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Glycine modulates N-methyl-D-aspartic acid induced learning facilitation in rats

R. Liljequist

Biomedicum, Department of Medical Pharmacology, University of Uppsala, Uppsala, Sweden

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Summary. Pretraining i.p. administration of N-methyl-D-aspartic acid (NMDA) at doses of 10 and 20mg/kg dose-dependently facilitated performance in a water T-maze learning task in rats. The effect of NMDA was inhibited by the competitive NMDA receptor antagonist CGP37849 [(DL)-E(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid] (CGP) at a dose of 6mg/kg, and by the NMDA receptor complex glycine site antagonist 1-hydroxy-3-amino-2-pyrrolidone (HA-966) at a dose of 10mg/kg. The NMDA site antagonist, when given alone, did not impair learning. The glycine precursor milacemide (2-N-pentylaminoacetamide HCl), at doses of 5 and 10mg/kg accelearted learning acquisition and its effect was antagonized by HA-966. The learning rate was impaired following the administration of NMDA 10mg/kg together with milacemide 5mg/kg when compared with the effect of 10mg/kg NMDA alone.

The administration of 5mg/kg NMDA was associated with an elevated tissue concentration of aspartate in the hippocampus, an effect which was antagonized by 6mg/kg of CGP. NMDA at doses of 10 and 20mg/kg elevated the concentration of glycine but decreased the concentration of aspartate, glutamate and glutamine in the cortex and aspartate in the hippocampus. The cortical effects of NMDA 10mg/kg were antagonized by 6mg/kg of CGP. Milacemide at the dose of 10mg/kg elevated glycine, aspartate, glutamate and taurine concentrations. The coadministration of 5mg/kg NMDA with 5mg/kg milacemide elevated the concentrations of glycine, glutamate and glutamine in the cortex and taurine in the hippocampus. These amino acid levels were higher than after administration of 5mg/kg either agent alone. The results demonstrate a dose-dependent facilitation effect on learning performance by NMDA and glycine receptor agonists. Antagonists at the NMDA and glycine sites counteracted the learning improvement of NMDA, and the glycine site antagonist the effect of milacemide.

Keywords: Amino acids – Learning-N-methyl-D-aspartic acid – Milacemide – CGP37849 – HA-966 – Amino acid concentrations

Introduction

Learning acquisition has been shown to be enhanced by low doses of N-methyl-D-aspartate (NMDA) receptor agonists (Flood et al., 1990) and by the glycine precursor milacemide (Christophe et al., 1983; Saletu and Grünberger, 1984; Saletu et al., 1986; Handelmann et al., 1989; Quartermain et al., 1991; Schwarz et al., 1992). Activation of the NMDA receptor has been suggested to be essential for the initiation of long-term potentiation, a physiological model for learning (Collingridge and Bliss, 1987; Harris et al., 1984). The positive effect of learning following the elevation of glycine concentrations is believed to result from its stimulatory action through the strychnine insensitive glycine receptor, associated with the NMDA receptor complex (Bonhaus et al., 1987; Johnson and Ascher, 1987; Monahan et al., 1989; Danysz et al., 1989; Krebs et al., 1989; Thomson et al., 1989). There are a number of factors which determine the activation of the NMDA receptor. Data from *in vitro* receptor binding studies indicate that the concentration of glutamate, aspartate and glycine to a great extent determine the degree of receptor activation and function. It is also well documented, that not only can glycine modify the binding of NMDA agonists and antagonists, but agonists of the NMDA receptor complex transmitter site can modify glycine binding (Fadda et al., 1988; Monaghan et al., 1988a,b; Kessler et al., 1989; Kaplita and Ferkani, 1990; Ransom and Deschener, 1990; Mugnaini et al., 1993). The role of glycine is considered so important that it is now accepted to be a co-agonist of the NMDA receptor (Kleckner and Dingledine, 1988). The nature of the interaction between these two agonists *in vivo* is not clear. Results from electrophysiological studies support the concept of negative allosteric interaction (Benveniste et al., 1990). The binding of an agonist at the glutamate recognition site *in vivo* appears to reduce the affinity of the glycine binding site for its ligand. The importance of an increase in central glycine concentrations is not clear since NMDA receptors appear to be saturated already at physiological glycine concentrations. The addition of extra glutamate or NMDA is able to further stimulate physiological responses.

In this study we have investigated how maze learning in adult rats is affected by NMDA and the glycine precursor milacemide alone and upon coadministration. We have also investigated the effect of the competitive NMDA antagonist CGP37849 (Fagg et al., 1990) and the glycine site antagonist HA-966 on learning, and whether the effect of NMDA and milacemide can be blocked by these antagonists. The effects of drug treatments on endogenous amino acid concentrations in the cortex and hippocampus were also measured.

Materials and methods

Animals

Adult male Sprague-Dawley rats weighing 260 ± 10 g were used. They were maintained on *ad lib* food and water under standard temperature- and humidity- controlled laboratory conditions. The experiments were carried out between 2 and 10 p.m. The dark period started at 5 p.m.

Parallel with the learning experiments, analogous drug treatments were performed in groups (4 animals in each group) of animals to measure their effect on brain concentrations of endogenous amino acids. On the 3rd day of drug treatment these rats were killed at that time from drug administration when learning experiments were started (45–60 min after drug injections). The rats were slightly anaesthesized with ether and killed by decapitation. Their cortices and hippocampi were dissected and stored at −70°C prior to analyses.

Apparatus and testing procedure

A water T-maze was used to test the learning acquisition. The maze was constructed of plastic material, painted grey, the arms were 36 cm long and the starting arm was 96 cm. The maze was enclosed by 56 cm high walls. The T-maze was put inside a circular water tank, filled with water at 25 ± 2°C. In one arm under water a transparent platform was fixed on which rats had to learn to climb.

Each rat was placed in the start arm and allowed to swim freely one of the two arms or back to the start alley. The cumulative time spent in, and the number of entries made into the arms (four paws within the second half of the arm or start box) were recorded. Most of the animals found the platform within 60 seconds. If the animal did not climb onto the platform within 120 seconds they were placed on it.

Four training trials were given each day for 3 consecutive days with 30 seconds between trials.

The experimental protocols were approved by the institutional ethical committee and met the quidelines of responsible governmental agency.

Analysis of endogenous amino acids and catecholamines

The concentration of endogenous amino acids alanine, aspartate, glutamate, glutamine, glycine and taurine, and the concentration of noradrenaline and dopamine with their metabolites (results not reported here) in the cortex and hippocampus were measured by HPLC according to the method described by Qureshi and Baig (1993) and Bednar et al. (1992).

Drugs and treatments

N-methyl-D-aspartic acid (Sigma, St. Louis, M.O.) (NMDA) was dissolved in 0.9% NaCl and injected intraperitoneally (i.p.) 45 minutes before training in doses of 5, 10 and 20 mg/kg. Milacemide (2-N-pentylaminoacetamide HCl), (kindly provided by Searle & Co., Belgium) was similarly dissolved in saline and injected i.p. in doses of 5 and 10 mg/kg 60 minutes prior to training. [(DL)-(E)-2-amino-4methyl-5-phosphono-3-pentenoic acid] CGP37849, (kindly provided by Ciba-Geigy, Switzerland) (CGP) 6 and 10 mg/kg, and (+)-HA-966 (R-(+)-3-amino-1-hydroxypyrrolid-2-one) (Tocris Neuramin, U.K.) 3 and 10 mg/kg, dissolved in saline, were injected 45 minutes prior to training. Control rats received saline, the same volume as that of the drug treatments. In learning experiments only CGP 6 mg/kg was used, as this agent tended to stimulate swimming activity at higher doses. HA-966 was used in learning experiments at a dose of 10 mg/kg which did not influence motor responses.

Statistics

Data were analyzed for main effects by using the one-way analysis of variance followed by individual post-hoc comparisons using Fisher Least Square Difference (PLSD) and Scheffe tests.

Results

Learning acquisition based on *error* scores is presented in Table 1 and results based on *time* scores in Table 2. One-way analysis of variance showed significant group differences in error scores on days 1 ($F = 13.35$; $p < 0.0001$) and 2 ($F = 3.21$; $p < 0.00059$).

On day 1, the groups with combined agonist-antagonist treatments performed most poorly. Thus the group which received 10mg/kg NMDA with 6mg/kg CGP made significantly more *errors* than did the control group and that given NMDA 10mg/kg. The group which received milacemide 10mg/kg made fewer errors than the group which received this treatment together with the antagonist HA-966.

On the second day, groups which received NMDA 10mg/kg, milacemide 10mg/kg, and NMDA 5mg/kg made significantly less errors than those who received saline. The combination of 10mg/kg NMDA with 5mg/kg milacemide resulted in an impaired performance compared with that by rats receiving NMDA 10mg/kg alone. Both NMDA 10mg/kg with CGP, and milacemide 10mg/kg with HA-966 treated rats made more errors than those given saline, NMDA 10mg/kg or milacemide 10mg/kg. All the mentioned individual group differences were significant at $p < 0.05$ probability level.

Table 1. Learning acquisition in T-maze, based on the number of errors (mean ± SEM)

	Errors		
	Day 1	Day 2	Day 3
Saline	3.00 ± 0.63	2.22 ± 0.49	0.50 ± 0.19
NMDA 5mg	1.75 ± 0.45	0.88 ± 0.64*	0.75 ± 0.49
NMDA 10mg	2.38 ± 0.50	0.63 ± 0.38*	1.00 ± 0.42
NMDA 20mg	2.25 ± 0.56	1.38 ± 0.38	1.13 ± 0.30
Mil 5mg	2.25 ± 0.49	1.13 ± 0.40	0.88 ± 0.52
Mil 10mg	1.75 ± 0.49	0.88 ± 0.40*	1.00 ± 0.50
NMDA 5mg+ Mil 5mg	2.25 ± 0.49	1.25 ± 0.65	1.00 ± 0.57
NMDA 5mg+ Mil 5mg	1.25 ± 0.25	0.75 ± 0.31*	0.50 ± 0.19
MNDA 10mg Mil 10mg	2.13 ± 0.35	2.75 ± 0.65†	1.25 ± 0.49
CGP 6mg	3.63 ± 0.60	1.75 ± 0.49	0.75 ± 0.25
NMDA 10mg+ CGP 6mg	11.13 ± 1.43*†	3.38 ± 0.63*†	2.38 ± 0.82
HA 10mg	2.50 ± 0.42	2.75 ± 0.59	1.38 ± 0.32
NMDA 10mg+ HA 10mg	3.38 ± 0.91	2.25 ± 0.77	1.13 ± 0.48
Mil 10mg+ HA 10mg	6.50 ± 1.24†#	4.38 ± 1.31*#	1.63 ± 0.57

Effect of intraperitoneal injections of N-methyl-D-aspartic acid (NMDA) in doses of 5, 10 and 20mg/kg, milacemide (Mil) in doses 5 and 10mg/kg, 6mg/kg CGP37849 (CGP) and 10mg/kg Ha-966 (HA), alone or combined, on acquisition of T-maze learning. Number of rats = 8 in each group. *$p < 0.05$ in comparison with saline group; †$p < 0.05$ in comparison with NMDA 10mg/kg; #$p < 0.05$ in comparison with Mil 10mg/kg.

Table 2. Learning acquisition in T-maze, based on the latency times (mean ± SEM seconds)

	Day 1	Day 2	Day 3
Saline	37.47 ± 7.22	15.36 ± 1.57	10.99 ± 2.58
NMDA 5mg	28.66 ± 2.07	12.94 ± 1.76	7.28 ± 0.77
NMDA 10mg	22.31 ± 2.76*	11.17 ± 1.31	8.8 ± 1.72
NMDA 20mg	21.28 ± 1.90*	10.03 ± 1.12*	10.28 ± 1.65
Mil 5mg	28.07 ± 2.33	9.44 ± 1.16*	6.81 ± 1.01
Mil 10mg	32.83 ± 4.96	8.90 ± 1.38*	8.69 ± 1.00
NMDA 5mg$^+$ Mil 5mg	27.25 ± 2.64	11.41 ± 1.54	9.69 ± 1.80
NMDA 5mg$^+$ Mil 10mg	26.12 ± 1.81*	10.04 ± 1.16	9.49 ± 1.13
NMDA 10mg$^+$ Mil 5mg	25.71 ± 3.56*	23.91 ± 4.91*†	8.55 ± 0.69
CGP 6mg	23.99 ± 2.55*	12.12 ± 1.23	8.33 ± 1.06
NMDA 10mg$^+$ CGP 6mg	33.99 ± 3.75†	16.08 ± 1.37	12.96 ± 2.20
HA 10mg	21.49 ± 1.81*	24.65 ± 3.70*	13.01 ± 0.92
NMDA 10mg$^+$ HA 10mg	32.97 ± 5.25	22.12 ± 5.46†	12.92 ± 1.39
Mil 10mg$^+$ HA 10mg	57.91 ± 7.97*$^\#$	24.75 ± 2.64*$^\#$	17.96 ± 4.76*$^\#$

Effect of intraperitoneal injections of N-methyl-D-aspartic acid (NMDA) in doses of 5, 10 and 20mg/kg, milacemide (Mil) in doses 5 and 10mg/kg, 6mg/kg CGP37849 (CGP) and 10mg/kg HA-966 (HA) , alone or combined, on acquisition of T-maze learning. Number of rats = 8 in each group. *$p < 0.05$ in comparison with saline group; $^\dagger p < 0.05$ in comparison with NMDA 10mg/kg; $^\# p < 0.05$ in comparison with Mil 10mg/kg.

One-way analysis of variance based on *time* scores showed significant effects on day 1 ($F = 5.29$; $p < 0.0001$), day 2 ($F = 5.37$; $p < 0.0001$) and day 3 ($F = 2.4$; $p < 0.008$).

On the first day, groups which received NMDA 10mg/kg, NMDA 20mg/kg, NMDA 10mg/kg with milacemide 5mg/kg and NMDA 5mg/kg with milacemide 10mg/kg, performed significantly faster than the saline group. CGP 6mg/kg and HA-966 10mg/kg treated rats also found the platform earlier than the saline group. The group which received milacemide 10mg/kg with its antagonist was the slowest. The combination of NMDA 10mg/kg with antagonist CGP differed significantly from NMDA 10mg/kg alone, as did milacemide 10mg/kg with HA-966 from milacemide 10mg/kg. Data from all these individual groups were significantly different, $p < 0.05$.

On the second day rats treated with NMDA 20mg/kg, milacemide 5, and 10mg/kg, swam faster than the saline group. Rats that received NMDA 10mg/kg with milacemide 5mg/kg, HA-966 alone, and milacemide 10mg/kg with HA-966, were slower than the saline group ($p < 0.05$). Similar to the errors scores, rats treated with a combination of NMDA 10mg/kg with milacemide 5mg/kg and HA-966 were slower than NMDA 10mg/kg alone.

On day three, the group treated with 10mg/kg milacemide together with HA-966, was still slower than the saline group.

Concentration of amino acids

The endogenous concentrations of alanine, aspartate, glutamate, glutamine, glycine, and taurine in the cortex and hippocampus are presented in Figs. 1 and 2. The treatments, given once a day for 3 days, caused significant differences ($p < 0.0001$) in the concentration of every amino acid measured (one-way analysis of variance for treatment effects, F-values varied between 5.21–27.57). Post-hoc comparisons revealed the following significant differences ($p < 0.05$ by Fisher PLSD or Scheffe).

Injection of 5 mg/kg/day NMDA for 3 days elevated the level of aspartate and glycine in the hippocampus. An increase in glycine but not in that of aspartate was found following the coadministration of 5 mg/kg NMDA with milacemide 5 mg/kg. The level of glycine in the cortex was elevated after the treatment of NMDA 5 mg/kg with 6 or 10 mg/kg of CGP.

The administration of NMDA 10 mg/kg elevated the concentration of taurine and glycine in the cortex. NMDA given with the antagonist CGP partially counteracted this increase in taurine and glycine levels. There was a decrease in cortical aspartate, glutamate and glutamine concentrations following the administration of 10 mg/kg NMDA. In the hippocampus the concentration of aspartate was decreased. The cortical effects of aspartate, glutamate and glutamine were counteracted by CGP 6 mg/kg.

NMDA 20 mg/kg influenced the cortical concentrations of similar amino acids as did 10 mg/kg and furthermore, increased concentrations of alanine in both the cortex and hippocampus. Concentrations of glutamate, glutamine and aspartate were also decreased in the hippocampus.

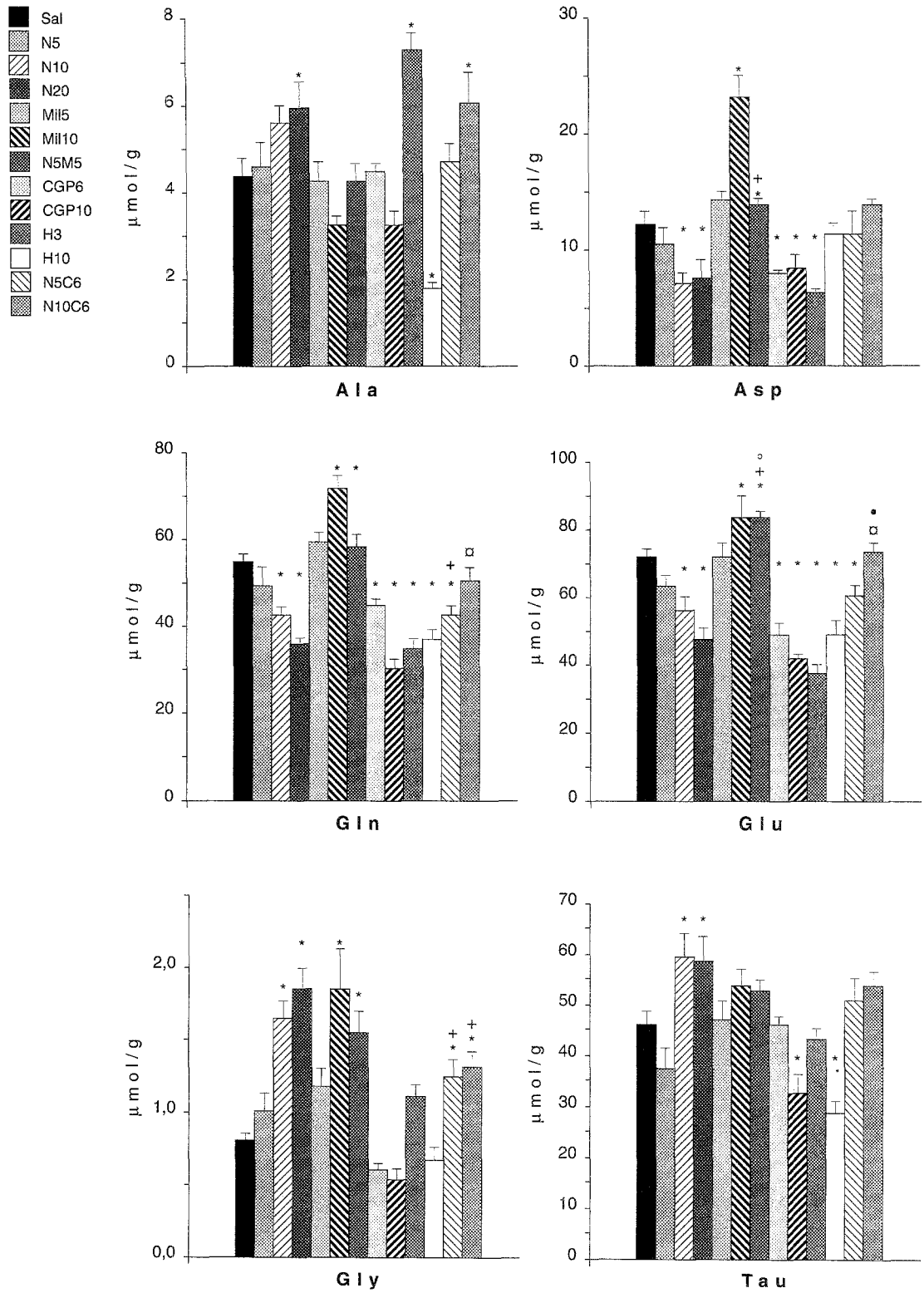
The administration of milacemide 5 mg/kg alone and in combination with NMDA 5 mg/kg elevated the concentration of alanine in the hippocampus. Identical combination resulted in a decrease of aspartate in the hippocampus. The fall in the hippocampal glutamate, but not aspartate, concentration was corrected by the combined administration of 5 mg/kg NMDA and milacemide.

The coadministration of 5 mg/kg NMDA with 5 mg/kg milacemide showed tissue NMDA and glycine concentrations corresponding to those achieved with milacemide administration alone. In contrast, a synergistic effect on the concentations of glycine in the cortex and hippocampus, and glutamate in the cortex, and taurine in the hippocampus was found. NMDA and milacemide together resulted in a higher elevation than either of these compounds alone.

The higher dose of milacemide, 10 mg/kg, was found to increase the levels of aspartate, glutamate, glutamine and glycine in the cortex, and aspartate, glutamate, glycine and taurine in the hippocampus.

The lower dose of the NMDA antagonist, 6 mg/kg of CGP resulted in a decrease in both of aspartate, glutamate and glutamine in the cortex and glutamate and glycine in the hippocampus. CGP 10 mg/kg decreased the concentration of aspartate, glutamine, glutamate and taurine in the cortex and alanine, aspartate, glutamate, glycine and taurine in the hippocampus.

Fig. 1. Concentrations (μmol/g dry weight) of alanine (*Ala*), aspartate (*Asp*), glutamate (*Glu*), glutamine (*Gln*), glycine (*Gly*) and taurine (*Tau*) in rat cortex following 3 days treatment with N-methyl-D-aspartic acid (*N*) in doses 5, 10 and 20 mg/kg, milacemide (*Mil*) in doses of 5 and 10 mg/kg, [(DL)-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid] CGP37849 (CGP) in doses of 6 and 10 mg/kg, and (+)-HA-966 (R-(+)-3-amino-1-hydroxypyrrolid-2-one) (H) in doses of 3 and 10 mg/kg. The compounds were given once a day intraperitoneally. NMDA 5 mg/kg was also given together with Mil 5 mg/kg (N5M5). N5C6 and N10C6 refer to the combined administration of NMDA 5 and 10 mg/kg with CGP 6 mg/kg. The rats were decapitated 45–60 min after the last injection. * $p < 0.05$ incomparison with saline (Sal) group; $^{+}$ $p < 0.05$ in comparison with N 5 mg/kg; • $p < 0.05$ in comparison with CGP 6 mg/kg; ◦ $p < 0.05$ in comparison with Mil 5 mg/kg; $^{\square}p < 0.05$ in comparison with N 10 mg/kg


Sal
N5
N10
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Mil5
Mil10
N5M5
CGP6
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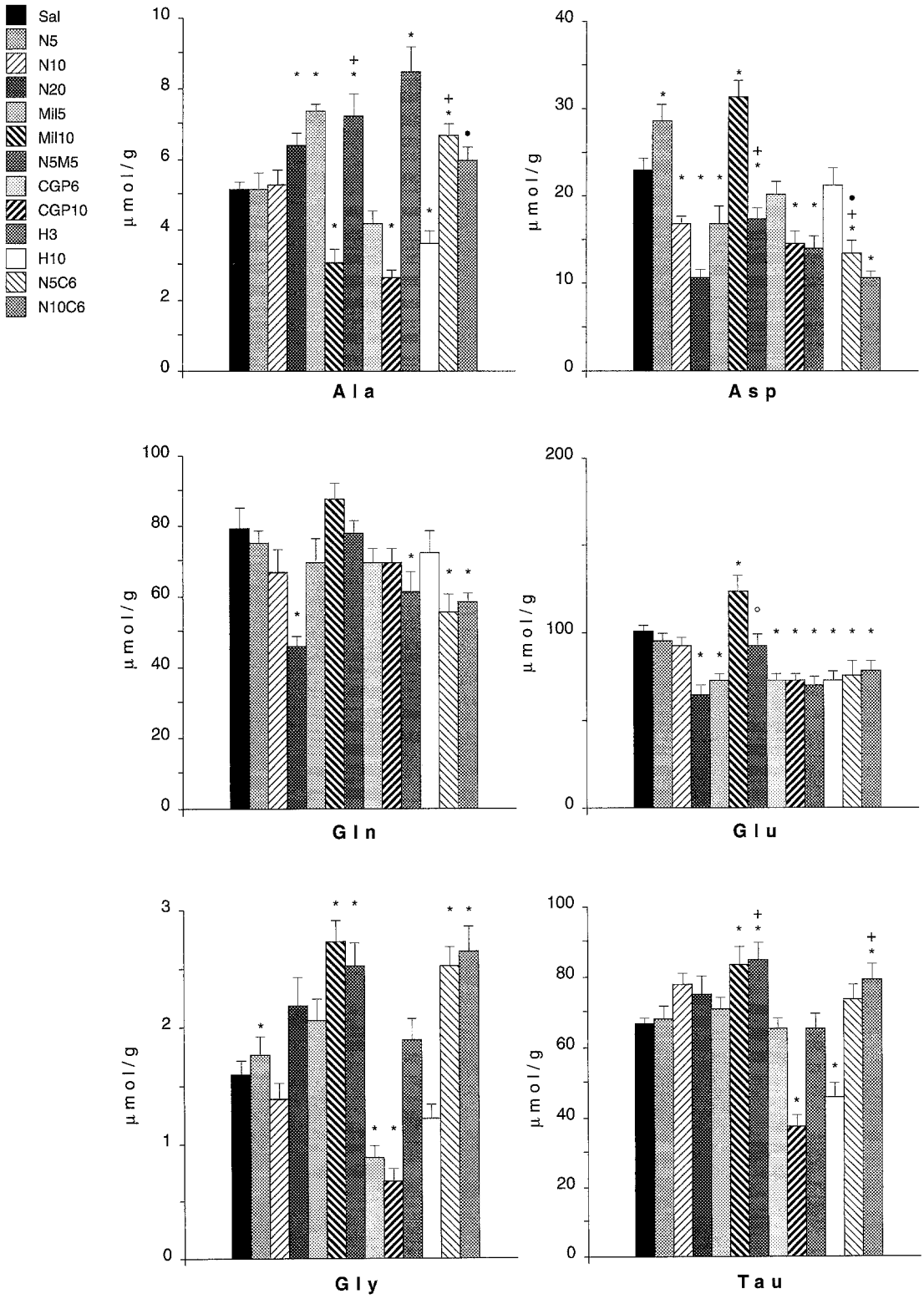
H3
H10
N5C6
N10C6
µmol/g
Ala
Asp
Gln
Glu
Gly
Tau


Sal
N5
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Glu
Gly
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The glycine site antagonist HA-966 at a dose of 3 mg/kg decreased the concentration of glutamate, glutamine and aspartate and elevated the content of alanine in both the cortex and hippocampus.

At 10 mg/kg, HA-966 administration resulted in a decrease of glutamate, glutamine, taurine and alanine concentrations in the cortex and glutamate, alanine and taurine levels in the hippocampus.

Discussion

NMDA receptor activation is thought to play a key role in the regulation of learning and memory function. This suggestion is mainly based on evidence from studies with antagonists of the NMDA receptor complex transmitters and ion-channel sites. The presence of both glutamate and glycine site agonists may be necessary for the activation and function of the NMDA receptor. In our present study, we confirm previously reported observations that NMDA and milacemide, within an optimized dose range, accelerate learning acquisition performance in a concentration-dependent manner (Saletu et al., 1986; Handelmann et al., 1989; Flood et al., 1990; Quartermain et al., 1991; Schwartz et al., 1992). Learning indices, both the number of errors and the time to complete a T-maze task, were influenced. Transmitter site and the glycine site antagonists blocked the learning facilitatory action of NMDA. The effect of milacemide was blocked by the glycine site antagonist HA-966. In this context aspartate, glutamate and glycine concentrations were co-regulated. The two highest doses of NMDA, 10 and 20 mg/kg, elevated cortical glycine levels. Milacemide at a dose of 10 mg/kg improved learning most effectively. Glycine levels, and those of the excitatory amino acids, taurine and alanine, were increased at that dose in the hippocampus. These results support an involvement of the NMDA receptor complex in learning and memory processes.

Brain amino acid concentrations were measured under conditions which simulated those of the learning experiments. The effect of drug treatments on amino acids was measured after 3 days. Compensatory mechanisms, i.e. endogenous synthesis, had already been activated which influenced the results. Endogenous tissue levels of aspartate and glycine did not strictly correlate

Fig. 2. Concentrations (μmol/g dry weight) of alanine (*Ala*), aspartate (*Asp*), glutamate (*Glu*), glutamine (*Gln*), glycine (*Gly*) and taurine (*Tau*) in rat hippocampus following 3 days treatment with N-methyl-D-aspartic acid (*N*) in doses 5, 10 and 20 mg/kg, milacemide (*Mil*) in doses of 5 and 10 mg/kg, [(DL)-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid] CGP37849 (*CGP*) in doses of 6 and 10 mg/kg, and (+)-HA-966 (R-(+)-3-amino-1-hydroxypyrrolid-2-one) (*H*) in doses of 3 and 10 mg/kg. The compounds were given once a day intraperitoneally. NMDA 5 mg/kg was also given together with Mil 5 mg/kg (N5M5). N5C6 and N10C6 refer to the combined administration of NMDA 5 and 10 mg/kg with CGP 6 mg/kg. The rats were decapitated 45–60 min after the last injection. * $p < 0.05$ in comparison with saline (Sal) group; $^{+}p < 0.05$ in comparison with N 5 mg/kg; • $p < 0.05$ in comparison with CGP 6 mg/kg; $\circ p < 0.05$ in comparison with Mil 5 mg/kg

with learning performance. However, the latter may be correlated with levels of recently synthesized amino acids which may be better indicators of neuronal activity than the basal amino acid pool. The lowest NMDA dose increased tissue levels of aspartate, yet did not tremendously improve the learning indices. The endogenous concentrations of aspartate decreased in a dose-dependent fashion following NMDA intraperitoneal administration in the dose range of 10–20 mg/kg, which may be attributed to the impairment in endogenous synthesis. At these doses the learning performance was significantly facilitated. NMDA is suggested to penetrate poorly into the central nervous system. A fraction sufficient to alter cognitive functions and endogenous concentrations of several amino acids reached the brain even following i.p. administration.

There was a close positive correlation between glutamine and glutamate concentrations, especially in the cortex. Aspartate displayed approximately the same pattern of effects in the same tissue. Lesions cause parallel reductions in the release and levels of both glutamate and aspartate (Schofield et al., 1983; Girault et al., 1986). Basal glutamine is supposed to be important in determining levels of new amino acids, especially glutamate, and has been proposed as a limiting factor in the synthesis and release of excitatory amino acids in neuronal stimulation (Szerb and O'Regan, 1985). L-glutamate is important in protein metabolism and energy production.

Milacemide, 2-N-pentylaminoacetamide, has been introduced as a glycine elevating compound. The B-type of monoamine oxidase converts the parent compound to glycinamide which is then catabolized to glycine. Peroral administration of milacemide 100 mg/kg has been shown to increase the concentration of glycine in rat brain (O'Brien et al., 1989). Our present results with rats show that milacemide at a dose of 10 mg/kg elevated the concentrations of glycine and that this dose was most effective at improving learning. At this dose aspartate and glutamate also reached peak levels.

The density of NMDA receptors is highest in the hippocampus of both rat and human tissues. Accordingly, the basal levels of aspartate, glutamate, glutamine and glycine were observed to be higher in the hippocampus than in cortex. There were some differences in the effects of the drugs between cortex and hippocampus. NMDA at a dose of 5 mg/kg elevated the aspartate concentration in the hippocampus but not in the cortex.

NMDA receptors were blocked using both a transmitter site and a glycine site antagonist. Besides their common anticonvulsant activity, these compounds have different *in vivo* effects. CGP causes motor stimulation, whereas HA-966 is a sedative and an anxiolytic agent (Dunn et al., 1992). Furthermore, there is a difference between NMDA antagonists and HA-966 in their effects on catecholamines, since HA-966 does not stimulate dopamine turnover (Hutson et al., 1991).

Both of these compounds were effective in blocking the memory actions following NMDA and milacemide. CGP alone impaired learning only at doses which started to stimulate motor activity – a property which deviates from those of the noncompetitive NMDA ion-channel blockers, such as MK-801. CGP has been shown to stimulate spontaneous motor activity in doses of

20mg/kg and higher (Maj et al., 1993). In the present study, doses greater than 10mg/kg CGP tended to cause the rats to swim straight over the platform in the swimming pool and impair their ability to stay on the platform during the resting period. The brain amino acids were decreased at a dose of 6mg/kg, which did not yet impair learning. CGP at a dose of 10mg/kg not only decreased NMDA but also reduced the concentration of glycine, alanine and taurine.

During the first day of combined administration of NMDA with CGP and milacemide with HA-966, the performance was surprisingly poor. Alone the antagonists facilitated rather than retarded performance, an effect shown under specified conditions (Mondadori, 1989; Weizkranz and Mondadori, 1991). The data on the first day of training reflect both learning and nonspecific performance factors. The combination of NMDA with CGP on second day resulted in an increased number of errors without an increase in time scores, which suggests that impaired learning was accompanied by high swimming speeds. In the hippocampus, the levels of aspartate and glutamate were low but the level of glycine was high after these combinations.

HA-966 alone had weak effects on memory, but counteracted the effect of milacemide on learning at the dose which decreased the concentrations of glutamine, glutamate, alanine and taurine. Poor performance following the combined administration of milacemide with HA-966, suggests that glycine is particularly important for memory. HA-966 is a weak partial glycine site agonist and as such may provide ideal conditions for inhibition of side effects associated with intensive NMDA receptor activation. It may block NMDA receptor function incompletely and therefore allow some activity at the transmitter site. Retention of step down passive avoidance has been shown to be unaffected at doses as high as 80mg/kg of HA-966 (Dunn et al., 1992). The anxiolytic actions of HA-966 could explain the low latency times seen on the second day. HA-966 had interesting effects on taurine and alanine levels in both the hippocampus and cortex. The reason for and significance of reduced taurine concentrations after the higher dose of HA-966, and elevation of alanine following the low dose seen in both the cortex and hippocampus remains to be further investigated.

Seizures and neurotoxic effects are associated with the potential use of excitatory amino acids as learning enhancers. In the NMDA dose range studied, no overt side effects were seen. It has been shown that glycine lowers the threshold for NMDA provoked convulsions (Singh et al., 1990). This kind of potentiation may explain the impairment of performance following the higher dose of NMDA when given together with milacemide. Our results emphasize that the memory effects of NMDA were more easily modified by glycine than the effects of glycine by NMDA. An interesting parallel concerning tissue concentrations of NMDA and glycine was found with electrophysiological measurements. NMDA and milacemide 5mg/kg, when coadministered, caused similar effects to those of milacemide alone on alanine and aspartate in the hippocampus, and aspartate and glutamine in the cortex. Synergistic effects of combined administration of NMDA and

milacemide were found on the concentrations of glycine, glutamate and taurine, which were elevated to levels higher than those achieved by either agent alone.

An increase in taurine levels was associated with the administration of excitatory amino acids, as indicated previously (Lehman et al., 1983). Taurine is an effective osmoregulator, and therefore its concentration can be increased by the presence of osmolar factors. However, Shibanoki et al., (1993) demonstrated using brain dialysis technique, that elevation of the hippocampal extracellular taurine concentration, following administration of NMDA i.p., was independent of osmolar changes.

In conclusion, the present study demonstrates that learning performance was dose-dependently improved by NMDA and milacemide and their effects antagonized by pharmacological antagonists at the NMDA transmitter and glycine sites. The analysis of brain amino acids revealed that NMDA increased glycine concentrations, and the effects of milacemide were associated with an elevation of NMDA, glutamate and glycine concentration. The present data stress the importance of glycine in physiological functions. The NMDA receptor may have more subunits than presently known (Monyer et al., 1992) which anticipates more selective effector sites.

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Author's address: Dr. R. Liljequist, Biomedicum Department of Medical Pharmacology, University of Uppsala, P.O. Box 593, S-75124 Uppsala, Sweden.

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