Differential Roles for mGluR1 and mGluR5 in the Persistent Prolongation of Epileptiform Bursts

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Received 13 July 2001; accepted in final form 21 September 2001

Merlin, Lisa R. Differential roles for mGlur1 and mGlur5 in the persistent prolongation of epileptiform bursts. J Neurophysiol 87: 621–625, 2002; 10.1152/jn.00579.2001. Transient activation of group I metabotropic glutamate receptors (mGlurS) with the selective agonist (S)-3,5-dihydroxyphenylglycine (DHPG) produces persistent prolongation of epileptiform bursts in guinea pig hippocampal slices, the maintenance of which can be reversibly suppressed with group I mGlur antagonists. To determine the relative roles of mGlur1 and mGlur5 in these group I mGlur-dependent induction and maintenance processes, subtype-selective antagonists were utilized. In the presence of picrotoxin, DHPG (50 μM, 20–45 min) converted interictal bursts into 1- to 3-s discharges that persisted for hours following washout of the mGlur agonist. 2-methyl-6-(phenylethynyl)-pyridine (MPEP, an mGlur5 antagonist; 25 μM) and (+)-2-methyl-4-carboxyphenylglycine (LY367385, an mGlur1 antagonist; 20–25 μM) each significantly suppressed the ongoing expression of the mGlur-in-duced prolonged bursts. However, LY367385 was more effective, reducing the burst prolongation by nearly 90%; MPEP only produced a 64% reduction in burst prolongation. Nevertheless, MPEP was more effective at preventing the induction of the burst prolongation; all 10 slices tested failed to express prolonged bursts both during and after co-application of DHPG with MPEP. Co-application of DHPG with LY367385, in contrast, resulted in significant burst prolongation (in 68% of slices tested) that was revealed on washout of the two agents. These results suggest that while both receptor subtypes participate in both the induction and maintenance of mGlur-mediated burst prolongation, mGlur1 activation plays a greater role in sustaining the expression of prolonged bursts, whereas mGlur5 activation may be a more critical contributor to the induction process underlying this type of epileptogenesis.

INTRODUCTION

Transient activation of group I metabotropic glutamate receptors (mGlurS) converts interictal bursts into seizure-length discharges (Merlin 1999; Merlin and Wong 1997), and in vivo studies reveal that selective group I mGlur agonists are convulsants (Camón et al. 1998; Thomsen and Dalby 1998). Moreover, group I mGlur-induced burst prolongation persists for hours following washout of the selective agonist, and group I mGlur antagonists reversibly suppress the persistent prolonged bursts (Merlin 1999; Merlin and Wong 1997). These findings implicate group I mGlurS not only in the expression of seizures but in the induction and maintenance of epileptogenesis, the process in which the neuronal cortical network becomes persistently predisposed to the production of seizure discharges.

The group I mGlurS are comprised of two receptor subtypes (mGlur1 and mGlur5), both of which are present in the hippocampal CA3 region (Blümcke et al. 1996; Fotuhi et al. 1994; Luján et al. 1996). Although both subtypes are linked to inositol-1,4,5-triphosphate (IP3) generation and calcium mobilization (Abe et al. 1992; Houamed et al. 1991; Masu et al. 1991), mGlur1 and mGlur5 generate different intracellular calcium responses (Kawabata et al. 1998). Furthermore, in kindling as well as human temporal lobe epilepsy studies, mGlur1 immunoreactivity and mRNA expression are upregulated while mGlur5 mRNA is either unchanged or downregulated (Akbar et al. 1996; Al-Ghouli et al. 1998; Blümcke et al. 2000), demonstrating independent regulatory control of the two receptors. Thus mGlur1 and mGlur5 may respond differently to similar stimuli, resulting in different long-term effects. Studies described herein therefore examine the contributions of each receptor subtype to the induction and maintenance of the persistent potentiation of epileptiform activities in the hippocampal slice. Portions of this work have appeared in abstract form (Merlin 2000).

METHODS

Guinea pigs 2–4 wk old were anesthetized with halothane and decapitated in accordance with standard protocols. The brain was placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 26 NaHCO3, 5 KCl, 1.6 MgCl2, 2 CaCl2, and 10 d-glucose. Transverse hippocampal slices (400 μm) were prepared with a Vibratome (Technical Products International) and then transferred to nylon mesh in an interface recording chamber (Fine Science Tools). Slices were oxygenated and humidified with 95% O2-5% CO2 at 35–36°C, perfused with ACSF, and incubated at least 1 h prior to recording.

Recordings from CA3 stratum pyramidale used 30- to 80- or 2- to 5-MΩ glass microelectrodes filled with 2 M potassium acetate or 2 M NaCl (for intracellular or extracellular recording, respectively). Data were amplified and digitized using an Axoclamp 2B and DigiData 1200 series interface (Axon Instruments) and analyzed using Clamp and SigmaPlot software. All chemicals were bath-applied. Picrotoxin (PTX, 50 μM), an antagonist of GABA_A receptor-mediated inhibition, was present throughout all experiments. (S)-3,5-dihydroxyphenyl-

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glycine (DHPG), (+)-2-methyl-4-carboxyphenylglycine (LY367385), and 2-methyl-6-(phenylethynyl)-pyridine (MPEP) were obtained from Tocris Cookson (Balwin, MO); all other chemicals were from Sigma (St. Louis, MO).

Sample sizes (n) refer to number of slices tested. Burst frequency (Hz) represents the reciprocal of interburst interval (seconds). Net burst prolongation was determined by subtracting the mean burst length induced in PTX ("control") from the prolonged bursts induced by DHPG. Data are reported as means ± SE. Statistical significance was determined using the Student’s t-test, paired when appropriate, using the baseline picrotoxin bursts in a given slice as its own control. Additionally, to compare across groups (LY367385 vs. MPEP), ANOVA with post-ANOVA Newman-Keuls multiple comparison test was performed. P < 0.05 was considered significant.

RESULTS

Contribution of mGluR1 vs. mGluR5 in the maintenance of DHPG-induced persistent prolonged bursts

Synchronized epileptiform discharges 320-525 ms in duration recurred regularly at 0.10–0.15 Hz in CA3 pyramidal cells in guinea pig hippocampal slices perfused with PTX. The addition of 50 µM DHPG (a group I mGluR agonist) (Ito et al. 1992) elicited a rapid-onset increase in burst frequency, reaching a maximum of 0.38 ± 0.02 Hz at approximately 10 min (n = 8) (also see Galoyan and Merlin 2000; Merlin et al. 1998). This was accompanied by a progressive increase in epileptiform burst duration that persisted on removal of the agonist (BD at 45 min DHPG, 1,631 ± 174 ms, n = 8; e.g., Fig. 1A). We have previously shown that the maintenance of DHPG-induced prolonged epileptiform bursts can be reversibly suppressed with group I mGluR antagonists (Merlin 1999; Merlin and Wong 1997). To determine whether this effect is primarily mediated by mGluR1 or mGluR5, experiments were performed using either the selective mGluR1 antagonist LY367385 (Clark et al. 1997) or MPEP, a selective antagonist for mGluR5 (Gasparini et al. 1999).

In the presence of LY367385 (20–25 µM), a nearly complete, reversible suppression of the prolonged bursts was seen, similar in appearance to that seen with the group I mGluR antagonists (+)-α-methyl-4-carboxyphenylglycine or (S)-4-carboxyphenylglycine (Figs. 1 and 2) (cf. Merlin and Wong 1997). LY367385 reduced the BD from 1,670 ± 201 to 508 ± 110, an 89% reduction of net prolongation over control length (n = 4; Fig. 2). In comparison, MPEP (25 µM) produced only a partial suppression of the prolonged persistent epileptiform bursts, with a significant reduction in mean BD (P < 0.05) but with a wide variability in burst length (e.g., Fig. 1). The mean

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BD just prior to MPEP application (at 30–60 min of DHPG washout) was 1,712 ± 233 ms; MPEP reduced the BD to 889 ± 148 ms, a 64% reduction of the net prolongation (n = 4; Fig. 2). ANOVA analysis revealed that the BD in MPEP was significantly different from those seen in LY367385 and the two sets of control picrotoxin bursts (P < 0.05).

### Contribution of mGluR1 vs. mGluR5 in DHPG-mediated induction of burst prolongation

Additional experiments were performed in which DHPG was applied in the presence of either MPEP or LY367385 (25 μM each). In all slices tested in the presence of MPEP, the induction of burst prolongation was markedly suppressed. The control burst duration in PTX + MPEP was 349 ± 20 ms; at 45–55 min of DHPG in the presence of MPEP, 411 ± 49 ms; after 1 h washout of DHPG and MPEP, 382 ± 56 ms (P > 0.05, n = 10) (Fig. 3B, I and 2). Nevertheless, the increase in burst frequency normally seen at 10 min of DHPG application was unaffected by the presence of MPEP, reaching a maximum frequency of 0.47 ± 0.03 Hz (n = 10; Fig. 3B1, bottom graph). Furthermore, burst prolongation could be elicited on reintroduction of DHPG in the absence of MPEP (Fig. 3B1, top graph).

DHPG application in the presence of LY367385 similarly elicited a prompt increase in burst frequency (0.35 ± 0.01 Hz at 10 min, n = 8; Fig. 3A1), and prolonged bursts were not expressed during co-application of DHPG and LY367385 (BD 418 ± 32 ms at 50 min DHPG in the presence of LY367385; P > 0.05 relative to control BD; n = 8). However, although in three slices BD remained unchanged at 1 h washout of the two agents (455 ± 25 ms, n = 3, P > 0.05), in five of eight slices, burst prolongation gradually appeared on washout (964 ± 196 ms at 1 h washout, n = 5, P < 0.05; Fig. 3A, I and 2).
**DISCUSSION**

**Potentiation of mGluR1-mediated responses underlies maintenance of prolonged bursts**

The data reveal that both mGluR1 and mGluR5 activation contribute independently to maintaining the persistent prolonged synchronized discharges (see Figs. 1 and 2). However, mGluR1 antagonist is much more effective at suppressing the persistent prolonged bursts, restoring the discharges to their control length in picrotoxin. This suggests that glutamate acting at mGlu1 receptors sustains the prolonged bursts. The burst prolongation may therefore be mediated predominantly by potentiation of mGluR1-mediated responses, similar to that shown accompanying both kindling-induced seizures and temporary lobe epilepsy (Akbar et al. 1996; Al-Ghoul et al. 1998; Blümcke et al. 2000). The involvement of mGluR5-mediated responses is less impressive (Figs. 1 and 2); this too is consistent with kindling studies, some of which demonstrate potentiation of responses (Keele et al. 2000) while others demonstrate mGluR5 down-regulation (Akbar et al. 1996) or lack of change (Blümcke et al. 2000).

**Activation of mGluR5 plays a critical role in the induction of long-lasting burst prolongation**

The potentiation of group I mGluR-mediated responses described in the preceding text is induced via transient group I metabotropic glutamate receptor expression (via antisense oligonucleotide injections) dramati- cally suppresses the rate of progression of kindled seizures, providing a selective and systemically active mGlu5 receptor antagonist. Nevertheless, prolonged bursts did appear with mGluR1 antagonist only serves to suppress seizure expression. Indeed, prolongation of epileptiform bursts was not elicited when DHPG was transiently applied in the presence of mGluR1 antagonist. Nevertheless, prolonged bursts did appear after the mGluR1 antagonist was removed (Fig. 3), revealing that the induction process stimulated by mGluR5 activation was able to proceed unimpeded, despite the suppressed expression of the seizure discharges. Given the stronger dependence on mGluR5 activation for induction of persistent prolonged bursts (Fig. 3), antagonism of mGluR5 may be required to accomplish the task of truly preventing epileptogenesis. Selective mGluR5 antagonists should therefore be studied for their potential clinical usefulness as antiepileptogenic agents.

The author thanks P. Huszár for helpful discussions. This work was funded by National Institute of Neurological Disorders and Stroke Grants NS-01901 and NS-40387.

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