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Inhibition of mGluR5 blocks hippocampal LTP in vivo and spatial learning in rats

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Abstract

Particular subtypes of metabotropic glutamate receptors (mGluRs) have been shown to be specifically involved in certain types of long-term synaptic plasticity and learning. We examined whether inhibition of mGluR5 by the specific noncompetitive antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) has any functional consequences on long-term potentiation in the dentate gyrus in vivo and on learning of a spatial alternation task. Intracerebroventricular application of 13.8 μg MPEP 30 min before tetanization resulted in a rapid decline of potentiation during the first 7 min and a significantly lower potentiation of the MPEP group as compared to controls. The same dose of the antagonist given 30 min before training of a Y-maze spatial alternation task caused a marked impairment of retention tested 24 h later. In contrast, MPEP had virtually no effects on retention if injected immediately after the training session. Our findings suggest an important function of mGluR5 during the initiation of synaptic plasticity and memory formation.

Keywords: Metabotropic glutamate receptors; mGluR5; MPEP; LTP; Synaptic plasticity; Learning and memory; Spatial alternation; Rat

1. Introduction

Metabotropic glutamate receptors (mGluRs) are now generally accepted to play a decisive role in a number of important brain functions. While the first period of mGluR research yielded valuable insights into the main actions of the three groups of mGluRs (I–III), the current research focuses on specific functions of the different mGluR subtypes. The mGluRs have been implicated in different forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) and in memory formation (for reviews, see Nakanishi, 1994; Riedel, 1996; Riedel et al., 1996; Conn and Pin, 1997). However, the understanding of the role of particular mGluR subtypes in those processes is rather limited.

During the last years, we have been interested in the function of Group I mGluRs in synaptic plasticity and learning. Studies with the Group I/II antagonist (*S*)-α-methyl-4-carboxyphenylglycine (MCPG) and the Group I antagonist (*S*)4-carboxyphenylglycine (4-CPG) (Davies et al., 1995; Sekiyama et al., 1996) lead us to conclude that Group I mGluRs have a specific role in synaptic plasticity and learning. While in the LTP studies, the strength of tetanization determined whether or not Group I mGluRs play a functional role, in the learning studies, the difficulty of the task appeared to be a critical variable. Therefore, activation of Group I mGluRs seemed to be of functional importance (i) if crucial cellular resources are limited, such as Ca^{2+} in LTP induction and (ii) under conditions of a conflicting learning situation or a high task difficulty (Balschun and Wetzel, 1998; Balschun et al., 1999; Wilsch et al., 1998).

Recent studies, using Group I mGluR knockout mice (Lu et al., 1997; Jia et al., 1998; Daniel et al., 1999; Chiamulera et al., 2001; Levenes et al., 2001) and subtype-specific agonists and antagonists (Cobb et al., 2000; Spooren et al., 2000a,b; Schulz et al., 2001; Tatarczynska et al., 2001) indicate a different involvement of Group I mGluR subtypes in synaptic plasticity and behavior. Thus,

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in the present study, we employed the specific, noncompetitive antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini et al., 1999; Spooren et al., 2001) to examine the functional consequences of mGluR5 inhibition on LTP in vivo and on the Y-maze spatial alternation task. Our findings lead us to conclude that activation of mGluR5 has an important function during the induction of synaptic plasticity in the dentate gyrus in vivo and the initial phase of memory formation.

2. Materials and methods

The experimental protocol was approved by the Ethics Committee of the State of Sachsen-Anhalt (Tierschutzkommission des Landes Sachsen-Anhalt).

2.1. Electrophysiological recordings in vivo

2.1.1. Surgical preparation

Eight-week-old male Wistar rats housed under standard laboratory conditions were prepared under pentobarbital anaesthesia (40 mg/kg ip) as previously described (Seidenbecher et al., 1997). Briefly, a monopolar recording electrode (coordinates AP −2.8, L 1.8 from bregma) and a bipolar stimulation electrode (coordinates AP −6.9, L 4.1), both made from lacquer-coated stainless-steel wire, were implanted stereotaxically into the hilus of the dentate gyrus and into the perforant path, respectively, in the right hemisphere. The electrodes were adjusted such that the population spike amplitude (PSA: difference between the first positive and negative deflections) was maximal. For drug application, a microcannula was chronically implanted in the right lateral ventricle (AP −0.8, L 1.6 from bregma; coordinates according to Paxinos and Watson 1998). All animals were allowed at least 8–10 days to recover from surgery.

2.1.2. Recording

Throughout each experiment, the animals could move freely in the experimental boxes ($40 \times 40 \times 40$ cm). The electrodes were connected by a flexible cable to a differential amplifier (Inhvers+, Science Products, Germany). The recorded responses were filtered by band-pass filters at 0.1 Hz and 5 kHz, transformed via an A/D interface (CED 1401, Cambridge Electronic Design, UK) and stored on-line in a PC. The stimulus intensity was adjusted to evoke 40% of the maximum population spike amplitude. To allow the detection of inhibitory and facilitatory effects on LTP a 'weak' LTP was generated by three bursts of 15 pulses, at 200 Hz (interburst interval 10 s, 0.2-ms duration each stimulus,) resulting in a potentiation that usually returned to baseline after 5–8 h (Seidenbecher et al., 1997). For each time point during the experiment, five responses, evoked every 10 s, were averaged. During baseline, recordings were collected every 10 min. After tetanization, recordings were taken at $t = 1$, 4, 7,10 and 15 min and then every 15 min up to 8 h.

2.2. Y-maze spatial alternation task

Adult male Wistar rats of our own breeding stock, weighing 270–320 g, were housed under standard laboratory conditions with light between 06:00 and 18:00 h and with free access to food and water. For the intracerebroventricular injection, a microcannula was chronically implanted in the right lateral ventricle under pentobarbital anaesthesia (40 mg/kg). Footshock-motivated right–left spatial alternation learning was tested in a computer-controlled Y-maze, as earlier described (Riedel et al., 1994; Balschun et al., 1999). At the beginning of the 40-trial training session, a footshock (0.7–1.3 mA, depending on individual sensitivity) was given in the start box, and the animal had to escape into the right alley (correct run, no footshock), whereas entry into the left alley (error) was punished. In the next trial, the former goal arm served as the start arm, and the animal had to run into the left alley to avoid punishment. In the third trial, the animal had to run into the right alley, and so on. Therefore, no handling between trials was necessary. The intertrial interval was 1 min. Twenty-four hours after the training session, retention of spatial alternation was tested using the same behavioural procedure as during training. As a sensitive measure of retention, % savings were calculated as follows: (training errors − retention test errors)/training errors.

2.3. Drugs

MPEP (13.8 μg; Tocris, Northpoint, UK) was dissolved in 0.9% saline and injected intracerebroventricularly at a total volume of 5 μl and a flow rate of 1 μl min^{-1} in 5 min, 30 min before tetanization or the learning session, respectively. Control rats received 5 μl 0.9% saline.

2.4. Data analysis

To test for group differences between the LTP time series, ANOVA with repeated measures was used. In the first step, a difference curve between the mean values of the MPEP and the NaCl group, respectively, was calculated. From this curve, the time interval that has to be tested by ANOVA was inferred. Thus, a time interval was chosen reaching from the maximal between-groups difference (7 min after tetanization) up to the time point where the difference fell below a certain criterion (20% difference).

For statistical analysis of behavioral data, nonparametric tests were preferred. To assess between-groups differences the Kruskal–Wallis H test and the Mann–Whitney U test were used. Within-groups differences were evaluated with the Wilcoxon matched pairs signed rank test. Statistical differences against zero were tested with the Wilcoxon median signed rank test.

3. Results

In the first set of experiments, we tested whether MPEP given 30 min before tetanization has any effect on LTP in the dentate gyrus in vivo. As depicted in Fig. 1, both groups attained about the same initial magnitude of potentiation (MPEP 230.31 ± 36.2, $n = 7$; NaCl 253.6 ± 24.3, $n = 12$). However, the MPEP group displayed a rapid decline of potentiation during the first 7 min, resulting in a reduction in amplitude by 97.3%, while the values of the control group were only diminished by 43.1% during that time. As a consequence, the values of the MPEP and control groups were statistically different for the following 6 h starting at 15 min after tetanization ($F = 5.002$, $P = .039$, ANOVA with repeated measures).

Because of the impairment of dentate LTP in vivo by MPEP, it was tempting to check whether intracerebroventricular application of the same dose may affect a hippocampus-dependent spatial learning paradigm such as the Y-maze spatial alternation (Balschun et al., 1999; Riedel et al., 1994). MPEP given intracerebroventricularly 30 min prior to training did not affect acquisition on Day 1 (no. of errors: MPEP 14.7 ± 1.0, $n = 14$; NaCl 15.7 ± 0.9, $n = 16$; $z = 0.73$, $P = .465$) (Fig. 2A). Although the difference of retention errors between the MPEP and NaCl group did not attain the significance level on Day 2 (no. of errors: MPEP 14.6 ± 0.7, NaCl 12.2 ± 1.0; $z = 1.85$, $P = .064$), an impairment of retention is clearly evidenced by the % savings difference between groups (MPEP 2.9 ± 5.8, $z = 0.12$, $P = .905$; NaCl 19.9 ± 7.1, $z = 2.06$, $P = .039$) (Fig. 2B). To exclude that the

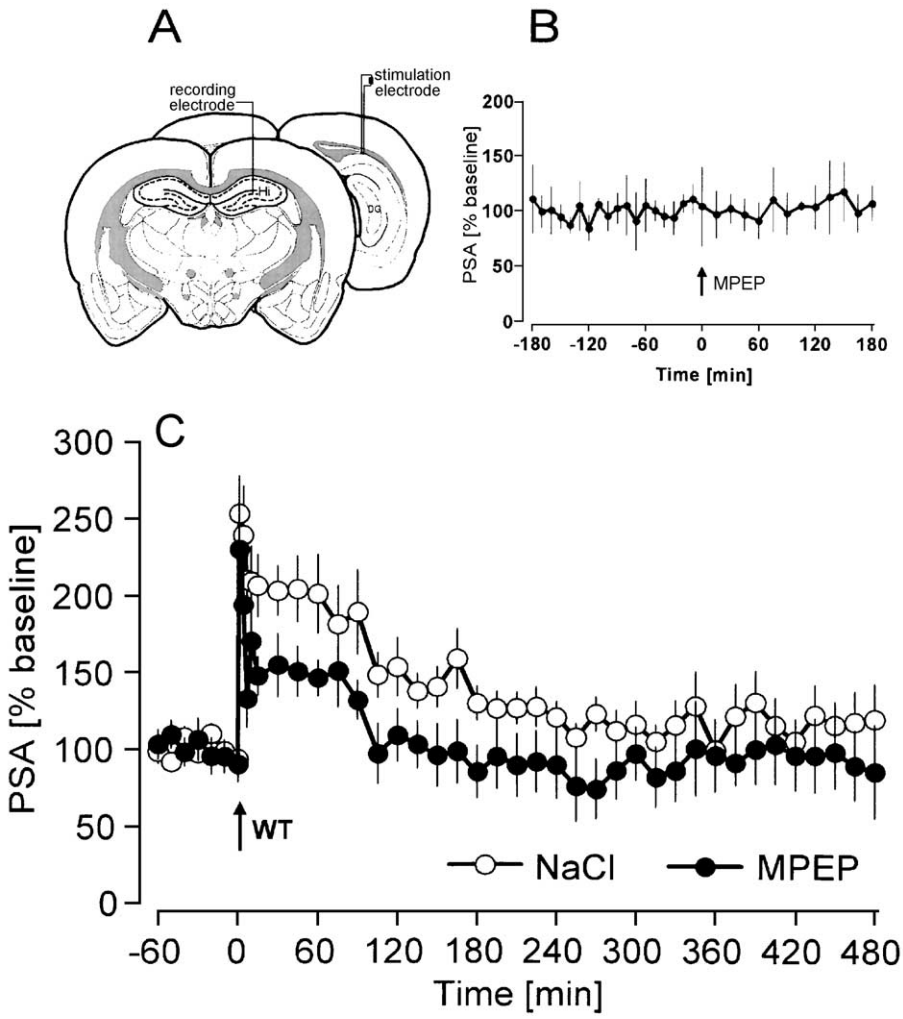

Fig. 1. The mGluR5 antagonist MPEP, applied intracerebroventricularly to freely moving rats, impaired an LTP induced by a weak tetanization paradigm (WT; three bursts of 15 pulses, 200 Hz, interburst interval 10 s, 0.2 ms pulse width). (A) Schematic diagram of electrode placement for the recording from the hilus (HI) of the dentate gyrus of the right hemisphere. (B) The intracerebroventricular application of MPEP (13.8 μg) did not affect baseline recordings ($n = 3$). (C) The same dose of MPEP applied 30 min before tetanization resulted in a significantly earlier decay of potentiation as compared with controls. Thus, the values of the MPEP and control group were statistically different for 6 h starting at 15 min after tetanization (MPEP: $n = 7$; NaCl: $n = 12$; $P = .039$, ANOVA with repeated measures). Mean ± S.E.M. is given.

observed amnesia following MPEP application was a state-dependent effect, in subsequent experiments, the drug was given twice, i.e. before the training and the retention sessions. As depicted in Fig. 2C,D, the results of these experiments resembled the previous ones. While no differences were discernible on Day 1 (no. of errors: MPEP/sd 15.1 ± 1.2, $n = 11$; NaCl/sd 15.2 ± 0.8, $n = 10$; $z = 0.11$, $P = .91$); on Day 2, the retention of the MPEP/sd group deteriorated (no. of errors: MPEP/sd 14.0 ± 1.17, NaCl/sd 11.7 ± 1.6; $z = 1.41$, $P = .159$) as clearly evidenced by the lack of significant savings of the MPEP/sd group (MPEP/sd 6.6 ± 7.9%, $z = 0.82$, $P = .412$; NaCl/sd 23.5 ± 9.7%, $z = 2.17$, $P = .030$).

In the next set of experiments, MPEP was applied immediately after training to examine whether the inhibition of retention is specific to the processes after training. As shown in Fig. 3A,B, under those conditions, no impairment of retention by MPEP could be detected and both groups yielded nearly identical results. Thus, there was a decrease in the number of errors from Day 1 (MPEP 15.9 ± 0.5, $n = 20$; NaCl 15.8 ± 0.6, $n = 20$) to Day 2 (MPEP 13.6 ± 1.0, NaCl 13.4 ± 0.9), resulting in significant % savings in both groups (MPEP 15.3 ± 5.4%, $n = 20$, $z = 2.20$, $P = .028$; NaCl 15.7 ± 5.9%, $n = 20$, $z = 2.14$, $P = .032$).

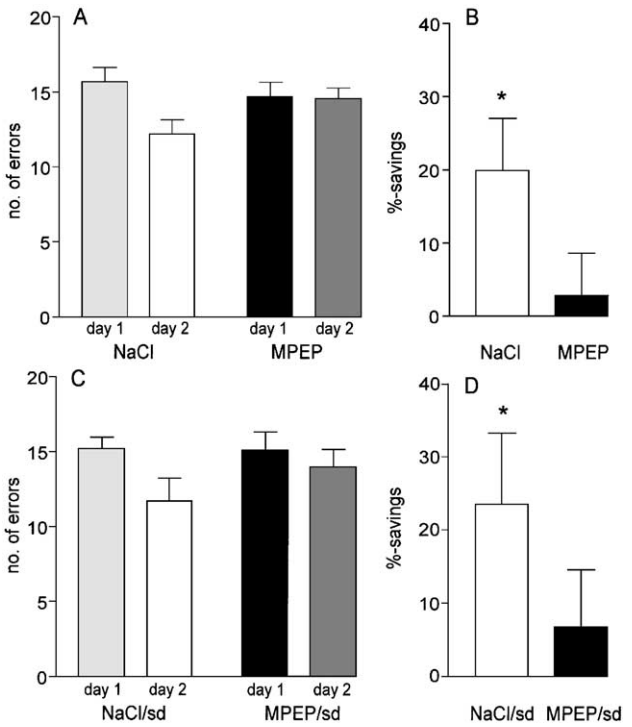

Fig. 2. The intracerebroventricular application of MPEP 30 before training caused a significant impairment of retention of the Y-maze spatial alternation task. (A, B) MPEP had no effect on acquisition but strongly impaired retention tested 24 h later (calculated as % savings; mean ± S.E.M.). * $P < .05$ (control group vs. MPEP; Mann–Whitney test). (C, D) The detrimental effect of MPEP on spatial alternation was not state-dependent as indicated by the low percentage of savings of the MPEP/sd group when the antagonist was applied not only before the acquisition session on Day 1 (as shown in A, B) but also before the retention test (Day 2). * $P < .05$ (control group; Wilcoxon test).

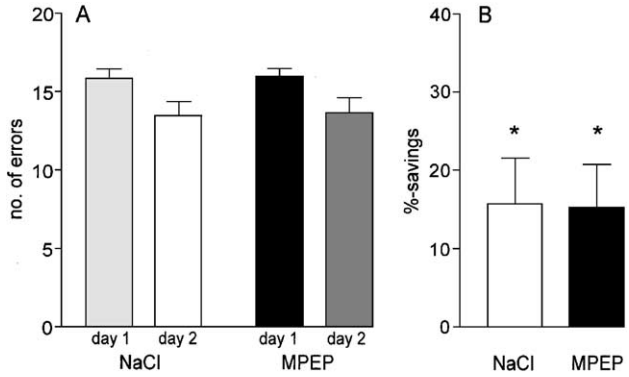

Fig. 3. Inhibition of mGluR5 by MPEP given immediately after training did not cause any changes in retention. (A, B) The retention scores of the MPEP group were indistinguishable from controls. Thus, both groups attained significant savings as shown in (B). * $P < .05$ (Wilcoxon test).

4. Discussion

In the present study, we used the model of a weak, unsaturated LTP (Seidenbecher et al., 1997) to detect alterations of potentiation after inhibition of mGluR5. Previous in vitro experiments indicated that Group I mGluRs may have a particular function during types of potentiation that are induced by weak tetanization protocols (Wilsch et al., 1998). Here, we describe that inhibition of mGluR5 by application of the selective antagonist MPEP (Gasparini et al., 1999) resulted in an impairment of LTP in the dentate gyrus in vivo. This is in agreement with findings in mutant mice where targeted deletion of mGluR5 caused a reduction of LTP in the dentate gyrus and the CA1 region (Lu et al., 1997). According to recent studies, mGluR5 appears to have a function in the potentiation of the NMDA component of LTP. CA1 neurons from mGluR5-deficient mice showed a complete loss of the NMDA-receptor-mediated component of LTP [LTP(NMDA)], but normal LTP of the AMPA-receptor-mediated component (Lu et al., 1997; Jia et al., 1998). Interestingly, the LTP(NMDA) deficit in mGluR5 mutant mice could be rescued by stimulating protein kinase C corroborating the tight link between the NMDA receptor and mGluR5 (Alagarsamy et al., 1999; De Blasi et al., 2001). However, in another study, application of the competitive mGluR5 antagonist LY344545 at a concentration that specifically prevented the DHPG-induced potentiation of NMDA responses failed to block LTP in the CA1 region (Doherty et al., 2000). The discrepancy may be caused by differences in the particular experimental protocols as suggested for the differential findings with other mGluR antagonist such as MCPG (see Wilsch et al.,

1998 for details). Furthermore, blockade of mGluR5 by MPEP was reported to antagonize the DHPG-induced suppression of evoked IPSCs and of the Ca^{2+}-activated potassium current (Mannaioni et al., 2001). Since we have found in previous experiments an inhibition of CA1-LTP in vitro after application of (*S*)-4-carboxyphenylglycine (4-CPG) at a dose where it predominantly acts at mGluR1 (Wilsch et al., 1998), the available experimental evidence supports distinct region- and 'protocol'-specific roles of mGluR5 and mGluR1 in the regulation of hippocampal excitability and LTP induction. The rapid decline of potentiation in the MPEP group reported here indicates an involvement of MPEP in posttetanic potentiation (PTP), an NMDA-independent type of short-term plasticity. In addition, the expression of LTP and /or the transition of PTP to LTP seems to be disturbed. Thus, the impairment of potentiation appears to be caused predominantly by an inhibition of mGluR5 rather than unspecific effects at the NMDA receptor (Spooren et al., 2001).

In the learning tests, application of MPEP 30 min before the training session induced a strong deficit in retention of the spatial alternation task. As indicated by the normal decrease of errors in the training session (data not shown), the antagonist given before training had virtually no effects on the acquisition of spatial alternation. In the retention test, however, MPEP rats displayed nearly the same number of errors as in the training session, resulting in % savings not statistically different from zero, i.e. a complete amnesia. The lack of state-dependent effects of MPEP classifies the observed deficit as a selective impairment of memory formation, which becomes discernible if the retention is tested 24 h later. This conclusion is in line with the impairment of water maze learning and context-dependent fear conditioning found in mGluR5 mutant mice (Lu et al., 1997). Since we did not observe any effect if MPEP was applied immediately after training, activation of mGluR5 may play a role in the initial signalling steps underlying memory formation. Other studies point to a delayed role of mGluR5 in memory formation. Rats trained in a combined context and cue conditioning paradigm displayed a continuous increase in mGluR5 protein expression in CA1 and, to a lesser extent, in dentate gyrus during the first days after training (Riedel et al., 2000). However, in light of the dramatic instantaneous regulation of mGluR5 by homologous and heterologous mechanisms (De Blasi et al., 2001), the physiological importance of this up-regulation of mGluR5 protein remains unclear. The contrasting effects of MPEP application before and after training are supported by previous studies with MCPG. Riedel et al. (1995) described a complete block of dentate LTP under almost the same experimental conditions as used here, if MCPG was given before but not after tetanization. Evaluated together, the electrophysiological findings and the results of the learning experiments suggest an important function of mGluR5 during the initiation of synaptic plasticity and memory formation.

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