The Metabotropic Glutamate Receptor mGluR3 is Critically Required for Hippocampal Long-term Depression and Modulates Long-term Potentiation in the Dentate Gyrus of Freely Moving Rats

Group II metabotropic glutamate receptors (mGluRs) play an important role in the regulation of hippocampal synaptic plasticity in vivo: long-term potentiation (LTP) is inhibited and long-term depression (LTD) is enhanced by activation of these receptors. The contribution, in vivo, of the individual group II mGluR subtypes has not been characterized. We analysed the involvement of the subtype mGluR3 in LTD and LTP. Rats were implanted with electrodes to enable chronic measurement of evoked potentials from medial perforant path-dentate gyrus synapses. Neither the selective mGluR3 agonist, N-acetylaspartylglutamate (NAAG), nor the antagonist β-NAAG, given intracerebrally, affected basal synaptic transmission. β-NAAG significantly inhibited LTD expression. NAAG exhibited transient inhibitory effects on the intermediate phase of LTD. Whereas NAAG altered paired-pulse responses, β-NAAG had no effect, suggesting that antagonism of mGluR3 prevents LTD via a postsynaptic mechanism, whereas agonist activation of mGluR3 modulates LTD at a presynaptic locus. NAAG impaired the expression of LTP, whereas β-NAAG had no effect. NAAG effects on LTP were blocked by EGLU, a selective group II mGluR antagonist. Our data suggest an essential role for mGluR3 in LTD, and a modulatory role for mGluR3 in LTP, with effects being mediated by distinct pre- and postsynaptic loci.

Keywords: EGLU, NAAG, paired-pulse, perforant path, synaptic plasticity

Introduction

The two main forms of activity-dependent hippocampal plasticity comprise long-term potentiation (LTP), a strengthening of synaptic efficacy (Bliss and Lomo, 1973), and long-term depression (LTD), a long-lasting decrease of synaptic transmission (Barrionuevo et al., 1980; Dudek and Bear, 1992).

Since the first descriptions of the involvement of metabotropic glutamate receptors (mGluRs) in LTP and LTD (Bashir et al., 1993; Bortolotto and Collingridge, 1993; Bolshakov and Siegelbaum, 1994), there has been much debate about the relevance of these receptors for synaptic plasticity (Bortolotto et al., 1994, 1999; Selig et al., 1995; Anwyl, 1999). However, it is now well established that mGluRs play a critical role in the induction and expression of synaptic plasticity in the hippocampus of intact animals (Bordi, 1996; Manahan-Vaughan, 1997, 1998, 2000; Holscher et al., 1997) and that they regulate hippocampus-dependent forms of learning (Balschun et al., 1999; Naie and Manahan-Vaughan, 2003). The mGluRs are therefore of particular significance for information processing in this structure.

The mGluRs are divided into three groups with eight subtypes according to their sequence homology and agonist binding (Conn and Pin, 1997). Group I receptors (mGluR1, mGluR5) are positively coupled to phospholipase C and are located postsynaptically. Group II mGluRs (mGluR2, mGluR3) are negatively coupled to adenylyl cyclase and are located presynaptically. Group III mGluRs (mGluR4, 6, 7, 8) are also negatively coupled to cAMP formation and located postsynaptically but exhibit different agonist preferences.

In vivo studies which examined the role of the different mGluR groups revealed that group I receptors are important for the stable induction of both LTP and LTD (Manahan-Vaughan, 1997). Group II and III receptors play an exclusive role in LTD (Manahan-Vaughan, 1997, 2000; Huang et al., 1997, 1999), serving only a modulatory function with regard to LTP (Manahan-Vaughan and Reymann, 1995; Manahan-Vaughan, 1998; Kulla et al., 1999; Lea et al., 2001).

Whereas substantial information now exists as to how the different mGluR groups contribute to synaptic plasticity, little is known about the involvement of the various mGluR subtypes in this phenomenon. Recent studies demonstrated a specific role for the group I receptor subtype mGluR5 in both LTP and LTD and learning processes (Naie and Manahan-Vaughan, 2004). There is a dearth of information about the role of group II and III receptor subtypes in these processes. Work with transgenic or knockout animals has revealed a role for the group III receptor mGluR7 in short-term potentiation in the CA1 region, whereas mice lacking the group II receptor mGluR2 exhibit impaired mossy fibre LTD (Yokoi et al., 1996; Bushell et al., 2002). Recently specific ligands were described for the group II mGluR subtype mGluR3 (Wroblewska et al., 1997; Lea et al., 2001) which make it possible to investigate the contribution of this subtype to the expression of synaptic plasticity. This subtype is of particular interest as it is expressed both on neurons (Ghose et al., 1997) and astrocytes (Wroblewska et al., 1998; Schools and Kimelberg, 1999). Activation of mGluR3 on neurons leads to enhanced expression of GABA receptor subunits (Ghose et al., 1997), whereas activation of astrocytic mGluR3 induces increased expression of the glutamate transporters, GLAST and GLT-1 proteins (Aronica et al., 2003). This suggests that mGluR3 may be of marked significance in the regulation of excitability in neuronal networks, as well as of synaptic plasticity. In vitro, pharmacological activation of mGluR3 leads to inhibition of LTP in the dentate gyrus (Lea et al., 2001). However, the role of this receptor in synaptic plasticity has never been characterized in vivo.

In the present study our goal was to elucidate whether mGluR3 contributes to the long-term expression of synaptic plasticity in freely moving rats. Both LTD and LTP were examined using methodology which enabled plasticity to be followed for >24 h. We could thus assess the relevance of this
receptor in the cellular processes which may underlie long-term memory formation.

**Materials and Methods**

**Surgical Preparation**

Seven- to eight-week-old male Wistar rats underwent implantation of a bipolar stimulating electrode and monopolar recording electrode (made from 0.1 mm diameter Teflon-coated stainless steel wire) into the medial perforant path and the dentate gyrus granule cell layer, respectively, as described previously (Manahan-Vaughan and Reymann, 1995). Briefly, under sodium pentobarbitone anaesthesia (Nembutal, 40 mg/kg, i.p., Serva, Germany) a hole was drilled in the skull for the recording electrode (1 mm diameter, 3.1 mm posterior to bregma, 1.9 mm lateral to the midline) and a second hole (1.5 mm diameter, 6.9 mm posterior to bregma, 4.1 mm lateral to the midline) was drilled for the stimulation electrode. The dura was pierced by both holes, and the stimulating electrode and recording electrode lowered into the medial perforant path and the dentate gyrus granule cell layer, respectively. Evoked field potentials were recorded via the implanted electrodes throughout the surgery. Additionally a cannula was implanted in the lateral cerebral ventricle at 0.5 mm posterior to bregma and 4.1 mm lateral to the midline to enable drug administration. Once the accurate depth of the electrodes were verified, the entire assembly was sealed and fixed to the skull with dental acrylic (Paladur, Heraeus Kulzer GmbH, Germany). The animals were allowed 10 days to recover from surgery before experiments were conducted. Throughout the experiments the animals could move freely. After the conclusion of experiments, the proper placement of the electrodes and cannula was verified by histological analysis.

**Measurement of Evoked Potentials**

Responses were evoked by stimulating at low frequency (5 or 15 min intervals, 0.2 ms stimulus duration, 16 000 Hz sample rate). For each time point five evoked responses at a 10 s interval were averaged. During electrophysiological recordings changes in population spike (PS) amplitude and field excitatory postsynaptic potential (fEPSP) slope were measured. The amplitude of the PS was measured from the peak of the first positive deflection of the evoked potential to the peak of the following negative potential. The field EPSP slope function was measured as the slope of the line connecting the start of the first positive deflection of the evoked potential with the peak of the second positive deflection of the evoked potential. The stimulation intensity was adjusted to evoke potentials which comprised 40% of the maximal population spike amplitude, defined by means of an input/output curve.

Paired-pulse (PP) stimulation was applied every 40 s with interstimulus intervals (ISI) of 20, 25, 40, 50, 100, 300 and 500 ms and 1 s in succession. Paired-pulse ratio was calculated as PS2/PS1 (where PS1 is the amplitude of the first population spike and PS2 is the amplitude of the second population spike) and expressed as a percentage. High-frequency stimulation (HFS; 200 Hz, 10 bursts of 15 stimuli, 0.2 ms stimulus duration, 10 s interburst interval) was given to evoke robust and persistent LTP. LTD was induced by low-frequency stimulation (LFS; 1 Hz, 900 pulses, 0.2 ms stimulus duration). The stimulation amplitude for all protocols was the same as that used for baseline recordings.

**Compounds and Drug Treatment**

L-Acetylaspartylglutamate (NAAG, 3 μg) and β-NAAG (61 μg) were obtained from Sigma, Deisenhofen, Germany. For injection they were dissolved in 0.9% sodium chloride (NaCl) solution. (2S)-α-ethylglutamic acid (EGLU, 100 nmol) was obtained from Tocris Cookson Ltd (Bristol, UK) and dissolved in a solution of 3% NaOH (1 M) and 97% NaCl (0.9%). The concentrations of the compounds applied were determined by their concentration-dependent effects on basal synaptic transmission — determined in the present study for NAAG and β-NAAG (data not shown) and in previous studies for EGLU (Manahan-Vaughan, 1997; Kulla et al., 1999). For plasticity-specific effects, the highest concentration that was found to have no effect on basal synaptic transmission was used. Compounds or vehicle (0.9% NaCl) were applied via a Hamilton syringe in a 5 μl injection volume over a period of 5 min. Injections were administered following measurement of the baseline for 30 min. In LTP and LTD experiments HFS or LFS was applied 30 min after drug/injection vehicle, with measurements then taken at t = 5, 10 and 15 min and then at subsequent 15 min intervals up to 4 h, with an additional measurement taken at 24 h post-HFS/LFS. The same protocol was followed for baseline experiments, except that no HFS or LFS was given. In PP experiments drugs were applied 30 min before PP stimulation was started.

**Data Analysis**

The baseline fEPSP or PS data were obtained by averaging the response to stimulation of the perforant path to obtain five sweeps at 10 s intervals, every 5 min over a period of 30 min. The data were then expressed as mean pre-injection baseline values in % ± SEM. Statistical significance was estimated using analysis of variance (ANOVA) with repeated measures. Between group analysis was conducted of the entire study in cases where basal synaptic transmission were examined, or of the time period after HFT or LFS, in the case of plasticity experiments. Post hoc Student’s tests were used to identify specific time-points at which a difference in evoked responses (between control- and drug-treated groups) became temporally evident. The probability levels interpreted as statistically significant were *P < 0.05, **P < 0.01 and ***P < 0.001.

**Results**

**mGluR3 Activation is Necessary for the Expression of LTD in Vivo**

To investigate whether the mGluR3 receptor plays a critical role in LTD, experiments were carried out in which the specific agonist NAAG (Wroblewska et al., 1997) or the antagonist β-NAAG (Lea et al., 2001) was administered 30 min before induction of LTD. The concentrations used (NAAG: 3 μg, n = 6; β-NAAG: 61 μg, n = 4) did not have any effect on basal synaptic transmission compared with vehicle-injected controls (n = 6, Fig. 1; ANOVA, NAAG-experiments: PS values: F(1,27) = 0.35, P = 0.55; fEPSP values: F(1,27) = 0.05, P = 0.82; ANOVA, β-NAAG-experiments: PS values: F(1,27) = 0.59, P = 0.44; fEPSP values: F(1,27) = 0.33, P = 0.56).

Robust LTD, which lasted for up to 24 h, was induced by LFS at 1 Hz (Fig. 2, n = 5). (In these experiments insertion of the guide cannula produced a transient change in basal recording values which recovered back to stable basal synaptic transmission before LFS application. This was likely due to the lower temperature of the solution. In subsequent experiments the solution was warmed before injection.) LFS given in the presence of vehicle produced a depression of PS values to 47 ± 8% of baseline levels and fEPSP values to 77 ± 5% at 5 min post LFS (t-test, P < 0.05; Fig. 2, n = 5). The level of depression remained unchanged up to 24 h where PS values were 38 ± 7% and fEPSP values 70 ± 4% of baseline levels (t-test, P < 0.05).

It was found that LTD expression was inhibited when the mGluR3 antagonist β-NAAG (n = 5) was injected before LFS (Fig. 2; ANOVA: PS values: F(1,27) = 107.12, P < 0.0001; fEPSP values: F(1,27) = 60.48, P < 0.0001 compared with vehicle treated controls). The effect became apparent at ~15 min after application of LFS.

Intriguingly, the mGluR3 agonist, NAAG, exhibited mild inhibitory effects on the maintenance of LTD (n = 10, Fig. 3). Here PS amplitude was significantly reduced 225 and 240 min after LFS had been applied (t-test, P < 0.05). fEPSP values were reduced from roughly 2 h after LFS onwards. By 24 h post-LFS no difference was seen in PS and fEPSP values however, indicating that these effects were transient. ANOVA indicated no overall
significant difference in the profile of PS responses when values in control and NAAG animals were compared after LFS. ANOVA determined that iEPSP responses were significantly different \(F(1.22) = 0, P < 0.05\).

**Paired-pulse Depression is Reduced after mGluR3 Receptor Activation**

Given the fact the mGluR3 is localized both presynaptically and postsynaptically, we investigated the locus of action of NAAG and \(\beta\)-NAAG. The influence of the mGluR3 ligands on the PP ratio at an interstimulus interval (ISI) range of 20–1000 ms was thus investigated to evaluate their action on presynaptic versus postsynaptic mGluR3.

Application of NAAG (3 μg) affected PP ratio by reducing PP depression at an ISI of 40 ms (Fig. 4, \(n = 20\), \(t\)-test: \(P < 0.05\)) supporting a presynaptic action of the agonist. Application of the antagonist \(\beta\)-NAAG (61 μg) elicited no change in PP expression suggesting that the antagonist acts on postsynaptic mGluR3 (Fig. 4, \(n = 11\)).

**MGlur3 Receptor Activation Reduces the Magnitude of LTP In Vivo**

To investigate the involvement of mGluR3 in LTP, we examined the effects of the specific agonist NAAG and the antagonist \(\beta\)-NAAG.

LTP in the dentate gyrus was induced by means of 200 Hz HFT of the medial perforant path. HFT given in the presence of vehicle produced a potentiation of PS values to 180 ± 10% of baseline level and iEPSP values to 170 ± 13% at 5 min post-HFT (\(t\)-test at 5 min post-HFT: \(P < 0.05\) compared with vehicle treated controls; Fig. 5, \(n = 11\)). This potentiation slightly declined to PS values of 157 ± 13% and iEPSP values at 145 ± 11% at 24 h post-HFT (\(t\)-test at 24 h post-HFT: \(P < 0.05\) compared with vehicle treated controls).

The independent application of the antagonist, \(\beta\)-NAAG, did not affect LTP compared with vehicle treated controls [Fig. 5, \(n = 8\); ANOVA: PS values: \(F(1.27) = 1.83, P = 0.18\); iEPSP values: \(F(1.27) = 0, P = 0.96\)]. The profile of LTP remained entirely unchanged in the presence of the antagonist, given in the same concentration that blocked LTD.

Application of a group II mGluR agonist inhibits the expression of LTP (Kulla et al., 1999); however, it is not known whether mGluR2 or mGluR3 contribute separately to this effect. We investigated if mGluR3 contributes to the inhibition of LTP seen by group II mGluR activation by applying NAAG, in our study. Following injection of NAAG (3 μg, \(n = 7\)) LTP was significantly inhibited compared with vehicle treated controls [Fig. 6; ANOVA: PS values: \(F(1.27) = 182.97, P < 0.0001\); iEPSP values: \(F(1.27) = 168.90, P < 0.0001\)]. Effects became apparent immediately after application of HFT. By 3.5 h post HFT, PS and iEPSP values showed no significant difference to basal levels of activity [ANOVA: PS values: \(F(1.27) = 0.66, P = 0.42\); iEPSP values: \(F(1.27) = 0.19, P = 0.66\)].

The inhibitory effects of NAAG on LTP may, however, have been elicited by non-mGluR3-mediated mechanisms. Therefore, we also examined whether the highly selective group II mGluR antagonist, EGLU (100 nmol, \(n = 8\); Thomas et al., 1996), could block NAAG's effects (Fig. 6). When EGLU was applied 30 min prior to NAAG, a significant inhibition of the inhibitory effects of NAAG on LTP were seen, thus confirming that NAAG was acting via group II mGluRs [ANOVA: PS values: \(F(1.27) = 105.87, P < 0.0001\); iEPSP values: \(F(1.27) = 124.85, P < 0.0001\)].

**Discussion**

The present study reveals an intriguing role of mGluR3 (a group II mGluR) in hippocampal synaptic plasticity. This receptor appears to be critically required for expression of LTD but not LTP: thus, LTD but not LTP was blocked by an mGluR3 antagonist. Most interestingly, postsynaptically expressed neuronal mGluR3 as well as presynaptically expressed mGluR3 appear to work together in regulating synaptic efficacy. Whereas activation of postsynaptic mGluR3 receptors are necessary for LTD, presynaptic mGluR3 receptors functions as modulators of both LTP and LTD.

**Characterization of Group II Metabotropic Glutamate Receptors in Synaptic Plasticity**

Group II mGluRs are subdivided into the subtypes mGluR2 and mGluR3. Both couple to adenylyl cyclase but are distinguished by their agonist coupling (Wroblewska et al., 1997; Conn and Pinn, 1997), the length of their carboxy terminus (Nakanishi,
1992) and their localization (Petralia et al., 1996). It was shown previously that agonist activation of group II mGluRs leads to inhibition of LTP and enhancement of LTD in the dentate gyrus in vivo (Manahan-Vaughan, 1998; Kulla et al., 1999). In the CA1 region, in vivo, LTD is reduced to short-term depression (STD) by group II mGluR antagonists (Manahan-Vaughan, 1997) whereas in the dentate gyrus STD was transformed to LTD by group II mGluR agonists (Manahan-Vaughan, 1998). In vitro, activation of mGluR3 receptors by the agonist NAAG leads to inhibition of hippocampal LTP (Lea et al., 2001) and induction of chemical LTD (Huang et al., 1999). Our data comprise the first characterization of the role of mGluR3 in the intact animal.

**LTD In Vivo Requires Activation of the mGluR3 Receptor**

In the dentate gyrus of freely moving rats robust LTD was induced by 1Hz LFS of the medial perforant path. We found that treatment with an mGluR3 specific antagonist leads to a significant inhibition of LTD expression, which suggests a critical role for this receptor in LTD. Group II mGluRs are negatively coupled to the cAMP-PKA-signalling cascade (Pin and Duvoisin, 1995), which leads to LTD promoting processes, e.g. dephosphorylation of AMPA receptors (Banke et al., 2000). Our PP study suggested that β-NAAG binds solely to postsynaptic mGluR3. Therefore it is feasible that β-NAAG exerts its action by interfering with postsynaptic cAMP-dependent processes which are essential for the long-term expression of LTD.

Interestingly, a transient inhibitory effect on the intermediate phase of LTD was seen when the mGluR3 agonist NAAG (Wroblewska et al., 1997) was applied. Chemical LTD, which occludes LFS-induced LTD, is induced by NAAG in vitro (Huang et al., 1999). Our expectation therefore was that activation of mGluR3 by the agonist NAAG would enhance LTD or leave LTD unchanged if the maximal degree of depression was already reached after LFS. To our surprise, we found a small but significant reduction of the intermediate phase of LTD, although no lasting effect on LTD occurred (24 h post LFS no effect by NAAG on LTD was evident compared with controls). The inhibitory effect of NAAG became apparent only for the fEPSP

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**Figure 2.** LTD in the dentate gyrus in vivo is dependent on mGluR3 receptor activation. (A, B) The mGluR3 antagonist β-NAAG (61 µg) significantly inhibits the expression of LTD (n = 5) compared with vehicle injected controls (n = 5). This applies for PS amplitude (A) as well as fEPSP slope (B). (C) Original analogue traces showing evoked responses in the dentate gyrus at three time points: (i) preinjection, (ii) t = 5 min and (iii) t = 24 h post-LFS. Analogues from vehicle baseline, vehicle LFS and β-NAAG LFS experiments are compared.
data, suggesting that the effect is mediated presynaptically. This possibility was supported by the effects on PP depression elicited by NAAG. However, the effects on LTD at this concentration of NAAG were very subtle, and not associated with an alteration of basal synaptic transmission in animals that received test-pulses only. This suggests that activation of presynaptic mGluR3 may be able to contribute to metaplasticity.

The interference of NAAG with the intermediate phase of LTD expression could be perhaps due to its inhibitory action on presynaptic GABA-release (Zhao et al., 2001): it was shown for the CA1 region that disinhibition of GABAergic regulation suppresses LTD (Hsu et al., 1999). Alternatively, effects may have been mediated by alterations of presynaptic glutamate release. If so, the effects were not strong enough to influence the NMDA receptor-dependent induction phase of LTD (Dudek and Bear, 1992; Manahan-Vaughan, 1997), but may have affected spillover of glutamate to the extrasynaptic space during LFS.

Group II mGluRs are localized extrasynaptically (Lujan et al., 1997). Thus, a reduction in glutamate release would be expected to influence the mGluR-dependent phase of LTD, which has been shown to occur roughly 90–120 min after LFS (Manahan-Vaughan, 1997).

**The mGluR3 Agonist NAAG and Antagonist β-NAAG Act at Different Synaptic Sites**

On neurons, group II mGluRs are predominantly presynaptically expressed, though they are also expressed postsynaptically (Ohishi et al., 1994; Petralia et al., 1996; Shigemoto et al., 1997). Astrocytic expression of mGluR3 also occurs (Wroblewska et al., 1998; Schools and Kimelberg, 1999). To ascertain if the selective mGluR3 ligands exert their effects pre- or postsynaptically, we conducted PP experiments with ISIs ranging from 20 to 1000 ms. The mGluR3 antagonist β-NAAG did not change...
PP responses for all ISI tested. This suggests a solely postsynaptic action of this ligand. However, one cannot exclude that possible effects on the PP ratio were masked because an effect would only be seen when tonic activation of presynaptic mGluR3 by glutamate was prevented by the antagonist. Thus the failure of β-NAAG to change PP expression could also indicate that tonic activation of presynaptic mGluR3 is negligible in this preparation because glutamate transporters function effectively in the intact brain.

In contrast to β-NAAG, the selective mGluR3 agonist NAAG reduced PP depression at an ISI of 40 ms, supporting a presynaptic action of the ligand. A presynaptic mode of action is supported by results from Sanabria et al. (2004) which suggest that the mGluR3 agonist NAAG suppresses synaptic transmission through presynaptic activation of group II mGluRs. However, Lea et al. (2001) saw no effect of NAAG on PP depression. This might be because they did not test the PP expression at the ISI of 40 ms which uncovered the difference in NAAG versus β-NAAG treatment in the current study. A previous study that examined PP depression at an ISI of 40 ms reported a reduction in PP depression by group II mGluR agonists (Brown and Reymann, 1995; but see also, Huang et al., 1999).

The effect of NAAG at an ISI of 40 ms suggests that the agonist affects presynaptic GABAergic transmission, since PP responses obtained in the range of 20–40 ms reflect the activation of GABA fast inhibitory postsynaptic potentials and subsequent inhibition of dentate gyrus granule cells (Albertson and Joy, 1987; DiScenna and Teyler, 1994; Moser, 1996). The finding is supported by the study of Zhao et al. (2001), which demonstrated that NAAG inhibits GABA-release via presynaptic mGluR3 activation on cortical interneurons. Thus the presynaptic effects of NAAG may be mediated not by neurally localized receptors but rather by interneuronally localized mGluR3. On the other hand, the antagonist effects appear to be mediated postsynaptically, suggesting that differences exist between pre- and postsynaptically expressed mGluR3.

**LTP In Vivo Does Not Critically Depend upon mGluR3**

Kulla et al. (1999) demonstrated that agonist activation of group II mGluRs inhibits LTP expression in the dentate gyrus in vivo. We were thus interested to clarify the role of mGluR3 in LTP expression in this subregion.

Interestingly, application of the mGluR3 antagonist, β-NAAG, did not change LTP expression at concentrations which affected LTD expression. Because β-NAAG seems to solely antagonize postsynaptic mGluR3, this suggests that postsynaptically located mGluR3 are not essential for LTP. This result is in line with the report that the group II mGluR antagonist EGLU leaves LTP in the dentate gyrus unaffected (Kulla et al., 1999).

The selective mGluR3 agonist NAAG inhibited the expression of LTP. This points to a modulatory role for mGluR3 in LTP expression. A presynaptic mode of action is most probable because the same concentration of NAAG that was effective against LTP also inhibited PP depression in our experiments. A presynaptic action of NAAG could comprise the inhibition of GABA release (Zhao et al., 2001; Turner and Salt, 2003). However, synaptic disinhibition is known to maintain LTP (Stelzer et al., 1994). Rather, activation of presynaptic mGluR3 could antagonize LTP-dependent mechanisms for the enhancement of glutamate release: it is known that activation of presynaptic mGluR can reduce transmitter release by inhibition of voltage-gated calcium channels (Chavis et al., 1994; Takahashi et al., 1996) and/or direct influence on the release machinery (Scanziani et al., 1995). However, an additional activation of postsynaptic mGluR3 by NAAG cannot be excluded. NAAG could reduce cAMP levels through the activation of postsynaptic mGluR3 (Tanabe et al., 1993) and consequently decrease PKA-dependent phosphorylation, which would in turn impair LTP expression. The effect of NAAG on LTP was weaker compared with the potency of effects seen with the general group II mGlu agonist 4C3HPG (Kulla et al., 1999). This suggests that not only mGluR3, but also mGluR2, contributes to the modulation of LTP expression.

**Does NAAG Act Specifically at mGluR3?**

NAAG may elicit non-specific effects that do no arise from mGluR3 activation. There are conflicting reports about NAAG affecting NMDA receptors: on the one hand, NAAG has been described as a low-potency agonist at the NMDA receptor (Westbrook et al., 1986; Valivullah et al., 1994). Additionally, it was suggested that NAAG may act as a partial agonist (Puttfarcken et al., 1993) or antagonist (Bergeron et al., 2004) at this receptor. On the other hand, Losi et al. (2004) found no evidence for agonistic or antagonistic activity of NAAG at NMDA receptors. These differences in results may derive from differences in the drug concentrations or test systems used.

To exclude the possibility that NAAG altered synaptic plasticity by a non-mGluR3-mediated mechanism, we examined in our study whether the highly selective group II mGluR...
antagonist EGLU (Thomas et al., 1996; Jane et al., 1996) prevents the effects on NAAG on LTP. In support of an mGluR3-mediated action of NAAG, we found that EGLU completely prevented the inhibition of LTP by NAAG. We can thus presume that the concentration of NAAG used in our study was specific for the agonism of mGluR3.

**Role of Metabotropic Glutamate Receptors in LTD**

This study demonstrated that antagonism of mGluR3 prevented LTD in the dentate gyrus in vivo. Other studies have shown that selective antagonists for group I or group III mGluRs can also fully block LTD in this hippocampal subfield (Camodeca et al., 1999; Klausnitzer et al., 2004). It seems likely that all three mGluR subgroups work together in the enablement of LTD in the dentate gyrus, and possibly that the ‘absence’ of one of these receptor groups cannot be compensated for by the remaining receptors. This likelihood is also supported by studies with transgenic animals, where knockout of mGluR1 or mGluR5, for example, will lead to impairments of synaptic plasticity even though the other members of the mGluR are functionally intact (Aiba et al., 1994; Lu et al., 1997).

**Conclusions**

In this study we described for the first time the importance of the mGluR3 receptor for synaptic plasticity in vivo. Our results suggest that postsynaptic mGluR3 is critically involved in the expression of LTD, whereas presynaptic mGluR3 regulates the degree of LTD and LTP expressed. Our study thus both reveals a distinct role for mGluR3 in the regulation of synaptic plasticity in vivo and suggests that defined mGluR3 subpopulations are involved in this phenomenon.

**Notes**

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Figure 6. LTP in the dentate gyrus in vivo is modulated by mGluR3 receptor activation. (A, B) The mGluR3 agonist NAAG (3 μg) significantly inhibits the expression of LTP (n = 7) compared with vehicle injected controls (n = 11). The selective group II mGluR antagonist EGLU (100 nmol) prevents the inhibitory effects of NAAG (3 μg) on LTP (n = 8). This is true for PS amplitude (A) and fEPSP slope (B). (C) Original analogue traces showing LTP responses in the dentate gyrus at three time points: (i) preinjection, (ii) t = 5 min and (iii) t = 24 h post-LFS. Analogues from vehicle baseline, vehicle LFS and β-NAAG LFS experiments are compared.

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