Epilepsia, **43**(Suppl. 5):179–183, 2002
Blackwell Publishing, Inc.

Determinants of Ictal Epileptiform Patterns in the Hippocampal Slice

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Summary: *Purpose:* The transition from an interictal to an ictal pattern of epileptiform activity is a strategic target for antiepileptic drug (AED) action. Both the muscarinic agonist pilocarpine and the selective group I metabotropic glutamate receptor (mGluR) agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) produce prolonged synchronous activity in the hippocampal slice that resembles ictal discharges. We evaluated the role of synaptic mechanisms and release of calcium from intracellular stores in the generation of prolonged ictal oscillations.

Methods: Pilocarpine (10 μ*M*) in 7.5 m*M* $[K^+]_o$ or DHPG (100 μ*M*) in 5 m*M* $[K^+]_o$ artificial cerebrospinal fluid (ACSF) were bath applied to hippocampal slices, and extracellular recordings were made from the CA3 region. The pattern of activity was characterized as ictal if prolonged oscillations of discharges occurred at >2 Hz lasting for >3 s. The pattern of epileptiform activity was characterized and compared with the pattern observed after bath application of pharmacologic agents.

Results: The AMPA/kainic acid (KA) glutamate receptor blocker DNQX (20 μ*M*) dampened and stopped ictal oscillations; however, antagonism of *N*-methyl-D-aspartate (NMDA) or γ-aminobutyric acid ($GABA_A$) receptors had minimal effects on ictal patterns. Ictal discharges were suppressed by dantrolene (30–100 μ*M*), which blocks release of calcium from intracellular stores, or thapsigargin (1–5 μ*M*), which inhibits the adenosine triphosphatase (ATPase) that maintains intracellular calcium stores. The L-type calcium channel antagonist nifedipine (1 μ*M*) blocked ictal activity produced by pilocarpine or DHPG.

Conclusions: Ictal discharges produced by pilocarpine or DHPG depended on intact synaptic transmission mediated by AMPA/KA receptors, release of calcium from intracellular stores, and L-type calcium channel activation. The results suggest that muscarinic and group I mGluRs activate a positive-feedback system that creates calcium oscillations and prolonged neuronal synchronization mediated by recurrent excitatory synaptic connections in the CA3 region of the hippocampus. **Key Words:** Pilocarpine—CA3—Metabotropic glutamate receptor—Inositol triphosphate—L-type calcium channel.

The hippocampal slice preparation has provided an understanding of the cellular mechanisms of epileptiform activity and can be used to study synchronous neuronal activity resembling epileptiform synchronization that occurs between seizures (interictal) or during a seizure (ictal) (1). In the hippocampal slice, the transition from an interictal to ictal pattern of activity depends on changes in extracellular ion concentrations (2,3), a reduction in extracellular space (2,3), and the presence of excitatory or inhibitory synaptic transmission (3–5).

We have found that agonists of muscarinic or group I metabotropic glutamate receptors (mGluRs) will produce prolonged (>2 s) epileptiform discharges that recur in a periodic fashion. We examined the role of synaptic transmission mediated by glutamate and γ-aminobutyric acid (GABA) receptors coupled to ion channels in the production of ictal-like activity produced by pilocarpine (3) or (R,S)-3,5-dihyroxyphenylglycine (DHPG), a group I mGluR agonist (6).

Both receptors are coupled to G proteins that activate phospholipase C and result in the production of inositol triphosphate (IP_3) and diacyl glycerol (DAG) (6–8). IP_3 leads to the release of calcium from intracellular stores (9). We investigated the role of calcium release from intracellular stores and influx of calcium through L-type calcium channels in the production of ictal discharges induced by pilocarpine or DHPG.

METHODS

Hippocampal slices, 500 μm thick, were prepared from adult male Sprague–Dawley rats (100–300 g). The

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slices were maintained in an interface chamber and superfused with artificial cerebrospinal fluid (ACSF) at 32–34°C and composed of (in m*M*): NaCl, 124; KCl, 5; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgSO_4$, 2; $NaHCO_3$, 26; and glucose, 10. After 1 h of ACSF incubation, the bathing solution was changed to one containing 100 μ*M* DHPG, selective group I mGluR agonist or 10 μ*M* pilocarpine in aCSF with a $[K^+]_o$ of 7.5 m*M,* a concentration that favors the occurrence of more prolonged discharges (3). Extracellular recordings were made from the CA3c or b region of the slice using micropipettes filled with 2 *M* NaCl and of 2- to 10-MΩ resistance. Slices were bathed in the pilocarpine ACSF for 1 h and DHPG for 2 h before recording activity.

Ictal discharges were operationally defined as synchronous extracellular field activity that occurred at 2 Hz for >3 s (Fig. 1). In some slices, briefer interictal (<500 ms) discharges occurred between prolonged ictal discharges. The duration of ictal discharges and time between discharges were measured in each slice. The bathing solution was then changed to one containing a pharmacologic agent in question. Slices were assessed for changes in epileptiform activity 30–60 min after the solution change. Multiple slices were sampled for each experiment, and care was taken to place the electrode in the same region for each evaluation. Movement of the recording site was associated with changes in the amplitude of the discharge but not the ictal duration or period between ictal discharges.

The effect on the epileptiform pattern was assessed for the following agents: DL-2-amino-5-phosphonovaleric acid (APV,100 μ*M*), bicuculline methiodide (BMI, 10 μ*M*), 6,7-dinitroquinioxaline-2,3-dione (DNQX, 20 μ*M*), dantrolene (10–100 μ*M*), nifedipine (0.1–1 μ*M*), and thapsigargin (1–5 μ*M*). Comparisons before and after drug application were made using Student's paired *t* test.

RESULTS

Blockade of ionotropic glutamate receptors

In all slices studied, blockade of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/KA glutamate receptor with 20 μ*M* DNQX stopped all epileptiform activity produced by pilocarpine (n = 8) (3) or DHPG (n = 17; Fig. 2). When the NMDA glutamate receptor was blocked with 100 μ*M* APV, pilocarpine-induced ictal patterns continued in most slices. Sixteen of 34 slices had ictal activity in the presence of pilocarpine, and 17, with the addition of APV. Some slices were converted from an ictal to an interictal pattern (n = 3), but some converted from an interictal to ictal pattern in the presence of APV (n = 4) (3).

DHPG-induced ictal patterns continued in 12 of 17 slices in the presence of APV (Fig. 2). The duration of the ictal discharge and the interval between ictal discharges in the slices that remained ictal did not change significantly (9.1 ± 2.1 s duration and 42.7 ± 7.6 s interval in control and 10.6 ± 1.9 s duration and 31.2 ± 4.5 s in the presence of APV).

Blockade of $GABA_A$-mediated inhibition

In the pilocarpine model of ictal discharges, blockade of the $GABA_A$ receptor with BMI did not alter the occurrence of ictal discharges, but did cause a significant prolongation of ictal duration (7.5 ± 0.4 to 9.5 ± 0.9 s; $p < 0.005$) (3). The ictal pattern of epileptiform activity

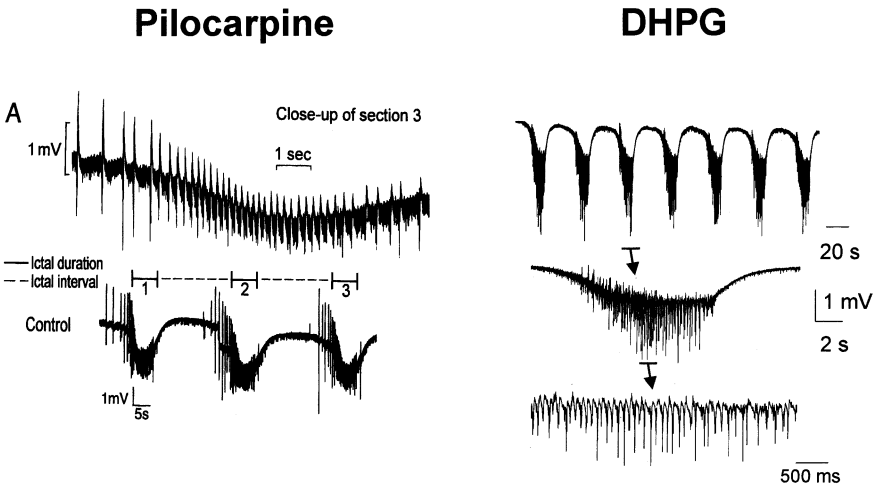

FIG. 1. Examples of ictal patterns of epileptiform activity produced by pilocarpine (10 μ*M*) in 7.5 m*M* $[K^+]_o$ or (RS)-3,5-dihydroxyphenylglycine (DHPG; 100 μ*M*) in 5 m*M* $[K^+]_o$ recorded from the CA3c region of the hippocampal slice. **Left:** In this slice exposed to pilocarpine, the pattern of activity included both interictal and ictal discharges. The interictal discharge was relatively brief (<100 ms) and occurred before the onset of the ictal oscillation. The ictal discharge consisted of an oscillation of field discharges at 4–6 Hz that resembled interictal discharges and was accompanied by a slow negative baseline shift. **Right:** In this slice the ictal discharges produced by DHPG were not accompanied by interictal discharges but were accompanied by a slow negative shift with an oscillation of population spikes occurring between 10 and 14 Hz. Interictal discharges occurred with ictal activity in DHPG as well as in pilocarpine, and pilocarpine also produced a pattern of recurrent ictal activity similar to that seen with DHPG.

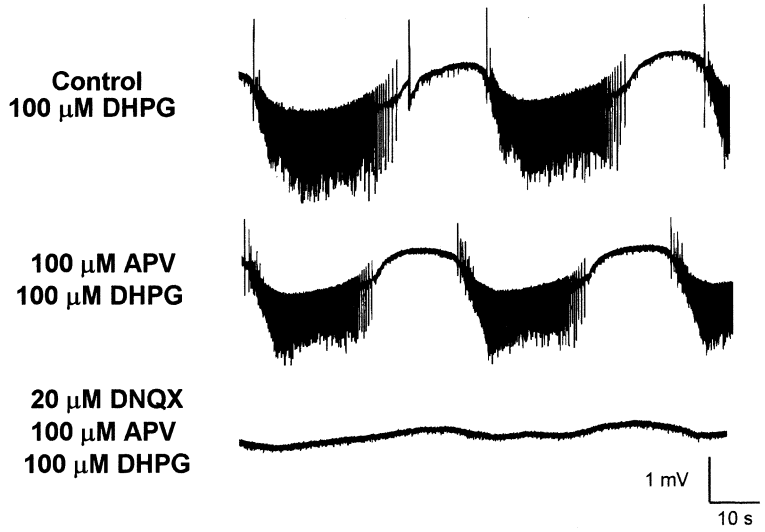

FIG. 2. Ictal activity was dependent on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainic acid (KA)-receptor activation. Representative traces from a hippocampal slice demonstrating recurrent ictal discharges. Although the amplitude was slightly reduced, the pattern of the ictal discharges was not changed by addition of *N*-methyl-D-aspartate–receptor blocker DL-2-amino-5-phosphonovaleric acid (100 μ*M*). Within 20 min of adding the AMPA/KA antagonist 6,7-dinitroquinioxaline-2,3-dione (DNQX), all epileptiform activity stopped.

produced by DHPG continued as ictal in all of the eight slices evaluated. The ictal duration (12.3 ± 1.2 s in control and 11.3 ± 1.3 s after BMI) and the interval between discharges (34.1 ± 6.6 s in control and 25 ± 3.7 s in BMI) did not change significantly.

Dantrolene suppresses ictal discharges

Dantrolene prevents the release of calcium from internal stores by interfering with calcium-induced calcium release mediated by ryanodine receptors (10,11). Pilocarpine-induced ictal activity was suppressed in a dose-dependent manner (Fig. 3) with ictal patterns converted to interictal patterns in seven of 11 slices by 100 μ*M* dantrolene (12). The ictal patterns produced by DHPG were also suppressed by dantrolene at similar concentrations. Nearly half the slices that originally displayed ictal patterns continued to show ictal patterns in the presence of 30 μ*M* dantrolene (n = 17), and all but one slice had ictal activity suppressed by 100 μ*M* (n = 13; Fig. 3).

Thapsigargin

Thapsigargin irreversibly blocks the adenosine triphosphatase (ATPase) that maintains intracellular calcium stores (11). Brief exposure to thapsigargin (1 μ*M*) caused four of 12 slices with pilocarpine-induced ictal discharges to stop displaying ictal discharges after a 30-min exposure. After wash of pilocarpine in 7.5 m*M* $[K^+]_o$ saline, only three slices continued to display ictal patterns of activity. At 5 μ*M* concentration, three of five slices displaying pilocarpine-induced ictal discharges converted to an interictal pattern with a 30-min exposure, and all were suppressed after wash (12).

Ictal activity produced by DHPG was suppressed by 3

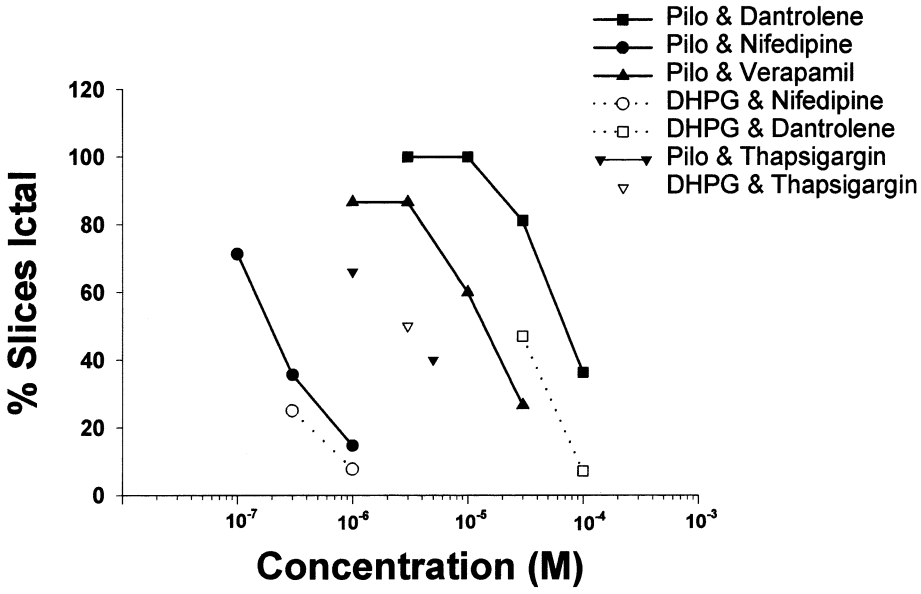

FIG. 3. Dose–response relation of ictal suppression. Data from the pilocarpine model are plotted using solid symbols, and data from (RS)-3,5-dihydroxyphenylglycine plotted with open symbols. The data points for thapsigargin represent percentage of slices that stopped demonstrating ictal discharges assessed at 30–40 min of exposure. With prolonged exposure, thapsigargin (1–5 μ*M*) inhibited all ictal discharges. The pharmacologic blockade of ictal discharges was similar for both models.

μM thapsigargin in half of the slices (six of 12) within 30 min, and all activity was suppressed in all slices after longer exposure. The effects did not reverse after return to ACSF containing DHPG without thapsigargin.

L-type calcium channel antagonist suppress ictal discharges

Pilocarpine-induced ictal discharges were suppressed by nifedipine, an L-type calcium channel blocker, in a dose-dependent manner (Fig. 3) (12). At a concentration of 1 μM, 11 of 13 slices with ictal patterns converted to interictal patterns of activity. Verapamil, a different class of L-type calcium channel blocker, had similar effects at higher concentrations (Fig. 3). Nifedipine at similar concentrations suppressed DHPG-induced discharges (Fig. 3). In both models, interictal discharges continued in the presence of nifedipine.

DISCUSSION

Ionotropic receptor channel contribution

Synaptic circuitry that involves fast excitatory synaptic transmission using the AMPA/KA glutamate receptor underlies the epileptiform synchronization of CA3 pyramidal neurons. DNQX suppressed all epileptiform activity produced by either pilocarpine or DHPG. Activation of the NMDA glutamate receptor was not required for the long-lasting sustained discharges. Discharges also were sustained in the presence of $GABA_A$-receptor blockade. These findings are in keeping with the results from others in which prolonged discharges produced by group I activation mGluR occurred in the presence of picrotoxin (13).

The findings differ from the high $[K^+]_o$ ictal discharges in the CA1 region that are sensitive to APV (2) or the long-lasting discharges produced by 0 Mg^{2+}, which are sensitive to GABA antagonists (4). Longer discharges in the 4-AP model that are mediated by GABAergic synaptic transmission also are sensitive to $GABA_A$ antagonism (14).

Requirement for calcium release from intracellular stores

A unique finding in this study is the requirement for release of calcium from intracellular stores in the generation of long-lasting ictal activity. Increasing evidence points to a complex intracellular system of calcium release that modifies synaptic transmission, second-messenger cascades, protein translation, and gene transcription (9). These systems are undoubtedly involved in the long-term changes associated with seizures and the process of epileptogenesis.

The mechanisms by which intracellular calcium release produces sustained neuronal synchrony are not clear. The synchronization requires a fast glutamatergic synaptic network, and a sustained intracellular calcium wave may underlie the ictal discharge. Both muscarinic and group I mGluR will cause calcium waves to occur in neurons (11,15,16) and glia (17,18). IP_3-receptor activation is enhanced by calcium that may come from ryanodine release (19). Increasing evidence points to a common intracellular calcium store that is released by either IP_3 or calcium activating the ryanodine receptor (9). Figure 4 depicts the positive feedback between the release of calcium by ryanodine and IP_3 receptor–mediated mechanisms.

L-type calcium channel involvement in ictal activity

Nifedipine suppressed ictal activity produced by either pilocarpine or DHPG. The suppression may be due to blockade of L-type channels activated by back-propagating action potentials or by synaptic potentials. Activation of dendritic calcium spikes may be important to boost postsynaptic excitatory potentials that are reduced in amplitude by muscarinic or group I mGluR activation (see later).

Alternatively, L-type channel blockers may inhibit nonspecific cationic channels that appear to be activated by either muscarinic or mGluRs (20,21). Evidence also points to the important role of L-type channels in replenishing intracellular calcium stores (11,22) and also may explain how L-channel blockade could stop ictal oscillations (Fig. 4).

Mechanisms of ictal activity produced by muscarinic and group I mGluRs

Both muscarinic and group I mGluRs cause membrane depolarization by decreasing potassium channel openings

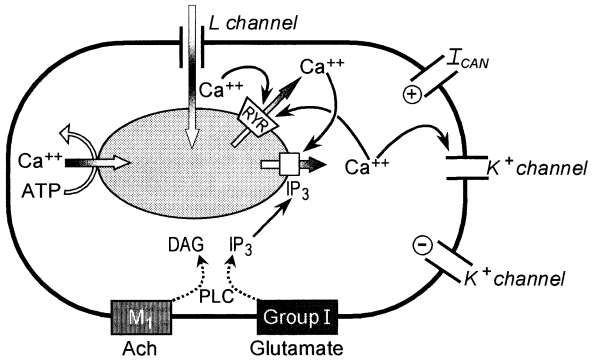

FIG. 4. Schematic diagram illustrating key components that may underlie calcium oscillations and ictal discharges. Both acetylcholine binding at muscarinic (M1) receptors or glutamate binding at group I receptors will activate phospholipase C (PLC) via G proteins, resulting in the production of diacyl glycerol (DAG) and inositol triphosphate (IP_3). In addition, both will result in closure of potassium channels and activate a nonspecific cation channel (I_{CAN}). These actions will result in depolarization and may not require activation of G proteins (29). The L-type calcium channel activates with depolarization that is produced by the synchronous synaptic input during either interictal or ictal discharges. Intracellular calcium stores are released by activation of the IP_3 receptor or calcium-dependent mechanisms via the ryanodine receptor complex (RYR). Intracellular calcium stores are replenished by an adenosine triphosphate–dependent mechanism that is blocked by thapsigargin (left) or through calcium that depends on L-type calcium channels (top). Calcium also opens potassium channels, and these may repolarize the neuron and terminate an ictal discharge.

or by activating a nonspecific cationic channel (7,8,23). The cationic current produced by mGluR activation desensitizes and may not be activated during prolonged exposure of DHPG required for ictal activity to occur (7,8). Depolarization will favor activation of L-type calcium channels and an increase in intracellular calcium that may contribute to replenishment of intracellular stores and activation of calcium-induced calcium release.

Both muscarinic and group I mGluRs inhibit synaptic transmission presynaptically (24,25), yet produce periods of prolonged synaptic synchronization. This paradox could result from the necessity for a releasable pool of transmitter required to drive the synaptic network (26). Interictal discharges may not evolve into more prolonged duration synchronization because of a reduction in releasable glutamate. Decrease of potassium currents postsynaptically will effectively lower the threshold for synaptic activation of regenerative responses and further promote synchrony when synaptic transmission is dampened presynaptically.

The other common mechanism of muscarinic and group I mGluR activation is production of IP_3 and release of calcium from intracellular stores. Our results point to the importance of calcium release from intracellular stores. We propose that the ictal oscillation is accompanied by a sustained calcium wave that recurs and underlies the ictal-duration measurement. The period between ictal discharges may depend on the need to replenish calcium stores or because of calcium activating potassium or other hyperpolarizing conductances.

Changes in intracellular calcium may contribute to epileptogenesis produced by muscarinic and group I mGluRs. Calcium release from intracellular stores activates transcriptional signals (9,27). Furthermore, group I mGluR activation leads to activation of protein synthesis of the fragile X–related protein that modulates local protein synthesis in the dendrite (28). Prolonged episodes of calcium influx can led to selective neuronal death observed in the hippocampus that then promotes reorganization and epileptogenesis.

Acknowledgment: This work was supported by VA office of Research. We thank Katherine Kleckner for help with preparation of the figures.

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