Long-Lasting Potentiation of Epileptiform Bursts by Group I mGluRs Is NMDA Receptor Independent

SAMVEL M. GALOYAN AND LISA R. MERLIN
Department of Neurology and Department of Physiology and Pharmacology, State University of New York Health Science Center at Brooklyn, Brooklyn, New York 11203

Galoyan, Samvel M. and Lisa R. Merlin. Long-lasting potentiation of epileptiform bursts by group I mGluRs is NMDA receptor independent. J. Neurophysiol. 83: 2463–2467, 2000. In CA3 pyramidal cells of guinea pig hippocampal slices, picrotoxin (50 μM) elicited spontaneous, rhythmically recurring epileptiform bursts 285–435 ms in duration. The addition of (S)-3,5-dihydroxyphenylglycine (DHPG, 50 μM, 90 min application), a selective group I metabotropic glutamate receptor (mGluR) agonist, resulted in a rapid-onset transient increase in burst frequency. This was followed by a slowly progressive increase in burst duration, with bursts reaching 1.5–3.8 s in duration at 90 min of DHPG application. The potentiation of epileptiform burst duration persisted at least 2 h after agonist removal. To determine whether N-methyl-D-aspartate (NMDA) receptor activation participates in the mGluR-induced potentiation of epileptiform bursts, experiments were carried out in the presence of d-2-amino-5-phosphonovaleric acid (APV, 50–100 μM), an NMDA receptor antagonist. Application of DHPG in the presence of APV resulted in a significantly enhanced transient increase in burst frequency. Nevertheless, when compared with the control described above, there was no significant alteration in the rate of development of the burst prolongation nor its persistence after washout. In other experiments, application of APV in the presence of fully developed mGluR-induced potentiated bursts (after 90 min washout of DHPG) resulted in no significant change in either burst frequency or duration. The data reveal that both induction and maintenance of group I mGluR-mediated potentiation of epileptiform discharges are NMDA receptor-independent processes, suggesting that epileptogenesis, when induced by group I mGluR activation, may occur and be sustained in the absence of NMDA receptor activation.

INTRODUCTION

Group I metabotropic glutamate receptor (mGluR) activation produces two effects on ongoing interictal activity in vitro: a rapid-onset, transient acceleration of interictal bursting and a slowly progressive, persistent prolongation of individual synchronized bursts into 1–12 s events (Merlin 1999; Merlin and Wong 1997; Merlin et al. 1998a; Taylor et al. 1995). The persistent increase in burst duration has a protein synthesis-dependent induction mechanism (Merlin et al. 1998a), which supports the conclusion that a long-term modification of cortical network properties has occurred. Such group I mGluR-mediated network plasticity may contribute to epileptogenesis, the process in which the network develops a long-lasting propensity for the production of seizure discharges. Experiments examining the mechanisms for synaptic and network plasticity in the hippocampus frequently demonstrate a requirement of N-methyl-D-aspartate (NMDA) receptor activation for induction to occur (Collingridge and Bliss 1987; Lynch et al. 1990; Morris et al. 1986; Slater et al. 1985; Stasheff et al. 1993; Swartzwelder et al. 1989; Young and Dragunow 1994). Potentiated NMDA receptor-mediated responses may also participate in sustaining the enhancement of network events (Traub et al. 1994, 1996). The studies presented herein address the role of NMDA receptor activation in the induction and maintenance of group I mGluR-mediated epileptogenesis. Portions of this work have appeared in abstract form (Merlin et al. 1998b).

METHODS

Guinea pigs (2–4 wk old) were anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane and decapitated. Brains were promptly placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 1.6 MgCl₂, 2 CaCl₂, 26 NaHCO₃, and 10 d-glucose. Hippocampi were dissected free and transverse slices 400 μm thick were prepared using a Vibratome (Technical Products International). Slices were placed on nylon mesh in an interface chamber and perfused with ACSF bubbled with 95% O₂,5% CO₂ at pH 7.4. Temperature was maintained at 35.5–36.0°C. Intracellular recordings were obtained from the CA3 stratum pyramidalis using thin-walled glass microelectrodes with tip resistance of 30–80 MΩ and filled with 2 M potassium acetate. Recordings were amplified and digitized (Axoclamp 2A and TL-1 DMA Interface, Axon Instruments). Drugs were applied via continuous bath perfusion. Picrotoxin (50 μM) was present throughout all experiments to elicit baseline epileptiform activity. (S)-3,5-dihydroxyphenylglycine (DHPG) and d-2-amino-5-phosphonovaleric acid (APV) were obtained from Tocris Cookson (Ballwin, MO). All other chemicals were obtained from Sigma (St. Louis, MO).

Burst duration (BD) was measured from the onset of primary burst depolarization until the end of the final secondary burst and did not include the postburst afterhyperpolarization. Instantaneous burst frequencies (Hz) represent the reciprocal of the interburst intervals (in seconds). Statistical significance was determined using Student’s t-test (paired t-test was used to evaluate changes in given slices over time; unpaired was used for differences between treatment groups). A P value <0.05 was considered significant. Data are reported as mean ± SE.

RESULTS

Effects of group I mGluR activation on picrotoxin-induced epileptiform activity

In the presence of picrotoxin, an antagonist of GABA_A receptor-mediated inhibition, CA3 pyramidal cells of guinea pig hippocampal slices expressed spontaneous synchronized...
FIG. 1. Induction of group I metabotropic glutamate receptor (mGluR)-mediated potentiation of epileptiform bursts in the presence of N-methyl-D-aspartate (NMDA) receptor antagonist. Picrotoxin (50 μM) induced baseline epileptiform activity and was present throughout all experiments. (S)-3,5-dihydroxyphenylglycine (DHPG, 50 μM) was applied for 90 min; time 0, beginning of DHPG washout. A: continuous intracellular recording from a single CA3 pyramidal cell. Each epileptiform discharge is represented in A1 by two symbols: ● representing burst duration and ○ representing burst frequency. For times indicated, corresponding traces are shown in A2 on two time scales; left and right calibration bars are for left and right traces, respectively. V_m for upper traces, −66 mV; middle traces, −68 mV; lower traces, −74 mV. B: representative example of DHPG-mediated induction of epileptiform burst potentiation in the presence of 50 μM 2-amino-5-phosphonovaleric acid (APV), an NMDA receptor antagonist. Changes in burst frequency and duration are displayed in B1 and B2 as in A1 and A2. V_m for upper and middle traces, −60 mV; lower traces, −54 mV. C: summary data comparing rate of development of DHPG-induced burst prolongation in the presence (●, n = 5) or absence (○, n = 6 up to 60 min, n = 5 at 90 min) of APV. Concurrent changes in burst frequency are also reported (●, APV present; ○, without APV). BD, burst duration; Hz, burst frequency. There is no significant difference in the two groups except for a transient enhancement of burst acceleration in APV at 10 min (*, P < 0.05, DHPG + APV vs. DHPG alone). D: summary graph demonstrating percent increase in BD over initial length of picrotoxin (pic)-induced epileptiform discharges (100 × [BD – BD_pico] / BD_pico). Time 0, initiation of washout period; 2 h of washout were monitored at 30-min intervals. Experiments with APV present during DHPG application (filled bars) are compared with experiments without APV (open bars). Error bars indicate SE. At 0–60 min, n = 6 for control group and 5 for APV; at 90–120 min, n = 5 for both groups.
bursts $321 \pm 10$ ms in duration recurring regularly at $0.13 \pm 0.01$ Hz ($n = 6$). The addition of $50 \mu$M DHPG, a selective group I mGluR agonist, resulted in a rapid-onset increase in the frequency of bursts (peak frequency $0.48 \pm 0.02$ Hz 10 min after introduction of agonist). This was accompanied by a mild decrease in burst duration (BD$_{10 \text{ min}}$ 250 ± 18 ms, $P < 0.01$, $n = 6$; Fig. 1A). Continued exposure to the agonist elicited a gradual increase in BD and a concurrent return of burst frequency to baseline (BD$_{2 \text{h wash}}$ 2264 ± 370 ms at 0.12 ± 0.02 Hz; $n = 5$). At 2 h of agonist removal, BD was still significantly prolonged over the initial picrotoxin-induced BD (BD$_{2 \text{h wash}}$ 269 ± 269 ms at 0.12 ± 0.01 Hz, 475 ± 80% potentiated, $n = 5$; Fig. 1A).

Effect of NMDA receptor antagonist on the induction of group I mGluR-mediated enhancement of epileptiform activity

To investigate the role of NMDA receptors in the induction of group I mGluR-mediated prolongation of epileptiform bursts, experiments were performed in the presence of 50–100 $\mu$M APV, an NMDA receptor antagonist (Fig. 1B). APV caused a significant reduction in the duration of picrotoxin-induced synchronized bursts from 343 ± 13 to 284 ± 22 ms ($P < 0.005$) without modifying the burst frequency (0.15 ± 0.01 Hz, $n = 5$). The subsequent application of the group I mGluR agonist DHPG in the presence of APV resulted in a markedly enhanced increase in burst frequency (peak frequency $0.66 \pm 0.07$ Hz at 10 min, $P < 0.05$ vs. slices exposed to DHPG alone for 10 min; Fig. 1, B and C) with no initial significant change in BD (208 ± 22 ms, $n = 5$). Nevertheless, at 90 min of DHPG exposure, the burst frequency returned to 0.11 ± 0.01 Hz and BD reached 2354 ± 389 ms (similar to that seen in the absence of APV, $P > 0.05$, $n = 5$). The BD remained significantly potentiated throughout a 2-h washout period (BD$_{2 \text{h wash}}$ 2326 ± 403 ms at 0.11 ± 0.01 Hz, 589 ± 132% potentiated, $n = 5$; Fig. 1, B and D). Comparison of these data with control experiments revealed that the presence of NMDA receptor antagonist during DHPG application did not impede the development of burst prolongation (Fig. 1C), nor did it affect the persistence of the epileptiform burst potentiation during 2 h of washout of the mGluR agonist (Fig. 1D).

Effect of NMDA receptor antagonist on the maintenance of persistent prolonged epileptiform bursts

A second set of APV experiments ($n = 4$) was designed to determine whether the activation of NMDA receptors is necessary for the maintenance of mGluR-induced prolonged discharges. In these experiments, NMDA receptor antagonist was introduced after 90 min of DHPG washout (Fig. 2). Baseline picrotoxin bursts were induced as usual (BD 390 ± 8 ms at 0.11 ± 0.02 Hz); 90 min of DHPG application prolonged the BD to 2453 ± 314 ms at 0.08 ± 0.01 Hz. At 90 min of agonist washout, there was no significant change in BD or burst frequency (1990 ± 332 ms and 0.10 ± 0.02 Hz, respectively, $n = 4$). On the addition of APV for 30 min, BD remained significantly potentiated as compared with the initial picrotoxin-induced bursts (1924 ± 285 ms at 0.10 ± 0.01 Hz, $P < 0.01$). Comparison of the persistent burst potentiation at 120 min of washout of DHPG in control slices with that seen at an equivalent time with 30 min of APV application revealed no significant difference between the two groups (Fig. 2B).

**DISCUSSION**

Enhancement of interictal burst frequency during NMDA receptor blockade is a transient effect

The depolarization that underlies the picrotoxin-induced interictal burst is largely carried by synaptic excitatory events mediated by $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors (Dingledine et al. 1986; Miles et al. 1984; Traub and Wong 1982; Traub et al. 1993). On blockade of NMDA receptors, the picrotoxin-induced interictal discharge was therefore significantly abbreviated. This phenomenon was a useful indicator of effective block of NMDA receptors.
Group I mGluR activation produces a transient acceleration of interictal epileptiform bursts (Merlin and Wong 1997; Merlin et al. 1998a). Similar mGluR-induced burst acceleration was observed in spinal cord locomotor burst frequency in the lamprey (Krieger et al. 1998) and was attributed to potentiation of NMDA-induced depolarizations. However, in our system, the acceleration of interictal bursting was markedly enhanced when induced in the presence of NMDA receptor antagonist (Fig. 1C), making it unlikely that potentiation of NMDA receptor–mediated responses accounts for the burst acceleration. Furthermore, the lack of a concurrent significant shortening of burst duration at the time of maximal acceleration (compared with control; Fig. 1C) makes the shortening of interictal bursts in APV an inadequate explanation for the enhancement of burst frequency. The mechanism for the frequency modification remains unclear. Nevertheless, the transient enhancement of interictal burst frequency had no enduring effect on the ultimate frequency or duration of the potentiated epileptiform bursts.

Induction of mGluR-mediated burst prolongation is not NMDA receptor dependent

The group I metabotropic receptor mGluR5 can be desensitized by phosphorylation (Gereau and Heinemann 1998). This desensitization can be reversed by an NMDA receptor–dependent dephosphorylation process (Alagarsamy et al. 1999). One might therefore expect NMDA receptor activation to be necessary for the prolongation of epileptiform bursts during group I mGluR activation. However, our data demonstrate that group I mGluR-mediated epileptogenesis will proceed unimpeded in the absence of concurrent NMDA receptor activation.

We previously demonstrated that transient group I mGluR activation during suppression of ionotropic glutamate receptor (iGluR)-mediated activity results in the appearance of persistent potentiated epileptiform bursts upon washout of the iGluR antagonists (Merlin 1999). Because the experiments previously reported were performed in the presence of both NMDA and AMPA receptor antagonists, all spontaneous synchronized bursting activity was suppressed during the DHPG application period, leaving no way to gauge the efficacy of the NMDA blockade. The current data, in contrast, allow the confirmation of effective NMDA antagonism (through the observation of shortened interictal bursts), thereby strengthening the conclusion that NMDA receptor activation is not necessary for group I mGluR-mediated induction of epileptogenesis. Furthermore, the design of the current experiments allows for assessment of the rate of development of the mGluR-induced modifications. The data reveal no delay in the development of the mGluR–mediated burst prolongation when induced in the absence of NMDA receptor activation (Fig. 1C), suggesting that activation of NMDA receptors does not significantly contribute to the potentiation induction process.

Group I mGluR-mediated epileptogenesis is not sustained by potentiation of NMDA responses

Enhancement of NMDA receptor–mediated responses will lengthen synchronized discharges, thereby encouraging the production of seizures (Traub et al. 1994, 1996). Because group I mGluR activation in the hippocampus can induce a long-lasting potentiation of NMDA receptor-mediated responses (Jia et al. 1998; O’Connor et al. 1994, 1995), one might hypothesize that potentiation of NMDA responses underlies the maintenance of mGluR-induced prolonged bursts. Here we show that NMDA receptor antagonists fail to suppress the prolonged bursts, demonstrating that NMDA potentiation does not significantly contribute to sustaining the persistent burst prolongation. In contrast, our previous data showed that group I mGluR antagonists are effective at reversibly shortening the persistent bursts (Merlin 1999; Merlin and Wong 1997). Taken together, the data support the conclusion that transient activation of group I mGluRs results in autopotentiation of group I mGluR-mediated responses, allowing endogenous glutamate to stimulate group I mGluRs, which results in the persistent expression of potentiated epileptiform discharges. This autopotentiation of group I mGluR-mediated responses is NMDA receptor independent and appears to be the major process underlying mGluR-mediated epileptogenesis.

This work was funded by National Institute of Neurological Disorders and Stroke Grant NS-01901 to L. R. Merlin.

Address for reprint requests: L. R. Merlin, State University of New York Health Science Center at Brooklyn, 450 Clarkson Ave., Box 29, Brooklyn, NY 11203.

Received 4 November 1999, accepted in final form 30 December 1999.

REFERENCES


