effect observed with this drug might be due to a nonspecific excitation of the nervous system, as is observed in poisoning and metabolic coma^(161,162), or due to its intrinsic agonist effects. However, the wide array of evidence reporting increases in "endogenous benzodiazepines" and BZR activity in HE favours the hypothesis that the action of flumazenil is due to the antagonism of these substances, and that they might indeed play a role in the pathogenesis of HE.

Still, it appears clear that there is a very large group of patients who do not respond to flumazenil therapy; moreover, the detection of increased levels of benzodiazepine-like ligands in HE patients is not totally consistent and does not always correlate clearly to the severity of the disease. Therefore, it appears that the activation of the BZR is one of several different mechanisms contributing to HE. This reaffirms the concept that HE is a multifactorial syndrome and emphasises the need of a wide therapeutic approach, in which benzodiazepine antagonists might come to play a role. More studies are thus necessary to determine which patients might benefit from treatment with flumazenil and to establish the true role of the BZR and its pharmacology in the pathogenesis and therapeutics of HE.

QUEVEDO J et al. Patogênese da encefalopatia hepática - um papel para os receptores de benzodiazepínicos? **Medicina, Ribeirão Preto, 32:** 82-96, jan./mar. 1999.

RESUMO: A encefalopatia hepática (EH) é uma síndrome multifatorial, na qual a função do sistema nervoso central está alterada devido às conseqüências metabólicas da disfunção hepática. Os dois principais componentes das doenças hepáticas que levam à EH são a diminuição no número de hepatócitos funcionantes e o rearranjo vascular, que leva à diminuição na fração de sangue, efetivamente detoxificado pelo fígado. Os sintomas da EH podem variar de déficits cognitivos leves até o coma profundo. Algum grau de morte neuronal pode ser observado em pacientes com EH, como conseqüência da cirrose hepática, ou, na EH avançada, da presença de edema cerebral. No entanto, a maior parte da síndrome neurológica é reversível com a compensação da doença hepática. A etiologia da EH não é totalmente conhecida e trata-se, provavelmente, de um processo multifatorial. Inicialmente, as teorias apontavam para o acúmulo de neurotoxinas que prejudicariam a função neuronal. Mais recentemente, anormalidades em vários sistemas de neurotransmissão foram propostos como causas potenciais da EH como, por exemplo, o aumento observado na neurotransmissão GABAérgica. Existe evidência de que este aumento esteja relacionado com o aumento da potenciação GABAérgica por substâncias de ação similar aos benzodiazepínicos, as quais se encontram aumentadas na EH. Com esta evidência em mente, foi tentada a terapia desta síndrome com flumazenil, um antagonista benzodiazepínico, o qual tem mostrado eficácia clínica em uma porcentagem variável de pacientes em estudos recentes. No entanto, ainda não há evidências conclusivas para sustentar uma relação causal entre o aumento de ligantes ao receptor de benzodiazepínicos e os sintomas da EH. É possível que esta relação exista em alguns, mas não em todos os pacientes com esta síndrome.

UNITERMOS: Encefalopatia Hepática. Benzodiazepinas. Receptores de GABA-A. Flumazenil.

REFERENCES

- 1 - LEIGH AD & CARD WI. Hepato-cellular degeneration: a case associated with postero-lateral column degeneration. **J Neuropathol Exp Neurol 8:**338-346, 1949.
- 2 - ZIEVE L et al. Shunt encephalomyelopathy. II. Occurrence of permanent myelopathy. **Ann Intern Med 53:** 33-52, 1960.
- 3 - VICTOR M et al. The acquired (non-wilsonian) type of chronic hepatocerebral degeneration. **Medicine (Baltimore) 44:** 345-396, 1965.
- 4 - READ AE et al. The neuropsychiatric syndromes associated with chronic liver disease and an extensive portal-systemic collateral circulation. **Q J Med 36:** 135-150, 1967.
- 5 - FINLAYSON MH & SUPERVILLE B. Distribution of cerebral lesions in acquired hepatocerebral degeneration. **Brain 104:** 79-95, 1981.
- 6 - TARTER RE et al. Hepatic dysfunction and neuropsychological test performance in alcoholics with cirrhosis. **J Stud Alcohol 47:** 74-77, 1986.
- 7 - CONN HO & LIEBERTHAL MM. **The hepatic coma syndrome and lactulose.** Williams & Wilkins, Baltimore, 1978.
- 8 - SCHAFER DF & JONES EA. Hepatic encephalopathy and the GABA neurotransmitter system. **Lancet 1:** 18-20, 1982.
- 9 - SHERLOCK S et al. Portal-systemic encephalopathy: neurological complications of liver disease. **Lancet 2:** 453-457, 1954.
- 10 - GAZZARD BG et al. Detection of hepatic encephalopathy. **Postgrad Med J 62:** 163-166, 1986.
- 11 - BASILE AS et al. The pathogenesis and treatment of hepatic encephalopathy: evidence for the involvement of benzodiazepine receptor ligands. **Pharmacol Rev 43:** 27-71, 1991.
- 12 - CONN HO et al. Comparison of lactulose and neomycin in the treatment of chronic portal systemic encephalopathy: a double blind controlled trial. **Gastroenterology 72:** 573-583, 1977.
- 13 - RIKKERS L et al. Subclinical hepatic encephalopathy: detection, prevalence and relationship to nitrogen metabolism. **Gastroenterology 75:** 462-469, 1978.
- 14 - GILBERSTADT SJ et al. Psychomotor performance defects in cirrhotics without overt encephalopathy. **Arch Intern Med 140:** 519-521, 1980.
- 15 - ADAMS RD & FOLEY JM. The neurological disorder associated with liver disease. **Res Publ Assoc Res Nerv Ment Dis 32:** 198-237, 1953.
- 16 - LEAVITT S & TYLER HR. Studies in asterix. **Arch Neurol 10:** 360-368, 1964.
- 17 - TARTER RE. Psychological deficits in chronic alcoholics. **Int J Addict 10:** 327-368, 1975.
- 18 - TARTER RE & ALTERMAN AI. Neuropsychological deficits in alcoholics: etiological considerations. **J Stud Alcohol 45:** 1-9, 1984.
- 19 - TARTER RE et al. Impact of cirrhosis on the neuropsychological test performance of alcoholics. **Alcohol Clin Exp Res 12:** 619-621, 1988.
- 20 - WEISSENBORN K et al. Neurophysiological assessment of early hepatic encephalopathy. **Electroencephalogr Clin Neurophysiol 75:** 289-295, 1990.

- 21 - DAVIES MG et al. The auditory P300 event-related potential: an objective marker of the encephalopathy of chronic liver disease. **Hepatology** **12**: 688-694, 1990.
- 22 - GITLIN N. Subclinical portal-systemic encephalopathy. **Am J Gastroenterol** **83**: 8-11, 1988.
- 23 - MORGAN MY et al. Lactitol and lactulose for the treatment of subclinical hepatic encephalopathy in cirrhosis patients. **J Hepatol** **8**: 208-217, 1989.
- 24 - WATANABE A et al. Clinical efficacy of lactulose in cirrhotic patients with and without subclinical hepatic encephalopathy. **Hepatology** **26**: 1410-1414, 1997.
- 25 - GOODAY R et al. Benzodiazepine receptor antagonism improves reaction time in latent hepatic encephalopathy. **Psychopharmacology** (Berlin) **119**: 295-298, 1995.
- 26 - BRUHA R et al. Flumazenil as a diagnostic test for latent hepatic encephalopathy. **J Hepatol** **20**: 844, 1994.
- 27 - AMODIO P et al. The effect of flumazenil on subclinical psychometric or neurophysiological alterations in cirrhotic patients: a double-blind placebo controlled-study. **Clin Physiol** **17**: 533-539, 1997.
- 28 - KAPCZINSKI F et al. Differential effects of flumazenil in alcoholic and nonalcoholic cirrhotic patients. **Psychopharmacology** (Berlin) **120**: 220-226, 1995.
- 29 - KAPCZINSKI F et al. Cognitive impairments of alcoholic cirrhotic patients: correlation with endogenous benzodiazepine receptor ligands and increased affinity of platelet receptors. **J Neurol Neurosurg Psychiatry** **60**: 676-680, 1996.
- 30 - ACKER W et al. The relationship between brain and liver damage in chronic alcoholic patients. **J Neurol Neurosurg Psychiatry** **45**: 984-987, 1982.
- 31 - ZANEROLI ML et al. Prevalence of brain atrophy in liver cirrhosis patients with chronic persistent encephalopathy: evaluation by computed tomography. **J Hepatol** **4**: 283-292, 1987.
- 32 - TARTER RE et al. Cerebral morphological abnormalities associated with non-alcoholic cirrhosis. **Lancet** **2**: 893-895, 1986.
- 33 - BERNTHAL RE et al. Cerebral CT scan abnormalities in cholestatic and hepatocellular disease and their relationship to neuropsychologic test performance. **Hepatology** **7**: 107-114, 1987.
- 34 - ARRIA AM et al. Improvement in cognitive functioning of alcoholics following orthotopic liver transplantation. **Alcohol Clin Exp Res** **15**: 956-962, 1991.
- 35 - TARTER RE et al. Subclinical hepatic encephalopathy. Comparison before and after orthotopic liver transplantation. **Transplantation** **50**: 632-637, 1990.
- 36 - NOREMBERG MD. The astrocyte in liver disease. In: FEDOROFF S & HERTZ L, eds. **Advances in cellular Neurobiology**, Academic Press, New York, v. 2, 1981.
- 37 - SYAPIN PJ & SKOLNICK P. Characterization of benzodiazepine binding sites in cultured cells of neural origin. **J Neurochem** **32**: 1047-1051, 1979.
- 38 - BENDER AS & HERTZ L. Binding of [³H]Ro 5-4864 in primary cultures of astrocytes. **Brain Res** **341**: 41-49, 1985.
- 39 - LAVOIE J et al. Studies of central and peripheral type benzodiazepine receptors in autopsied brain tissue from cirrhotic patients with hepatic encephalopathy. In: BUTTERWORTH RF & POMIER-LAYRARGUES G, eds. **Hepatic encephalopathy. Pathophysiology and treatment**. Humana Press, Clifton, p. 305-318, 1989.
- 40 - JONES EA & GAMMAL SH. Hepatic encephalopathy. In: ARIAS IM, et al., eds. **The liver: biology and pathobiology**. Raven Press, New York, p. 985-1005, 1988.
- 41 - ALBRECHT J et al. Retinal gliopathy accompanying thioacetamide-induced liver insufficiency: light and electron microscopic observations. **Acta Neuropathol** (Berlin) **96**: 57-66, 1998.
- 42 - SHERLOCK S. Hepatic encephalopathy. In: SHERLOCK S, ed. **Diseases of the liver and biliary system**. Blackwell, Oxford, p. 95-115, 1989.
- 43 - LUX HD. Ammonium and chloride extrusion: hyperpolarizing synaptic inhibition in spinal motoneurons. **Science** **173**: 555-557, 1971.
- 44 - AHMED K et al. Effects of methanethiol on erythrocyte membrane stabilization and on Na⁺, K⁺, -adenosine triphosphatase: relevance to hepatic coma. **J Pharmacol Exp Ther** **228**: 103-108, 1984.
- 45 - HAWKINS RA & MANS AM. Brain energy metabolism in hepatic encephalopathy. In: BUTTERWORTH RF & POMIER-LAYRARGUES G, eds. **Hepatic encephalopathy. Pathogenesis and treatment**. Humana Press, Clifton, p. 159-176, 1989.
- 46 - MIGLIO F et al. Rifaximin, a non-absorbable rifamycin, for the treatment of hepatic encephalopathy. A double-blind, randomised trial. **Curr Med Res Opin** **13**: 593-601, 1997.
- 47 - SCHENKER S & HOYUMPA AM. Pathophysiology of hepatic encephalopathy. **Hosp Pract** **19**: 99-121, 1984.
- 48 - ZIEVE L et al. A benzodiazepine antagonist does not alter the course of hepatic encephalopathy or neural GABA binding. **Metab Brain Dis** **2**: 201-205, 1987.
- 49 - GABUZDA G et al. Reversible toxic manifestations in patients with cirrhosis of the liver given cation-exchange resins. **N Engl J Med** **246**: 124-130, 1952.
- 50 - TORDA C. Ammonium ion content and electrical activity of the brain during the preconvulsive and convulsive phases induced by various convulsivants. **J Pharmacol Exp Ther** **107**: 197-203, 1953.
- 51 - LOCKWOOD AH et al. The dynamics of ammonia metabolism in man: effects of liver disease and hyperammonemia. **J Clin Invest** **63**: 449-460, 1979.
- 52 - VAN LEEUWEN et al. Ammonia production and glutamine metabolism in the small and large intestine of the rat and the influence of lactulose and neomycin. In: KLEINBERGER P et al, eds. **Advances in hepatic encephalopathy and urea cycle diseases**. S. Karger, Basel, p.154-162, 1984.
- 53 - INAGAKI et al. Histochemical demonstration of Cl⁻-ATPase in rat spinal motoneurons. **Brain Res** **419**: 375-378, 1987.
- 54 - LUX HD et al. The action of ammonium ion on postsynaptic inhibition in spinal motoneurons. **Exp Brain Res** **11**: 431-447, 1970.

- 55 - LLINAS R & BAKER R. A chloride dependent inhibitory postsynaptic potential in cat thalamic motoneurons. **J Neurophysiol** **35**: 484-492, 1972.
- 56 - RAABE W & GUMNIT RJ. Disinhibition in cat motor cortex by ammonia. **J Neurophysiol** **38**: 347-355, 1975.
- 57 - THEORET Y & BOSSU JL. Effects of ammonium salts on synaptic transmission to hippocampal CA1 and CA3 pyramidal cells *in vivo*. **Neuroscience** **16**: 807-821, 1985.
- 58 - RAABE W. Neurophysiology of ammonia intoxication. In: BUTTERWORTH R & POMIER-LAYRARGUES G, eds. **Hepatic encephalopathy. Pathogenesis and treatment**. Humana Press, Clifton, p. 49-77, 1989.
- 59 - MCKHANN GM & TOWER DB. Ammonia toxicity and cerebral oxidative metabolism. **Am J Physiol** **200**: 420-424, 1961.
- 60 - HINDFELT B et al. Effect of acute ammonia intoxication on cerebral metabolism in rats with porto-caval shunts. **J Clin Invest** **59**: 386-394, 1977.
- 61 - LAI JCK & COOPER AJL. Brain-ketoglutarate dehydrogenase complex: kinetic properties, regional distribution and effects of inhibition. **J Neurochem** **47**: 1376-1386, 1986.
- 62 - LOWRY OH & PASSONNEAU JV. Kinetic evidence for multiple binding sites of phosphofructokinase. **J Biol Chem** **241**: 2268-2279, 1966.
- 63 - HAGHIGHAT N & McCANDLESS DW. Effect of ammonium chloride on energy metabolism of astrocytes and C6-glioma cells *in vitro*. **Metab Brain Dis** **12**: 287-298, 1997.
- 64 - STAUCH S et al. Oral L-ornithine-L-aspartate therapy of chronic hepatic encephalopathy: results of a placebo-controlled double-blind study. **J Hepatol** **28**: 856-864, 1998.
- 65 - DASANI B, et al. Analysis of risk factors for chronic hepatic encephalopathy: the role of *Helicobacter pylori* infection. **Am J Gastroenterol** **93**: 726-731, 1998.
- 66 - STAHL J. Studies of the blood ammonia in liver disease: its diagnostic, prognostic and therapeutic significance. **Ann Intern Med** **58**: 1-24, 1963.
- 67 - OPOLON P. Large-pore hemodialysis in fulminant hepatic failure. In: BRUNNER G & SHMIDT FW, eds. **Artificial liver support**. Springer-Verlag, Berlin, p. 141-146, 1980.
- 68 - ITZHAK Y & NOREMBERG MD. Ammonia-induced upregulation of peripheral-type benzodiazepine receptors in cultured astrocytes labeled with [³H]PK 11195. **Neurosci Lett** **177**: 35-38, 1994.
- 69 - ITZHAK Y et al. Acute liver failure and hyperammonemia increase peripheral-type benzodiazepine receptor binding and pregnenolone synthesis in mouse brain. **Brain Res** **705**: 345-348, 1995.
- 70 - HA JH & BASILE AS. Modulation of ligand binding to components of the GABA-A receptor complex by ammonia: implications for the pathogenesis of hyperammonemic syndromes. **Brain Res** **720**: 35-44, 1996.
- 71 - MICHALAK A & BUTTERWORTH RF. Selective increases of extracellular brain concentrations of aromatic and branched-chain amino acids in relation to deterioration of neurological status in acute (ischemic) liver failure. **Metab Brain Dis** **12**: 259-269, 1997.
- 72 - SMITH B & PROCKOP DJ. Central nervous system effects of the ingestion of L-tryptophan by normal subjects. **N Engl J Med** **267**: 1338-1341, 1962.
- 73 - HUET PM et al. Blood-brain transport of tryptophan and phenylalanine: effect of portacaval shunt in dogs. **Am J Physiol** **241**: G163-G169, 1981.
- 74 - SILK DBA. Branched chain amino acids in liver disease: fact or fantasy **Gut** **27**: 103-110, 1986.
- 75 - MASSERANO JM et al. The role of tyrosine hydroxylase in the regulation of catecholamine synthesis. In: TRENDELENBURG U & WEINER N, eds. **Catecholamines**, Springer Verlag, Berlin, v. 2, p.427-456, 1989.
- 76 - JAMES JH et al. Hyperammonemia, plasma amino acid imbalance and blood-brain amino acid transport: a unified theory of portal-systemic encephalopathy. **Lancet** **2**: 772-775, 1979.
- 77 - SAAVEDRA J. False neurotransmitters. In: TRENDELENBURG U & WEINER N, eds. **Catecholamines**, Springer Verlag, Berlin, v. 2, p. 457-480, 1989.
- 78 - KOPIN IJ et al. Effect of false transmitters on norepinephrine synthesis. **J Pharmacol Exp Ther** **170**: 246-252, 1969.
- 79 - TRENDELENBURG U. Classification of sympathomimetic amines. In: TRENDELENBURG U & WEINER N, eds. **Catecholamines**, Springer Verlag, Berlin, v. 2, p.336-362, 1989.
- 80 - U'PRICHARD DC et al. Binding characteristics of a radio-labelled agonist and antagonist at central nervous system alpha noradrenergic receptors. **Mol Pharmacol** **13**: 454-473, 1977.
- 81 - FISCHER JE & BALDESSARINI RJ. Pathogenesis and therapy of hepatic coma. **Prog Liver Dis** **5**: 363-397, 1971.
- 82 - CASCINO A et al. Plasma and cerebrospinal fluid amino acid patterns in hepatic encephalopathy. **Dig Dis Sci** **27**: 828-832, 1982.
- 83 - CUILLERET C et al. Changes in brain catecholamine levels in human cirrhotic encephalopathy. **Gut** **21**: 565-569, 1980.
- 84 - ZIEVE L & BRUNNER G. Encephalopathy due to mercaptans and phenols. In: McCANDLESS DW, ed. **Cerebral energy metabolism and metabolic encephalopathy**. Plenum Press, New York, p. 179-201, 1985.
- 85 - DJURICIC BM et al. Phasic recognition of edema caused by ischemia. In: GO KG & BAETHMAN A, eds. **Recent progress in the study of brain edema**. Plenum Press, New York, p. 491-498, 1984.
- 86 - CHALLENGER F & WALSHE M. Methyl mercaptan in relation to foetal hepaticus. **Biochem J** **59**: 372-375, 1955.
- 87 - TANGERMAN A et al. New methods for the release of volatile sulphur compounds from human serum: its determinants by Tenax trapping and gas chromatography and its application in liver disease. **J Lab Clin Med** **106**: 175-182, 1985.
- 88 - BLOOM HJ et al. Methanethiol metabolism and its role in the pathogenesis of hepatic encephalopathy in rats and dogs. **Hepatology** **11**: 682-689, 1990.
- 89 - WHITE RP & SAMSON FE. Effect of fatty acid anions on the electroencephalogram of unanesthetized rabbits. **Am J Physiol** **186**: 271-274, 1956.
- 90 - WILCOX HG et al. Plasma long chain fatty acids and esterified lipids in cirrhosis and hepatic encephalopathy. **Am J Med Sci** **276**: 293-303, 1978.
- 91 - LAI JCK et al. Plasma short-chain fatty acids in fulminant hepatic failure. **Clin Chim Acta** **78**: 305-310, 1977.

- 92 - FARAJ BA et al. Evidence for central hypertyraminemia in hepatic encephalopathy. **J Clin Invest** **67**: 395-402, 1981.
- 93 - WINDUS-PODEHL G et al. Encephalopathic effect of phenol in rats. **J Lab Clin Med** **101**: 586-592, 1983.
- 94 - BRUNNER G et al. On the role of free phenols in the blood of patients in hepatic failure. In: BRUNNER G & SCHMIDT FW, eds. **Artificial liver**. Springer-Verlag, Heidelberg, p. 25-31, 1981.
- 95 - ZAKI AEO et al. Experimental studies of blood brain barrier permeability in acute hepatic failure. **Hepatology** **4**: 359-363, 1983.
- 96 - SPIEGEL R & AEBI HJ. **Psychopharmacology: an introduction**. John Wiley & Sons, New York, 1983.
- 97 - GOLDSTEIN GW. The role of brain capillaries in the pathogenesis of hepatic encephalopathy. **Hepatology** **4**: 565-567, 1984.
- 98 - BASSET ML et al. Increased brain uptake of GABA in a rabbit model of hepatic encephalopathy. **Gastroenterology** **98**: 747-757, 1990.
- 99 - HOROWITZ ME et al. Increased blood-brain transfer in a rabbit model of acute liver failure. **Gastroenterology** **84**: 1003-1011, 1983.
- 100 - MANS AM et al. Correlation of plasma and brain amino acids and putative neurotransmitter alterations during acute hepatic coma in the rat. **J Neurochem** **32**: 285-292, 1979.
- 101 - LAL S et al. Cerebrospinal fluid homovanilic acid, 5-hydroxyindoleacetic acid, lactic acid and pH before and after probenecid in hepatic coma. **Clin Neurol Neurosurg** **77**: 142-154, 1974.
- 102 - BENGTTSSON F et al. Hepatocerebral dysfunction and brain serotonin. In: BUTTERWORTH RF, POMIER-LAYRARGUES G, eds. **Hepatic encephalopathy. Pathogenesis and treatment**. Humana Press, Clifton, p. 355-387, 1989.
- 103 - ZIMMERMAN C et al. Hepatic encephalopathy in thioacetamide-induced acute liver failure in rats: characterization of an improved model and study of amino acidergic neurotransmission. **Hepatology** **9**: 594-601, 1989.
- 104 - BRADFORD HF & WARD HK. Glutamine as a metabolic substrate for isolated nerve-endings: inhibition by ammonium ions. **Biochem Soc Trans** **3**: 1223-1226, 1975.
- 105 - SCHAFER DF et al. Visual evoked potentials in a rabbit model of hepatic encephalopathy. I. Sequential changes and comparisons with drug-induced comas. **Gastroenterology** **86**: 540-545, 1984.
- 106 - BASSET ML et al. Amelioration of hepatic encephalopathy by pharmacological antagonism of the GABA/benzodiazepine complex in a rabbit model of fulminant hepatic failure. **Gastroenterology** **93**: 1069-1077, 1987.
- 107 - BARALDI M & ZANEROLI ML. Experimental hepatic encephalopathy: changes in the binding of GABA. **Science** **216**: 427-429, 1982.
- 108 - SCHAFER DF et al. Gamma-aminobutyric acid and benzodiazepine receptors in animal models of fulminant hepatic failure. **J Lab Clin Med** **102**: 870-880, 1983.
- 109 - PAPPAS SC & GORDON VP. Altered brain GABA binding and synaptic plasma membrane composition in cirrhosis: a role in the pathogenesis of chronic hepatic encephalopathy. **Hepatology** **6**: 1106, 1986.
- 110 - MADDISON JE et al. Brain GABA receptor binding is normal in rats with thioacetamide induced hepatic encephalopathy despite elevated plasma GABA-like activity. **Gastroenterology** **93**: 1062-1068, 1987.
- 111 - BASILE AS et al. The GABA-A receptor complex in an experimental model of hepatic encephalopathy: evidence for elevated levels of an endogenous benzodiazepine receptor ligand. **J Neurochem** **53**: 1057-1063, 1989.
- 112 - SCHAFER DF et al. Acute hepatic coma and inhibitory neurotransmission: increase in GABA levels in plasma and receptors in brain. **Gastroenterology** **79**: 1123, 1980.
- 113 - SCHAFER DF et al. Colonic bacteria: a source of γ -aminobutyric acid in blood. **Proc Soc Exp Biol Med** **167**: 301-303, 1981.
- 114 - MINUK GY et al. Serum γ -aminobutyric acid levels in patients with hepatic encephalopathy. **HepatoGastroenterology** **32**: 171-174, 1985.
- 115 - TRABER PG et al. Electron microscopic evaluation of brain in rabbits with galactosamine-induced fulminant hepatic failure: ultrastructure and integrity of the blood-brain barrier. **Hepatology** **7**: 1272-1277, 1987.
- 116 - BARALDI M et al. Supersensitivity of benzodiazepine receptors in hepatic encephalopathy due to fulminant hepatic failure in the rat: reversal by a benzodiazepine antagonist. **Clin Sci** **67**: 167-175, 1984.
- 117 - LEONG DK et al. Densities of binding sites for the "peripheral-type" benzodiazepine receptor ligand 3 H-PK11195 are increased in brain 24 hours following portacaval anastomosis. **Metab Brain Dis** **9**: 267-273, 1994.
- 118 - SAMSON et al. Cerebral uptake of benzodiazepines measured by positron emission tomography in hepatic encephalopathy. **N Engl J Med** **316**: 414-415, 1987.
- 119 - BASILE AS et al. Brain concentrations of 1,4-benzodiazepines are elevated in patients with fulminant hepatic failure. **Hepatology** **12**: 76, 1990. Abstract.
- 120 - LAL S et al. Muscarinic, benzodiazepine, GABA, chloride channel and other binding sites in frontal cortex in hepatic coma in man. **Prog Neuropsychopharmacol Biol Psychiatry** **11**: 243-250, 1987 -
- 121 - FERENCI P et al. Changes in cerebral receptors for γ -aminobutyric acid in patients with hepatic encephalopathy. **Liver** **8**: 225-230, 1988.
- 122 - BASILE AS & GAMMAL S. The involvement of benzodiazepine receptors in hepatic encephalopathy: implications for treatment with benzodiazepine receptor antagonists. **Clin Neuropharmacol** **11**: 401-422, 1988.
- 123 - MULLEN KD et al. Could endogenous benzodiazepine ligands contribute to hepatic encephalopathy. **Lancet** **1**: 457-459, 1988.
- 124 - MCKERNAN RM et al. Which GABA_A-receptor subtypes really occur in the brain? **Trends Neurosci** **19**: 139-143, 1996.
- 125 - ROTHSTEIN JD. Benzodiazepine-receptor ligands and hepatic encephalopathy: a causal relationship? **Hepatology** **19**: 248-250, 1994.
- 126 - WIDLER P et al. Increased benzodiazepine-like activity is neither necessary nor sufficient to explain acute hepatic encephalopathy in the thioacetamide-treated rat. **Hepatology** **18**: 1459-1464, 1993.

- 127 - AVALLONE R et al. Endogenous benzodiazepine-like compounds and diazepam binding inhibitor in serum of patients with liver cirrhosis with and without overt encephalopathy. **Gut** **42**: 861-867, 1998.
- 128 - POMIER-LAYRARGUES G et al. Flumazenil in cirrhotic patients in hepatic coma: a randomized double-blind placebo-controlled crossover trial. **Hepatology** **19**: 32-37, 1994.
- 129 - GROENEWEG M et al. Effect of flumazenil on the electroencephalogram of patients with portosystemic encephalopathy. Results of a double blind, randomised, placebo-controlled multicentre trial. **Electroencephalogr Clin Neurophysiol** **98**: 29-34, 1996.
- 130 - GYR K et al. Evaluation of the efficacy and safety of flumazenil in the treatment of portal systemic encephalopathy: a double blind, randomised, placebo controlled multicentre study. **Gut** **39**: 319-324, 1996.
- 131 - BARBARO G et al. Flumazenil for hepatic encephalopathy grade III and IVa in patients with cirrhosis: an Italian multicenter double-blind, placebo-controlled, cross-over study. **Hepatology** **28**: 374-378, 1998.
- 132 - YURDAYDIN C et al. Gut bacteria provide precursors of benzodiazepine receptor ligands in a rat model of hepatic encephalopathy. **Brain Res** **679**: 42-48, 1995.
- 133 - MULLEN KD et al. "Endogenous" benzodiazepine activity in physiological fluids of patients with hepatic encephalopathy. **Lancet** **336**: 81-83, 1990.
- 134 - OLASMAA M et al. Endogenous benzodiazepine ligands in human and animal hepatic encephalopathy. **J Neurochem** **55**: 2015-2023, 1990.
- 135 - OLASMAA M et al. Endogenous benzodiazepines in hepatic encephalopathy. **Lancet** **1**: 491-492, 1989.
- 136 - BASILE AS et al. Elevated brain concentrations of 1,4-benzodiazepines in fulminant hepatic failure. **N Engl J Med** **325**: 473-478, 1991.
- 137 - MACDONALD GA et al. Cerebral benzodiazepine receptor binding *in vivo* in patients with recurrent hepatic encephalopathy. **Hepatology** **26**: 277-282, 1997.
- 138 - HOYUMPA M & SCHENKER S. Influence of liver disease on the disposition and elimination of drugs. In: SCHIFF L & SCHIFF ER, eds. **Diseases of the liver**. JB Lippincott, Philadelphia, p. 709-719, 1982.
- 139 - ROTHSTEIN JD et al. Release of endogenous benzodiazepine receptor ligands (endozepines) from cultured neurons. **Neurosci Lett** **143**: 210-214, 1992.
- 140 - TINUPER P et al. Idiopathic recurring stupor. A case with possible involvement of the GABAergic system. **Ann Neurol** **31**: 503-506, 1992.
- 141 - ROTHSTEIN JD & GUIDOTTI A. Endozepines: non-benzodiazepine endogenous allosteric modulators of GABA receptors. In: IZQUIERDO I & MEDINA JH, eds. **Naturally occurring benzodiazepines - Structure, distribution and function**. Ellis Horwood, New York, p. 115-130, 1993.
- 142 - BASILE AS et al. Relationship between plasma benzodiazepine receptor ligand concentration and severity of hepatic encephalopathy. **Hepatology** **19**: 112-121, 1994.
- 143 - ZENEROLI ML et al. Benzodiazepine-like compounds in the plasma of patients with fulminant hepatic failure. **Scand J Gastroenterol** **33**: 310-313, 1998.
- 144 - GACAD et al. Detection of benzodiazepine-like activity in hepatic encephalopathy requires an initial lipophilic extraction procedure! **Hepatology** **20**: 544-546, 1994.
- 145 - ZENEROLI ML et al. Changes in endogenous benzodiazepine-like compound levels during the course of fulminant hepatic failure: potential effects of decreased renal function. **Ital J Gastroenterol Hepatol** **29**: 456-458, 1997.
- 146 - SCOLLO-LAVIZZARI G & STEIMAN E. Reversal of hepatic coma by benzodiazepine antagonist Ro 15-1788. **Lancet** **1**: 1324, 1985.
- 147 - JONES EA. Benzodiazepine receptor ligands and hepatic encephalopathy: further unfolding of the GABA story. **Hepatology** **14**: 1286-1290, 1991.
- 148 - BANSKY G et al. Effects of the benzodiazepine receptor antagonist flumazenil in hepatic encephalopathy in humans. **Gastroenterology** **97**: 744-750, 1989.
- 149 - MEIER R & GYR K. Treatment of hepatic encephalopathy with the benzodiazepine antagonist flumazenil: a pilot study. **Eur J Anaesthesiol** p. 139-146, 1988. Suppl 2.
- 150 - BURKE DA et al. Reversal of hepatic coma with flumazenil with improvement in visual evoked potentials. **Lancet** **1**: 505-506, 1988.
- 151 - PIDOUX B et al. Electroencephalographic study of the effect of a benzodiazepine antagonist in hepatic encephalopathy. **Neurophysiol Clin Fr** **19**: 469-476, 1989.
- 152 - GRIMM G et al. Improvement of hepatic encephalopathy treated with flumazenil. **Lancet** **2**: 1392-1394, 1988.
- 153 - SUTHERLAND LR & MINUK GY. Ro 15-1788 and hepatic failure. **Ann Intern Med** **108**: 158, 1988.
- 154 - VAN DER RIJST CCD et al. Flumazenil therapy for hepatic encephalopathy: a double-blind cross-over study. **Hepatology** **10**: 590, 1989.
- 155 - MEIER R et al. Persisting benzodiazepine metabolites responsible for the reaction to the benzodiazepine antagonist flumazenil in patients with hepatic encephalopathy. **Gastroenterology** **101**: 274-275, 1991.
- 156 - KLOTZ U & WALKER S. Flumazenil and hepatic encephalopathy. **Lancet** **1**: 155-156, 1989.
- 157 - AMARAL OB et al. Flumazenil and hepatic encephalopathy. **Hepatology** **29**: 1338-1339, 1999.
- 158 - WALZ R et al. Encefalopatia hepática e o sistema GABAérgico: o papel dos benzodiazepínicos endógenos. **Psiquiatr Biol** **3**: 25-34, 1995.
- 159 - FERENCI P et al. Successful long-term treatment of portal-systemic encephalopathy by the benzodiazepine antagonist flumazenil. **Gastroenterology** **96**: 240-243, 1989.
- 160 - PEROUX JL et al. Chronic hepatic encephalopathy treated by long-term intravenous administration of flumazenil in ambulatory care. **Gastroenterol Clin Biol** **20**: 106-108, 1996.
- 161 - HOGER J et al. Diagnostic utility of flumazenil in coma with suspected poisoning: a double-blind randomized controlled study. **BMJ** **301**: 1308-1311, 1990.
- 162 - RITZ R et al. Use of flumazenil in intoxication patients with coma. A double-blind placebo-controlled study in ICU. **Intensive Care Med** **16**: 242-247, 1990.

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