

Group I metabotropic glutamate receptor activation produces prolonged epileptiform neuronal synchronization and alters evoked population responses in the hippocampus

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Received 1 October 2002; received in revised form 1 January 2003; accepted 8 January 2003

Abstract

Glutamate activates a class of receptors coupled to G-proteins that initiate second messenger cascades, change ion channel function, cause release of calcium from intracellular stores, and produce long-term changes in synaptic strength. We used the CA3 region of the adult rat hippocampal slice to evaluate group I metabotropic glutamate receptor (mGluR) activation on epileptiform activity and the population response recorded extracellularly evoked by stratum radiatum stimulation. The selective group I mGluR agonist (*R,S*)-3,5-dihydroxyphenylglycine (DHPG) accelerated the rate of bicuculline-induced interictal discharges at concentrations of 10 and 30 μ M. At a concentration of 100 μ M, DHPG produced prolonged recurrent discharges that last more than 2 s and consisted of an oscillation of the field potential at 2–20 Hz that resembled electrographic seizure activity (ictal). DHPG (100 μ M) when bath-applied alone for 30–120 min produced both ictal and interictal discharges that persisted following removal of DHPG from the bathing solution. DHPG (100 μ M) reduced the amplitude of the first population spike evoked by stratum radiatum stimulation and changed the relationship of paired evoked population spikes from suppression of the second response relative to the first to facilitation of the second response at interpulse intervals of 15 and 25 ms. To test the possibility that a reduction of the first evoked population spike and loss of inhibition of a second evoked population spike generated prolonged ictal discharges, we used 4-aminopyridine (4-AP 50 μ M) to enhance synaptic transmission. 4-AP converted ictal discharges produced by DHPG to an interictal pattern of synchronous activity, reversed the DHPG-induced reduction in the first evoked population spike, and changed paired-pulse facilitation to inhibition. Reversing the changes of evoked population neuronal activity produced by group I mGluR activation favored interictal patterns of epileptiform activity. These results confirm that group I mGluR activation promotes epileptiform activity in the hippocampus and support the hypothesis that a lower efficacy of synaptic transmission favors the generation of prolonged synchronization of neurons that underlies seizures.

Published by Elsevier Science B.V.

Keywords: Dihydroxyphenylglycine (DHPG); 4-Aminopyridine; Paired-pulse inhibition; CA3

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1. Introduction

Besides activating ionotropic receptors that are coupled directly to ion channels, glutamate also binds to

receptors that are coupled to G-proteins that activate second messengers (Conn and Pin, 1997; Hermanns and Challiss, 2001). These receptors are characterized by a seven transmembrane region and have homology to the GABA_B receptor (Tanabe et al., 1992). They are activated under conditions of prolonged or high glutamate release as occurs with tetanic stimulation, seizures, or trauma. mGluRs play a role in both long-term potentiation (Wilsch et al., 1998) and depression (Fitzjohn et al., 2001; Xiao et al., 2001) as well as in the generation of gamma oscillations (Whittington et al., 1995).

The mGluR are classified as group I, II, or III based on molecular and pharmacological properties (Conn and Pin, 1997; Hermanns and Challiss, 2001). Group I receptors are primarily located at postsynaptic sites (Lujan et al., 1996) and change membrane excitability by reducing potassium currents (Charpak et al., 1990; Guerineau et al., 1994) or activating cationic currents (Guerineau et al., 1995; Bianchi et al., 1999) and also result in the release of calcium from intracellular stores coupled to IP₃ receptors (Fagni et al., 2000; Rae et al., 2000). In the hippocampus, group I mGluRs depress evoked synaptic transmission at excitatory (Fitzjohn et al., 2001; Fass et al., 2002) and inhibitory synapses (Desai et al., 1994).

We investigated the effect of (*R,S*)-3,5-dihydroxyphenylglycine (DHPG), a selective group I mGluR agonist on epileptiform activity induced by bicuculline methiodide (BMI). DHPG alone produces epileptiform discharges that resembled interictal and more prolonged ictal discharges (Taylor et al., 1995; Chuang et al., 2001). We evaluated effects of group I mGluR activation on the population spike generated by stimulation of stratum radiatum of the CA3 region that activates feed forward and recurrent synaptic circuitry and allows for assessment of relative inhibition versus facilitation of a second paired response. The results showed that DHPG suppressed the first population spike but enhanced the amplitude of the second response relative to the first. To determine if this relationship correlated with the ictal pattern of epileptiform discharges produced by DHPG, we enhanced synaptic transmission with 4-aminopyridine (4-AP) in the presence of DHPG and evaluated effects on stratum radiatum stimulation and the pattern of epileptiform activity.

2. Methods

2.1. Slice preparation

Adult male Sprague–Dawley rats (150–300 g) were anesthetized with ether or pentobarbital (60–75 mg/kg), decapitated, and the brain transferred quickly to iced artificial cerebrospinal fluid (ACSF). Hippocampal slices (400–500 μm thick) were prepared using a McIlwain tissue chopper or a vibratome (Leica or Technical Products International) and placed in an interface recording chamber. The slices were superfused with ACSF that was composed of (in mM): NaCl 124, KCl 5, NaH₂PO₄ 1.25, CaCl₂ 2, MgSO₄ 2, NaHCO₃ 26, glucose 10. The ACSF was bubbled with 95% O₂–5% CO₂ and warmed to 32–35 °C. Slices were incubated for 1 h before a convulsant or other drug was added.

In a separate set of experiments, slices were prepared and incubated in small bottles containing 10–20 cc of ACSF at room temperature and bubbled with 95% O₂–5% CO₂. After 30–60 min, DHPG was added to the ACSF to produce a concentration of 100 μM, and the slices incubated for 90–120 min. The slices were then transferred to the interfaced chamber and superfused with control ACSF.

2.2. Recording of epileptiform activity

Extracellular recordings were made from the CA3 region (CA3c or b) with microelectrodes filled with 2 M NaCl. Slices were monitored for spontaneously occurring epileptiform discharges and that activity was characterized as interictal or ictal. Interictal epileptiform activity was defined as brief (less than 500 ms) discharges that occurred at rates less than 1 Hz. Ictal epileptiform activity was defined as synchronous field discharges that lasted for more than 3 s and with a population oscillation of >2 Hz. Activity was recorded digitally for 3–5 min depending on the pattern of activity (3 min for just interictal activity and 5 min for ictal patterns). Interictal discharges produced by 10 μM BMI were recorded before and after bath application of the selective group I agonist DHPG (10–100 μM). Multiple slices were assessed but the electrode position was held constant in one slice during the solution

change. Other slices were recorded from before and after drug application with movement of the recording electrode between solution changes. Care was taken to record from the same general region. Changes in the position of the electrode produced small changes in the amplitude of the discharge but the pattern of epileptiform activity was not affected by electrode position. The pattern of epileptiform activity was characterized in terms of interictal rate and ictal pattern by the duration and interval between ictal discharges 20–40 min after drug application, or, for slices incubated in DHPG, more than 60 min after transfer to ACSF in the interface chamber.

2.3. Paired-pulse stimulation

Paired-pulse stimulation of the associational pathways of the CA3 region was delivered by a bipolar stimulating electrode placed in stratum radiatum of the CA3 region between the upper blade of the dentate granule cell layer and stratum lucidum. Extracellular recordings were made from the CA3 pyramidal cell layer. The stimulus intensity was adjusted to the lowest intensity that produced the maximal population spike. The population spike that occurred during the population excitatory potential was used to characterize the output of the synaptic circuitry of the CA3 region. The population spike of the first evoked response that represents the synchronous activation of action potentials in a large population of neurons was compared to a second evoked response at interpulse intervals of 15, 25, 200, and 350 ms. The population spikes were measured as the mean amplitude of the two positive phases of the response measured from the maximum negative component of the population spike (Sayin and Rutecki, 1997). For these experiments, the position of the stimulating and recording electrodes was maintained. The data were analyzed by comparing the ratio of the second to the first population spike as a percentage. Normalized ratios of the evoked population spikes (P2/P1) that were less than 100% were regarded as paired-pulse inhibition, and ratios greater than 100% were considered paired-pulse facilitation. At interpulse intervals of 15–25 ms, the response is normally inhibition that is mediated by activation of GABA_A synaptic transmission (Sayin et al., 2001).

2.4. Drugs used

Racemic *R,S*-DHPG was obtained from Tocris Cookman. BMI and 4-AP were obtained from Sigma.

2.5. Statistics

All values represent means \pm S.E.M. and significance was assigned as a $P < 0.05$. Data were compared with Student's paired *t*-test or an ANOVA when distributed normally. When data were not distributed normally, a Mann–Whitney rank sum test or an ANOVA on ranks was used to compare data.

3. Results

3.1. Group I metabotropic glutamate receptor activation and bicuculline-induced interictal discharges

When GABA_A inhibition is blocked by BMI (10 μ M), spontaneous epileptiform bursting occurs in the hippocampal slice that is analogous to interictal spike activity (Rutecki et al., 1985). Bath application of the selective group I mGluR agonist DHPG accelerated the rate of discharges at concentrations of 10 and 30 μ M ($n = 17$ slices, Fig. 1). The interictal discharge rate increased from a control rate of 0.26 ± 0.07 Hz to 0.58 ± 0.08 Hz in the presence of 10 μ M DHPG and 1.12 ± 0.15 Hz in 30 μ M DHPG. At 100 μ M, DHPG, the pattern of discharges changed in four of eight slices to one that included more prolonged synchronous activity that resembles an ictal or seizure discharge (Fig. 1).

3.2. Epileptiform activity produced by DHPG

Bath application of DHPG (100 μ M) produced both interictal and ictal discharges in the absence of any other convulsant in hippocampal slices. The ictal activity took time to evolve (30–60 min), but the pattern of epileptiform activity was stable after a 90-min exposure. In eight preparations, an average of $65.9 \pm 10\%$ of slices demonstrated spontaneous epileptiform activity (Fig. 2A). Of all slices, 12% demonstrated only interictal discharges (6 of

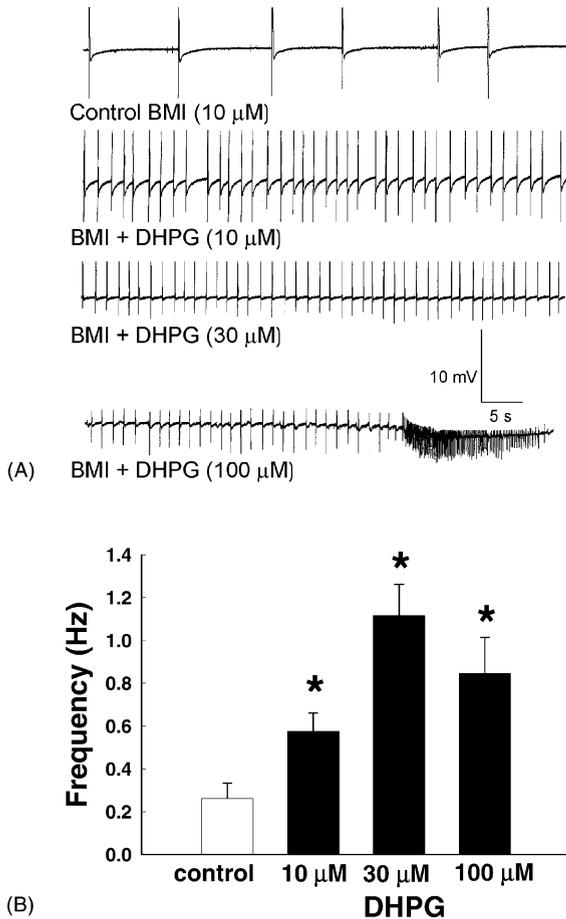


Fig. 1. Group I mGluR activation accelerated BMI-induced epileptiform discharges. (A) Traces show spontaneously occurring interictal epileptiform discharges that are produced by 10 μM BMI. The interictal rate was enhanced by DHPG (10 and 30 μM). At 100 μM , DHPG caused four of eight slices to display ictal epileptiform activity (oscillations that lasted >3 s and occurred at >2 Hz). (B) Relationship between DHPG concentration and interictal discharge rate. The rate increased significantly at all three concentrations. Asterisk (*) represents $P < 0.05$ compared to control using Bonferroni post hoc analysis following ANOVA.

50 slices), 52% demonstrated ictal activity, and 34% had no spontaneous population epileptiform activity. The average frequency of interictal discharges in slices that only displayed interictal activity was 0.32 ± 0.03 Hz. For slices that had ictal patterns of activity, the average duration of the ictal discharge was 6.45 ± 0.4 s and the interval between discharges was 32.0 ± 4.5 s.

We found that exposure to DHPG produced epileptiform activity that continued following removal of the agonist. Slices incubated in DHPG for 90–120 min and then transferred to an ACSF bathing solution continued to demonstrate spontaneously occurring epileptiform discharges for more than 3 h. In 22 slices, 50% of slices continued to display ictal discharges and 27% interictal activity at times greater than 1 h after removal of the agonist. The average duration of ictal discharges was 10.1 ± 1.3 s and the interval between discharges was 34.7 ± 4.6 s. The average duration of ictal discharges in the slices that had been exposed to DHPG was significantly longer than the slices bathed in DHPG ($P < 0.01$, Mann–Whitney rank sum test).

3.3. Group I mGluR alteration of paired-pulse responses

Stimulation of the stratum radiatum activates a circuit that includes feed forward and feedback inhibition and anti- and orthodromic activation of CA3 pyramidal neurons. We measured the paired-pulse response to determine network excitability as defined by the ratio of population spike amplitude (P2/P1) as a function of interpulse interval. Control responses were collected for 15 min and then the ACSF was changed to one containing DHPG (100 μM).

Of 14 slices tested, 3 could not be used because of loss of ability to generate a population spike in the presence of DHPG, even with increasing the stimulus intensity. In the other 11 slices, the first evoked population spike was depressed but the ratio of the second to first demonstrated a reduction of paired-pulse inhibition (Fig. 3). This change was significant for the 15 ms interpulse paired-pulse response at 30 min after bath application (in control ACSF the second population spike was $56.5 \pm 5.0\%$ the amplitude of the first population spike and the relationship of the second to first population spike changed to $105.3 \pm 13.1\%$ in the presence of DHPG). The population spike amplitude was depressed significantly after 60 min (a reduction to $38.8 \pm 6.6\%$ of the control population spike amplitude). The loss of paired-pulse inhibition and resultant facilitation was noted at both 15 and 25 ms inter-pulse intervals (Fig. 3C). No significant differences were noted at longer inter-pulse intervals.

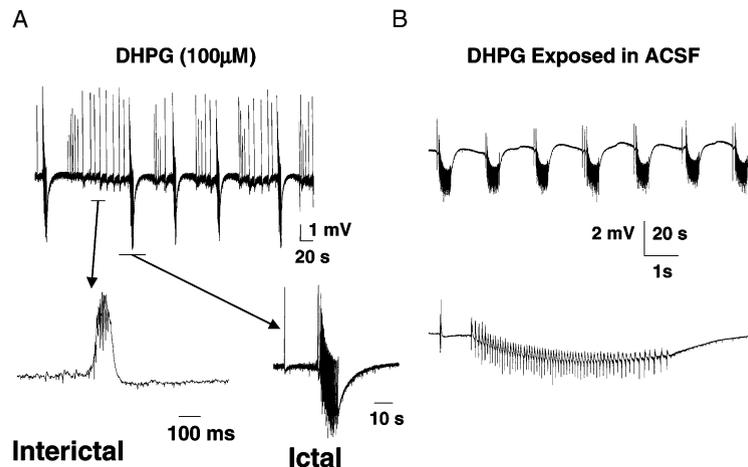


Fig. 2. Examples of epileptiform activity produced by DHPG. (A) Recordings from the CA3 region of a slice in the presence of DHPG demonstrates interictal and ictal activity. (B) Recordings from a slice that had been exposed to DHPG for 2 h then bathed in ACSF for greater than 1 h. In this slice, the epileptiform activity was predominantly ictal and consisted of recurring population oscillations of 4–8 Hz lasting 8–10 s.

3.4. Reversal of ictal discharges and PPI by enhancement of synaptic transmission with 4-aminopyridine

The above results suggested that ictal activity was more likely to occur when population spike generation was dampened. Because the initial population spike was reduced in amplitude and could not be increased by increasing stimulus intensity, we hypothesized that a reduction in evoked excitability as measured by population spike amplitude may favor ictal or prolonged synchronous discharges. To test this idea we assessed the effect of increasing synaptic transmission with 4-AP (50 μ M).

Ictal discharges observed in 11 slices in the presence of DHPG (100 μ M) converted to interictal patterns in 7 with the addition of 4-AP (50 μ M, Fig. 4A). In the four slices that continued to display ictal activity in the presence of 4-AP and DHPG, the duration and interval between discharges demonstrated a non-significant change with a trend to shortening and reduced frequency of ictal discharges (duration 7.8 ± 1.0 s in DHPG versus 4.2 ± 0.9 s in the presence of 4-AP; interval 23.9 ± 7.1 in DHPG and 62.3 ± 29.3 s in the presence of 4-AP).

We assessed the paired-pulse response produced by stratum radiatum stimulation and the effect of DHPG and 4-AP. The paired-pulse population spike ratio re-

sponse measured at a 15-ms interval was $43.8 \pm 7.0\%$ in control slices, and following exposure to DHPG (100 μ M), the paired-pulse response was $118 \pm 11.7\%$ representing facilitation rather than inhibition. The first population spike was depressed to $35 \pm 6\%$ of the original control amplitude ($n = 5$, Fig. 4). Co-application of 4-AP (50 μ M) reversed the DHPG effects. The first evoked population spike approached the original amplitude ($69 \pm 18\%$) and paired-pulse inhibition was restored (the second population spike being $59.2 \pm 6\%$ of the first).

4. Discussion

We describe a paradoxical change in network excitability of the CA3 region of the hippocampus produced by group I mGluR activation. A reduction in evoked population responses was associated with a loss of inhibition and resultant facilitation of population spike amplitude generated by a second stimulus at interpulse intervals of 15 and 25 ms. GABA_A-mediated inhibition reduces the amplitude of the second population response at these interpulse intervals (Sayin et al., 2001). Presynaptic changes that reduce the probability of release also can favor facilitation at these interpulse intervals (Dobrunz and Stevens, 1997). Furthermore, ictal discharges

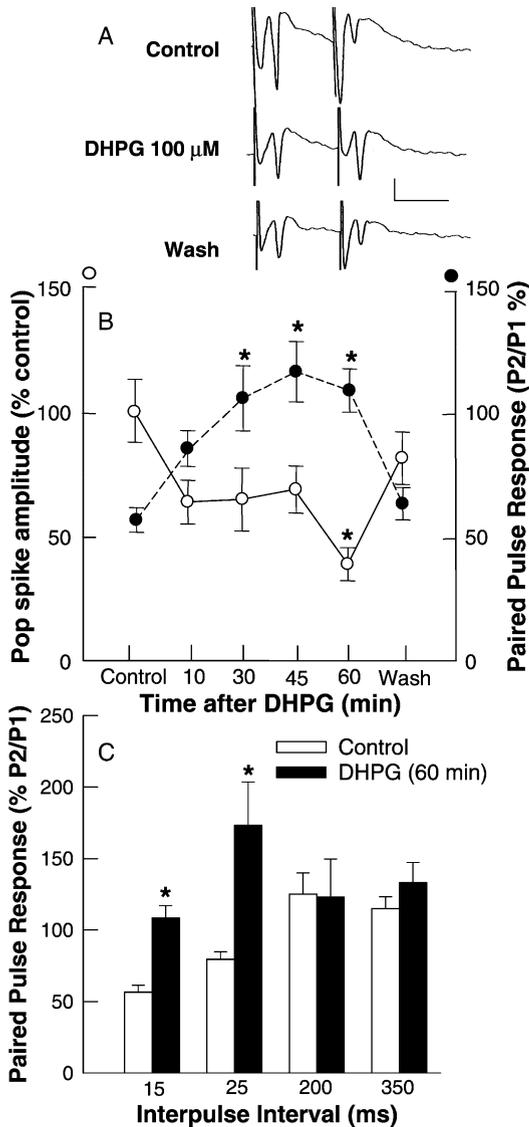


Fig. 3. DHPG reduced paired-pulse inhibition and evoked population spike amplitude. (A) Recordings from the stratum pyramidale in CA3 region of a hippocampal slice before, 60 min after exposure to 100 μ M DHPG, and following wash to control ACSF. DHPG reduced both the amplitude of the first evoked population spike and the inhibition of the second population spike expressed as a percentage of the first. The scale bar represents 1 mV and 10 ms. (B) DHPG reduced the amplitude of the first evoked population spike and the change became significant at 60 min (open circle, left axis, $n = 11$ slices, except 30 and 60 min data from six slices, (*) ANOVA with Bonferroni post hoc analysis $P < 0.05$). The paired-pulse response that was inhibitory at 15 ms in control ACSF was converted to facilitation in the presence of DHPG after 30 min (closed circle, right axis, (*) ANOVA on ranks with

produced by group I mGluR activation were converted to interictal discharges when evoked responses were restored toward baseline conditions by the co-application of 4-AP.

4.1. mGluR effects on disinhibited slices

In slices that were disinhibited, we found that group I mGluR activation using DHPG resulted in the acceleration of interictal discharges at low concentrations and prolonged discharges at higher concentrations, as observed in previous studies (Merlin and Wong, 1997). These results suggest that the enhanced frequency of interictal discharges produced by DHPG was not dependent on alteration of GABA_A-mediated inhibition. The acceleration in rate of interictal discharges most likely results from group I mGluR activation reducing one or more potassium currents (Rutecki and Yang, 1997). The group I and II agonist, ACPD, produces similar effects that are associated with a decrease in the amplitude of after hyperpolarization that follows interictal discharges (Rutecki and Yang, 1997).

4.2. mGluR-induced epileptiform activity

We found that group I mGluR activation results in epileptiform activity that resembles both interictal and ictal discharges and are similar to findings reported by others (Taylor et al., 1995; Chuang et al., 2001). The pattern of ictal discharges produced by DHPG is not altered by GABA_A blockade (Taylor et al., 1995; Rutecki et al., 2002) and differs from more physiologic oscillations produced by tetanic stimulation that depend on interneuron activation and GABA_A receptor activation (Whittington et al., 1995). The ictal-like discharges were comparable to those produced by elevated $[K^+]_0$ and pilocarpine (Rutecki and Yang, 1998; Rutecki et al., 2002). In both cases the ictal discharges depended on AMPA/KA receptor activation and occurred in the presence of NMDA receptor blockade (Rutecki et al., 2002).

Dunn's post hoc analysis $P < 0.05$). The inhibitory relationship recovered following wash to control saline. (C) DHPG (60 min exposure) converted paired-pulse inhibition to facilitation at 15 and 25 ms interpulse intervals without any significant change at longer interpulse intervals (asterisk (*) represents $P < 0.05$ by paired Student's t -test for 15 ms and by Mann-Whitney rank sum test for 25 ms).

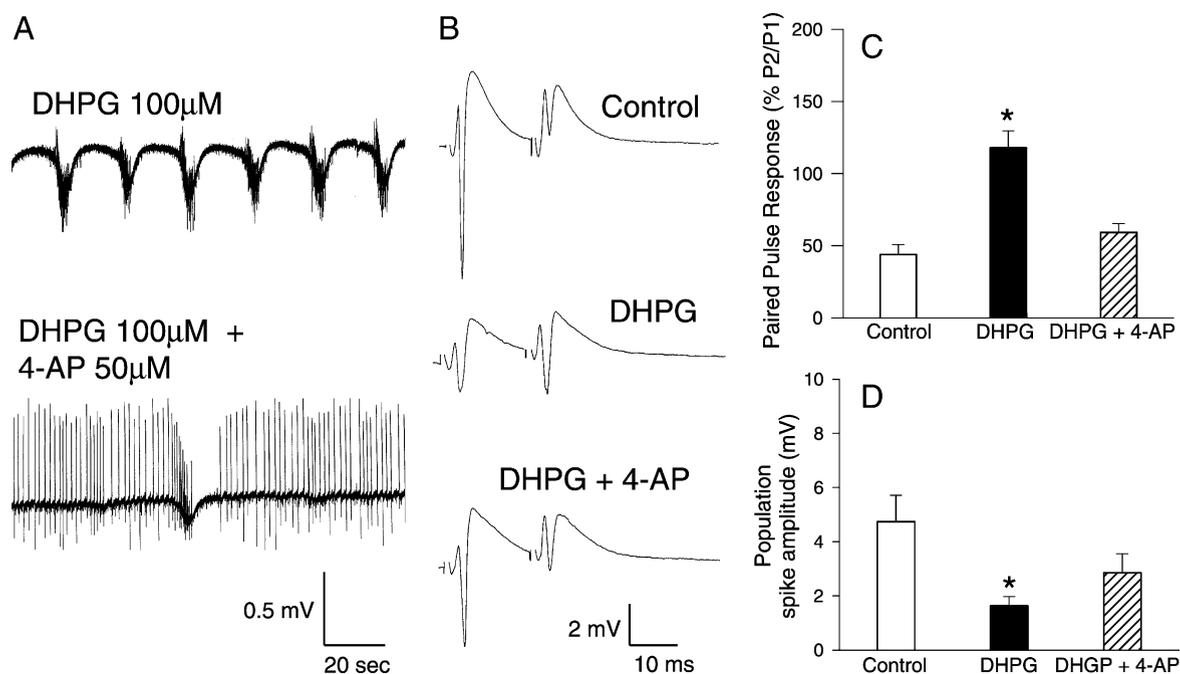


Fig. 4. 4-AP converted ictal epileptiform patterns to interictal activity and reversed effect of DHPG on paired-pulse responses. (A) Ictal pattern of epileptiform activity produced by prolonged exposure of 100 μM DHPG was converted to an interictal pattern in the presence of 50 μM 4-AP. (B) Paired-pulse responses recorded in CA3 stratum pyramidale following stratum radiatum stimulation in control ACSF, after 60 min exposure to DHPG (100 μM), and following co-application of DHPG and 4-AP (50 μM) for 30 min. The amplitude of the first population spike is reduced in the presence of DHPG and this effect was reversed by co-application of 4-AP. (C) DHPG resulted in a significant reduction in paired-pulse inhibition that was reversed by 4-AP ($n = 5$ slices, $P < 0.05$ by ANOVA with Bonferroni post hoc analysis). (D) 4-AP also reversed the reduction in the amplitude of the first evoked population spike produced by DHPG application ($n = 5$ slices, $P < 0.05$ by ANOVA with Bonferroni post hoc analysis).

A notable difference compared to pilocarpine was the persistence of DHPG-induced ictal activity with removal of the agonist. The group I mGluR have been shown to mediate LTP (Bortolotto et al., 1994; Wilsch et al., 1998) and LTD (Huber et al., 2000; Fitzjohn et al., 2001; Xiao et al., 2001) in the hippocampus and to produce prolongation of picrotoxin-induced epileptiform discharges that does not reverse with removal of the agonist (Merlin and Wong, 1997). LTD and the long-lasting effect of prolonging picrotoxin-induced discharges is protein synthesis-dependent (Merlin et al., 1998; Huber et al., 2000). Our findings complement these studies and demonstrate a long-lasting change in network excitability that persists after the removal of the agonist. Taken together the findings support the potential role of the group I mGluR in epileptogenesis.

4.3. Paired-pulse effects

DHPG resulted in a reduction in the first evoked population spike amplitude produced by stratum radiatum stimulation. The reduction in population spike amplitude could result from presynaptic inhibition of transmitter release (Fitzjohn et al., 2001) or a postsynaptic decrease in AMPA receptors (Snyder et al., 2001; Xiao et al., 2001). A loss of paired-pulse inhibition at 15 and 25 ms interpulse intervals suggests a relative loss of GABA_A-mediated circuit inhibition or an increase in facilitation produced by a decrease in the probability of synaptic release. Stimulus-evoked inhibition is reduced by DHPG in CA1 neurons (Desai and Conn, 1991) and mGluRs reduce excitatory drive onto hilar border interneurons (Doherty and Dingledine, 1998). These actions may explain our findings on a

decrease paired-pulse inhibition. Furthermore, a reduction in probability of release by DHPG favors facilitation of a second response (Fass et al., 2002) that could favor spike generation for the second stimulus compared to the first stimulus.

The effect on paired-pulse responses is similar to that produced by pilocarpine (Sayin and Rutecki, 1997), a muscarinic agonist that is commonly used to produce status epilepticus followed by the development of chronic recurrent seizures. Muscarinic activation also results in a decrease in both excitatory and inhibitory stimulus-evoked synaptic transmission (Valentino and Dingledine, 1981; Williams and Johnston, 1990; Behrends and Ten Bruggencate, 1993). Like group I agonists, muscarinic agonists also enhance membrane excitability by decreasing several different potassium conductances and enhancing non-specific cation conductance (Bianchi and Wong, 1994; Guerineau et al., 1994; Guerineau et al., 1995; Fraser and MacVicar, 1996). Both muscarinic and group I mGluR activation increase the occurrence of spontaneous inhibitory synaptic potentials but are associated with a decrease in stimulus-evoked responses (Behrends and Ten Bruggencate, 1993; Mannaioni et al., 2001). The increased spontaneous activity may reflect what occurs at seizure onset more accurately than stimulus-evoked population responses. The dissociation of spontaneous and stimulus-evoked synaptic transmission may relate to a presynaptic inhibitory action and a postsynaptic enhancement of excitability. Such conditions appear to enhance prolonged discharges in the CA3, an area that typically is biased to interictal discharges that prevent ictal activity (Barbarosie and Avoli, 1997).

Dampening synaptic transmission may result in a paradoxical increase in network excitability by altering the relative balance of facilitation and inhibition. A decrease in the probability of release at all glutamate terminals would depress excitatory synaptic drive on interneurons, resulting in a loss of paired-pulse inhibition. A decrease in probability of release would favor facilitation of excitatory recurrent synapses of CA3 neurons. Presynaptic inhibition in combination with postsynaptic increased excitability produced by decreases in potassium currents or activation of cationic currents favor abnormal synchronization and may also underlie the genesis of other physiologic oscillations.

4.4. 4-AP reversal of PPI and ictal discharges

The CA3 region tends to demonstrate brief interictal discharges that may result in depletion of presynaptic glutamate stores and prevent the occurrence of prolonged ictal discharges. Another discharge cannot occur until releasable stores are replenished (Staley et al., 1998). Presynaptic inhibition of glutamate release on both pyramidal and interneurons may conserve releasable transmitter stores and allow for longer lasting synchronization and ictal epileptiform activity.

To test the role of diminished synaptic strength in generating ictal discharges, we evaluated the effects of 4-AP on ictal discharges produced by group I mGluR activation. 4-AP reverses the depression of synaptic vesicular release in synaptosome preparations produced by group I mGluR activation (Herrero et al., 1998). Our results demonstrated that increasing synaptic release with 4-AP restored paired-pulse inhibition and converted ictal discharges to interictal activity. 4-AP also reversed the reduction of the first population spike amplitude produced by DHPG. 4-AP is a convulsant that enhances both inhibitory and excitatory synaptic transmission in the CA3 region of the hippocampus (Rutecki et al., 1987), and at this concentration reduces I_D , an inactivating potassium current that controls repetitive action generation and synaptic release (Storm, 1998). Furthermore, in combined entorhinal-hippocampal slices, 4-AP induced interictal discharges in CA3 inhibit more prolonged ictal discharges (Barbarosie and Avoli, 1997).

Taken together, the data support the hypothesis that prolonged ictal oscillations require a combination of changes in physiology that lead to sustained synchronization. In the case of mGluR activation that may mimic the increased glutamate concentrations that occur during seizures and models of epileptogenesis, a reduction in synaptic drive, both inhibitory and excitatory, facilitates ictal discharges. Seizure generation may be enhanced paradoxically by reducing the strength of synaptic connections in the CA3 region of the hippocampus.

Acknowledgements

We would like to thank Lew Haberly, Matt Jones, Bob Pearce, and Tom Sutula for comments on drafts of

this manuscript, and Kathryn Kleckner for help with the art work. This work was supported by a Merit Review grant from VA research.

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