PKC phosphorylates GluA1-Ser831 to enhance AMPA receptor conductance

Meagan A. Jenkins and Stephen F. Traynelis*
Department of Pharmacology; Emory University School of Medicine; Atlanta, GA USA

Keywords: AMPA receptor, GluA1, GluR1, PKC, phosphorylation

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; PKC, protein kinase C; CaMKII, Ca2+/calmodulin dependent protein kinase II; TARP, transmembrane AMPA receptor regulatory protein; SAP97, synapse-associated protein 97; A-kinase-anchoring protein 79; PP2B, calcineurin

Submitted: 09/29/11
Revised: 11/03/11
Accepted: 11/03/11
http://dx.doi.org/10.4161/chan.18648
*Correspondence to: Stephen F. Traynelis; Email: strayne@emory.edu

AMPARs mediate fast excitatory synaptic transmission in the brain, and are dynamically regulated by phosphorylation of multiple residues within the C-terminal domain. CaMKII phosphorylates Ser831 within the AMPA receptor GluA1 subunit to increase single channel conductance, and biochemical studies show that PKC can also phosphorylate this residue. In light of the discovery of additional PKC phosphorylation sites within the GluA1 C-terminus, it remains unclear whether PKC phosphorylation of Ser831 increases GluA1 conductance in intact receptors. Here, we report that the purified, catalytic subunit of PKC significantly increases the conductance of wild-type GluA1 AMPA receptors expressed in the presence of stargazin in HEK293T cells. Furthermore, the mutation GluA1-S831A blocks the functional effect of PKC. These findings suggest that GluA1 AMPA receptor conductance can be increased by activated CaMKII or PKC, and that phosphorylation at this site provides a mechanism for channel modulation via a variety of protein signaling cascades.

Introduction

Ligand-gated, AMPA-selective ionotropic glutamate receptors are integral membrane proteins that mediate fast, excitatory neurotransmission.1 The central pore of the AMPA receptor is formed by four large independent subunits that assemble as a homomeric or heteromeric dimer of dimers.2 Each individual subunit possesses an intracellular carboxy terminal domain, located downstream of the third membrane spanning helix, that contains distinct phosphorylation sites that regulate receptor gating, trafficking and localization.3-5 Biochemical studies have identified Ser831 within the C-terminal domain of the GluA1 subunit as a phosphorylation target of both CaMKII and PKC.6,8,9 GluA1-Ser831 phosphorylation by CaMKII does not appear to increase synaptic localization of GluA1-containing receptors,11,13 but it does increase the recombinant homomeric GluA1 receptor current response to glutamate by increasing single channel conductance.12,14 Although both CaMKII- and PKC-dependent increases in GluA1 activity rely on Ser831,15-17 it is unclear whether PKC modulates the channel in a similar manner to CaMKII. We thus set out to test whether PKC can also increase AMPA receptor conductance via phosphorylation of GluA1-Ser831 in functional AMPA receptors in the presence of accessory TARP subunits. We find that inclusion of the purified, catalytic subunit of PKC in the patch pipette intracellular solution significantly enhances conductance of intact homomeric GluA1 AMPA receptors co-assembled with a TARP accessory subunit.

Results and Discussion

PKC has previously been shown to phosphorylate the GluA1 C-terminus,18 and Roche and colleagues used phosphopeptide mapping to identify GluA1-Ser831 as one specific target residue of PKC phosphorylation.19 Derkach et al. (1999) provided the first evidence that phosphorylation at this site by CaMKII enhances the single channel conductance.
The current response with a graded waveform was generated by slowly washing glutamate from the patch, and $\gamma_{\text{MEAN}}$ was determined from variance analysis of the current response (Fig. 1B and C). Inclusion of the purified, catalytic subunit of PKC within the patch pipette solution increased the $\gamma_{\text{MEAN}}$ of recombinant homomeric wild-type GluA1-L497Y AMPA receptors in HEK cells to 13.5 ± 1.1 pS (Table 1 and Fig. 1C; n = 14, p < 0.001 by Student's t-test). Thus, in order to study the effects of PKC at GluA1-Ser831 in isolation, a mutant GluA1 AMPA receptor construct was used that expressed alanine substitutions at both Ser818 and Thr840. In addition, GluA1-Ser845 was mutated to alanine to prevent phosphorylation by endogenous PKA. For simplicity, this mutant is referred to as GluA1-LY-ASAA (GluA1-L497Y,S818A, S845A). PKC also enhanced the conductance of the GluA1-LY-ASAA mutant receptor. When the purified, catalytic fragment of PKC was included in the patch pipette solution, $\gamma_{\text{MEAN}}$ was increased from 9.8 ± 1.6 pS (n = 6) to 17.7 ± 1.8 pS (Table 1; n = 6, p < 0.01 by ANOVA). Although GluA1-Ser818 and GluA1-Ser845 are both phosphorylated by PKC, their effects were not additive, with the PKC dose-dependently increasing the conductance of GluA1-LY-ASAA to 17.7 ± 1.8 pS (n = 6, p < 0.01 by ANOVA).
Phosphorylation at Ser831 is then mediated primarily by CaMKII rather than PKC.14 Our results suggest that phosphorylation of GluA1-Ser831 by either PKC or CaMKII can enhance AMPA receptor conductance.3,14 In addition, these data confirm that GluA1 Ser831 is a substrate for PKC in functional full length receptors.

Examining this phenomenon is of particular importance in light of the report that PKC may be as physiologically relevant for GluA1 phosphorylation as PKA.15,27 When it has been suggested that A-kinase-anchoring protein 79 (AKAP79) targeting of PKC to GluA1 via synapse-associated protein 97 (SAP97) is not important in regulation of GluA1 in some systems,22 the data presented here show that PKC can phosphorylate GluA1 to enhance conductance.

Materials and Methods

Molecular biology. The coding region of the GRIA1 gene was placed in the CMV-based mammalian expression vector pRK5 (BD PharMingen), and this construct was used for transient expression of GluA1 in HEK293T cells. The GluA1-S818A, S831A, T840A, S845A mutations, as...
well as the non-desensitizing mutant L497Y, were inserted into GluA1 containing constructs using the QuikChange mutagenesis method according to the Stratagene protocol. cDNA sequences were verified by DNA sequencing (SeqWright, Fisher Scientific). Stargazin (rat) was maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 100 units/mL of penicillin, and 100 μg/mL of streptomycin on polystyrene culture dishes (or 8 mm glass coverslips coated with poly-D-lysine) in a humidified atmosphere of 5% CO2, 95% O2, at 37°C. To protect against excitotoxicity by endogenous glutamate in the media, transfected cells were grown in DMEM supplemented with 200 μM NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione), a non-selective AMPA receptor antagonist (Sigma-Aldrich). Pipettes had a tip resistance of 2–6 MΩ. External recording solution for all experiments comprised of (in mM) 150 NaCl, 10 HEPES, 3 KCl, 1 CaCl2, 1 MgCl2, pH 7.4; 5 gluconic acid, 110 CsOH, 30 CsCl, 4 NaCl, 5 HEPES, 4.37 EGTA, 2.1 CaCl2, 2.37 MgCl2, 0.1 spermine (Sigma-Aldrich), 4 ATP, 0.3 GTP, for some experiments A TP was omitted from the internal solution. The pH was adjusted to 7.3 with CO2. For some experiments the intracellular solution was supplemented with the catalytic fragment of PKC purified from rat brain (20 μM; Sigma-Aldrich). Pipettes had a tip resistance of 4–6 MΩ. External recording solution for all experiments comprised of (in mM) 150 NaCl, 10 HEPES, 3 KCl, 1 CaCl2, 1 MgCl2, pH 7.4, 310–330 mM Os. Currents were recorded at room temperature (23°C) at a holding potential of +60 mV with an HEKA EPC9 amplifier, filtered at 5 kHz (−3dB), and digitized with a sampling rate of 20 kHz.

References

1. Traynelis SF, Wollmuth LP, McIntosh FS, Nusser Z, Okada R, et al. Glutamate receptor ion channel structure, regulation, and function. Pharmacol Rev 2010; 62:465–96. PMID:20774469; http://dx.doi.org/10.1124/pr.110.109821.

2. Soderling TR. A molecular perspective on structural and regulatory mechanisms of AMPA receptor-glutamate receptor interaction. Nature 2005; 435:1052–8; PMID:15858532; http://dx.doi.org/10.1038/nature03684.

3. Shepherd JD, Huganir RL. The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu Rev Cell Dev Biol 2005; 21:65–94. PMID:15626117; http://dx.doi.org/10.1146/annurev.cellbio.21.012804.111351.

4. Tien A, Asidoff H, Solgohci M, Zhang W, Wada K, Howe JR, et al. Stargazin modulates AMPA receptor gating and trafficking by distinct domains. Neuron 2005; 43:151–62. PMID:15985532; http://dx.doi.org/10.1016/j.neuron.2005.06.013.

5. Shepherd JD, Huganir RL. The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu Rev Cell Dev Biol 2005; 21:65–94. PMID:15626117; http://dx.doi.org/10.1146/annurev.cellbio.21.012804.111351.

6. Rogers CD, Martin GS, Rizzuto RD, Huganir RL, Malinow R. Synaptic incorporation of AMPA receptors requires cyclic AMP-stimulated protein kinase A-dependent mechanisms. Neuron 2003; 39:425–35. PMID:14544521; http://dx.doi.org/10.1016/S0896-6273(03)00499-5.

7. Bansal TG, Brotan D, Lee H, Huganir RL, Schwarzbauer JE. The cell biology of synaptic plasticity: AMPA receptor trafficking. Annu Rev Neurosci 2000; 23:489–527. PMID:10806773; http://dx.doi.org/10.1146/annurev.neuro.23.1.489.

8. Barria A, Millie D, Doitchinov V, Griffin LI, Sudhof TC. Regulatory phosphorylation of AMPA-type glutamate receptors by Ca2+/calmodulin-dependent protein kinase II. Science 1997; 278:2042–5. PMID:9179270; http://dx.doi.org/10.1126/science.278.5321.2042.

9. Mammen AL, Kameyama K, Roche KW, Huganir RL. Regulatory phosphorylation of AMPA-type glutamate receptors by Ca2+/calmodulin-dependent protein kinase II. Science 1997; 278:2042–5. PMID:9179270; http://dx.doi.org/10.1126/science.278.5321.2042.

10. Brien RJ, Mammen AL, Bernhardt J, Berezin E, Ruben SA, et al. AMPA receptor phosphorylation at a Ca2+-calmodulin-dependent protein kinase II regulatory phosphorylation site on GluR1. Neuron 1997; 19:372–82. PMID:9297873; http://dx.doi.org/10.1016/S0896-6273(97)80029-9.

11. Boehm J, Malinow R. AMPA receptor phosphorylation during synaptic plasticity. Biochem Soc Trans 2005; 33:995–96. PMID:16266617; http://dx.doi.org/10.1042/bst051395.

12. Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Johnson RC, et al. Mechanisms of Ca2+-calmodulin-dependent kinase II regulation of AMPA receptor gating. Neuron 2004; 43:755–62. PMID:15551582; http://dx.doi.org/10.1016/j.neuron.2004.01.018.

13. Barria A, Millie D, Doitchinov V, Griffin LI, Sudhof TC. Regulatory phosphorylation of AMPA-type glutamate receptors by Ca2+/calmodulin-dependent protein kinase II. Science 1997; 278:2042–5. PMID:9179270; http://dx.doi.org/10.1126/science.278.5321.2042.

14. Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Johnson RC, et al. Mechanisms of Ca2+-calmodulin-dependent kinase II regulation of AMPA receptor gating. Neuron 2004; 43:755–62. PMID:15551582; http://dx.doi.org/10.1016/j.neuron.2004.01.018.

15. Mammen AL, Kameyama K, Roche KW, Huganir RL. Regulatory phosphorylation of AMPA-type glutamate receptors by Ca2+/calmodulin-dependent protein kinase II. Science 1997; 278:2042–5. PMID:9179270; http://dx.doi.org/10.1126/science.278.5321.2042.

16. Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Johnson RC, et al. Mechanisms of Ca2+-calmodulin-dependent kinase II regulation of AMPA receptor gating. Neuron 2004; 43:755–62. PMID:15551582; http://dx.doi.org/10.1016/j.neuron.2004.01.018.

17. Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Johnson RC, et al. Mechanisms of Ca2+-calmodulin-dependent kinase II regulation of AMPA receptor gating. Neuron 2004; 43:755–62. PMID:15551582; http://dx.doi.org/10.1016/j.neuron.2004.01.018.

18. Banul J, Kang MK, Johnson RC, Esteban JA, Huganir RL. Mechanisms of AMPA receptor phosphorylation during synaptic plasticity. Biochem Soc Trans 2005; 33:995–96. PMID:16266617; http://dx.doi.org/10.1042/bst051395.

19. Banul J, Kang MK, Johnson RC, Esteban JA, Huganir RL. Mechanisms of AMPA receptor phosphorylation during synaptic plasticity. Biochem Soc Trans 2005; 33:995–96. PMID:16266617; http://dx.doi.org/10.1042/bst051395.

20. Banul J, Kang MK, Johnson RC, Esteban JA, Huganir RL. Mechanisms of AMPA receptor phosphorylation during synaptic plasticity. Biochem Soc Trans 2005; 33:995–96. PMID:16266617; http://dx.doi.org/10.1042/bst051395.
19. Lin DT, Makino Y, Sharma K, Hayashi T, Nieuw B, Takahira K, et al. Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. Nat Neuroscience 2009; 12:979-87; PMID:19503082; http://dx.doi.org/10.1038/nn.2351
20. Lee HK, Takahara K, Kawaiyama K, He K, Yu S, Bennett L, et al. Identification and characterization of a novel phosphorylation site on the GluR1 subunit of AMPA receptors. Mol Cell Neurosci 2005; 30:86-94; PMID:15989797; http://dx.doi.org/10.1016/j.mcn.2004.06.005
21. Brooks DI, Tavalin SJ. Ca2+/calmodulin-dependent protein kinase II inhibits AMPA receptor signaling in GluR1-AMPA receptors. J Biol Chem 2011; 286:6697-706; PMID:21156788; http://dx.doi.org/10.1074/jbc.M110.183558
22. Hooks N, Langberg LK, Scott JD. Distinct enzyme combinations in AKAP signaling complex permit functional diversity. Nat Cell Biol 2005; 7:1066-73; PMID:16225861; http://dx.doi.org/10.1038/ncb1315
23. Nikolosova YA, Jeon Y, Banister AJ, Tavalin SJ, Colledge M, Ca2+/calmodulin-dependent protein kinase II interacts to disrupt association with AKAP79/150 and modulate N-methyl-D-aspartate receptor (NMDAR) activity. J Biol Chem 2010; 285:5253-61; PMID:19858198; http://dx.doi.org/10.1074/jbc.M109.839909
24. Milstein AD, Zhou W, Kanthasamy A, Hack DS, Nicoll RA. TARPs subserve differentially and dose-dependently control extrasynaptic AMPA receptor gating. Neuron 2007; 55:905-18; PMID:17880996; http://dx.doi.org/10.1016/j.neuron.2007.08.022
25. Jin R, Bedis TG, Mann JR, Traynelis SF, Gouaux E. Structural basis for partial agonist action at ionotropic glutamate receptors. Nat Neuroscience 2005; 8:109-18; PMID:15972125; http://dx.doi.org/10.1038/nn1391
26. Traynelis SF, Jaramillo F. Getting the most out of noise in the central nervous system. Trends Neuroscience 1998; 21:137-45; PMID:9554720; http://dx.doi.org/10.1016/S0166-2236(98)01238-7
27. Tavalin SJ, Colledge M, Hell JW, Langeberg LK, Huganir RL, Scott JD. Regulation of GluR1 by the A-kinase anchoring protein 79 (AKAP79) signaling complex shares properties with long-term depression. J Neuroscience 2002; 22:3046-51; PMID:11938087

© 2012 Landes Bioscience. Do not distribute.