Role of Synaptic Metabotropic Glutamate Receptors in Epileptiform Discharges in Hippocampal Slices

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Lee, Angela C., Robert K. S. Wong, Shih-Chieh Chuang, Hee-Sup Shin, and Riccardo Bianchi. Role of synaptic metabotropic glutamate receptors in epileptiform discharges in hippocampal slices. J Neurophysiol 88: 1625–1633, 2002; 10.1152/jn.00143.2002. Application of group I metabotropic glutamate receptor (mGluR) agonists elicits seizure discharges in vivo and prolonged ictal-like activity in vitro. In this study we examined 1) if group I mGluRs are activated synaptically during epileptiform discharges induced by convulsants in hippocampal slices and, if so, 2) whether the synthetically activated mGluRs contribute to the pattern of the epileptiform discharges. The GABAA receptor antagonist bicuculline (50 μM) was applied to induce short synchronized bursts of ~250 ms in mouse hippocampal slices. Addition of 4-aminopyridine (4-AP; 100 μM) prolonged these bursts to 0.7–2 s. The mGluR1 antagonist (S)-(+)-amino-4-carboxy-2-methylbenzeneacetic acid (LY 367385; 25–100 μM) and the mGluR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP; 10–50 μM), applied separately, significantly reduced the duration of the synchronized discharges. The effects of these antagonists were additive when applied together, suggesting that mGluR1 and mGluR5 exert independent actions on the epileptiform bursts. In phospholipase C β1 (PLCβ1) knockout mice, bicuculline and 4-AP elicited prolonged synchronized discharges of comparable duration as those observed in slices from wild-type littermates. Furthermore, mGluR1 and mGluR5 antagonists reduced the duration of the epileptiform discharges to the same extent as they did in the wild-type preparations. The results suggest that mGluR1 and mGluR5 are activated synthetically during prolonged epileptiform discharges induced by bicuculline and 4-AP. Synchronous activation of these receptors extended the duration of synchronized discharges. In addition, the data indicate that the synaptic effects of the group I mGluRs on the duration of epileptiform discharges were mediated by a PLCβ1-independent mechanism.

INTRODUCTION

Periodic hypersynchronized discharges of large groups of neurons are the main trait of various epileptic conditions (for recent reviews see Delgado-Escueta et al. 1999; McCormick and Contreras 2001). Based on their time course and on the extent of neurons involved, these discharges are referred to as interictal bursts and ictal discharges. Intercritical bursts are approximately 100–200 ms in duration and are localized to restricted brain regions, whereas ictal discharges last for seconds to minutes and spread to large regions of the brain. Electrophysiological recordings from hippocampal slices and computer simulation data have revealed that key cellular and synaptic properties in the generation of interictal bursts are intrinsic burst firing and activation of ionotropic glutamate receptors at recurrent synapses between pyramidal cells (Miles et al. 1984; Traub and Wong 1982; Wong et al. 1986). In contrast, the critical factors that precipitate seizures and that are involved in the maintenance of ictal discharges remain to be elucidated.

Metabotropic glutamate receptors (mGluRs) have been shown to be critically involved in plastic events, such as long-term potentiation and long-term depression of synaptic transmission (for reviews see Anwyl 1999; Bortolotto et al. 1999; Braunewell and Manahan-Vaughan 2001), and changes associated with epilepsy. Changes in the expression of mGluRs (Blümcke et al. 2000) and in their function (Dietrich et al. 1999; Nagerl et al. 2000) have been found in the human hippocampus of temporal lobe epilepsy. In vivo data from animal models of epilepsy have shown modulatory roles of mGluRs on ictal activity during seizures (Dalby and Thomsen 1996; Tizzano et al. 1995). mGluRs are coupled to G proteins and include three groups: group I mGluRs, which are positively coupled to the phospholipase C and the inositol-1,3,5-trisphosphate/diacylglycerol second messenger system, and group II and group III mGluRs, which are negatively coupled to the adenyl cyclase (for review see Conn and Pin 1997; De Blasi et al. 2001). Group I mGluRs sustain or promote seizures (Camon et al. 1998; Chapman et al. 1999, 2000), whereas group II and group III mGluRs suppress seizures via presynaptic inhibition of glutamate release (Attwell et al. 1998; Gasparini et al. 1999a). Studies on in vitro brain slices suggest that activation of group I mGluRs plays a critical role in the transition of intercritical bursting into ictal activity (Merlin and Wong 1997; see DISCUSSION) and in the maintenance of the prolonged synchronized discharges (Holmes et al. 1996; Merlin and Wong 1997). Thus we have recently proposed that synaptic activation of mGluRs causes the emergence of ictal activity (Wong et al. 1999).

In most studies, group I mGluRs have been stimulated by...
direct application of agonists to the preparation. Although this approach has revealed that the activation of these receptors is sufficient to generate ictal-like activity in an otherwise “normal” preparation (Taylor et al. 1995), it remains to be seen whether, and in what conditions, synaptic release of glutamate can stimulate group I mGlURs to the extent that it causes the emergence of ictal-like discharges.

It has been shown that combined application of the convulsants bicuculline and 4-aminopyridine (4-AP) reliably induces ictal-like discharges in rat entorhinal cortex–hippocampal slices (Brückner et al. 1999). Since bicuculline and 4-AP do not have known direct effects on mGlURs, we have used this treatment (see METHODS) to induce epileptiform discharges and we asked if mGlURs were activated by synthaptically released glutamate. Also, by using selective antagonists for the two group I mGlUR subtypes, we tested the specific role of synaptic mGlUR1 and mGlUR5 in sustaining the prolonged discharges.

METHODS

Animals

Four- to 10-week-old wild-type and transgenic mice that were knocked out for phospholipase C β1 were used. Homozygous and wild-type littersmates were obtained from crosses of C57BL/6J(N8)PLCB1+/+ and 129S4/SvJae(N8)PLCB1+/-.. PCR analysis of DNA from tail samples was performed to determine the genotypes as described previously (Kim et al. 1997). Animal care and handling were carried out according to institutional guidelines (SUNY Health Science Center, Brooklyn, NY).

Slice preparation

Mice were anesthetized with halothane and their brains were quickly removed and placed in ice-cold dissection solution. The dissection solution had the same composition as the artificial cerebrospinal fluid (aCSF; see following text) except for lower Ca2+ (0.5 mM CaCl2) and higher Mg2+ (8 mM MgCl2) concentrations. The hippocampus was isolated and transverse hippocampal slices (400 μm thick) were prepared using a Lancer Vibratome 1000 (The Vibratome Company, St. Louis, MO). In some experiments, extracellular recordings from the CA3 region were obtained with low-resistance microelectrodes (30–40 MΩ) containing potassium acetate (2 M). Recordings were performed in current-clamp mode and amplified with an Axoclamp-2A amplifier (Axon Instruments, Union City, CA). In some experiments, extracellular recordings from the CA3 region were obtained with low-resistance microelectrodes (4–10 MΩ) filled with NaCl (0.5 M) or aCSF. Electrical signals were displayed on an oscilloscope (DSO 400, Gould Instruments, Valley View, OH) and were sent to a chart recorder (TA240, Gould Instruments) and to a computer with pClamp 8.0 software (Axon Instruments) for off-line analysis. A digital stimulator (PG 4000, Cygnus Technology, Delaware Water Gap, PA) connected to the amplifier allowed for intracellular injection of square-wave current pulses. Hyperpolarizing current pulses (∼0.3 to −0.7 nA; 100–200 ms) were injected to monitor the bridge balance and to calculate the cell input resistance. Cells included in this study had average stable resting membrane potential of approximately −65 mV, input resistance of ∼40 MΩ, and action potential height > 65 mV. In some experiments, cells were held at −65 mV through DC current injection and the threshold for action potential firing was measured as the membrane potential at the elicitation of the first action potential by depolarizing current pulses. The amplitude of the current pulses (0.05 to 0.25 nA; 100 ms) was chosen as to elicit action potentials approximately 50% of the time.

Pharmacological agents

Epileptiform discharges were induced by adding the convulsants bicuculline (50 μM) and 4-aminopyridine (4-AP; 100 μM) to the perfusing solution. Bicuculline is a GABA_A receptor antagonist (Curtis et al. 1970) and it is reported to also block SK channels (Johnson and Seutin 1997; Khawaled et al. 1999) and glycine receptors (Shirasaki et al. 1991). 4-AP blocks the transient potassium currents I_A and I_F (Ficker and Heinemann 1992; Storm 1987, 1988; Wu and Barish 1992) and a subtype of I_F channels (Ficker and Heinemann 1992). The following group I mGlUR antagonists were added to the perfusate at the indicated concentrations: the selective mGlUR1 antagonist (S)-(+) rac-4-carboxy-2-methylbenzenacetic acid (LY 367385) at 25–100 μM (IC_{50} for mGlUR1 = 8.8 μM; IC_{50} for mGlUR5 > 100 μM) (Clark et al. 1997); and the selective group I mGlUR5 antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP) at 10–50 μM (IC_{50} for mGlUR5 = 0.036 μM; IC_{50} for mGlUR1 > 100 μM) (Gasparini et al. 1999b). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), except for the mGlUR antagonists, which were purchased from Tocris Cookson (Ellisville, MO).

Data analysis

Electrophysiological recordings were analyzed off-line on chart paper and on a computer with Clampfit 8 software (Axon Instruments). Burst duration was measured from the beginning of the first action potential to the end of the last one for each burst in intracellular recordings. Burst frequency was obtained from the intervals between the beginnings of the first action potential of two successive bursts. For these measurements most cells (15/25) were kept at a level more hyperpolarized (∼68 to −90 mV) than their resting membrane potential by injecting a constant hyperpolarizing current (−0.2 to −1.0 nA) to suppress spontaneous action potentials so that synchronized bursts could be distinguished from intrinsic firing. Measurements from the same cell in different drug conditions were taken at approximately the same membrane potential. After a minimum of 30 min of perfusion with each blocker to reach steady-state conditions, measurements were taken from 2- to 10-min periods of recording. Data were reported as mean ± SE and those displayed as histograms were analyzed with Sigma Plot 5.0 (SPSS Science, Chicago, IL). Student’s t-tests for paired or unpaired data were used for statistical comparisons and the level of significant difference was set at P < 0.05.

RESULTS

Induction of prolonged synchronized bursts in mouse hippocampal slices

Intracellular recordings were performed on CA3 pyramidal cells of mouse hippocampal slices in vitro. The resting membrane potential of these neurons was −65.4 ± 1.2 mV (mean ± SE; n = 25) and the input resistance was 40.4 ± 2.6 MΩ (n = 22). After 10–20 min of perfusion with bicuculline,
short-duration bursts (150–270 ms) of action potentials riding on a depolarizing envelope appeared at regular intervals ranging from 15 to 90 s (Fig. 1A). On average, the duration of these interictal-like bursts was 255.2 ± 23.4 ms (range 140–480 ms; Fig. 1C, Bic; n = 16) and the interburst interval was 35.1 ± 2.7 s (range 20–56 s; n = 16). Combined application of bicuculline and of 4-aminopyridine has been shown to produce ictal-like events in brain slices (Brückner et al. 1999). We added 4-AP (100 μM) to slices perfused with bicuculline. After 20–30 min, prolonged discharges (890–2120 ms) occurring at regular intervals of 15–25 s appeared (Fig. 1B). A prolonged discharge consisted of an initial burst followed by afterdischarges riding on a 15–40 mV depolarization. In 16 experiments, the average duration of the prolonged discharge was 1139.1 ± 93.2 ms (range 710-2050 ms; Fig. 1C, Bic + 4-AP), significantly longer than that of interictal bursts (255.2 ± 23.4 ms; n = 16; P < 0.001). The interval between prolonged discharges (11.5 ± 1.5 s; range 5–25 s; n = 16) was significantly shorter than that between interictal bursts (P < 0.001). Prolonged discharges were observed for 2 h in three experiments and in all cases they occurred with a stable pattern for the duration of the recordings.

Both the interictal bursts recorded in bicuculline and the prolonged discharges observed after addition of 4-AP were synchronized population bursts because 1) their frequency did not change at different levels of the membrane potential (n = 11) and 2) they were recorded with extracellular electrodes as field potentials in the CA3 region (n = 4; not shown).

Effects of group I mGluR antagonists on burst prolongation

We used selective antagonists for mGluR1 and mGluR5 to determine whether either or both of the group I mGluR subtypes were involved in the maintenance of prolonged bursts induced by bicuculline and 4-AP.

The mGluR1 selective antagonist LY 367385 was applied to test the role of mGluR1. After induction of prolonged discharges with bicuculline and 4-AP (Fig. 2A), addition of LY 367385 (100 μM) to the perfusate reduced the burst duration from 820–1050 to 340–460 ms (Fig. 2, A and B). LY 367385 at 100 μM shortened the bursts by 66.2 ± 6.7% (n = 5). The same agent at 25 μM shortened the burst by 61.1 ± 8.8% (n = 3). The effects of LY 367385 at 100 μM were not significantly different from those produced at 25 μM. This finding together with previous data indicating that the IC 50 of LY 367385 is 8.8 μM (Clark et al. 1997) suggest that LY 367385 at 25 μM is near the saturating dosage for antagonizing the synaptically activated mGluR1. The combined data for LY 367385 at the two concentrations show that the average burst duration before and after the mGluR1 antagonist was 1133.7 ± 117.9 and 401.6 ± 61.2 ms, respectively (Fig. 2C; n = 8; P < 0.001). The interburst interval was also significantly decreased to 3.9 ± 0.6 s by the mGluR1 antagonist (P < 0.05). This suggests that synaptically released glutamate activated mGluR1 to extend the epileptiform discharges.

To test the role of mGluR5, we applied the selective antagonist MPEP to slices with synchronized prolonged discharges.
Figure 3 shows that MPEP (50 μM) also reduced the burst duration from 820–1550 to 620–980 ms (Fig. 3, A and B). In five experiments, MPEP at 50 μM concentration shortened the burst by 53.8 ± 8.0%. At 10 μM, MPEP reduced the burst duration by 50.5 ± 5.7% (n = 3). The effects of MPEP at the two concentrations tested were not significantly different. On average, the burst duration before and after MPEP at 10 or 50 μM was 1144.5 ± 155.9 and 526.7 ± 75.6 ms, respectively (Fig. 3C; n = 8; P < 0.01). MPEP also shortened the interburst interval (5.5 ± 0.9 s; P < 0.05). These results suggest that...
mGluR1 antagonist induced additional reduction to 22.3% of the bursts recorded in bicuculline and 4-AP (in A; 990.5 ± 90.4 ms; in B: 1013.3 ± 115.8 ms).

synaptically activated mGluR5 is also involved in sustaining the prolonged discharges.

To examine whether the synaptically activated mGluR1 and mGluR5 exerted independent effects on the epileptiform bursts, the antagonists for the two receptor subtypes were applied sequentially to the same slices. In a first group of experiments, MPEP (50 μM) was added to slices that were perfused with LY 367385 (100 μM) for 45–80 min. LY 367385 reduced the burst duration to 33.8 ± 6.7% of control values (i.e., burst duration in the presence of bicuculline and 4-AP). Subsequent addition of MPEP produced further significant shortening of the burst to 22.7 ± 4.8% of control (Fig. 4A; n = 5; P < 0.05). In the second group, LY 367385 (100 μM) was applied 45–80 min after the addition of MPEP (50 μM) to the perfusate. MPEP decreased the burst duration to 46.2 ± 8.0% of control and subsequent application of the mGluR1 antagonist induced additional reduction to 22.3 ± 8.7% (Fig. 4B; n = 5; P < 0.05). Thus the effects of the subtype antagonists were additive (Fig. 4, A and B, right histogram bars). The burst durations after application of both antagonists in the two groups of experiments were not significantly different (in MPEP after LY 367385: 227.7 ± 50.9 ms; in LY367385 after MPEP: 201.3 ± 61.8 ms; P = 0.75). Also, these values were not significantly different from the duration of interictal bursts recorded in the presence of bicuculline alone in the same slices (278.3 ± 55.5 and 234.2 ± 44.4 ms; P = 0.65 and P = 0.17 for the two groups of experiments, respectively).

In three experiments, we tested the reversibility of the mGluR antagonist effects. After 45–65 min of perfusion with both LY 367385 (100 μM) and MPEP (50 μM), the duration of the bursts recorded in bicuculline + 4-AP (1040.7 ± 77.4 ms) was significantly shortened (265.3 ± 61.5 ms; n = 3; P < 0.05). Following washout of the mGluR antagonists for 60–90 min, the burst duration again increased to 581.7 ± 51.7 ms (washout vs. mGluR antagonists, P < 0.01; washout vs. bicuculline + 4-AP, P = 0.027). This indicates that the shortening effect of the mGluR antagonists on the burst duration was partially reversible.

In six experiments, possible effects of the mGluR antagonists on the resting membrane potential, input resistance, and firing threshold of CA3 pyramidal cells were monitored before and 40- to 60-min after addition of the antagonists (Table 1). None of these cellular properties were significantly changed by either one of the group I mGluR antagonists.

Epileptiform burst activity in phospholipase C β1 knockout mice

In hippocampal slices, we have recently shown that the induction of ictal-like discharges by group I mGluR agonist application requires functional PLCβ1 (Chuang et al. 2001). We recorded from hippocampal slices of PLCβ1 knockout mice to test whether this enzyme is involved in mediating the effects of the synaptically activated mGluR1 and mGluR5 on the epileptiform discharges.

The resting membrane potential (−65.3 ± 2.2 mV; n = 9) and input resistance (38.8 ± 3.3 MΩ; n = 9) of CA3 pyramidal cells in PLCβ1-deficient slices (PLCβ1−/−) were not significantly different from those of wild-type CA3 neurons. In PLCβ1−/− slices, bath application of bicuculline (50 μM) induced interictal-like bursts (Fig. 5A) that were similar to those recorded from wild-type animals. Subsequent addition of 4-aminopyridine (100 μM) significantly prolonged the bursts from 246.6 ± 10.7 to 954.7 ± 96.8 ms (Fig. 5, B and C, filled histograms; n = 9; P < 0.001) and shortened the interval between bursts from 33.6 ± 6.4 to 9.6 ± 1.2 s (n = 9; P < 0.01). The duration of prolonged bursts recorded in PLCβ1−/− slices was not significantly different from that recorded in wild-type slices (PLCβ1−/−, 954.7 ± 96.8 ms, n = 9; wild-type, 1139.1 ± 93.2 ms, n = 16; P = 0.22). Similarly, the interval between the prolonged bursts in PLCβ1−/− slices (9.6 ± 1.2 s) and that in wild-type slices (11.5 ± 1.5 s) were not significantly different (P = 0.42).

The involvement of group I mGluRs in the prolonged burst activity observed in PLCβ1−/− slices was again tested with selective antagonists. Similarly to that observed in wild-type slices, antagonists of either mGluR1 (LY 367385, 100 μM, n = 5; Fig. 6A) or mGluR5 (MPEP; 50 μM, n = 4; Fig. 6B) significantly suppressed the duration of prolonged discharges recorded in the presence of bicuculline and 4-AP. Also, the interburst interval was decreased by LY 367385 (7.8 ± 1.6 s; P < 0.05) and by MPEP (6.5 ± 2.1 s; P < 0.05). These results suggest that activation of both mGluR subtypes extended the

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Values are means ± SE from 3 cells before and after LY 367385 (100 μM) and from 3 cells before and after MPEP (50 μM). V_mem, resting membrane potential; R_mem, membrane input resistance; FT, firing threshold.
epileptiform discharges via a PLCβ1-independent cellular mechanism.

DISCUSSION

The results of this study indicate that, during prolonged synchronized discharges in the presence of the convulsants bicuculline and 4-aminopyridine: 1) group I mGluRs were activated by synaptically released glutamate and 2) synaptic activation of both subtypes of group I mGluRs prolonged the epileptiform discharges through a phospholipase C-independent mechanism.

Role of group I mGluRs in prolonged synchronized discharges

In the presence of GABA_A receptor blockers, hippocampal slices generate interictal bursts that are not affected by antagonists of mGluRs (Merlin et al. 1995). Addition of 4-aminopyridine to disinhibited slices elicits prolonged synchronized discharges (Fig. 1) (Brückner et al. 1999). Known actions of 4-AP are the blockade of transient K^+ currents (I_A, I_D, and a subtype of I_K) (Ficker and Heinemann 1992; Storm 1987, 1988; Wu and Barish 1992) and the increase of presynaptic Ca^{2+} entry (Jones and Heinemann 1987; Qian and Saggau 1999; Schubert and Heinemann 1988). Through these actions, 4-AP increases transmitter release (Buckle and Haas 1982; Rutecki et al. 1987). The enhanced release of glutamate could exert two postsynaptic actions: 1) potentiation of ionotropic glutamate receptor-mediated responses (Perreault and Avoli 1991) and 2) recruitment of mGluR-mediated responses. Previous studies showed that prolonged synchronized discharges induced by 4-AP in amygdales neurons (Arvanov et al. 1995) and in hippocampal CA1 pyramidal cells (Martin et al. 2001) are not blocked by the broad-spectrum mGluR antagonist α-methyl-4-carboxyphenylglycine. These results suggest that the enhancement of postsynaptic ionotropic glutamate receptor-mediated responses in itself is sufficient to sustain prolonged discharges under certain conditions. In contrast to these previous findings, we observed that group I mGluRs are also activated to sustain the prolonged epileptiform discharges elicited by 4-AP in the CA3 region of the mouse hippocampus. The difference in our findings with those reported previously could be due to the different brain regions studied by the different groups. For example, we have recently shown that pyramidal cells in CA1 and CA3 regions of the mouse hippocampus responded differently to the group I mGluR agonist stimulation (Chuang et al. 2002).

The data from this study also show that, when the mGluR-mediated responses were blocked, the prolonged bursts recorded in 4-AP reverted to the length of interictal bursts recorded in bicuculline. This suggests that the enhancement of postsynaptic iGluR-mediated responses by 4-AP did not contribute to burst prolongation. Our data do not provide an explanation for this lack of contribution of enhanced iGluR-mediated response. Possibly, the duration of the enhanced iGluR-responses may be self-limiting because of negative feedback mechanisms that are also enhanced by 4-AP. These mechanisms include Ca^{2+}-activated K^+ currents and postsynaptic GABA_B responses.
The direct effects of 4-AP on cell excitability (blockade of K⁺ channels; see METHODS) may also explain the increase in burst frequency compared with that observed in the presence of bicuculline alone. The shortening of the bursts by subsequent addition of group I mGluR antagonists reduced the refractoriness following each burst. These effects allowed for the observed further decrease of the interburst interval.

**Cellular mechanisms for group I mGluR-mediated prolongation of burst activity**

Our data show that the effects of antagonists of mGluR1 and of mGluR5 were additive, suggesting that both subtypes of group I mGluRs contribute to produce prolonged discharges. Group I mGluR-induced epileptiform discharges can also be elicited by the addition of a specific agonist to hippocampal slices (Taylor et al. 1995; Merlin and Wong 1997). Recent experiments demonstrated that the induction and maintenance of epileptiform discharges in this model also involve a contribution of both the group I mGluR subtypes (Merlin 2002).

Synchronized events examined in cortical tissue following disinhibition are typically 100–500 ms in duration (Delgado-Escueta et al. 1999; McCormick and Contreras 2001; Prince 1978). These events are considered comparable to interictal spikes in epilepsy. One difference between interictal and ictal discharges is in their durations. The lengthening of the burst in ictal discharges (>1 s) may engage excitatory mechanisms other than disinhibition alone. In this context, the mGluR-dependent excitation that prolongs the interictal spikes caused by disinhibition could be a mechanism involved in the interictal-to-ictal transition.

In previous studies, synchronized bursts of 1 to 12 s in duration have been elicited by a direct action of group I mGluR agonists and have been considered to resemble ictal events (Merlin and Wong 1997; Taylor et al. 1995). The major difference between the agonist-induced ictal-like bursts and the synaptically elicited prolonged discharges reported in this study is in the dependency on phospholipase C (PLC) β1 activation. The ictal-like discharges cannot be elicited in PLCβ1 knockout preparations (Chuang et al. 2001), whereas the prolonged synchronized discharges described in this paper were unaffected by the absence of PLCβ1 (Fig. 5). Thus it appears that the mGluR-dependent excitation consists of two components. The PLCβ1-independent component activated by addition of 4-aminoypyridine provides the initial prolongation of interictal spikes. The recruitment of the PLCβ1-dependent component further extends the mGluR-mediated excitatory process to sustain the ictal-like discharges. A PLCβ1-dependent, depolariza-
tion-activated current induced by group I mGluR agonists has been identified as a cellular process contributing to the ictal-like discharges (Chuang et al. 2001).

Finally, while we have shown that synaptically activated group I mGluRs prolong burst firing via a PLCβ1-independent mechanism, the cellular processes involved have not yet been identified. Several possibilities exist, including (1) the suppression of the background K⁺ current (Chuang et al. 2001), (2) the blockade of AHP currents (Young et al. 2000), and (3) the activation of inward currents via a G protein-independent (Heuss et al. 1999) or G protein-dependent (Tozzi et al. 2001) and protein-tyrosine-kinase-dependent (Heuss et al. 1999; Tozzi et al. 2001) intracellular pathways.

REFERENCES


