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# Memantine, a Noncompetitive NMDA Receptor Antagonist Improves Hyperammonemia-Induced Encephalopathy and Acute Hepatic Encephalopathy in Rats

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The aim of this study was to investigate the possible role of *N*-methyl-D-aspartate (NMDA)-receptor overactivity in two different experimental rat models of encephalopathy: subacute encephalopathy caused by severe hyperammonemia in portacaval-shunted rats (AI-PCS rats) and acute hepatic encephalopathy caused by complete liver ischemia (LIS rats). The effect of the noncompetitive NMDA-receptor antagonist memantine (intraperitoneal [i.p.] 10-20 mg/kg bw or intravenous [i.v.] 5 mg/kg bw) was studied on the severity of encephalopathy by assessment of clinical grading and electroencephalogram (EEG) spectral analysis, on plasma ammonia concentrations, amino acid concentrations in cerebrospinal fluid (CSF), intracranial pressure (ICP), and brain water content. Both rat models developed encephalopathy within 3 to 6 hours, associated with increased CSF glutamate and aspartate concentrations and increased ICP and brain water content. Memantine administration in AI-PCS and LIS rats resulted in a significant improvement in clinical grading and less slowing of EEG activity ( $P < .05$ ), and smaller increases in CSF glutamate ( $P < .05$ ) concentrations. Moreover, ICP and brain water content were significantly lower in memantine-treated AI-PCS rats than in untreated AI-PCS rats ( $P < .05$ ). Memantine had no significant effect on ICP and brain water content in LIS rats, and on ammonia concentrations in both models. These results indicate that NMDA-receptor activation might be involved in the pathogenesis of hyperammonemia-induced encephalopathy and of acute hepatic encephalopathy caused by LIS. (HEPATOLOGY 1997;25:820-827.)

The pathogenesis of hepatic encephalopathy (HE) is multifactorial and has not yet been completely elucidated. Ammonia is assumed to play an important role by either directly or indirectly affecting brain metabolism and/or neurotransmission (for review, see Butterworth et al.<sup>1</sup>). Increased brain glutamine concentrations are related to increased ammonia concentrations, and it has been suggested that brain glutamine more than brain ammonia is implicated in the patho-

genesis of HE.<sup>2-7</sup> Moreover, elevated brain glutamine concentrations can induce cell swelling, resulting in brain edema,<sup>8,9</sup> a terminal event in patients with fulminant hepatic failure.<sup>10</sup>

Elevation of cerebral extracellular and cerebrospinal fluid (CSF) concentrations of glutamate and aspartate has also been observed in experimental<sup>9,11-14</sup> and clinical<sup>15-17</sup> HE, and it has been hypothesized that these two excitatory neurotransmitters are implicated in the pathogenesis of HE. Glutamate and aspartate can bind to different types of excitatory receptors (metabotropic and ionotropic), of which the *N*-methyl-D-aspartate (NMDA) receptor has been best characterized.<sup>18</sup> A fast increase in extracellular glutamate and aspartate concentrations observed in (sub)acute liver failure can result in an overstimulation of the NMDA receptor. This NMDA-receptor overactivity leads to an increased influx of different ions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$ ), finally resulting in cell swelling and/or neuron degeneration.<sup>19,20</sup>

This study was designed to investigate the role of NMDA-receptor activity in hyperammonemia-induced encephalopathy and liver ischemia-induced HE, two experimental rat models that both are associated with intracranial hypertension. Therefore, the noncompetitive NMDA-receptor antagonist memantine<sup>21-24</sup> was administered to portacaval-shunted (PCS) rats with an ammonium-acetate infusion and to rats with complete liver ischemia. The severity of encephalopathy was quantified by clinical grading and electroencephalogram (EEG) spectral analysis. Plasma concentrations of ammonia and memantine, and CSF concentrations of amino acids, were assessed. Intracranial pressure was measured with a pressure transducer connected to a cisterna magna cannula, and, in addition, brain water content was assessed at the end of the experiment.

## MATERIALS AND METHODS

### Animals and Surgical Procedures

Male Wistar rats (200-300 g; HSD, Zeist, the Netherlands; 12-hour light cycle: 8 AM-8 PM) were fed standard laboratory chow (RMH 1410 Hope Farms, Woerden, The Netherlands) and water *ad libitum*. Animal welfare was in accordance with the guidelines of the University of Amsterdam.

Two different experimental groups were studied: 1) PCS rats with an ammonium-acetate infusion (AI-PCS rats), and 2) rats with liver ischemia (LIS rats).

**AI-PCS Rats.** One week before the ammonium-acetate infusion, a PCS was performed in rats under ether anesthesia according to the technique of Lee et al.<sup>25</sup> On the day of the infusion, the carotid artery and the jugular vein of the PCS rats were cannulated under ether anesthesia to take blood samples from the artery and to give an intravenous (i.v.) ammonium-acetate infusion or an i.v. injection of memantine. In the case of intraperitoneal (i.p.) injection of memantine, an i.p. catheter was also placed. After recovery from anesthesia, the PCS rats received a bolus injection of ammonium-acetate (0.5 mmol/kg body weight) after which an ammonium-acetate infusion of 2.8 mmol/kg body weight per hour was given for 6 hours. The pH of the ammonium-acetate solutions was 7.3 to 7.4.

Abbreviations: HE, hepatic encephalopathy; CSF, cerebrospinal fluid; NMDA: *N*-methyl-D-aspartate; PCS, portacaval shunting; EEG, electroencephalogram; AI-PCS, PCS rats with ammonium-acetate infusion; LIS, rats with liver ischemia; i.v., intravenous; i.p., intraperitoneal; MDF, mean dominant frequency; ICP, intracranial pressure.

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TABLE 1. Stages in Experimental HE

Clinical grade 0	Normal behavior
Clinical grade 1	Mild lethargy
Clinical grade 2	Decreased motor activity, poor gesture control, diminished response to pain stimuli
Clinical grade 3	Severe ataxia, no spontaneous righting reflex
Clinical grade 4	No righting reflex to pain stimuli
Clinical grade 5	No reaction to pain stimuli

**LIS Rats.** A ligation of the hepatic artery was performed under ether anesthesia 1 week after PCS surgery. After devascularization, the carotid artery was cannulated and an i.p. catheter was placed for memantine and glucose (5%) administration. In both experimental groups, body temperature was kept constant between 36°C and 37°C by a heating lamp, because Traber et al. showed that normothermic devascularized rats developed brain edema, whereas hypothermic devascularized rats did not.<sup>26</sup>

Memantine (1-amino-3,5-dimethyladamantane hydrochloride; Akatinol memantine) was a generous gift from Merz and Co., GmbH (Frankfurt/Main, Germany), and was dissolved in 0.9% saline in a concentration of 5 mg/mL. It was administered to the AI-PCS rats 1 hour after the start of the ammonium-acetate infusion, either intravenously (5 mg/kg bw) or intraperitoneally (10 mg/kg body weight). LIS rats received only an i.p. injection of memantine of 10 to 20 mg/kg body weight after loss of the righting reflex (i.e., clinical grade 3). Control experiments consisted of 0.9% saline administration in a volume similar to memantine. (Because the results from i.p. and i.v. administration of 0.9% saline were similar, results from both administrations were combined and used as data from one control group for the AI-PCS rats. Control LIS rats received i.p. administration of 0.9% saline).

### Experimental Procedures

Two different series of experiments were performed in AI-PCS rats and LIS rats:

In the first series of experiments, the development of encephalopathy was determined by clinical grading of encephalopathy and EEG spectral analysis (n = 7 per group, except control LIS rats: n = 5). Encephalopathy was graded clinically according to the level of consciousness, using the five grades shown in Table 1.

Five days before a PCS was performed, four golden skull electrodes were implanted, and, on the day of the experiment, EEG spectral analysis was performed for four EEG power band regions within the range of 1 to 26.5 Hz.<sup>27</sup> After Fourier transformation, the so-called power density spectra were obtained, and, in addition, the EEG left index and mean dominant frequency (MDF) were calculated. EEG left index was defined as the logarithm of the ratio between the power of the low frequency (1-7.4 Hz) and the high frequency (13.5-26.5 Hz). Normal rats show an EEG left index of approximately 0.60, and rats in coma show approximately 0.80-0.90. During the development of encephalopathy, MDF will decrease from 16 to 18 Hz to 11 to 13 Hz (our observations).

At the end of the first series of experiments, rats were exsanguinated under complete ether anesthesia. The brain was promptly removed and frozen in liquid nitrogen. They were stored at -70°C until determination of memantine concentrations.

In the second series of experiments (n = 7 per group, except control LIS rats: n = 5), amino acids in CSF were assessed and intracranial pressure (ICP) was measured. (In addition, clinical grading of encephalopathy was also measured in this series of experiments).

A cisterna magna cannula was implanted according to the technique of Boer et al.<sup>28</sup> 1 day before the experiment. After the rat was anesthetized (30 mg/kg nembutal i.p.), a stainless-steel cannula (outside diameter 0.8 mm) was positioned by hand and fixed to the skull by a round plate soldered at an angle of 45° using dental cement.

Aliquots of 50 µL CSF from the cisterna magna cannula were taken for amino acid analysis at the start and end of the experiment. CSF samples were frozen in liquid nitrogen immediately after sampling and stored at -80°C until required for analysis of amino acids.

The cisterna magna cannula was also used to measure the ICP at the start and end of the experiment, just before CSF samples were taken. The cisterna magna cannula was connected with a pressure transducer (Viggo-Spectramed DTX/Plus disposable transducer kit, Ohmeda, Singapore), which monitored the ICP.

At the end of the second series of experiments, rats were exsanguinated under complete ether anesthesia. The cerebrum was promptly removed and the wet weight was measured on an analytical balance (Mettler PM400, Mettler Toledo Ag, Urdorf, Switzerland), after which it was placed in an oven at 160°C for 72 hours. After this, dry weight was measured and brain water content was calculated by subtracting dry from wet brain weights.

### Biochemical Parameters

In all series of experiments, blood samples were taken from the carotid artery to measure blood ammonia concentration (0.05 mL every hour) or plasma memantine concentration (1.0 mL at the end of the experiment). Additionally, in the LIS rats, blood glucose was measured every hour (Haemo-Glukotest, Boehringer Mannheim, Mannheim, Germany). Mean arterial blood pressure was measured every 2 hours via the carotid artery that was connected to a pressure transducer (Viggo-Spectramed DTX/Plus disposable transducer kit).

Ammonia concentration was measured with the Blood Ammonia Checker II (Kyoto Daiichi Kagaku Co., Ltd., Kyoto, Japan).<sup>29</sup> Amino acid concentrations were measured after deproteinization by high-pressure liquid chromatography analysis as described by van Eijk et al.<sup>30</sup> Memantine concentration was measured by gas chromatography/mass spectroscopy as described by Danysz et al.<sup>31</sup>

### Statistical Analysis

Data are expressed as means ± SEM. Statistical analyses were performed using the SPSS/PC+ Statistical Software Package, version 5.0 (SPSS Inc., Chicago, IL). Repeated measures MANOVA was used for comparison of differences in blood ammonia concentrations, clinical grade, EEG left index, MDF, and ICP, in the presence or absence of memantine (Figs. 1, 2, 3, 5, and 6). The results of clinical grading are analyzed separately for both series of experiments. ANOVA (one-way procedure) was used for differences between the experimental groups in Table 2 and Fig. 4. When the ANOVA procedure showed statistical significance, the Scheffé procedure was used for individual group comparison. Levels of significance were set at  $P < .05$ .

## RESULTS

**General Aspects.** Blood ammonia concentration in AI-PCS rats reached a steady-state concentration of approximately 1,000 µmol/L within 1 hour after the start of the ammonium-acetate infusion. This steady state was maintained during the rest of the infusion (Fig. 1A). Memantine administration (either i.p. or i.v.) did not affect the blood ammonia levels in these rats. The mean arterial pressure increased during the infusion from approximately 100 mm Hg to approximately 140 mm Hg in all AI-PCS rats (data not shown).

Blood ammonia in treated and untreated LIS rats also reached concentrations of approximately 1,000 µmol/L, but it increased gradually to values of 2,000 µmol/L when severe encephalopathy had developed (Fig. 1B). Mean arterial pressure in LIS rats showed, similar to AI-PCS rats, a gradual increase from approximately 100 to 150 mm Hg, and blood glucose concentrations remained between 6 and 10 mmol/L (data not shown).

**Severity of Encephalopathy.** Clinical grading of encephalopathy and EEG spectral analysis showed that the AI-PCS rats developed encephalopathy within the 6 hours of ammonium-acetate infusion (Fig. 2). However, when rats were treated with memantine (either i.p. or i.v.), a significantly smaller increase in clinical grade was observed (Fig. 2A;  $P < .05$ ). Additionally, separate analysis of the subgroups (rats with a cisterna magna cannula or skull electrodes) showed significant differences in clinical grade ( $P < .05$ ). For this reason, the data were pooled together in Figs. 2 and 3. Administration of this NMDA-receptor antagonist further resulted in a positive effect on EEG activity. After both i.p. and i.v. injection, EEG left index showed a smaller increase, in which the EEG left index after i.p. administration was significantly lower than untreated rats (Fig. 2B;  $P < .05$ ). The MDF showed a significantly smaller decrease in both treated groups compared with controls (Fig. 2C;  $P < .0001$ ).

LIS rats developed encephalopathy within 3 to 6 hours after devascularization. The experiment was ended within 7 hours (when clinical grade 5 was reached), either by "spontaneous" death (60%) or by killing (40%). Memantine was administered at a dose of 10 to 20 mg/kg body weight i.p. when righting reflex disappeared (clinical grade 3), and, as shown in Fig. 3, the NMDA-receptor antagonist induced a significant improvement in clinical grading ( $P < .05$ ), in EEG left index ( $P < .05$ ) and in MDF ( $P < .01$ ), during the registration period of 3 hours. After this, the clinical manifestations of the LIS rats deteriorated comparable with that in LIS rats without memantine. However, none of the treated LIS rats died spontaneously when clinical grade 5 was reached.

In addition, EEG spectral analysis was performed during 4 hours in normal rats that received memantine (i.p. 10 mg/kg body weight). EEG left index did not change after memantine administration: the initial value of  $0.73 \pm 0.01$  was simi-

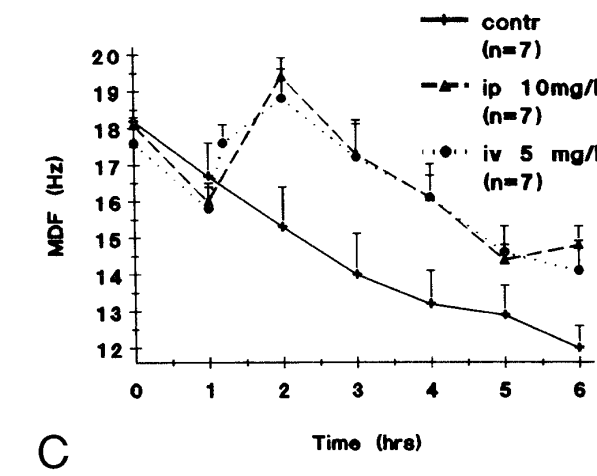
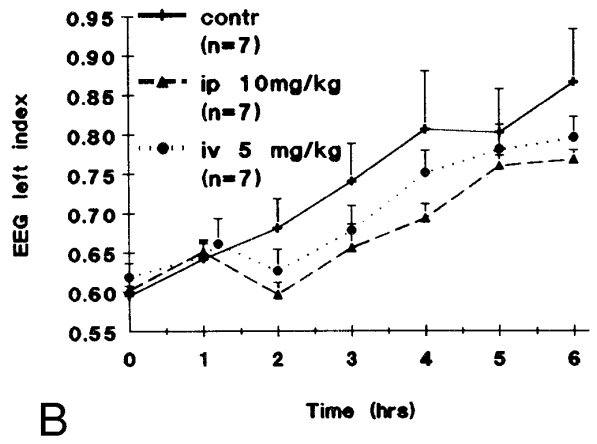
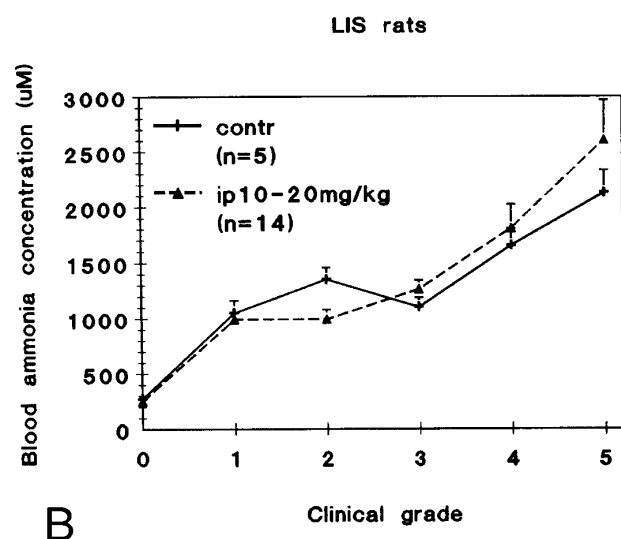
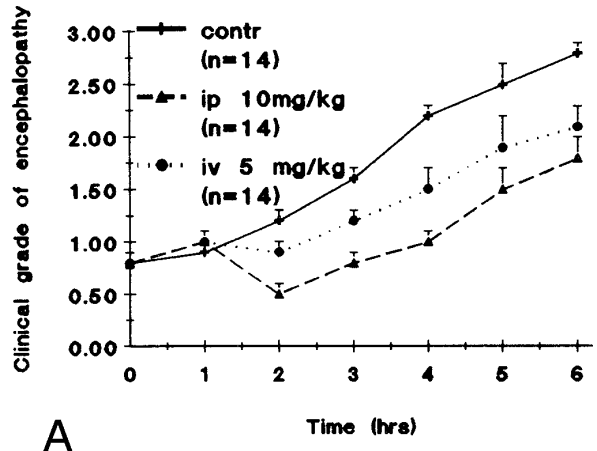
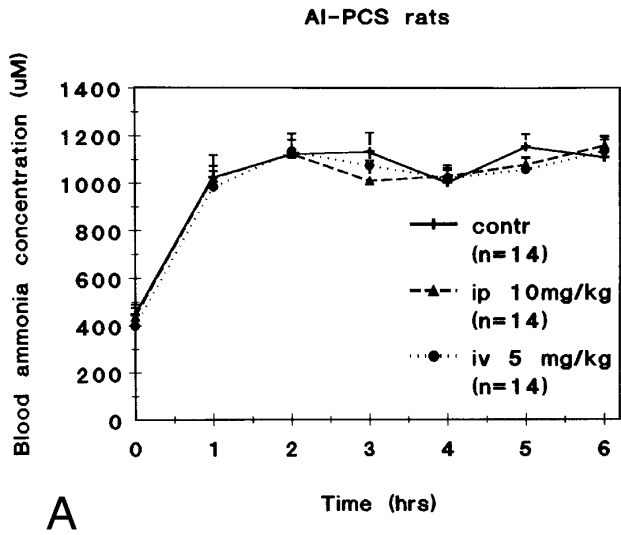


FIG. 1. The effect of memantine administration (i.p. 10-20 mg/kg body weight or i.v. 5 mg/kg body weight) on blood ammonia concentration in (A) AI-PCS rats and (B) LIS rats. (X-axis in AI-PCS rats is expressed as time whereas x-axis in LIS rats is expressed as clinical grade.) Values are expressed as means  $\pm$  SEM. Statistical analysis was performed by means of repeated MANOVA. Blood ammonia concentrations were not significantly different in memantine-treated rats compared with control rats.

FIG. 2. The effect of memantine administration (i.p. 10 mg/kg body weight or i.v. 5 mg/kg body weight) in AI-PCS rats on (A) clinical grade of encephalopathy, (B) EEG left index, and (C) MDF. Values are expressed as means  $\pm$  SEM. Statistical analysis was performed by means of repeated MANOVA. Clinical grade and EEG left index were significantly lower in memantine-treated rats compared with controls ( $P < .05$ ). MDF was significantly higher in memantine-treated rats compared with controls ( $P < .0001$ ).

lar at 1 hour after memantine administration and had a value of  $0.74 \pm 0.2$  at 4 hours. MDF changed from  $15.2 \pm 0.4$  Hz to  $17.1 \pm 0.6$  Hz at 1 hour after memantine administration and was  $16.0 \pm 0.7$  Hz at 4 hours.

**Memantine Concentrations in Plasma and Brain.** Intraperitoneal injection of 10 mg/kg body weight memantine, given

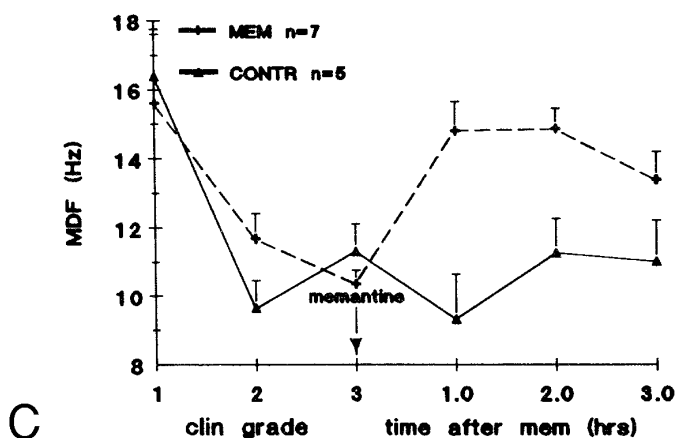
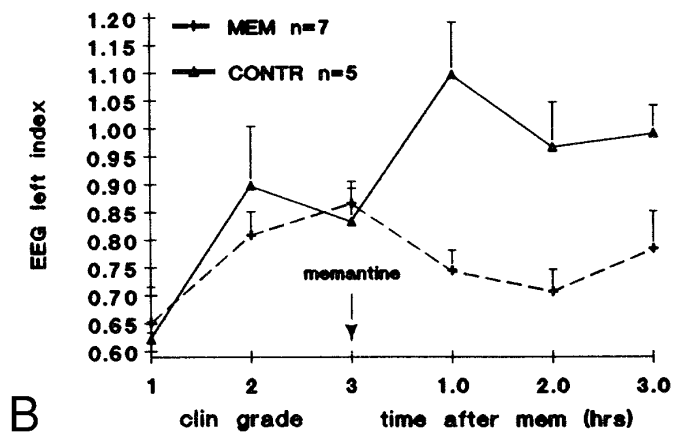
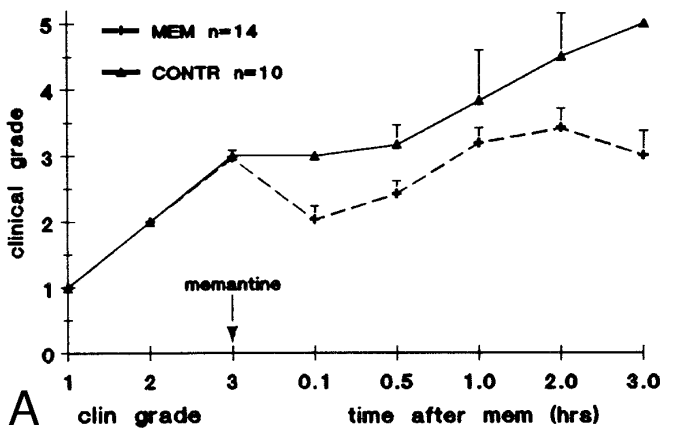


FIG. 3. The effect of memantine administration (i.p. 10-20 mg/kg body weight) in LIS rats on (A) clinical grade of encephalopathy, (B) EEG left index, and (C) MDF. X-axes are expressed as clinical grade until memantine is administered. From the time point of administration time is given after memantine administration. Values are expressed as means  $\pm$  SEM. Statistical analysis was performed by means of repeated MANOVA. Clinical grade and EEG left index were significantly lower in memantine-treated rats compared with controls ( $P < .05$ ). MDF was significantly higher in memantine-treated rats compared with controls ( $P < .01$ ).

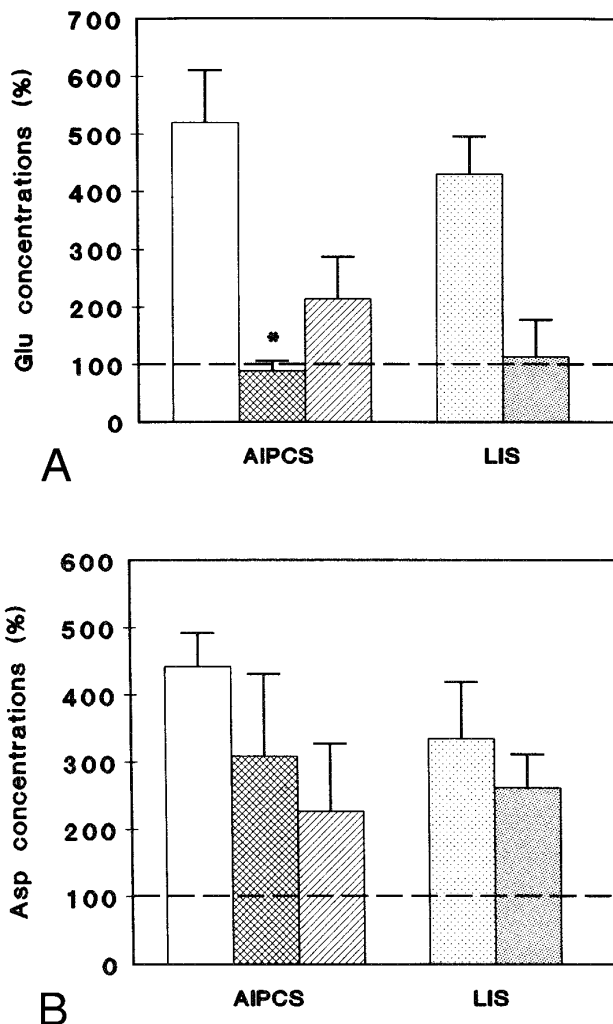


FIG. 4. The effect of memantine administration (i.p. 10-20 mg/kg body weight or i.v. 5 mg/kg body weight) on (A) CSF concentrations of glutamate, and (B) CSF concentrations of aspartate in AI-PCS rats and LIS rats at the end of the experiment (expressed as a percentage of initial value in each rat, means  $\pm$  SEM). Statistical analysis was performed by one-way ANOVA for differences between the experimental groups (which was statistically significant for CSF glutamate concentrations). The Scheffé procedure was used for individual group comparison for glutamate concentrations. \* $P < .05$  i.p.-treated AI-PCS rats versus control AI-PCS rats.  $\square$  control AI-PCS (n = 5);  $\otimes$  i.v. 10 mg/kg (n = 5);  $\text{▨}$  i.v. 5 mg/kg (n = 6);  $\text{▩}$  control LIS (n = 4); and  $\text{▧}$  i.v. 10-20 mg/kg (n = 4).

1 hour after the start of the infusion, resulted in a higher plasma memantine concentration measured at the end of the experiment (i.e.,  $334 \pm 32$  ng/mL) compared with i.v. injection of 5 mg/kg body weight memantine (i.e.,  $196 \pm 16$  ng/mL). Memantine concentration in brain was  $5.74 \pm 0.63$   $\mu$ g/g brain wet weight (n = 4 in AI-PCS rats with i.v. injection of memantine).

**Amino Acid Concentrations in CSF.** CSF concentrations of glutamate and aspartate were elevated in AI-PCS and LIS rats at the end of the experiment (Fig. 4A and 4B). Memantine administration in both rat models resulted in a smaller increase in CSF glutamate concentrations ( $P < .05$  in i.p.-treated AI-PCS rats versus control AI-PCS rats) and a tendency toward a smaller increase in CSF aspartate concentrations. Other amino acids did not show any significant differences after memantine administration (data not shown).

**ICP and Brain Water Content.** After ammonium-acetate infusion, ICP increased in all AI-PCS rats from approximately 2 mm Hg to variable values of 5 to 20 mm Hg (Fig. 5). How-

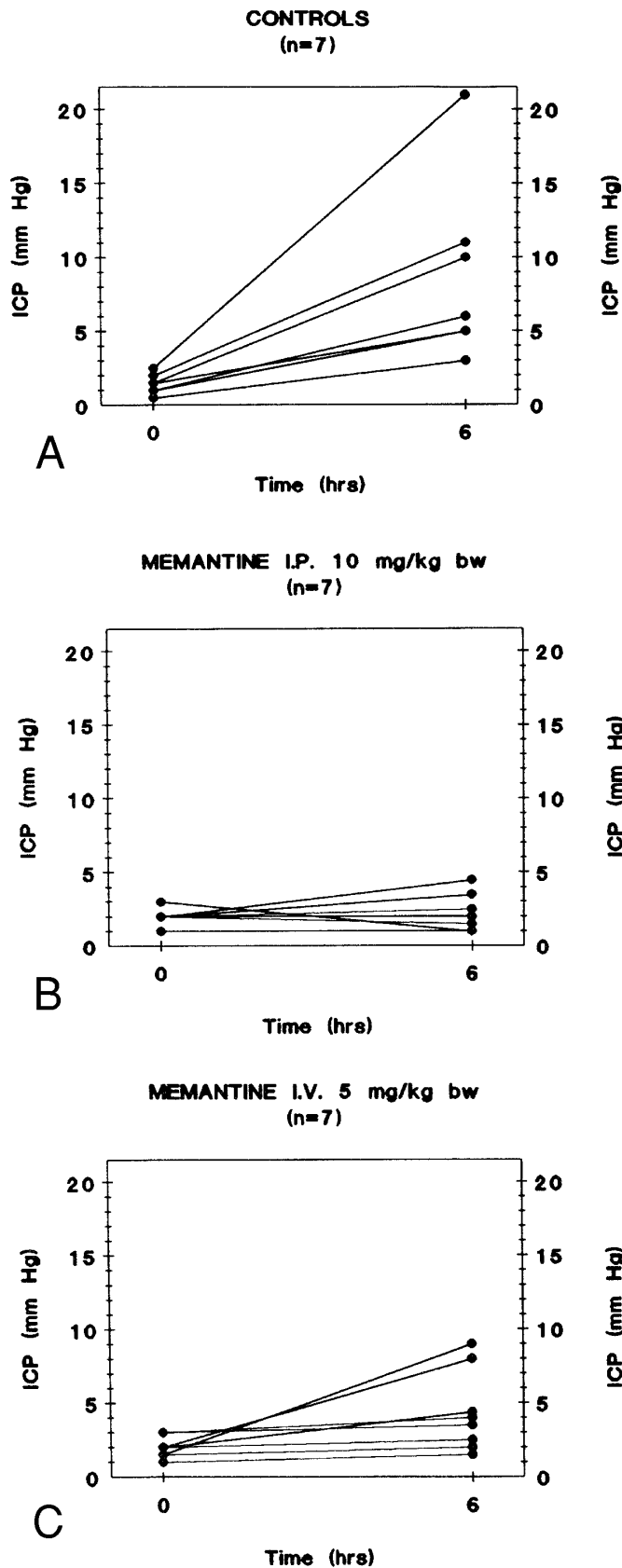


FIG. 5. The effect of memantine administration ([B] i.p. 10 mg/kg body weight or [C] i.v. 5 mg/kg body weight) on ICP in AI-PCS rats. Each line represents one individual rat. Statistical analysis was performed by repeated MANOVA. ICP was significantly different in memantine i.p.-treated rats compared with (A) controls ( $P < .01$ ).

ever, ICP in AI-PCS rats with i.p. administration of memantine remained constant with a maximal value of 5 mm Hg, and was significantly lower than in controls ( $P < .01$ ). Although ICP in AI-PCS rats with i.v. administration of memantine was not significantly lower than in controls, only two of the seven rats reached an ICP of 8 and 9 mm Hg. Memantine administration in LIS rats did not result in a lower ICP (Fig. 6). ICP of LIS rats with and without memantine increased from approximately 3 mm Hg to values of 10 to 25 mm Hg.

As shown in Table 2, brain water content of AI-PCS rats is significantly higher than brain water content in normal rats.<sup>9</sup> Although the difference is small, LIS rats show an even higher brain water content than AI-PCS rats. Memantine administration resulted in a significantly lower brain water content in AI-PCS rats only, compared with untreated rats.

## DISCUSSION

The exact pathophysiological mechanism of HE is still a matter of debate, but it is believed to be multifactorial. Previ-

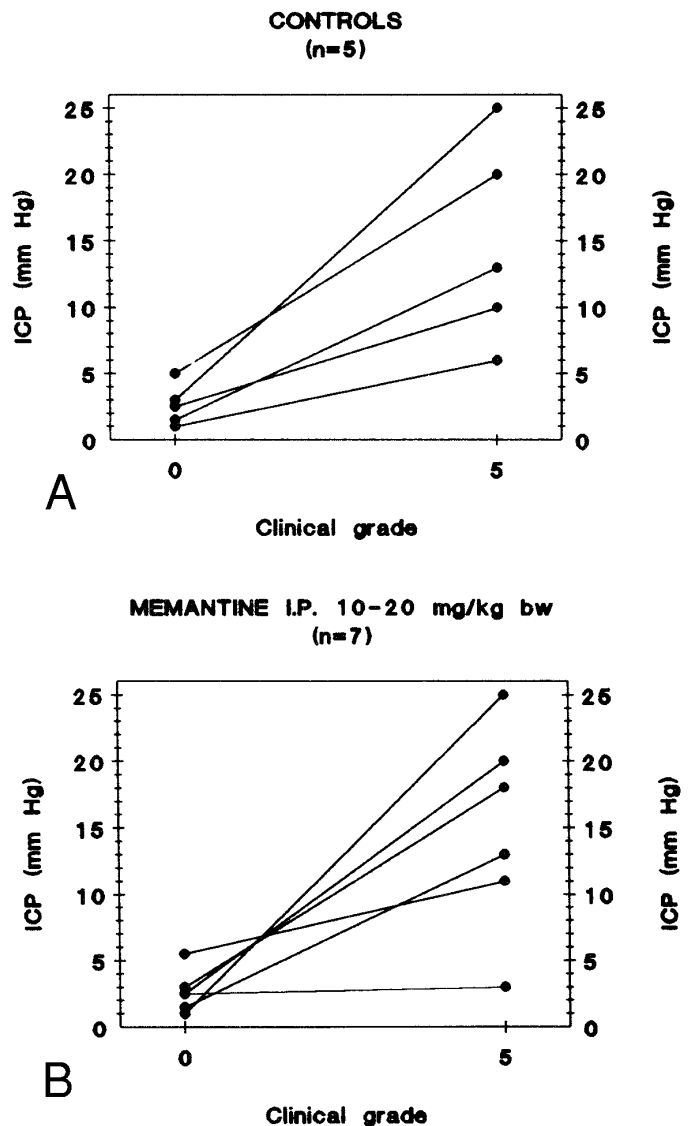


FIG. 6. The effect of memantine administration ([B] i.p. 10-20 mg/kg body weight) on ICP in LIS rats. Each line represents one individual rat. Statistical analysis was performed by repeated MANOVA. ICP was not significantly different in memantine-treated rats compared with controls (A).

TABLE 2. Brain Water Content in Normal Rats, AI-PCS Rats, and LIS Rats

Normal (n = 3)	AI-PCS			LIS	
	Control (n = 7)	MEM i.p. (n = 7)	MEM i.v. (n = 7)	Control (n = 4)	MEM i.p. (n = 4)
80.90 ± 0.07	81.96 ± 0.14*	81.31 ± 0.10†	81.24 ± 0.14†	82.43 ± 0.21*	82.64 ± 0.25

NOTE. One-way ANOVA was used for differences between the experimental groups (which was statistically significant). The Scheffé procedure was used for individual group comparisons. Data are expressed in percentages as means ± SEM. Memantine was administered to AI-PCS rats either i.p. 10 mg/kg body weight or i.v. 5 mg/kg body weight, and to LIS rats i.p. 10-20 mg/kg body weight.

Abbreviation: MEM, memantine.

\*  $P < .05$  versus normal rats.

†  $P < .05$  versus control AI-PCS rats.

ous data of our experimental model of hyperammonemia-induced encephalopathy in rats have suggested a role for elevated extracellular concentrations of glutamate and aspartate in brain.<sup>9</sup> The increase in these excitatory amino acids may finally result in NMDA-receptor overactivity. This overstimulation of NMDA-receptor activity can induce cell swelling and/or neuronal degeneration.<sup>19,20</sup> Cell swelling is caused by an increased influx of sodium, chloride, and water, and neuronal degeneration is the result of an increased influx of calcium. Because neuronal damages are not common in HE, we assume that astrocyte swelling is the most important negative consequence of NMDA-receptor overstimulation in encephalopathy. The mechanism by which NMDA-receptor overstimulation induces astrocyte swelling can be twofold: 1) indirectly by increasing the release of glutamate from neurons (shown by Bustos et al.<sup>32</sup>), possibly resulting in astrocyte swelling,<sup>33</sup> or 2) directly by increasing the ion influx ( $\text{Na}^+$ ,  $\text{Cl}^-$ ) into the astrocyte, if NMDA receptors are present on its membrane.<sup>34</sup> However, the presence of NMDA receptors on astrocytes has been suggested but not yet been proven.<sup>35</sup> If the NMDA-receptor-mediated cell swelling together with the suggested cell swelling induced by increased concentrations of intracellular brain glutamine<sup>8</sup> is not restricted, life-threatening intracranial hypertension will finally develop.

To test the possible role of NMDA-receptor overactivity in two different rat models of encephalopathy (i.e., hyperammonemia-induced encephalopathy and acute HE), the effect of the noncompetitive NMDA-receptor antagonist memantine was studied in AI-PCS rats and LIS rats. Memantine has been used in neurological diseases like Parkinson's disease, dementia, and coma.<sup>36-38</sup> The therapeutic effect of memantine is caused by selective blocking of the open ion-channel of the NMDA receptor<sup>21,23,39,40</sup> in a noncompetitive way, and its fast kinetics and voltage dependency are probably responsible for the lack of side effects at therapeutically used doses. It therefore seemed worthwhile to study this lipophilic drug, with high affinity for the CNS,<sup>41</sup> in experimental encephalopathy. If NMDA-receptor overactivity does play a role in the pathogenesis of hyperammonemia-induced encephalopathy and acute HE, blockade of the NMDA receptor could be able to attenuate encephalopathy in both models.

Intraperitoneal and intravenous administration of memantine resulted in plasma concentrations of  $334 \pm 32$  ng/mL and  $196 \pm 16$  ng/mL at the end of the experiment, respectively. Because the CSF/plasma ratio of memantine has a value of 0.5,<sup>42</sup> plasma concentrations of about 200 ng/mL will result in CSF concentrations of about 100 ng/mL (ie, approximately 0.5  $\mu\text{mol/L}$ ). The  $K_d$  value of memantine for the NMDA receptor at normal resting membrane potentials is approximately 1  $\mu\text{mol/L}$  in rats,<sup>21,40</sup> indicating that the doses of memantine used in our study could be expected to inhibit NMDA-receptor activity by at least 50% to 80%.

In agreement with our hypothesis, both i.p. and i.v. administration of memantine in AI-PCS rats and i.p. administration in LIS rats attenuated the clinical manifestations of encephalopathy (i.e., clinical grade and EEG activity)

significantly. These results suggest that overstimulation of the NMDA-receptor activity is implicated in the pathogenesis of hyperammonemia-induced encephalopathy and acute HE. In addition, the improvement in EEG activity after NMDA-receptor blockade observed in both models suggests that NMDA-receptor activation is associated with changes in EEG activity. Indications of excitatory amino acid-induced alterations in EEG activity have been reported by Kuchiwaki et al.<sup>43</sup>

Despite high blood ammonia concentrations, brain water content and ICP were significantly decreased in AI-PCS rats after memantine administration. Assuming a possible link between neurological signs and cell swelling, this suggests that the improvement in clinical manifestations of encephalopathy in AI-PCS rats may be at least partially caused by a reduction in cell swelling. However, memantine did not affect brain water content or ICP in LIS rats, although clinical manifestations were improved. In this case, however, clinical grading and EEG spectral analysis were measured directly after memantine administration, whereas ICP and brain water content were measured only at the end of the experiment, the time point at which the clinical manifestations of the treated LIS rats were similar to control LIS rats. Thus, it may be possible that NMDA-receptor blockade initially did result in a reduction of ICP and brain water content, which could result in an improvement of clinical manifestations of encephalopathy. Ultimately, however, the blockade was insufficient with resultant increased ICP, increased brain water content, and deterioration of clinical manifestations.

Other possible explanations for the absence in reduction of brain water content or ICP in LIS rats are: 1) differences in the type of brain edema (e.g., more cytotoxic in the LIS rats), and 2) relatively more contribution of glutamine-induced osmotic swelling than NMDA-receptor overactivity. However, we cannot exclude the possibility that NMDA-receptor blockade would have reduced cell swelling in LIS rats if memantine was administered earlier. In that case, we assume that memantine was administered to these rats at the moment that brain edema was no longer reversible.

Memantine treatment further resulted in a smaller or no increase in CSF concentrations of glutamate. This can be explained by the fact that stimulation of NMDA-receptor activity also promotes glutamate release, which is in agreement with the data of Bustos et al.<sup>32</sup> They reported that the addition of NMDA to the dialysate perfusion induces a release of glutamate and aspartate, measured by *in vivo* brain dialysis in rats. After administration of MK801, another noncompetitive NMDA-receptor antagonist, the increase of glutamate and aspartate in brain dialysate was inhibited, indicating that NMDA-receptor activity enhances the release of glutamate and aspartate.

The stimulation of glutamate release by NMDA-receptor activity might be mediated by nitric oxide (NO), which is increased after NMDA-receptor activity by stimulation of NO synthase. Montague et al.<sup>44</sup> showed that NMDA-receptor-mediated NO production and its diffusion into the extracellu-

lar compartment induces a release of glutamate. Blockade of NO synthase resulted in an inhibition of glutamate release.

Several explanations are possible for the increase in extracellular glutamate and aspartate concentrations in HE. Initially, the increase of these excitatory amino acids has been explained by an inhibition of the re-uptake by increased ammonia concentrations in synaptosomes.<sup>45</sup> However, we have shown in a previous study that glutamate and aspartate concentrations in CSF of PCS rats with severe hyperammonemia were significantly higher than in normal rats with similar increased brain and CSF concentrations of ammonia.<sup>9</sup> This implied that the increase in extracellular glutamate and aspartate could not only be caused by increased brain ammonia concentrations. We suggested that these amino acids are released as a consequence of cell swelling, probably caused by the significantly higher increase in brain glutamine concentrations in PCS rats than in normal rats. This explanation is in agreement with other *in vitro* studies that showed that aspartate and glutamate are released after cell swelling.<sup>46,47</sup> However, the release of glutamate can also be induced indirectly by NMDA-receptor activity that results in NO production as mentioned above. Apparently, the release of glutamate by overactivity of the NMDA receptor plays a more dominant role under our experimental conditions, because memantine was able to reduce extracellular glutamate concentrations to control values.

In conclusion, the present data indicate that overstimulation of NMDA-receptor activity by increased extracellular concentrations of the excitatory amino acids, glutamate and aspartate, contribute to the pathogenesis of hyperammonemia-induced encephalopathy and acute-HE. Furthermore, the results suggest that noncompetitive NMDA-receptor antagonists like memantine are of potential therapeutic value for this syndrome.

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