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The influence of NMDA, a potent agonist of glutamate receptor, on behavioral activity of rats with experimental hyperammonemia evoked by liver failure

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Summary. The study was designed to investigate the effects of NMDA receptor agonist on the behavioral activity in rats with experimental hyperammonemia. The experiments were performed on adult male Wistar rats. Experimental hyperammonemia was induced by intraperitoneal injections of tioacetamide (TAA, 200 mg/kg) for three consecutive days. Rats treated with saline (0.9%) served as control. Stimulation of the NMDA glutamatergic receptor was evoked by *ip.* injection of agonist N-methyl-D-aspartate acid (NMDA) in a dose of 30 mg/kg thirty minutes before experiments. Memory motivated affectively was evaluated in the passive avoidance responses. The speculative influence of the treatment on anxiety and motor activity was tested in elevated plus-maze and in open field respectively.

To show change of NMDA receptor function after various doses of agonist, the seizures evoked by N-methyl-D-aspartate acid was carried out. This experiment showed that with rise of dose of NMDA time to appear of convulsions was contracted in rats with hyperammonemia as well as in control rats. Dose of NMDA caused convulsions was three times as less in rats with hyperammonemia than dose in control. Time of duration of convulsions was proportional to applied dose of NMDA and it lengthened with rise of agonist's dose in both groups of studied animals.

Furthermore, we observed that NMDA increased motor activity of control rats in open field test, but not in rats with hyperammonemia (treated tioacetamide). Hyperammonemia did not have significant influence on motor activity and on a passive avoidance latency. The NMDA given in control and in hyperammonemia, increased acquisition, consolidation and recall of a passive avoidance responses. Moreover, NMDA had anxiogenic-like profile in elevated plus-maze.

In rats with hyperammonemia NMDA had no influence on locomotor activity but it significantly increased memory in a passive avoidance responses. Furthermore, we observed that reactivity of NMDA glutamate receptor in rats with hyperammonemia was higher than in control rats.

Keywords: NMDA – Hyperammonemia – Behavior – Rats

Introduction

In the mammalian brain, glutamate is the most prominent excitatory neurotransmitter. Glutamate receptors are widely distributed throughout different regions on the brain and can be divided into two majors types: ionotropic and metabotropic. Ionotropic glutamate receptors (iGluRs) are intristic ligand-gated ion channels for sodium and calcium cations whereas metabotropic glutamate receptors (mGluRs) are coupled to intracellular signal transduction pathways via membrane bound G proteins.

In recent years much attention has been concentrated on the relationship between neurochemistry and behavior (Myhrer et al., 1992). Within this research the role of the glutaminergic N-methyl-D-aspartate (NMDA) receptor has received special interest. Because NMDA receptors are believed to be involved in long-term potentiation (LTP), this receptor type seems to be very important of learning and memory (Davis et al., 1988; Mondadori et al., 1988; Morris, 1986; Olton, 1988).

Both the glutamate-nitric oxide-cGMP pathway and the NMDA receptor-dependent LTP are involved in some forms of learning and memory formation (Bliss et al., 1993; Danysz et al., 1995; Chen et al., 1997; Meyer et al., 1998). It is well know, that pretraining administration of NMDA receptor antagonists impairs the acquisition of the passive avoidance task (Riekkinen et al., 1996; Schmidt et al., 1997), while postraining administration also impairs memory in passive avoidance tasks (Cestari et al., 1997).

It is well known that ammonia at elevated concentrations is toxic to the brain (Plum, 1976; Gjedde, 1978). Ammonia can exert diverase effects on nervous tissue, including membrane depolarization (Fan and Szeb, 1993; Raabe, 1990), alternation of intracellular pH (Gilette, 1983), increased conversion of glutamate to glutamine and respiratory enzymes (Cooper and Plum, 1987).

Hyperammonemia affects normal brain functioning in many ways; it may disturb basic cell functions, electrophysiology and biochemical neurotransmission (Plum, 1976; Gjedde, 1978; Hoyumpa, 1979; Zieve, 1981; Benjamin, 1982). However, the molecular mechanisms by which hyperammonemia leads to impaired cerebral function have not been clarified (Muñoz et al., 2000). It has been reported that chronic hyperammonemia impairs NMDA receptor associated signal transduction pathways (Hermenegildo et al., 1998). On the other hand, Monfort et al. (2002), observed that acute intoxication with large doses of ammonia leads to activation of NMDA receptors *in vivo*.

The purpose of our study was to investigate an influence of NMDA an agonist of glutamate receptor on behavior in rats with hyperammonemia.

Material and methods

Animals

The study was conducted on white, male Wistar rats weighing 200–220 g. They were housed in cages (55 × 40 × 20 cm), 8 animals per cage, in an air conditioned room under 12 h light/12 h dark cycle beginning at 07 h. The animals were fed standard diet, food and water were freely accessible. The experimental procedures applied in this study were in compliance with the Board for Ethic Affairs and Supervision over Research on Animals and Individuals, Medical Academy of Białystok.

Hyperammonemia induced by liver failure

Experimental hyperammonemia was induced by Tioacetamide Sigma (USA). The intraperitoneal injections of tioacetamide (TAA) at the dose of 200 mg/kg per rat, as a freshly prepared TAA solution were given for three consecutive days. In this work the blood ammonia level was measured by Blood Ammonia test. In rats treated TAA a marked increase of blood ammonia was observed. It increased about 153% to control rats (blood ammonia level in rats treated TAA was 176 μmol/l, in control rats it was 57.4 μmol/l).

Drugs

NMDA Tocris (UK) at the dose of 15 mg/kg or 30 mg/kg per rat were injected *ip.* The injections, as a freshly prepared NMDA solution, were given 30 min before the open field and elevated plus maze tests or before the trial on the second day of the experiment in acquisition stage and on the 3rd day of the test in recall of the passive avoidance situation. In consolidation of passive avoidance responses NMDA was injected immediately after the trial on the 2nd day of experimenting passive avoidance situation. The control rats received 0.9% NaCl Polfa (Poland) *ip.*

After termination of each experiment, all animals were anesthetized with chloral hydrate at the dose of 0.4 g/kg per rat and after that they were killed by decapitation.

Before behavioral tests NMDA receptor reactivity to various doses of agonist was examined.

Seizures evoked by NMDA

In our examinations we had regard to NMDA convulsive property with increase receptor activity. This test was experimented to show change of NMDA receptor function after various doses of agonist (NMDA). NMDA at the doses of 15, 30, 60, 90, 100, 110, 120 mg/kg per rat were injected *ip.* The injections, as a freshly prepared NMDA solution were given for rats with hyperammonemia and for control rats as well.

Time of appear of convulsions as well as time of duration of them in dependence from dose NMDA was estimated.

Behavioral tests

All experiments were carried out in a quiet, diffusely lit room between 08 h and 13.00 p.m. with each group equally represented at the times of testing. Rats were randomly allocated to treatment groups and used only once. Passive avoidance responses were selected to estimate acquisition, consolidation and recall memory. Moreover, the putative influence of the treatment on anxiety and motor activity was tested in elevated plus-maze and in open field, respectively.

Passive avoidance responses training

The response was induced using the one-trial-learning method of Ader et al. The apparatus consisted of a 6 × 25 cm platform illuminated with a 25 W electric bulb connected through a 6 × 6 cm opening with a dark compartment (40 × 40 × 40 cm). The floor of the cage was made of metal rods 3 mm in diameter, spaced at 1 cm. The investigation took advantage of the natural preference of rats to stay in dark compartments. The test lasted 3 days. On the first day, after 2 min of habituation in the dark compartment, rats were immediately removed. Two similar trials, at an interval of 2 min, were carried out on the second day. After the first trial rats were allowed to stay in the dark compartment for 10–15 s. In the second trial when a rat entered the dark compartment it received a foot shock (0.25 mA, 3 s) delivered through the metal rods. The presence of the passive avoidance was checked 24 h later. Rats were placed on the illuminated platform once more and latency to enter the dark compartment was measured, with the cut time of 300 s. To determine the effect drug treatment on retrieval, according to the protocol proposed by Matthies NMDA was administrated on the third day 30 min before retention test. To determine NMDA effect on consolidation, treated drug was given immediately after the completion of induction of passive avoidance.

Locomotor and exploratory activity

The open field test was used for estimation of locomotor activity of rats. The apparatus consisted of a square with 100 × 100 cm white floor, which was divided by 8 lines into 25 equal squares, and surrounded by white wall, 47 cm high. Four plastic bars, 20 cm high, were located at four different line crossing in the central area of the floor. A single rat was placed inside the apparatus for 1 min of adaptation. Subsequently, crossing, rearings and bar approaches were counted manually for 5 min. NMDA was given 30 min before the test.

Elevated "plus" maze

The maze (constructed of grey coloured wooden planks) consisted of two open arms, 50 cm (length) × 10 cm (width) and two closed arms, 50 cm (length) × 10 cm (width) × 40 cm (height), covered with a removable lid, such that the open or closed arms were opposite to each other. The maze was elevated to a height of 50 cm from the floor. 30 minutes after injection a native rat was placed for the 5 min in a pretest arena (60 × 60 × 35 cm, constructed from the same material) prior to exposure to the maze. This step allows the facilitation of exploratory behavior. The experimental procedure was similar to that described by Pellow et al. Immediately after the pretest exposure rats were placed in the centre of the elevated "plus" maze facing one of the open arms. During the 5 min test period the following measurements were taken: the number of entries into the open and closed arms and the time spent in the open and closed arms. An entry

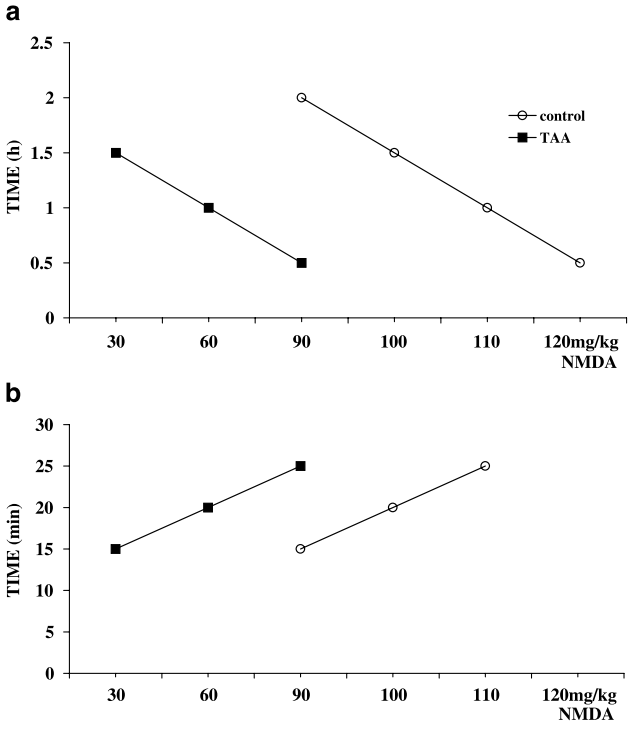

Fig. 1a. Seizures evoked by NMDA. Time of appears of convulsions in dependence of dose of NMDA. Control group received N-methyl-D-aspartic acid in doses from 15 mg/kg to 120 mg/kg. Rats with hyperammonemia received NMDA in doses from 15 mg/kg to 90 mg/kg. **b** Seizures evoked by NMDA. Time of duration of convulsions in dependence of dose of NMDA

was defined as all four feet into one arm. An increase in open arm entries and increase in time spent in open arms is indicative of potential anxiolytic activity, as rats naturally prefer the closed arms. NMDA was given 30 min before pretest.

Statistical analysis

The statistical significance of the results was computed by analysis of one-way variance (ANOVA) followed by t-student and by Newman-Keulus tests, except for passive avoidance behavior was assessed with Mann-Whitney ranking test. F – rations, degrees of freedom and p – values are reported only for significant differences. In all comparisons between particular groups a probability of 0.05 or less was considered significant.

Results

NMDA receptor reactivity test (Fig. 1a, b)

NMDA gave *ip.* in doses from 30 mg/kg to 120 mg/kg induced convulsions in both groups of animals. In rats with hyperammonemia single convulsions performed after NMDA in dose 30 mg/kg, and generalized convulsions in all rats performed after NMDA in dose 90 mg/kg. In control rats single convulsions performed after NMDA in dose 90 mg/kg, and generalized convulsions in all rats performed after NMDA in dose 120 mg/kg NMDA.

Time of duration of convulsions was proportional to applied dose of NMDA and it lengthened with rise of agonist's dose in both groups of studied animals.

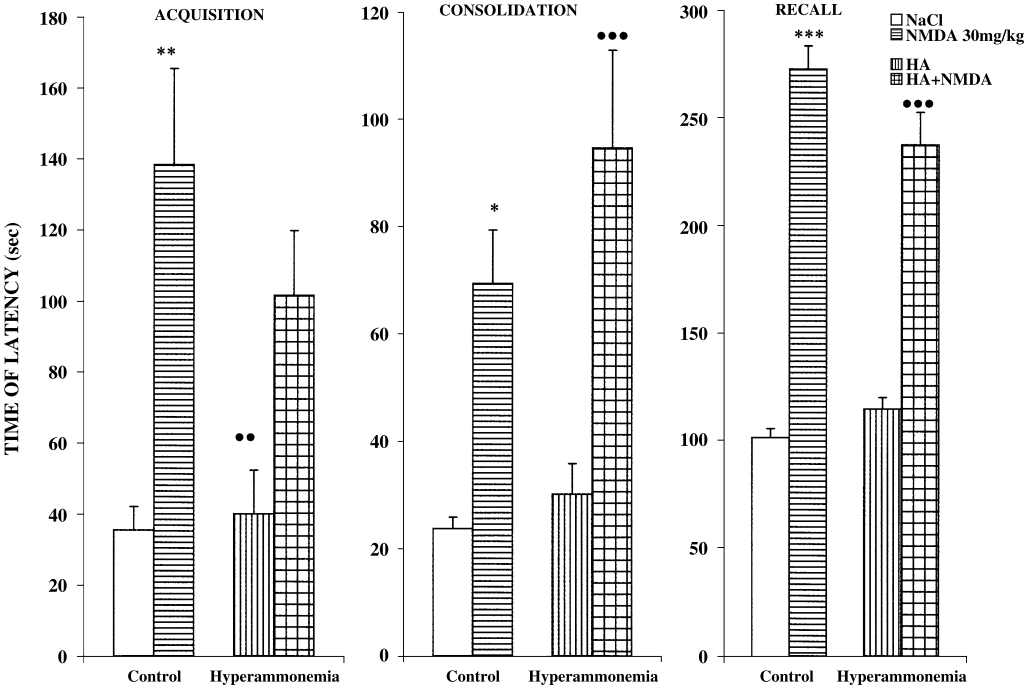

Fig. 2. Influence of N-methyl-D-aspartic acid (NMDA) at the dose of 30 mg/kg on acquisition, consolidation and recall of passive avoidance in rats with hyperammonemia. Control group received 0.9 NaCl. Columns represent means ± SEM of the results from 9–10 subjects. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs NaCl; $\bullet\bullet p < 0.01$; $\bullet\bullet\bullet p < 0.001$ vs hyperammonemia

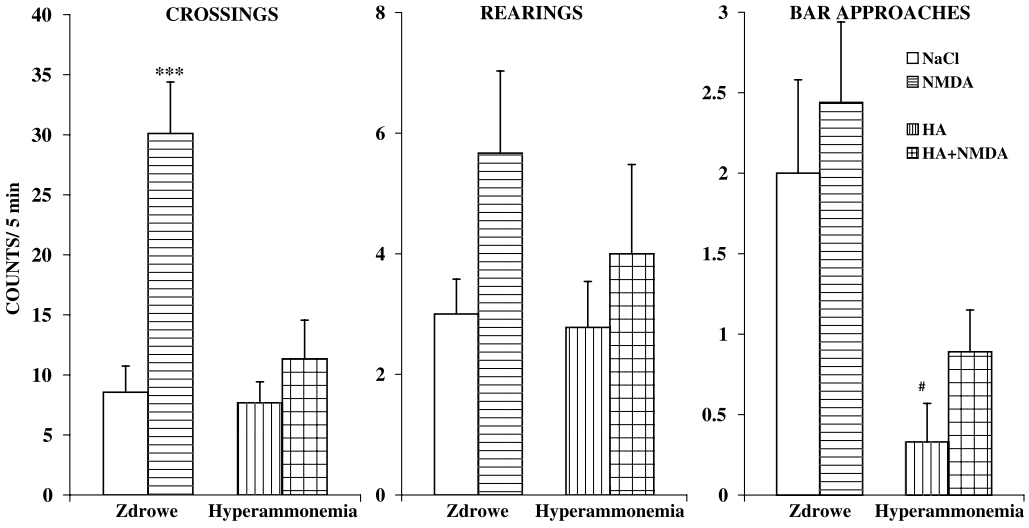

Fig. 3. Influence of N-methyl-D-aspartic acid (NMDA) at the dose of 30 mg/kg on locomotor and exploratory activity in the open field test in rats with hyperammonemia. Control group received 0.9 NaCl. Columns represent means ± SEM of the results from 9–10 subjects. $^{***}p < 0.001$ vs NaCl

Influence of NMDA on acquisition, consolidation and recall of passive avoidance in rats with hyperammonemia (Fig. 2)

NMDA given in a dose of 30 mg/kg significantly increased passive avoidance latency in control and in rats with hyperammonemia. Data showed that hyperammonemia had no influence on acquisition, consolidation and recall and had no change on influence of NMDA on memory in this test.

Influence of NMDA on locomotor and exploratory activity in rats with hyperammonemia in the open field test (Fig. 3)

NMDA given alone significantly increased locomotor activity in this test, but not in rats with hyperammonemia (TAA).

We observed that hyperammonemia had no influence on locomotor activity of rats measured by the number of crossings, rearings and bar approaches in the open field test.

Influence of NMDA on activity in the elevated plus maze test in rats with hyperammonemia (Fig. 4a, b)

The ANOVA test showed that there were significant differences between the groups in the time spent in the closed arms and open arms, and number of open arms entries. NMDA (alone and in rats with hyperammonemia) had anxiogenic-like profile, because it increased the time spent in the closed arms and decreased the time

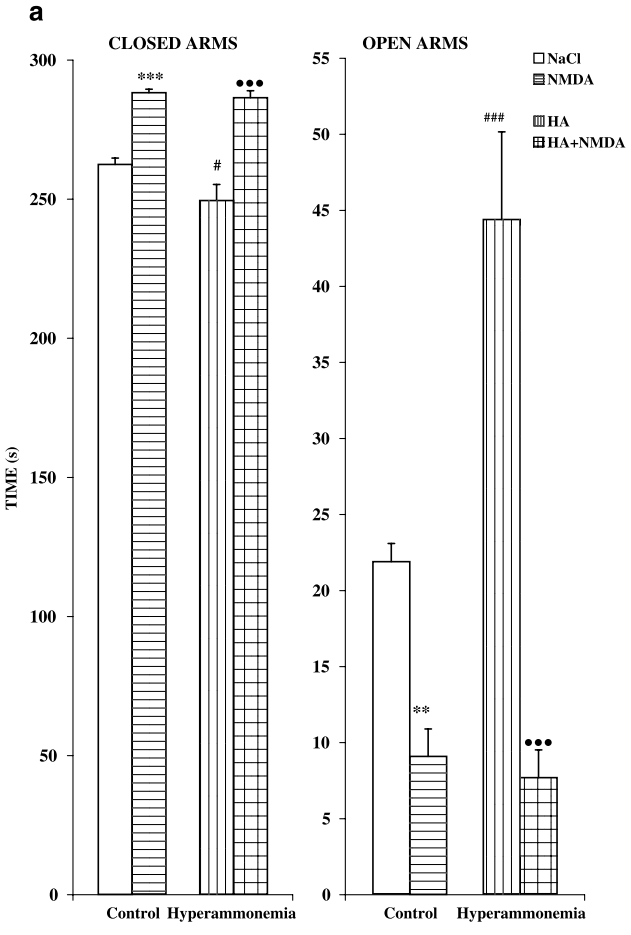

Fig. 4a, b. Influence of N-methyl-D-aspartic acid (NMDA) at the dose of 30 mg/kg on activity in the elevated plus maze test in rats with hyperammonemia. Control group received 0.9 NaCl. Columns represent means ± SEM of the results from 9–10 subjects. $^{**}p < 0.01$; $^{***}p < 0.001$ vs NaCl; •••$p <$ 0.001 vs hyperammonemia; $^{\#}p < 0.05$; $^{\#\#\#}p < 0.001$ vs groups without hyperammonemia

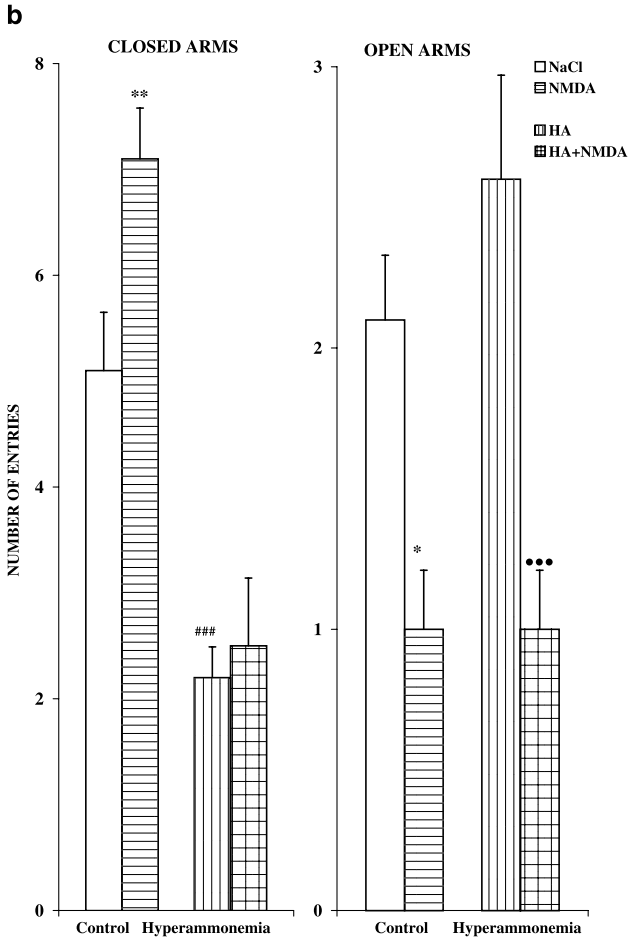

Fig. 4 (*continued*)

spent in the open arms and the number of entries to the open arms of the maze comparing to saline-treated group.

Hyperammonemia in rats produced opposite effects in plus-maze behavior. The parameters showing significant effect of hyperammonemia vs. the control were as follows: increased time spent in the open arms, and decreased time spent in the closed arms of the maze comparing to the control group. This action suggests anxiolytic-like effect of hyperammonemia.

NMDA had anxiogenic-like profile in elevated plus-maze, because it increased the time spent in the closed arms and decreased the time spent in the open arms in both HA and control rats.

Discussion

It is known that it is correlation between NMDA convulsive property and increase receptor activity. Seizures evoked by NMDA was carried out to show change of NMDA receptor function after various doses of agonist in both groups of studied animals. Our study showed that with rise of dose of NMDA time of appear of convulsions was contracted in both groups of animals. Dose of NMDA generated convulsions was three times as less in rats with hyperammonemia than dose in control rats. Time of duration of convulsions was proportional to applied dose of NMDA and it lengthened with rise of agonist's dose in both groups of studied animals. Results of this test showed that reactivity of NMDA receptor in hyperammonemia was altered.

Considering that all cognitive effects had to be expressed by psychomotor activity, the effect of the treatments on the general locomotor and exploratory behavior was checked in open field. We observed that hyperammonemia had no influence on crossings and rearings in locomotor activity but it significantly decreased the number of bar approaches. In our study we noticed a tendency to an increase in a number of crossings, rearings and bar approaches after the administration of NMDA at the dose of 30 mg/kg, but not in rats with hyperammonemia. These data are partially agree with previous observations (Aquilar et al., 1999) where hyperammonemia did not affect the motor behavior of rats exposed postnatally to ammonia. The number of bar approaches significantly decrease in rats with hyperammonemia seems to be subsequent to lower exploratory activity in those animals.

However, it is well reported that glutamate is critically involved in the control of motor behavior in the basal ganglia and that in rodents, the blockade of NMDA receptors increases motor activity (Schmidt et al., 1997). In contrast, our passed data showed that blockade NMDA by MK-801 have not changed the locomotor activity in the open field test (Car, Oksztel, Wiśniewski, 2000; Car et al., 2001).

On the other hand Carter et al. have observed that NMDA increased motor activity in rats (Carter et al., 1988). It is possible that the hyperammonemia induced in this experiment affects specifically some NMDA receptor-associated transduction pathways but not those involved in the control of motor activity.

Anxiety could also be an important factor in the experiments, so it was evaluated in elevated plus maze. We have observed that hyperammonemia produced anxiety enhancement in animals manifested by a increase number of entries into the open arms, and the time spent in these arms, which might suggest an anxiolytic-like activity. On the contrary to influence of hyperammonemia, the administration of NMDA evoked decrease of entries into open arms and the time spent in this arms. Our data showed that NMDA produced anxiogenic effects. The anxiolytic-like effect of hyperammonemia was inhibited by NMDA.

The NMDA receptor appears to play a crucial role in learning and memory, with calcium influx through the cationic channel being a critical signal transduction event for long-term potentiation, the putative cellular basis of learning (Patel et al., 1998). This crucial role of the glutamatergic system in learning processes has been indicated by numerous studies in which antagonists of NMDA receptors have been shown to disrupt acquisition of various learning tasks (Danysz et al., 1995).

It is know, that chronic hyperammonemia impairs the response of NMDA receptors to its antagonists in long-lasting rats pre- and neonatally exposed *in vivo* to ammonemia (Minana et al., 1995). Chronic moderate hyperammonemia impairs the neuronal glutamate-nitric oxide-cyclic GMP pathway both in cultured neurons and in the rat *in vivo* (Hermenegildo et al., 1998). Chronic hyperammonemia also impairs the induction of NMDA receptor-dependent long-term potentiation (LTP) in the CA1 region of the hippocampus (Muñoz et al., 1996).

When we examined behavior in passive avoidance situation, we found that acute hyperammonemia had no influence on the passive avoidance task. The NMDA at the dose of 30 mg/kg significantly facilitated acquisition, consolidation and recall in hyperammonemia and so in control rats. This data may agree with the theory, that acute intoxication with large doses of ammonia leads to activation of NMDA in rat brain *in vivo* (Hermnegildo et al., 2000). Also, it is necessary to take into consideration that acute hyperammonemia without influence on memory in rats can be a compensative mechanism to memory dysfunction following after chronic exposure to ammonia.

There are differences between acute and chronic exposure to ammonia on glutamate transport in brain glial cells. Chronic hyperammonemia elevates level of extracellular glutamate and the level of intracellular Glu is lower (Mort et al., 2001). A reduction in glutamate uptake when ammonia levels raised has been seen in cultured astrocytes after prolonged exposure to ammonia (Bender and Noremberg, 1996), in synaptosomes from animals with experimental liver failure (Oppong et al., 1995), and in brain slices from patients dying in hepatic encephalopathy (Schmidt et al., 1990). The data suggest that in rats with liver failure and in cultured astrocytes exposed to ammonia, there is reduced expression on the glial glutamate transporters GLT-1 (the main Glu transporter in the brain) and GLAST, suggesting that the increased extracellular glutamate level may result from decreased transporter expression (Haugeto et al., 1996; Knecht et al., 1997; Norenberg et al., 1997; Zhou and Norenberg, 1999).

Contrary, several reports indicate that acute hyperammonemia showed increased concentrations of the excitatory Glu in the cortical brain dialysate (de Knegt et al., 1994). During experimental hyperammonemia the glutamate concentration in the extracellular space and also basal glutamate levels in brain were increased (de Knegt, 1994).

Also, it is necessary to take into consideration that hyperammonemia leads to increase level of Glu. In this manner, Glu which is in excess may accumulate about NMDA receptors and this situation probably leads to hyperactivity of this receptors and sets the course of our data. Furthermore, increase in basal glutamate levels and hyperactivity of NMDA receptors in acute hyperammonemia supposed to be a compensative mechanism of the deficits of cognitive functions in future time.

It is known that NMDA receptors are involved in some effects of glutamatergic system on learning and memory (Morris, 1986; Davis et al., 1988; Mondadori et al., 1988; Olton, 1988). In our study we have shown that NMDA receptors may have an influence on cognitive functions in rats with hyperammonemia.

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References

Ader R, Weijenen JAWM, Moleman P (1972) Retention of passive avoidance response as function of the intensity and duration of electric shock. Psychon Sci 26: 125–129

Aguilar MA, Mirarro J, Felipo V (2000) Chronic moderate hyperammonemia impairs active and passive avoidance behavior and conditional discrimination learning in rats. Experimental Neurology 161: 704–713

Benjamin AM (1982) Ammonia. In: Plajtha A (ed) Handbook of neurochemistry, vol 1, 2nd edn. Plenum Press, New York

Bliss TVP, Collingride GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361: 31–39

Car H, Oksztel R, Wiśniewski K (2000) NMDA receptor antagonists are involved in behavioral activities of (S) 3,5-DHPG. 5th ECNP Regional Meeting St Petersburg Russia 15–17 May 2000. Eur Neuropsychopharmacol 10[Suppl 2] abstracts s 90 P 6.09

Car H, Oksztel R, Nadlewska A, Wiśniewski K (2001) NMDA receptor antagonists change behavioral activity of rats treated with (S)-4CPG. Pol J Pharmacol 53: 331–339

Carter CJ, L'Heureux R, Scatton B (1988) Differential control by N-methyl-D-aspartate and kainate of striatal dopamine release *in vivo*: a trans-striatal dialysis study. J Neurochem 51: 462–468

Chen J, Zhang S, Zuo P, Tadg L (1997) Memory-related changes of nitric oxide synthase activity and nitrite level in rat brain. NeuroReport 8: 1771–1774

Cooper AJ, Plum F (1987) Biochemistry and physiology of brain ammonia. Physiol Rev 67: 440–519

Danysz W, Zajączkowski W, Parsons CG (1995) Modulation of learning processes by inotropic glutamate receptor ligands. Behav Pharmacol 6: 455–474

Davis S, Butcher SP, Morris RGM (1988) The role of NMDA receptors in certain kinds of learning. In: Cavalheiro EA, Lehman J, Turski L (eds) Frontiers in excitatory amino acid research. Liss, New York, pp 385–392

de Knegt RJ, Schalm SW, van der Rijt CCD, Fekkes D, Dalm E, Hekking-Weyma I (1994) Extracellular brain glutamate during acute liver failure and during acute hyperammonemia simulating acute liver failure: an experimental study based on *in vivo* brain dialysis. J Hepatol 20: 19–26

Fan P, Szerb JC (1993) Effects of ammonium ions on synaptic transmission and on responses to quisqualate and N-methyl-D-aspartate in hippocampal CA1 pyramidal neurons *in vitro*. Brain Res 632: 225–231

Gillette R (1983) Intracellular alkalization potentiates slow inward current and prolonged bursting in a molluscan neuron. J Neurophysiol 49: 509–515

Gjedde A, Lockwood AH, Duffy TE, Plum F (1978) Cerebral flow and metabolism in chronically hyperammonemic rats: effect of an acute ammonia challenge. Ann Neurol 3: 325–330

Haugeto O, Ullenswang K, Levy LM, Chaudhry FA, Honore T, Nielsen M, Lehre KP, Danbolt NC (1996) Brain glutamate transporters from homomultimers. J Biol Chem 271: 27715–27722

Hermenegildo C, Montoliu C, Liansola M, Munoz MD, Gaztelu JM, Minana MD, Felipo V (1998) Chronic hyperammonemia impairs glutamate-nitric oxide-cyclic GMP pathway in cerebellar neurons in culture and in rat *in vivo*. Eur J Neurosci 10: 3201–3209

Hermenegildo C, Monfort P, Felipo V (2000) Activation of NMDA receptors in rat brain *in vivo* following acute ammonia intoxication. Characterisation by *in vivo* brain microdialysis. Hepatology 31: 709–715

Hoyumpa AM, Desmon PV, Avant GR, Roberts RK, Schenker S (1979) Hepatic encephalopathy. Gastroenterology 76: 184–189

Huang R, Kala G, Murthy CRK, Hertz L (1994) Effects of chronic exposure to ammonia on glutamate and glutamine interconversion and compartmentation in homogeneous primary cultures of mouse astrocytes. Neurochem Res 19: 257–265

Marcaida G, Minana MD, Burgal M, Grisolia S, Felipo V (1995) Ammonia prevents activation of NMDA receptors in rat cerebellar neuronal cultures. Eur J Neurosci 7: 2389–2396

Meyer RC, Knox J, Purwin DA, Spangler EL, Ingram DK (1998) Combined stimulation of the glycine and polyamine sites of the NMDA receptor attenuates NMDA blockade-induced learning deficits of rats in an 14-unit T-maze. Psychopharmacology 135: 290–295

Minana MD, Marcaida G, Grisolia S, Felipo V (1995) Prenatal exposure of rats to ammonia impairs NMDA receptor function and affords delayed protection against ammonia toxicity and glutamate neurotoxicity. J Neuropathol Exp Neurol 54: 644–650

Mondadori C, Ortman R, Petschke F, Buerki H, D'Amato F, Meisburger J-G, Fagg GE (1988) Task-dependent impairment of facilitation of learning by NMDA receptor blockade. In: Cavalheiro EA, Lehman J, Turski L (eds) Frontiers in excitatory amino acid research. Liss, New York, pp 419–426

Monfort P, Kosenko E, Erceg S, Canales JJ, Felipo V (2002) Molecular mechanism of acute ammonia toxicity: role of NMDA receptors. Neurochem Int 41: 95–102

Morris RGM, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor agonist, AP5. Nature 319: 774–776

Mort D, Marcaggi P, Grant J, Atwell D (2001) Effect of acute exposure to ammonia on glutamate transport in glial cells isolated from the salamander retina. J Neurophysiol 86: 836–844

Muñoz MD, Gaztelu JM, Felipo V (1996) Ammonia prevents NMDA-dependent LTP in the *in vitro* rat hippocampus. MD Second Meeting of European Neuroscience, European Neuroscience Association, Strasbourg, France

Muñoz MD, Monfort P, Gaztelu JM, Felipo V (2000) Hyperammonemia impairs NMDA receptor-dependent lg-term-potentiation in the CA1 of rat Hippocampus *in vitro*. Neurochem Res 4: 437–441

Norenberg MD, Huo Z, Neary JT, Roing-Cantesano A (1997) The glial glutamate transporter in hyperammonemia and hepatic encephalopathy: relation to energy metabolism and glutamatergic neurotransmission. Glia 21: 124–133

Olton DS, Wenk GL (1988) Animal models to investigate the role of NMDA receptors in learning and memory. In: Cavalheiro EA, Lehman J, Turski L (eds) Frontiers in excitatory amino acid research. Liss, New York, pp 379–388

Patel N, Spangler EL, Greig NH, Yu Q-S, Ingram DK, Meyer RC (1988) Phenserine, a novel acetylocholinesteraze inhibitor, attenuates impaired learning of rats in a 14-unit T-maze induced by blockade of the N-methyl-D-aspartate receptor. NeuroReport 9: 171–176

Plum F, Hidfelt B (1976) The neurological complications of liver disease. In: Vinket PJ, Bruyn GW (eds) Handbook of clinical neurology, metabolic and deficiency diseases of the central nervous system, vol 27. Elsevier Press, North-Holland, pp 349–377

Pook PCK, Brise EF, Jane DE, Jones AW, Jones PLSJ, Mewett KN, Sunter DC, Udvarhelyi PM, Wharton B, Watkins JC (1993) Differential actions of the metabotropic glutamate receptor antagonists 4C-PG and M4C-PG at L-AP4-like receptors in neonatal rat spinal cord. Br J Pharmacol Proceed [Suppl 108]: 87

Raabe W (1990) Effects of NH^+_4 on reflexes in the cat spinal cord. J Neurophysiol 64: 565–574

Riekkinen M, Stefanski R, Kuitunen J, Riekkinen P (1996) Effects of combined block of α1-adrenoreceptors on spatial and passive avoidance behavior in rats. Eur J Pharmacol 300: 9–16

Schmidt RD, Grzelak ME, Coffin VL (1997) Reduction of dizocilpine and scopolamine-induced deficits in avoidance responding by SCH 54338, a metabolite of felbamate. Pharmacol Biochem Behav 58: 657–664

Schmidt WJ, Kretschmer BD (1997) Behavioral pharmacology of glutamate receptors in the basal ganglia. Neurosci Biobehav Rev 21: 381–392

Thomsen C, Boel E, Suzdak PD (1994) Actions of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family. Eur J Pharmacol Molec Pharm 267: 77–84

Waniewski RA (1992) Physiological levels of ammonia regulate glutamine synthesis from extracellular glutamate in astrocyte cultures. J Neurochem 58: 167–174

Zhou B, Norenberg MD (1999) Ammonia downregulates GLAST mRNA glutamate transporter in rat astrocyte cultures. Neurosci Lett 276: 145–148

Zieve L (1981) The mechanism of hepatic coma. Hepatology 1: 360–365

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