Spatial-Temporal Patterning of Metabotropic Glutamate Receptor-mediated Inositol 1,4,5-Triphosphate, Calcium, and Protein Kinase C Oscillations

PROTEIN KINASE C-DEPENDENT RECEPTOR PHOSPHORYLATION IS NOT REQUIRED*

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The metabotropic glutamate receptors (mGluRs), mGluR1a and mGluR5a, are G protein-coupled receptors that couple via Gi to the hydrolysis of phosphoinositides, the release of Ca2+ from intracellular stores, and the activation of protein kinase C (PKC). We show here that mGluR1/5 activation results in oscillatory G protein coupling to phospholipase C thereby stimulating oscillations in both inositol 1,4,5-triphosphate formation and intracellular Ca2+ concentrations. The mGluR1/5-stimulated Ca2+ oscillations are translated into the synchronized repetitive redistribution of PKCβII between the cytosol and plasma membrane. The frequency at which mGluR1a and mGluR5a subtypes stimulate inositol 1,4,5-triphosphate, Ca2+, and PKCβII oscillations is regulated by the charge of a single amino acid residue localized within their G protein-coupling domains. However, oscillatory mGluR signaling does not involve the repetitive feedback phosphorylation and desensitization of mGluR activity, since mutation of the putative PKC consensus sites within the first and second intracellular loops as well as the carboxyl-terminal tail does not prevent mGluR1a-stimulated PKCβII oscillations. Furthermore, oscillations in Ca2+ in the presence of PKC inhibitors, which blocked PKC/βII redistribution from the plasma membrane back into the cytosol. We conclude that oscillatory mGluR signaling represents an intrinsic receptor/G protein-coupling property that does not involve PKC feedback phosphorylation.

Metabotropic glutamate receptors (mGluRs)† are members of the G protein-coupled receptor superfamily (GPCRs) that are activated by the excitatory amino acid glutamate and play an essential role in regulating neural development and plasticity (1, 2). The activation of Group I mGluRs (mGluR1 and mGluR5) leads to increases in membrane-bound diacylglycerol and intracellular inositol 1,4,5-trisphosphate (InsP3) concentrations, the release of Ca2+ from intracellular stores, and the activation of protein kinase C (PKC) isoforms (1, 2). Group I mGluR activation gives rise to repetitive base-line separated Ca2+ transients (oscillations) (3–6), which can also be organized into intercellular Ca2+ waves (7). Ca2+ oscillations in response to mGluR activation are observed in immature neuronal cultures, developing neocortex, astrocytes, and heterologous cell cultures (3–6). In response to glutamate, mGluR1a and mGluR5a stimulate Ca2+ oscillations at distinct frequencies (4). Low frequency Ca2+ oscillations are stimulated by mGluR1, whereas mGluR5 stimulates Ca2+ oscillations at a higher frequency (4). The difference in the frequency of mGluR1a-versus mGluR5a-stimulated Ca2+ oscillations is proposed to occur as the consequence of mGluR1/5 subtype-specific differences in the feedback phosphorylation by PKC (3). However, it is unknown whether mGluR-stimulated Ca2+ oscillations are effectively translated into the cyclical activation of PKC leading to the repetitive desensitization of mGluR-mediated responses.

The oscillatory activation of the release of Ca2+ from intracellular stores in response to GPCR stimulation represents a fundamental mechanism of cell signaling in both non-excitable and excitable cells. Ca2+ oscillations not only fine tune gene expression during inflammatory immune responses (8) but are also involved in both the activation of Ca2+/calmodulin-dependent protein kinase II and the selective activation of different transcription factors (9, 10). Moreover, Ca2+ oscillation at different frequencies may be critically important for differential gene transcription in developing systems, since the transcription factor NF-xB is activated by low frequency Ca2+ oscillations, whereas higher frequency Ca2+ oscillations are needed to switch on the transcription factor NF-AT (10). It is also reported that Ca2+ oscillations cause sustained activation of mitochondrial metabolism and may be decoded by PKCy (11, 12).

In the present study, we explore the mechanism(s) underlying oscillatory mGluR Ca2+ signaling and test two hypotheses. 1) mGluR-stimulated Ca2+ oscillations are translated into the repetitive activation and plasma membrane translocation of PKC, protein kinase C; PCR, polymerase chain reaction; C-tail, carboxyl-terminal tail.
PKC. 2) PKC oscillations contribute directly to oscillatory receptor/G protein-coupling. We find that repetitive G protein activation by mGluR1a and mGluR5a results in the cyclical activation of PLC resulting in oscillations in InsP3 formation and Ca2+ release from intracellular stores. These mGluR-stimulated Ca2+ oscillations are effectively translated into the synchronized repetitive cytosol to plasma membrane translocation of PKCII. However, the mutation of 10 putative PKC phosphorylation consensus sites within the intracellular domains of mGluR1a reveals that PKC phosphorylation does not underlie oscillatory mGluR signaling. Furthermore, mGluR1 and mGluR5 each stimulate InsP3, Ca2+, and PKCII oscillations at distinct frequencies, a response that is determined by the charge of a single amino acid residue within the G protein-coupling domain of these receptors. We conclude that oscillatory mGluR signaling represents an intrinsic receptor/G protein coupling property that does not require PKC feedback phosphorylation.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes were obtained from Promega and New England Biolabs. pDsRed1-C1, pEGFP-C2, and pEGFP-C3 expression vectors were purchased from CLONTECH. The QuickChange™ Site-directed Mutagenesis kit was from Stratagene. The Marathon-Ready™ human brain, cerebellum, and the human universal QuickClone™ cDNA libraries were also obtained from CLONTECH. Human embryonic kidney cells (HEK 293) were from American Tissue Culture Collection. Fetal bovine serum was purchased from HyClone Laboratories Inc. Gentamicin, minimal essential medium, Hanks’s modified Eagle’s medium, and 0.05% trypsin containing 0.5 mM EDTA were acquired from Life Technologies, Inc. The calcium indicators, Oregon Green 488 BAPTA-1 AM and Calcium Orange, as well as BAPTA-AM were obtained from Molecular Probes. Quisqualate was purchased from Tocris Cookson Inc.; thapsigargin was obtained from Calbiochem, and EGTA and staurosporine were from Sigma. All other biochemical reagents were acquired from Sigma, Fisher, and VWR Scientific.

GFP and DsRed1 Plasmid Constructs—To construct DsRed1-
PKCβII cDNA was first amplified by PCR from the plasmid pBK-CMV containing the GFP-PKCβII construct (13). The 5′ and 3′ primers used in this PCR contained XhoI and BamHI restriction site sequences, respectively. The PCR product generated was subsequently digested with the restriction enzymes XhoI and BamHI, and the PKCβII cDNA was cloned into the corresponding sites of the vector pDsRed1-C1 (CLONTECH). To construct GFP-PH PLC-β1, the phospholipase C-β1 (PLC-β1) cDNA was amplified by PCR and used as the template for generating the 522-base pair fragment, which contained the PH domain (14). The 5′ and 3′ primers used in this reaction contained HindIII and XbaI restriction site sequences, respectively. The PHPLC-β1-containing PCR product was digested with the appropriate restriction enzymes and was cloned into the corresponding sites of the vector pEGFP-C3 (CLONTECH).

Site-directed Mutagenesis—PCR-based site-directed mutagenesis was performed using the QuickChange™ Site-directed Mutagenesis Kit (Stratagene). For mGluR1α the following mutations were made at position 854: D854T, D854A, and D854G. For mGluR5a the following mutations were made at position 840 (this corresponds to position 854 in mGluR1α): T840D and T840G. In addition, mGluR1α PKC consensus site mutants were constructed to create four mutants: Loop 1 (T620A and S627G), Loop 2 (T679A, S689G, and T695A), Loop 1/2 (T620A, S627G, T679A, S689G, and T695A), and C-tail (S892A, S940A, S950G, S1068A, and S1108A). Mutations and sequence integrity were confirmed by DNA sequencing.

Confocal Microscopy—HEK 293 cells were transiently transfected using a modified calcium phosphate method as described previously (15). Following transfection the cells were reseeded on collagen-coated 35-mm glass-bottomed culture dishes. Prior to visualization, the cells were washed three times with HEPES-buffered salt solution, HBSS (1.2 mM KH2PO4, 5 mM NaHCO3, 20 mM HEPES, 11 mM glucose, 116 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 2.5 mM CaCl2, pH 7.4) and incubated for 1 h at 37 °C in HBSS.

Confocal microscopy was performed on a Zeiss LSM-510 laser-scanning microscope using Zeiss 63×1.4 numerical aperture oil immersion lens. Enhanced GFP and Oregon Green 488 BAPTA fluorescence was visualized with excitation at 488 nm and emission 515–540 nm emission filter set. DsRed1 and Calcium Orange fluorescence were visualized with excitation at 543 nm and emission 590–610 nm filter set. For calcium imaging by confocal microscopy, following the manufacturer’s instructions, the cells were preloaded for 30 min with 10 μM Oregon Green 488 BAPTA-1 AM or Calcium Orange. Fluorescent signals were collected sequentially every 6.8–12.5 s using the Zeiss LSM software time scan function.

Data Analysis—PKC translocation time courses and Ca2+ oscillations were recorded as time series of 100–200 confocal images for each experiment. Image analysis was performed using the Zeiss LSM-510 analysis software and defined as the relative change in cytoplasmic fluorescence intensity over time in a 5-μm diameter region of interest. The time courses for GFP-PKCβII, GFP-PHPLC-α1, and DsRed1-PKCβII
Translocation responses as well as increases in intracellular Ca²⁺ concentrations were plotted using GraphPad Prism.

RESULTS

mGluR-stimulated PKCβII Cytosol to Plasma Membrane Oscillations—Because mGluR1α and mGluR5α stimulate Ca²⁺ oscillations, we sought to test whether a ubiquitously expressed conventional PKC isoform (PKCβII) (16) also exhibits cyclical patterns of cytosol to plasma membrane translocation in response to mGluR activation. Fig. 1, A and C, shows confocal images of GFP-tagged PKCβII (GFP-PKCβII) distribution in the absence (0 s) and presence of 100 μM quisqualate (20–140 s) in mGluR1α and mGluR5α-expressing HEK 293 cells. In response to the continued activation of mGluR1α and mGluR5α, GFP-PKCβII repetitively redistributes from the cytosol to the plasma membrane (Fig. 1, A and C). Three examples of the patterns of GFP-PKCβII plasma membrane oscillations in response to either mGluR1α or mGluR5α activation are shown in Fig. 1, B and D, respectively. GFP-PKCβII plasma membrane translocations occur at a frequency of 31 ± 13 oscillations/h (1 oscillation every 70–300 s, mean = 116 ± 48 s) in response to mGluR1α activation and 90 ± 28 oscillations/h (1 oscillation every 25–60 s, mean = 40 ± 12 s) in response to mGluR5α activation (Table I).

Synchronous mGluR-stimulated Ca²⁺ and GFP-PKCβII Oscillations—Because mGluR activation stimulates oscillations in intracellular free Ca²⁺ concentrations, we investigated whether mGluR1/5-stimulated GFP-PKCβII oscillations are synchronized with mGluR1/5-induced Ca²⁺ oscillations. In response to mGluR1α activation with 100 μM quisqualate, increases in intracellular free Ca²⁺ concentration are followed rapidly by the translocation of DsRed1-PKCβII to the plasma membrane (Fig. 2A, 20 s and 120 s). When intracellular free Ca²⁺ concentrations fall to base line, DsRed1-PKCβII redistributes back into the cytosolic compartment of each cell (Fig. 2A, 70 s). Analysis of concomitant Ca²⁺ and DsRed1-PKCβII oscillations reveals that the frequency of Ca²⁺ oscillation induced by mGluR1α (Fig. 2B) activation is mirrored exactly by the repetitive translocation of GFP-PKCβII between the cytosol and plasma membrane. Detailed examination of a single Ca²⁺ and DsRed1-PKCβII response (Fig. 2B, dashed box) reveals that the rise in intracellular free Ca²⁺ concentrations slightly precedes the plasma membrane translocation of DsRed1-PKCβII (Fig. 2C). Similarly, the release of DsRed1-PKCβII from the plasma membrane back into the cytosol following the inactivation of the Ca²⁺ transient is also slightly delayed (Fig. 2C). PKCβII oscillations in response to mGluR5α activation are also coupled to Ca²⁺ oscillations (Fig. 3).

GFP-PKCβII Oscillations Are Regulated by the Release of Ca²⁺ from Intracellular Stores—The dependence of mGluR1/5-stimulated GFP-PKCβII oscillations on intracellular free Ca²⁺ concentrations was examined by treating HEK 293 cells with the caged Ca²⁺ chelator, BAPTA-AM (Fig. 4, A and B). The treatment of cells with 50 μM BAPTA-AM to chelate intracellular free Ca²⁺ effectively blocked both mGluR1α- and mGluR5α-stimulated GFP-PKCβII oscillations (Fig. 4, A and B). The role of intracellular Ca²⁺ stores in promoting GFP-PKCβII oscillations was investigated using thapsigargin to deplete intracellular Ca²⁺ stores (17). Thapsigargin treatment resulted in a transient increase in GFP-PKCβII localized to the plasma membrane of mGluR1α-expressing cells but eventually resulted in the inhibition of both mGluR1α- and mGluR5α-stimulated GFP-PKCβII oscillations (Fig. 4, C and D). Finally, the addition of 2.5 mM EGTA to mGluR5α-expressing cell cultures to chelate extracellular Ca²⁺ had very little effect on mGluR5α-stimulated GFP-PKCβII oscillations but significantly reduced the periodicity of GFP-PKCβII translocation responses to mGluR1α activation (Fig. 4, E and F). These observations indicate that both intracellular and extracellular Ca²⁺ regulate mGluR1α but not mGluR5α-stimulated translocations in GFP-PKCβII.

Oscillatory Patterns of mGluR1/5 G Protein Coupling—Phosphatidylinositol 4,5-bisphosphate (PIP₂), the precursor for the second messenger InsP3, is metabolized in response to the activation of GPCRs coupled to the activation of PLC (18). Consequently, the hydrolysis of PIP₂ and InsP₃ formation represents a receptor-proximal event upstream of the release of Ca²⁺ from intracellular stores. Changes in both PIP₂ and InsP₃ levels can be measured in single cells using the pleckstrin homology (PH) domain of PLC-1 fused to GFP (GFP-PH PLC-1α) as a fluorescent indicator (14, 19). Therefore, we utilized GFP-PH PLC-1α to assess whether mGluR1α- and mGluR5α-stimulated Ca²⁺ and PKC oscillations might be associated with oscillatory G protein coupling to PLC.

In the absence of mGluR1α activation, GFP-PH PLC-1α is localized at the plasma membrane (Fig. 5A, 0 s) due to the interaction of PLC-1α PH domain with membrane PIP₂ (20). In response to mGluR1α activation, GFP-PH PLC-1α is released from the plasma membrane and accumulates in the cytosol of the cell (Fig. 5A, 150 s). The release of GFP-PH PLC-1α from the plasma membrane is the consequence of both PIP₂ hydrolysis and the high affinity of the PLC-1α PH domain for InsP₃ (19). Subsequently, as the mGluR-mediated response inactivates, GFP-PH PLC-1α is observed to redistribute back to the plasma membrane (Fig. 5A, 300 s). The persistent exposure of both mGluR1α (Fig. 5B) and mGluR5α (Fig. 5C) to 100 μM quisqualate results in oscillatory increases in intracellular InsP₃ as measured by the cyclical movement of GFP-PH PLC-1α between
the plasma membrane and cytosol. The oscillations in GFP-PH<sub>PLC-β1</sub> between the plasma membrane and cytosol in response to mGluR1α stimulation are also matched by the synchronous translocation of DsRed1-PKC<sup>β</sup>II at the plasma membrane (Fig. 5D); similar results are obtained for mGluR5α (data not shown).

**PKC Phosphorylation and mGluR1α Oscillatory Signal-**ing—It is possible that mGluR1α- and mGluR5α-stimulated PKC oscillations between the cytosol and plasma membrane may be sufficient to mediate repetitive cycles of mGluR phosphorylation. To investigate whether PKC-mediated phosphorylation contributes to oscillatory mGluR1α signaling, we mutated the 10 putative PKC phosphorylation consensus sites localized within Loop 1 (T620A and S627G), Loop 2 (T679A, S689G, and T695A), Loop 1/2 (T620A, S627G, T679A, S689G, and T695A), and the C-tail (S892A, S940A, S950G, S1068A, and T695A), and we tested the resulting mutants for their ability to stimulate oscillation in PKC<sup>β</sup>II distribution between the cytosol and plasma membrane (Fig. 6A). The mutation of the PKC phosphorylation consensus sites in Loop 1, Loop 2, Loop 1/2, and the C-tail of mGluR1α did not prevent mGluR1α-stimulated GFP-PKC<sup>β</sup>II oscillations (Fig. 6B–E). mGluR1α mutant-stimulated GFP-PKC<sup>β</sup>II oscillation frequencies were not significantly different from those observed following the stimulation of the wild-type mGluR1α (31 ± 13 oscillations/h versus 20 ± 7 to 28 ± 8 oscillations/h) (Table I). The time courses of the representative traces shown in Fig. 6B–E, match the time courses for wild-type mGluR1α-stimulated GFP-PKC<sup>β</sup>II oscillations shown in Fig. 1B. Mutation of all 10 putative PKC phosphorylation consensus sites resulted in an mGluR1α mutant that was completely uncoupled from G protein (data not shown).

To examine further the role of PKC phosphorylation in mGluR1α-stimulated Ca<sup>2+</sup> oscillations and GFP-PKC<sup>β</sup>II oscillations, we treated mGluR1α-expressing cells with two PKC inhibitors, staurosporine, a broad spectrum protein kinase inhibitor that inhibits multiple protein kinases, and bisindolylmaleimide I (Bis I), a PKC-selective inhibitor. The treatment of HEK 293 cells with 1 μM staurosporine inhibited mGluR1α-stimulated GFP-PKC<sup>β</sup>II oscillations resulting in the accumulation of GFP-PKC<sup>β</sup>II at the plasma membrane (Fig. 7A, top panel) but did not block mGluR1α-stimulated Ca<sup>2+</sup> oscillations in the same cell (Fig. 7A, bottom panel). The treatment of mGluR1α-expressing cells with 1 μM Bis I resulted in the gradual accumulation of GFP-PKC<sup>β</sup>II at the plasma membrane and the eventual inhibition of GFP-PKC<sup>β</sup>II oscillations (Fig. 7B, top panel) but had no effect on mGluR1α-stimulated Ca<sup>2+</sup> oscillations in the same cell (Fig. 7B, bottom panel). Identical results were observed following the staurosporine and Bis I treatment of mGluR5α-expressing cells (data not shown). These data provide additional evidence that mGluR phosphorylation by PKC is not required for mGluR-stimulated oscillations in intracellular Ca<sup>2+</sup> concentrations.

**Patterns of GFP-PKC<sup>β</sup>II Translocation Elicited by mGluR1α/5 Mutants**—It is proposed that mGluR subtype-specific Ca<sup>2+</sup> oscillations may be regulated by differences in PKC phosphorylation of a PKC consensus site in mGluR5α (Thr-840) that is not conserved at the analogous position in mGluR1α.
PKC phosphorylation consensus site (3) increases the frequency of mGluR1a-D854T to reconstitute a (Fig. 6A). The substitution of threonine residue 840 for an aspartic acid residue in mGluR5a (mGluR5a-T840D) reduces the mGluR5a-stimulated GFP-PKCβII oscillation frequency from 90 ± 28 to 38 ± 8 oscillations/h (Fig. 8A and Table I). In contrast, the replacement of aspartic acid residue 854 with a threonine residue (mGluR1a-D854T) to reconstitute a PKC phosphorylation consensus site (3) increases the frequency of mGluR1a-mediated GFP-PKCβII oscillations by 3-fold from 31 ± 13 to 94 ± 27 oscillations/h (Fig. 8B) (Table I). A similar response is obtained for mGluR-stimulated Ca^{2+} oscillations (data not shown). Taken alone, these data are consistent with the idea that the PKC-mediated phosphorylation accelerates the rate of mGluR-stimulated Ca^{2+} and PKC oscillations. However, the substitution of aspartic acid residue 854 with an alanine in mGluR1a not only increases the frequency of agonist-stimulated GFP-PKCβII oscillations to 92 ± 27 oscillations/h but also results in spontaneous GFP-PKCβII oscillations (Fig. 8B and Table I). Because the substitution of alanine and glycine residues at aspartic acid residue 854 supports mGluR5a-like oscillation patterns, it is unlikely that repetitive phosphorylation and dephosphorylation of threonine residue 840 can account for the observed mGluR subtype differences in Ca^{2+} and GFP-PKCβII oscillation frequencies.

**DISCUSSION**

Spontaneous oscillations in intracellular Ca^{2+} concentrations play a critical role in neuronal development (21, 22). Endogenous glutamate acting at Group I mGluRs is responsible for these spontaneous oscillations in the neocortex (6). Moreover, during long-term potentiation in the hippocampus, the plasma membrane translocation of PKCβ is dependent upon mGluR activation (23). Therefore, it is essential to understand how mGluR signaling patterns are decoded by downstream effector enzymes. In the present study, we find that mGluR-stimulated oscillations in intracellular Ca^{2+} concentrations are faithfully recapitulated by the synchronous cytosol to plasma membrane translocation of PKCβII. The oscillatory patterning of the release of Ca^{2+} from intracellular stores and PKCβII activation by mGluRs is mediated by the repetitive activation of PLC, resulting in oscillations in InsP_{3} formation. The activation of G_{q}-mediated signaling by mGluR1a and mGluR5a occurs at distinct frequencies that are regulated by the identity of a single amino acid residue found within a conserved stretch of amino acids within the mGluR1/5 G protein-coupling domains. However, the spatial-temporal dynamics of mGluR signaling does not involve PKC-mediated receptor phosphorylation because Ca^{2+} oscillations continue in the presence of PKC inhibitors that block PKCβII oscillations. Taken together, our data indicate that oscillatory mGluR signaling may represent a unique intrinsic receptor property.

Two potential mechanisms have been proposed to explain the generation of base-line separated Ca^{2+} oscillations in response to GPCR activation. The receptor-controlled model of Ca^{2+} oscillations suggests that Ca^{2+} oscillations arise in response to receptor-mediated oscillations in InsP_{3} formation and require feedback receptor phosphorylation by PKC (24). Alternatively, the Ca^{2+}-induced Ca^{2+} release model of Ca^{2+} oscillations proposes that low levels of InsP_{3} stimulate local rises in intracellular Ca^{2+} concentrations that sensitize the InsP_{3} receptor, giving rise to a Ca^{2+} spike. Subsequently, inhibition of InsP_{3} receptors at the higher concentrations of intracellular Ca^{2+} attained at the peak of the Ca^{2+} release limits the duration of the Ca^{2+} spike (25).

Until now, mGluR-stimulated oscillations in intracellular Ca^{2+} concentrations have been best modeled by a receptor-controlled mechanism requiring cyclical mGluR phosphorylation and dephosphorylation events (3, 4). In particular, rapid mGluR5a-mediated Ca^{2+} oscillations are proposed to involve the phosphorylation of a threonine residue at position 840 that forms a PKC phosphorylation consensus site that is not conserved in mGluR1a (3). In contrast to the above models, we find that mGluR-stimulated Ca^{2+} oscillations do not require feedback PKC phosphorylation. This conclusion is based on three
lines of experimental evidence. First, the mutation of putative PKC phosphorylation consensus sites localized within the intracellular domains of mGluR1a does not prevent mGluR1a-stimulated oscillations in PKC distribution. Second, the treatment of HEK 293 cells with protein kinase C inhibitors results in the accumulation of PKC at the plasma membrane without blocking concomitant Ca\(^{2+}\) oscillations in the same cells. Third, the reconstitution of a PKC phosphorylation consensus site in...

![Diagram](image_url)
mGluR1a at position 854 is not required to establish a mGluR5a-like oscillation frequency. Taken together, these observations do not support the feedback phosphorylation component of the receptor-controlled model of mGluR-stimulated Ca\(^{2+}\) oscillations. Furthermore, the observation that mGluR stimulation evokes receptor subtype-specific repetitive activation of PLC also argues against a Ca\(^{2+}\)-induced Ca\(^{2+}\) release model for mGluR-mediated Ca\(^{2+}\) oscillations.

We propose a model of oscillatory mGluR signaling that is independent of PKC-mediated feedback receptor phosphorylation but that is dependent upon the repetitive coupling of mGluRs to G proteins leading to oscillations in InsP\(_3\) formation, release of Ca\(^{2+}\) from intracellular stores, and PKC activation (Fig. 9). In support of this model, we find that mGluR-
stimulated InsP₃ formation mirrors mGluR subtype-specific PKC oscillation frequencies. Furthermore, the identity of a single amino acid residue within the highly conserved G protein-coupling domain of Group I mGluRs regulates the frequency of PLC activity, Ca²⁺ release, and PKC activation. The oscillation frequency is dependent upon the nature of the amino acid residue found at either position 854 of mGluR1a or 840 of mGluR5a. Alanine and threonine residues at the corresponding positions in mGluR1a and mGluR5a support rapid mGluR-stimulated oscillations, whereas a charged aspartic acid residue reduces the frequency of mGluR/G protein coupling. Therefore, the localization of an aspartic acid residue at position 854 of mGluR1a antagonizes and/or slows mGluR1a coupling to G proteins. We propose that the observed differences in the oscillatory coupling of mGluR1a and mGluR5a represent intrinsic differences in the rate at which mGluR1a and mGluR5a reassociate to the Gα-dependent activation of PLC. However, we cannot definitively exclude the possibility that either 1) Ca²⁺ feedback regulation of the InsP₃ receptor contributes in part to the basal rate of mGluR-stimulated Ca²⁺ oscillations or 2) differences in the rate of coupling are determined by observed differences in mGluR1a and mGluR5a sensitivity to extracellular Ca²⁺.

Several studies have reported the cytosol to plasma membrane translocation of GFP-tagged conventional PKC isoforms in response to GPCR activation (13, 26–28). The presumption has been that the inactivation phase of PKCβII redistribution from the plasma membrane back into the cytosol is associated with receptor desensitization (13). However, our data show that the inactivation of mGluR-stimulated PKCβII plasma membrane localization does not appear to be associated with PKCβII-mediated receptor phosphorylation. Furthermore, the translocation of PKCβII back into the cytosol occurs subsequent to the return of intracellular Ca²⁺ concentrations to basal levels (Fig. 2C). Also, we find that the treatment of cells with PKC inhibitors following the initiation of mGluR-stimulated oscillations in Ca²⁺ and PKCβII results in the plasma membrane accumulation of PKCβII rather than the redistribution of the enzyme back into the cytosol. These observations are consistent with recent data showing that the autophosphorylation of threonine residue 641 and serine residue 660 is not only important for the activation of PKCβII but is also required for the dissociation of the activated kinase from the plasma membrane (28, 29). Taken together, these observations indicate that the decoding of mGluR-stimulated Ca²⁺ oscillations also involves PKC autophosphorylation.

In conclusion, the temporal regulation of Ca²⁺ signaling represents a universal mechanism exploited by both excitable and non-excitable cells to regulate specific cellular responses. Ca²⁺ oscillations in response to mGluR activation are observed in immature neuronal cultures and developing neocortex (6). During neuronal development, Ca²⁺ spikes regulate neurotransmitter and channel expression, neuronal differentiation of the neural crest, the extension and path finding of growth cones, and synapse formation and elimination (30–33). These developmental events are regulated, at least in part, by the activation of Group I mGluRs (6, 33, 34). In the mature brain, repetitive mGluR-stimulated increases in intracellular Ca²⁺ concentrations may contribute to increases in neuronal excitability associated with memory and learning. In particular, mGluR-stimulated increases in intracellular free Ca²⁺ concentrations and PKC activation are implicated in long term depression in the cerebellum (35). In the hippocampus, Group I mGluR activation is required for the transient translocation of PKCγ during long term potentiation (24). Furthermore, long term potentiation is substantially reduced in both PKCγ and mGluR1 knockout mice (36, 37). Consequently, the delineation of the molecular mechanisms by which Ca²⁺ oscillations are decoded in both a receptor subtype- and effector isoform-specific manner will be essential for understanding the role of mGluR signaling during development and in the adult brain. Because PKCα and PKCγ isoforms are also activated in a Ca²⁺-dependent manner, it is likely that the oscillatory regulation of these PKC isoforms will also contribute to mGluR1a modulation of neuronal activity during neuronal development and in the adult brain. It will also be of interest to determine whether novel and atypical PKC isoforms are activated and oscillate in response to mGluR activation.

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