Supersensitivity of benzodiazepine receptors in hepatic encephalopathy due to fulminant hepatic failure in the rat: reversal by a benzodiazepine antagonist

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Summary
1. Benzodiazepine receptors were studied in rats with hepatic encephalopathy due to fulminant hepatic failure induced by galactosamine. [3H]-Diazepam binding studies on brain synaptic membranes of rats with mild and severe encephalopathy show a significant increase in the number of receptors in both stages of coma.
2. [3H]Diazepam binding to synaptic membrane preparations from rats in the mild or severe stage of encephalopathy hyper-responded to the stimulatory effect of γ-aminobutyric acid (GABA) applied in vitro at doses which for control rat preparations were in a subthreshold range. The effect of GABA was shown to be specific, since it was blocked by bicuculline methiodide. The sensitivity of benzodiazepine receptors in hepatic encephalopathy to nanomolar concentrations of GABA, which induced a significant increase in their affinity, seems to indicate a functional supersensitivity of benzodiazepine receptors in vivo in both mild and severe stages of encephalopathy.
3. The phenomena described may be attributed to a partial degeneration of nerve terminals in hepatic encephalopathy, leading to a supersensitivity of benzodiazepine receptors, which parallels the previously described denervation supersensitivity of GABA receptors present in this animal model of fulminant hepatic failure. These findings may account for the brain hypersensitivity to sedatives administered to patients with liver disease.
4. The administration in vivo of a benzodiazepine antagonist, 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one, counteracted the hypersensitivity of benzodiazepine receptors in the mild stage of encephalopathy. In fact, electrophysiological studies with visually evoked potential recordings in rats in the mild stage of encephalopathy 30–60 min after the administration of the drug showed a recovery of the wave pattern, and this electrophysiological finding was substantiated by the demonstration that both the number and the affinity of benzodiazepine receptors were strongly reduced.

Key words: γ-aminobutyric acid, benzodiazepine receptors, bicuculline methiodide, [3H]diazepam binding, galactosamine-HCl, hepatic encephalopathy, 2-phenylpyrazolo[4,3-c]quinolin-3(5H)-one, visually evoked potentials.

Abbreviations: GABA, γ-aminobutyric acid; VEP, visually evoked potential.

Introduction
Clinical observation of patients with acute or chronic severe liver disease has given rise to the concept that these patients are supersensitive to administration of sedatives. In 1972 Fessel & Conn [1] pointed out that 12% of iatrogenic episodes of encephalopathy in liver cirrhosis were due to the administration of chlordiazepoxide. Since neither
increased sensitivity of the brain to this drug was hypothesized [4, 5]. Evidence for such a hypothesis was given by Branch et al. [4], who found that, at similar plasma concentrations of diazepam, patients with liver cirrhosis showed an increased electroencephalographic response to diazepam in comparison with normal subjects. On the other hand, an indirect demonstration that both of the mechanisms are involved is the fact that oxazepam, whose biotransformation is not greatly altered in liver disease [6-8], is considered to possess a wide margin of therapeutic safety in liver disease patients; however, studies on the responsiveness of the brain to this drug are still incomplete.

We have described in an animal model of hepatic encephalopathy, due to fulminant hepatic failure induced by a single injection of galactosamine-HCl into rats [9], how γ-aminobutyric acid (GABA) receptors in the brain undergo pathological changes [10-12]. In the mild stage of hepatic encephalopathy, we detected an increased number of GABA binding sites, which we interpreted as an expression of a denervation supersensitivity phenomenon at GABA-ergic synapses in the central nervous system, since the same phenomenon has been described for GABA receptors after chemical or surgical induction of local neuronal lesions in the striato-nigral pathway [13-15]. This concept is further supported by the demonstration of a reduction of glutamate decarboxylase activity in several brain areas of rats with hepatic encephalopathy [16]. This supersensitivity in hepatic encephalopathy has been substantiated by the finding that GABA receptors are hypersensitive to bicuculline methiodide inhibition both in vitro [12] and in vivo [17]. An increase in the number of GABA receptors, during an unspecified degree of encephalopathy in a rabbit model of fulminant hepatic failure, has also been reported by others [18-20] and attributed to an increase in the brain of a gut-derived GABA. However, evidence for such an increase in GABA in the brain based on appropriate methodological approaches has never been provided by the authors of these reports, since, as pointed out by Hoyumpa & Schenker [21] and by us [22], an increase in GABA entering the brain cannot be assumed from the accumulation of a nonmetabolized GABA isomer. As further evidence of continued degenerative processes in hepatic encephalopathy we found that selection of high-affinity GABA receptors in the severe stage of coma was due to an apparent loss of the low-affinity GABA receptors [10-12]. More recently evidence has been provided that a similar phenomenon seems to be present in a dog model of chronic liver disease induced by the administration of dimethylnitrosamine followed by portocaval anastomosis [23].

In view of these results we decided to investigate whether GABA-ergic denervation supersensitivity may also be extended to benzodiazepine receptors in hepatic encephalopathy, since pharmacological, electrophysiological and neurochemical experiments have demonstrated a functional interaction between benzodiazepine and GABA receptors in the mammalian central nervous system [24-27]. Radioligand binding studies using tritiated GABA and benzodiazepine performed on cloned neuroblastoma and glioma cells in culture have indicated that GABA and benzodiazepine receptors reside on the same cell membranes [28, 29]. Furthermore, radiographic and immunochemical techniques have provided evidence that benzodiazepine receptors are mainly located at GABA-ergic synapses [30]. In addition the occupation of GABA recognition sites by specific agonists enhances the affinity of benzodiazepine receptors [31, 32] whereas benzodiazepine increases the number of high-affinity GABA binding sites in brain membranes [33]. In the brain benzodiazepine receptors are part of a supramolecular entity, the 'GABA receptor unit' [29], which includes high- and low-affinity GABA recognition sites, benzodiazepine binding sites, Cl⁻ ionophores [34] and endogenous modulators [35]. These modulators are represented by a neuropeptide termed GABA-modulin [28], which inhibits non-competitively [³H]GABA specific binding and GABA stimulation of benzodiazepine binding sites [36], and by a polypeptide termed diazepam-binding inhibitor (DBI), which competitively inhibits the binding of benzodiazepines to their recognition sites [37] and antagonizes the benzodiazepine-elicited increase in the maximal binding value of GABA to its high-affinity receptors.

It seemed therefore rational to extend our knowledge of the pathology of GABA receptors in hepatic encephalopathy to benzodiazepine receptors, with the aim of seeking a biochemical explanation for the hypothesized hypersensitivity of patients with hepatic encephalopathy to sedative administration.

Further, the discovery of new compounds which are benzodiazepine antagonists, such as ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazolo[1,5a][1,4]benzodiazepine-3-carboxylate (Ro 15 1788) [38] and 2-phenylpyrazolo[4,3-c]-quinolin-3(5H)-one (CGS 8216) [39], prompted
Animal model

Hepatic encephalopathy was induced in Sprague-Dawley albino rats (100-125 g body weight) (Charles River Como, Italy) by intraperitoneal injection of galactosamine-HCl (3 g/kg) (Sigma Chemical Co., St. Louis, U.S.A.) as previously described [9-12, 40]. Approximately 80% of the animals developed fulminant hepatic failure as a result of massive necrosis of the liver within 48 h. They showed progressive encephalopathy, characterized in the mild stage by a long period of stupor, poor righting reflexes and diminished response to pain. This stage of encephalopathy proved to be reversible, since some of the animals recovered. The severe stage is characterized by unconsciousness, flaccidity and areflexicity to painful stimuli, leading to death 3.5-4 days after the injection of the toxin. Hepatic encephalopathy was divided into mild and severe stages on the basis of the pattern of visually evoked potential (VEP) recordings, since this method of evaluation has been demonstrated to be reliable and sensitive enough to discriminate different stages of unconsciousness [9, 22, 40]. Briefly, in order to make VEP recordings from the two cortical electrodes, fixed to the skull with dental cement, were permanently implanted under ether anaesthesia. VEP recordings were made 3 days after the surgical procedure, before injection of galactosamine, and during the development of hepatic encephalopathy. As previously described [9], VEP recordings in normal rats are characterized by four waves, positive and negative, labelled P1, N1, P2, N2. The peak-to-peak amplitudes of P1-N1 and N1-P2 and the latency of these waves were used to distinguish mild and severe coma: for the mild stage there was a 50% reduction in P1-N1 amplitude without change in the latencies and, in the severe stage, a 75% reduction in P1-N1 amplitude associated with a 30% decrease in the latency of N2 occurred.

Although it is well established that D-galactosamine is rapidly metabolized by the liver after intraperitoneal administration [41] or after intravenous injection [42], and that less than 0.05% of the injected dose reaches the brain [42], we have tested whether galactosamine injected intravenously in rats (3 g/kg) is able to directly interfere with the characteristics of brain benzodiazepine receptors. These rats (n = 5) were killed 1 h after the single injection of galactosamine before the onset of the hepatic lesion. With the same aim, the rats injected intraperitoneally with galactosamine without developing fulminant hepatic failure (and consequently not encephalopathic) were killed 3.5-4 days later, in parallel with controls and with rats which developed hepatic encephalopathy, in order to perform [3H]diazepam binding studies on brain membrane preparations.

Control rats (n = 10) and rats in mild (n = 12) or severe (n = 10) stages of hepatic encephalopathy were killed by decapitation. The brains were immediately removed and homogenized in 30 vol. of sucrose solution (0.32 mol/l). The crude synaptic membrane fractions were prepared by the method of Enna & Snyder [43], resuspended in Tris-HCl buffer (50 mmol/l), pH 7.4, and washed twice with the same buffer. The membranes were stored at -20°C until they were used 3-5 days later. On the day of the assay the frozen membranes were thawed and centrifuged. The pellet was suspended in 30 vol. of Tris-HCl buffer, incubated at 37°C for 30 min and subsequently washed three more times. After the last washing the pellet was resuspended in an appropriate volume of buffer to give a membrane preparation containing 200-300 μg of protein in 1 ml. The [3H]diazepam binding assay was performed by adding 800 μl of membrane suspension to glass test tubes containing Tris-HCl buffer or unlabelled clonazepam (1 μmol/l; Hoffmann-La Roche, Basel, Switzerland) and an appropriate amount of radioligand ([3H]diazepam, specific radioactivity 87 Ci/mmol; New England Nuclear, Dreieichenhain, West Germany). The total incubation volume was 1 ml. After incubation at 0-4°C for 45 min, the reaction was stopped by rapid filtration through Whatman GF/B glass-fibre filters followed by two washings with 5 ml of ice-cold Tris-HCl buffer. For Scatchard analyses [3H]diazepam concentrations ranged from 0.7 to 23 nmol/l. [3H]Diazepam specific binding was calculated by subtracting binding in the presence of clonazepam (1 μmol/l) from total binding determined in its absence. Tritium on the filters was determined by conventional scintillation counting (Packard Tri-Carb 350) in 8 ml of Instagel (Packard Instruments, Milano, Italy). Protein concentrations were determined by the method of Lowry et al. [44] with bovine serum used as the standard.

GABA-stimulated [3H]diazepam binding studies

The stimulatory effect of GABA on [3H]-diazepam was studied in vitro in two sets of
experiments. The effect of increasing concentrations of GABA on a fixed dose of $[^{3}H]$diazepam (1.5 nmol/l) was first studied on membranes from control rats ($n = 8$) and of rats with mild ($n = 8$) and severe encephalopathy ($n = 8$). Since it has been reported that the threshold dose of GABA required to increase the binding of benzodiazepines to their binding sites in well-washed synaptic membrane preparations of normal rats ranges between 0.1 and 1 pmol/l [31, 32, 45-47], concentrations of GABA lower than the above-mentioned threshold dose (from 1 pmol/l to 1 pmol/l) were added to the $[^{3}H]$diazepam binding assay in order to test the sensitivity of benzodiazepine receptors to GABA stimulation in membranes of rats with hepatic encephalopathy. Moreover, the specificity of the effect exerted by GABA on the $[^{3}H]$diazepam binding was tested by adding in vitro the specific GABA antagonist, bicuculline methiodide (0.1 mmol/l) [32, 46]. On the basis of the results of these experiments, the stimulatory effect of GABA on $[^{3}H]$diazepam saturation curves (0.7-23 nmol/l) was studied to determine whether or not GABA at 10 nmol/l, which does not change the affinity constants of $[^{3}H]$diazepam binding in control membranes, was able to affect the affinity constant in membranes of rats with hepatic encephalopathy. In this set of experiments $[^{3}H]$diazepam binding assay was performed as described above with the exception that, in order to keep the final volume of the assay constant at 1 ml, an appropriately decreased amount of membrane suspension was added to the test tubes.

**Statistical analysis**

All statistical analyses were performed by using Student's $t$-test.

**Results**

$[^{3}H]$Diazepam binding studies

The mean Scatchard plots obtained by averaging the values of five saturation curves of $[^{3}H]$diazepam binding to synaptic membrane preparations indicate, as shown in Fig. 1, that an increase in the number of benzodiazepine receptors is associated with both the mild and the severe stages of hepatic encephalopathy. No significant change in the dissociation constants was detected. The mean $(\pm$ SD) kinetic characteristics derived from the Scatchard plots of each experiment were as follows: controls, $K_D = 10.1 \pm 0.43$ nmol/l, $B_{max} = 1.20 \pm 0.15$ pmol/mg of protein; mild encephalopathy, $K_D = 9.8 \pm 0.52$ nmol/l, $B_{max} = 1.88 \pm 0.13$ pmol/mg of protein ($P < 0.01$ vs controls); severe encephalopathy, $K_D = 9.5 \pm 0.64$ nmol/l, $B_{max} = 2.0 \pm 0.21$ pmol/mg of protein ($P < 0.01$ vs controls).

Rats receiving galactosamine, but not developing fulminant hepatic failure and consequently not encephalopathic, had normal $[^{3}H]$diazepam binding kinetic characteristics (data not shown), indicating that galactosamine per se does not affect brain benzodiazepine receptors. The same

Studies with 2-phenylpyrazolo[4,3-c]quinolin-3-(5H)-one (CGS 8216)

Galactosamine-treated rats ($n = 16$), selected as being in a mild stage of encephalopathy by means of VEP recordings, were divided into two groups. Group 1 was injected intraperitoneally with sodium chloride solution (154 mmol/l: saline) and group 2 was similarly injected with CGS 8216 (10 mg/kg; CIBA-Geigy, Summit, NJ, U.S.A.). In parallel, another two groups of eight normal rats each were injected intraperitoneally with saline or CGS 8216 (10 mg/kg).

VEP recordings were made from all the above four groups 30 and 60 min after treatment. All the rats were then killed by decapitation and synaptic brain membranes prepared for $[^{3}H]$diazepam binding saturation curves performed as described above. CGS 8216 was chosen in this preliminary study as its pharmacological action is longer than that of Ro 15-1788 [39].

**Fig. 1.** Scatchard plot analysis of $[^{3}H]$diazepam binding to synaptic membrane preparations from brains of normal rats (△) and of rats with mild (○) and severe (●) hepatic encephalopathy. Each point represents the mean value of five different saturation curves (0.7-23 nmol/l).
finding was obtained in rats with circulating galactosamine after an intravenous injection, thus excluding a direct interference of this toxin with the [3H]diazepam kinetic characteristics.

**GABA-stimulated [3H]diazepam binding studies**

Fig. 2 shows dose-response curves of the enhancement of [3H]diazepam binding (1.5

nmol/l) in response to GABA (1 pmol/l-1 µmol/l) added in vitro to synaptic membrane preparations from normal rats and from rats in mild and severe stage of hepatic encephalopathy.

These experiments performed on GABA-stimulated [3H]diazepam binding reveal a hypersensitivity of the benzodiazepine recognition sites in mild and severe hepatic encephalopathy to the addition in vitro of GABA. In fact, increased concentrations of GABA enhanced [3H]diazepam binding to membranes of rats in mild and severe stages of encephalopathy by 2-2.5 times the binding in control membranes. This enhancing effect of GABA on [3H]diazepam binding seems to be rather specific since, as shown in Fig. 2, it was blocked by the addition to the incubation mixture of bicuculline methiodide.

A further demonstration that benzodiazepine receptors become supersensitive during the development of hepatic encephalopathy is provided by [3H]diazepam saturation curves in the presence or absence of GABA at 0.01 µmol/l, as shown in Table 1, which summarizes the characteristics of [3H]diazepam and of GABA-stimulated [3H]diazepam binding to brain membranes of control rats and rats with mild and severe encephalopathy in this set of experiments. In fact, the addition of an amount of GABA which does not affect the dissociation constant (K_D) in normal membranes significantly increases the affinity of [3H]diazepam for its receptors in the membranes of rats with both mild and severe hepatic encephalopathy, as indicated by the reduction in K_D.

**Studies with 2-phenylpyrazol[4,3-c]quinolin-3(5H)-one (CGS 8216)**

As previously described [9, 12, 40], VEP recordings from normal rats consist of four waves

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<tr>
<th>TABLE 1. Kinetic characteristics of [3H]diazepam receptors in brain membranes of control rats and of rats with mild and severe hepatic encephalopathy: effect of GABA stimulation in vitro (mean ±1 SD)</th>
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<tr>
<td>[3H]Diazepam binding by brain membrane preparations was determined at 0.7–23 nmol/l, both in the absence and presence of clonazepam (1 µmol/l), and the kinetic characteristics were calculated from Scatchard plots derived from [3H]diazepam saturation curves as described in the text, with either 100 µl of Tris–HCl buffer (None) or 100 µl of Tris–HCl buffer containing unlabelled GABA added to the incubation tubes. Student's t-test: ***P &lt; 0.01 vs None mild encephalopathy, **P &lt; 0.01 vs None severe encephalopathy, *P &lt; 0.001 vs control values.</td>
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<tr>
<th>Addition</th>
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<th>K_D (nmol/l)</th>
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<tr>
<td></td>
<td>Controls</td>
<td>Hepatic encephalopathy</td>
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<td>None</td>
<td>10.7 ± 0.25</td>
<td>10.1 ± 0.52</td>
<td>10.2 ± 0.78</td>
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<td>GABA (10 nmol/l)</td>
<td>10.0 ± 0.13</td>
<td>8.0 ± 0.36***</td>
<td>8.2 ± 0.51**</td>
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with peak-to-peak amplitudes $P_1-N_1 \ 60.0 \pm 4.5 \mu V$ and $N_1-P_2 \ 75.2 \pm 15.0 \mu V$, and latencies $P_1 \ 10.5 \pm 3.5$, $N_1 \ 38.2 \pm 4.3$, $P_2 \ 72.3 \pm 12.7$ and $N_2 \ 194.6 \pm 14.2 \text{ ms (Fig. 3a)}$. The mild stage of hepatic encephalopathy is characterized by a decrease in $P_1-N_1$ amplitude (mean $\pm \text{SD}$: $24.8 \pm 3.5 \mu V$) without substantial changes in the latencies (Fig. 3b). VEP recordings from rats in the mild stage of encephalopathy 30 min after the injection of CGS 8216 demonstrated a recovery of the $P_1-N_1$ amplitudes (mean $\pm \text{SD}$: $57.4 \pm 4.6 \mu V$), as shown in Fig. 3c. The recovery persisted at the second recording 60 min after injection of CGS 8216.

Animals showing mild encephalopathy treated with CGS 8216 passed from a stuporous state, immobility and lower reaction to pain stimuli, to an apparent normal status with a recovery of spontaneous motility and of sensitivity to painful stimuli during the 60 min observation.

VEP recordings from normal rats after CGS 8216 did not show any appreciable change, indicating that this compound is devoid of any intrinsic activity in normal conditions. VEP recordings from rats in the mild stage of encephalopathy injected with saline remained abnormal during the 60 min study.

As shown in Fig. 4, the above-described effects of CGS 8216 injection in rats with mild hepatic encephalopathy on the VEP pattern and on behaviour, were further substantiated by a profound change of the kinetic characteristics of $[^3H]$diazepam binding performed in vitro on brain membranes obtained from these rats. In this set of experiments the characteristics of $[^3H]$diazepam in membranes of normal rats injected with saline were $K_D \ 10.1 \pm 0.36 \text{ mmol/l}$ and $B_{\text{max}} \ 1.18 \pm 0.15 \text{ pmol/mg of protein}$, and the $K_D$ and the $B_{\text{max}}$ of rats with mild encephalopathy injected with saline were $9.8 \pm 0.65 \text{ mmol/l}$ and $1.81 \pm 0.06 \text{ pmol/mg of protein}$ respectively. The injection of CGS 8216 into normal rats resulted in a change in both $K_D$, $13.9 \pm 0.47 \text{ mmol/l}$ ($P<0.01$ vs controls), and in $B_{\text{max}}$, $0.91 \pm 0.09 \text{ pmol/mg of protein}$, thus confirming the mixed-type inhibition properties of CGS 8216 previously reported [39, 48].

The inhibitory effect of CGS 8216 on $[^3H]$diazepam binding performed on membranes of rats in the mild stage of hepatic encephalopathy treated with the drug was magnified, since the affinity was greatly reduced ($K_D \ 20.5 \pm 1.75 \text{ mmol/l}$, $P<0.001$ vs controls and saline-injected rats with mild hepatic encephalopathy) and the maximum number of binding sites was also con-

![FIG. 3. Typical VEP patterns recorded in a rat in (a) the basal condition, (b) mild stage of hepatic encephalopathy before, and (c) 30 min after intraperitoneal injection of CGS 8216 (10 mg/kg).](image)

![FIG. 4. Scatchard plot analysis of $[^3H]$diazepam binding to synaptic membrane preparations from brains of normal rats (○), of rats with mild hepatic encephalopathy (△), of normal rats (●) and of rats with mild hepatic encephalopathy (▲) injected intraperitoneally with CGS 8216 (10 mg/kg). Each point represents the mean value of three separate saturation curves (0.7–23 mmol/l).](image)
considerably reduced ($P_{\text{max}}$, 0.61 ± 0.03 pmol/mg of protein, $P < 0.001$ vs controls and saline-injected rats with mild hepatic encephalopathy).

The results of this experiment seem further to indicate that the benzodiazepine receptors present in brain membranes of rats with mild hepatic encephalopathy are indeed hypersensitive, since they are not only stimulated by subthreshold doses of GABA but also more extensively inhibited than controls by an antagonist such as CGS 8216.

Discussion

In this study experiments were undertaken to characterize the kinetic properties of benzodiazepine receptors in an animal model of hepatic encephalopathy in which an increase in GABA receptors followed by a selection of distinct high-affinity recognition sites has been demonstrated. The present demonstration that the number of benzodiazepine receptors in brain membranes of rats in mild hepatic encephalopathy is increased in parallel with the increase in GABA receptors might indicate that both types of receptors undergo the same fate in this pathological condition. Bearing in mind that benzodiazepine recognition sites are part of the GABA receptor complex, it is likely that the increase in benzodiazepine receptors in the postsynaptic membranes of comatose rats is attributable to a reactive compensatory phenomenon which follows degenerative processes, as described for the GABA receptors. This hypothesized degeneration could be due to circulating toxins such as ammonia, mercaptans and short chain fatty acids acting synergistically [40, 49–52], which accumulate in the brain from the failing liver during hepatic encephalopathy.

There is experimental evidence in favour of the degenerative hypothesis, since a denervation supersensitivity for GABA and benzodiazepine recognition sites has been described after kainic acid-induced degeneration of the striato-nigral pathway [13–15, 53]. The increase in GABA and benzodiazepine binding sites described in those studies has been attributed to degeneration of GABA-ergic neurons, since a marked decrease in the marker enzyme glutamate decarboxylase was found in those conditions [13, 15, 53]. On the other hand, gliosis and degeneration of neurons has been described as occurring during hepatic encephalopathy [54], and neuropsychiatric disturbances and epilepsy resulting from brain atrophy have been reported as sequelae in patients surviving fulminant hepatic failure [55, 56]. Further, the finding that benzodiazepine receptors are still increased in the severe stage of hepatic encephalopathy, in which low-affinity GABA receptors are completely absent [10, 12, 22], corroborates the idea that benzodiazepine receptors are coupled with high-affinity GABA receptors [28, 29, 33, 34].

An increased number of benzodiazepine receptors has been briefly reported by others in a rabbit model of hepatic encephalopathy, but without any characterization of the benzodiazepine receptors [18–20]. Our demonstration that benzodiazepine receptors in the synaptic membranes of rats with hepatic encephalopathy revealed a hypersensitivity to the addition of GABA in vitro, which increases their affinity, is an important index for the prediction of the functional status of benzodiazepine and GABA receptors in vivo.

To summarize, the present study supports the concept that in galactosamine-induced hepatic encephalopathy there is an increase in the number of benzodiazepine receptors. Extrapolation from results of experiments in animals to human situation is always uncertain, but assuming that the same phenomenon may be present in human fulminant hepatic failure, the above finding might account for the described brain supersensitivity to sedative administration of patients with liver disease.

The present demonstration that CGS 8216 can temporarily antagonize the hypersensitivity of the GABA-benzodiazepine receptor system in experimental hepatic encephalopathy may be regarded as a promising result in the attempt to counteract some of the neurological disturbances which characterize this multifactorial pathology, at least in its early stages.

Acknowledgments

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