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Identification of a Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in non-*N*-methyl-D-aspartate glutamate receptors

(ligand-gated ion channel/protein phosphorylation/*Xenopus* oocytes/patch clamp)

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ABSTRACT **Glutamate receptor ion channels are colocalized in postsynaptic densities with Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase II), which can phosphorylate and strongly enhance non-*N*-methyl-D-aspartate (NMDA) glutamate receptor current. In this study, CaM-kinase II enhanced kainate currents of expressed glutamate receptor 6 in 293 cells and of wild-type glutamate receptor 1, but not the Ser-627 to Ala mutant, in *Xenopus* oocytes. A synthetic peptide corresponding to residues 620–638 in GluR1 was phosphorylated *in vitro* by CaM-kinase II but not by cAMP-dependent protein kinase or protein kinase C. The ^{32}P-labeled peptide map of this synthetic peptide appears to be the same as the two-dimensional peptide map of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) glutamate receptors phosphorylated in cultured hippocampal neurons by CaM-kinase II described elsewhere. This CaM-kinase II regulatory phosphorylation site is conserved in all AMPA/kainate-type glutamate receptors, and its phosphorylation may be important in enhancing postsynaptic responsiveness as occurs during synaptic plasticity.**

Neural tissues are the most abundant sources for many protein kinases and phosphatases, and phosphorylation of ion channels is an important mechanism for modulating the excitability of neurons (1, 2). The major excitatory neurotransmitter in mammalian brain is glutamate, and the recent cloning of the glutamate receptor (GluR) family of ion channels makes them available for studies of their regulation by phosphorylation. Three categories of GluR channels have been identified: α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)-type GluR1–4, kainate-type GluR5–7 and associated proteins KA-1 and KA-2, and *N*-methyl-D-aspartate (NMDA)-type GluRs (3, 4). All of these ion channels have consensus phosphorylation sites on predicted intracellular domains for numerous protein kinases.

Phosphorylation of GluRs may be physiologically important since considerable evidence supports a regulatory role for Ca^{2+}-dependent protein kinases in long-term potentiation (LTP) (5, 6), a model of cellular learning and memory, at glutamatergic synapses. Current evidence (7) indicates that the enhanced synaptic responsiveness that occurs during expression of LTP is due to an increased presynaptic release of glutamate (8, 9) and an enhanced postsynaptic responsiveness (10, 11). The postsynaptic responsiveness is largely mediated by AMPA-type GluRs since they become progressively more responsive to iontophoretically administered AMPA after induction of LTP (10).

We have been investigating whether phosphorylation of AMPA-type GluRs by Ca^{2+}/calmodulin-dependent protein kinase II (CaM-kinase II) may enhance their responsiveness. This is an attractive hypothesis since GluRs are colocalized in the postsynaptic density (PSD) (12) with CaM-kinase II, the major PSD protein in forebrain (13). Induction of LTP, which requires postsynaptic influx of Ca^{2+} through NMDA receptors (14), produces a stable activation of CaM-kinase II (15) and protein kinase C (PKC) (16). Ca^{2+} influx through NMDA receptors in cultured hippocampal neurons gives a prolonged activation of CaM-kinase II, through an autophosphorylation mechanism, whereas the activation of PKC is very transient (17).

Our previous studies have shown that CaM-kinase II in isolated rat forebrain synaptosomes and PSDs is a strong catalyst for *in vitro* phosphorylation of GluRs (18). In cultured hippocampal neurons, activation of CaM-kinase II or PKC increases ^{32}P-labeling of AMPA-type GluRs (19). Furthermore, infusion of stably activated CaM-kinase II into hippocampal neurons gives a 3-fold enhancement of AMPA-type current (18). The present study was initiated to extend these results by (*i*) demonstrating the regulatory effect of CaM-kinase II on specific, expressed GluRs, and (*ii*) identifying the regulatory site in AMPA-type GluRs phosphorylated by CaM-kinase II. Since this phosphorylation site is conserved in all cloned non-NMDA GluRs, CaM-kinase II may fulfill an important physiological role in modulating GluR responsiveness.

MATERIALS AND METHODS

Expression of GluRs in 293 Cells and Whole-Cell Recordings. The cDNAs for GluR1 (flop) and GluR6 were gifts from M. Hollmann and S. Heinemann of the Salk Institute (20). Both cDNAs were ligated into the *Eco*RI sites of the pSFFV-Neo mammalian cell expression vector (21), and correct orientation was determined by *Apa* I digestion for GluR1 and *Eco*RV digestion for GluR6. The transformed human embryonic kidney 293 cells were seeded at 5×10^4 cells on poly(L-lysine)-coated (10 μg/ml) glass coverslips (diameter, 31 mm) and were transfected with 3 μg of plasmid per dish using the calcium phosphate method as modified by Chen and Okayama (22). Whole-cell recordings (23) were made 36–60 hr after transfection from cells (diameters, ≈30 μm) attached to the coverslip. Patch pipettes had resistances of 3–7 MΩ when filled with the following control solution: 120 mM cesium glutamate or gluconate/20 mM CsCl/1 mM $CaCl_2$/11 mM EGTA/10 mM Hepes, pH 7.3 (adjusted with CsOH)/4 mM MgATP.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate; CaM, calmodulin; CaM-kinase II, Ca^{2+}/CaM-dependent protein kinase II; GluR, glutamate receptor; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PSD, postsynaptic density.

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After formation of the whole-cell configuration, the series resistance was 15.8 ± 0.8 MΩ (n = 15). Control solution plus CaM-kinase II buffer contained 9 vol of control solution plus 1 vol of CaM-kinase II autothiophosphorylation reaction mixture (see below) without the CaM-kinase II, whereas the experimental test solutions also contained the CaM-kinase II. CaM-kinase II (2 μM) was autothiophosphorylated for 10 min at 5°C in 50 mM Hepes, pH 7.5/0.5 mM $CaCl_2$/6 μM calmodulin (CaM)/10 mM $Mg(OAc)_2$/0.4 mM adenosine 5′-[γ-thio]triphosphate/bovine serum albumin (1 mg/ml), which resulted in 60–70% Ca^{2+}-independent activity. The CaM-kinase II, or an identical control buffer lacking CaM-kinase II, was diluted 1:10 into the pipette solution. Extracellular solution contained 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 10 mM D-glucose, and 5 mM Hepes (pH 7.2). A dual-barrel pipette delivery system operated at a hydrostatic pressure of 20 cm of water was positioned 50–100 μm from the cell. Extracellular solution was applied through one barrel, whereas extracellular solution plus 100 μM L-glutamate or kainate was applied for 5–20 ms through the other barrel. The flow through the pipette was controlled electronically through PCLAMP software by a three-way valve. Recordings were made at room temperature with a band width of 0–2 kHz and sampled for analyses at 2–4 kHz.

Mutagenesis and *in Vitro* Transcription of GluR1 cDNA. Oligonucleotide-directed mutagenesis of Ser-627 to Ala (Ser627Ala) was performed by ligating the GluRI cDNA into the replicative form of M13mp18 at the *Eco*RI site and using the M13 Muta-Gene kit from Bio-Rad. The mutant primer was 5′-pGAG AGG ATG GTG GCT CCC ATT G-3′, where GCT encodes alanine at position 627. The point mutant was confirmed by dideoxynucleotide sequencing (24).

Wild-type and Ser627Ala mutant GluR1 mRNAs were prepared *in vitro* with the mCAP mRNA capping kit from Stratagene. After transcription, template DNAs were degraded by 10 units of RNase-free DNase. The RNAs were extracted with phenol/chloroform and then precipitated with ethanol in the presence of 0.3 M NaOAc (pH 5.0). The RNAs were suspended in RNase-free 10 mM Tris·HCl, pH 7.5/0.1 mM EDTA and adjusted to 2 μg/μl.

Recordings from Oocytes. The harvesting, injection, and incubation of oocytes (stages V–VI) from *Xenopus laevis* were performed as described (25). Oocytes were injected with 100 ng of wild-type or mutant GluR1 RNA 3–4 days before recordings were made. Electrodes, with resistances of <0.5 MΩ, were filled with 3 M KCl and inserted into oocytes. Oocytes were voltage-clamped with either single or dual electrodes using the Axoclamp 2A amplifier. Unless otherwise indicated, the oocytes were maintained at a holding potential of −60 mV and continually bathed in the following solution: 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Hepes (pH adjusted to 7.5 with KOH). Kainate (100 μm) (Research Biochemicals, Natick, MA) was dissolved in bathing solution and applied by a manually controlled perfusion system consisting of parallel-mounted glass capillary tubes (internal diameter, 1 mm) with the tips positioned 1 mm directly in front of the oocyte. The flow rate was ≈5 ml/min. Kainate was applied for 10 s at 3- to 5-min intervals. CaM-kinase II (2 μm), which was autothiophosphorylated (18) 5–30 min prior to use, was pressure injected (Picospritzer general valve) into oocytes via a separate electrode. The pressure (10–15 psi; 1 psi = 6.9 kPa) and duration (3–8 ms) were calibrated to inject 50 nl. For controls (buffer), the same volume of solution containing everything except CaM-kinase II was injected.

Kainate response curves were fitted to the following equation:

$$X = 100[\text{kainate}]^n/[K^n + (\text{kainate})^n],$$

where X is the response amplitude normalized to the amplitude obtained with 1 mM kainate in the same oocyte, K is the dissociation constant (apparent) for kainate, and n is the Hill coefficient.

Current–voltage (I/V) curves were constructed by normalizing the peak current amplitude at the various potentials vs. its amplitude at −90 mV. All averages are presented as means ± SEM with statistical significance determined by use of the Student t test.

Phosphorylation and Peptide Mapping of GluR1 Synthetic Peptide. A synthetic peptide corresponding to residues 620–638 of GluR1 plus a C-terminal Lys (Leu-Thr-Val-Glu-Arg-Met-Val-Ser-Pro-Ile-Glu-Ser-Ala-Glu-Asp-Leu-Ala-Lys-Gln-Lys) was synthesized by Macromolecular Resources (Fort Collins, CO) and purified by reverse-phase HPLC. The C-terminal Lys was added to ensure that the peptide would bind to phosphocellulose in the protein kinase assay. Peptide purity and concentration were determined by mass spectroscopy and amino acid composition. Two-dimensional peptide mapping of tryptic digests of ^{32}P-labeled GluR1 peptide was performed as described (18) using the Hunter thin-layer electrophoresis system (HTLE-7000; C.B.S. Scientific, Del Mar, CA).

RESULTS

Regulation of GluR6 in 293 Cells. We chose to express the cDNAs for GluR1 and GluR6 as representatives of the AMPA-type and kainate-type GluRs, respectively, to determine the regulatory effects of CaM-kinase II-mediated phosphorylation. We recorded kainate currents of up to 2 nA from

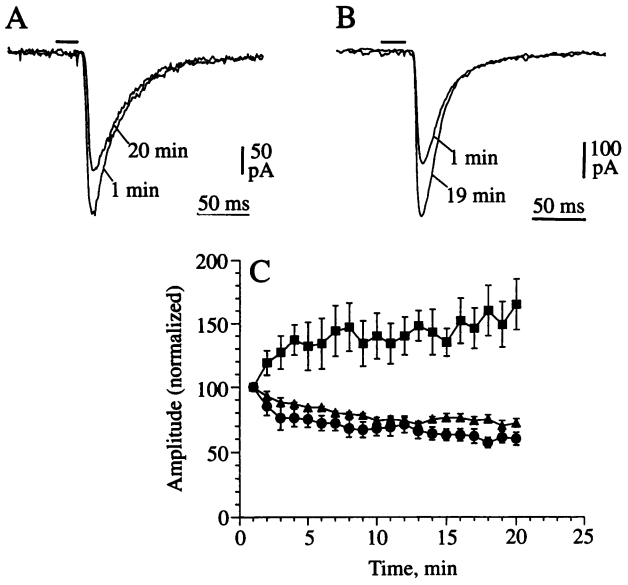

FIG. 1. Effect of CaM-kinase II on GluR6 in 293 cells. (*A*) Tracings illustrate rundown of whole-cell current observed upon application at 1 and 20 min of 100 μM L-glutamate for 20 ms (solid bar) in 293 cells expressing GluR6. Patch pipette contained control solution including CaM-kinase II buffer. (*B*) Similar tracings are shown from another cell except the patch pipette also contained 200 nM autothiophosphorylated CaM-kinase II, and rather than rundown there was an enhancement of current. (*C*) Current amplitudes at the indicated times were normalized to the 1-min value for GluR6-expressing cells in which the patch pipette contained control solution without kinase buffer (●; n = 4) or control solution with kinase buffer (▲; n = 4) or solution with 200 nM autothiophosphorylated CaM-kinase II (■; n = 6). Data combine results using either 100 μM L-glutamate or 100 μM kainate as both agonists gave essentially identical results. In all experiments, the holding potential was −80 mV. Results are plotted as means ± SEM, and enhancement by CaM-kinase II is significant ($P < 0.05$) for 7–20 min.

≈40% of the individual 293 cells expressing GluR6. Fig. 1 illustrates whole-cell patch-clamp recordings from 293 cells expressing GluR6 without (Fig. 1*A*) or with (Fig. 1*B*) activated CaM-kinase II in the pipette. Even though the patch pipette in the control cells contained Mg^{2+}ATP, there was 30–40% rundown of current over the 20 min. Similar results were obtained by using 100 μM either L-glutamate or kainate as agonist. However, when activated CaM-kinase II was present with the Mg^{2+}ATP in the pipette, there was an enhancement of agonist-dependent current at 20 min to 165% ± 18% (n = 6; $P < 0.05$) of the 1-min value (Fig. 1*C*). When compared to the controls, the CaM-kinase II enhancement at 20 min was ≈2-fold. The CaM-kinase II was activated by autothiophosphorylation, which converted it to a largely (60–70%) Ca^{2+}-independent form that is resistant to protein phosphatases (18).

Regulation of GluR1 in Oocytes. We wanted to pursue the effect of CaM-kinase II on GluR1 since the physiological role for AMPA-type receptors is better established than for kainate-type receptors. We chose to express GluR1 (flop) in *Xenopus* oocytes, since previous studies have shown that measurable currents can be consistently recorded with this system (20). In our experiments, we typically obtained currents of 50–300 nA in response to 100 μM kainate from oocytes 3–4 days after injecting 100 ng of GluR1 RNA (Fig. 2*A*). In general, the current amplitudes were stable upon repeated application (10 s each) of 100 μM kainate for up to 90 min. Kainate currents were reversibly blocked by 95% using 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (non-NMDA antagonist) (26) (n = 3; data not shown).

When oocytes expressing GluR1 were injected with activated CaM-kinase II, there was a progressive increase in the amplitude of the kainate current (Fig. 2 *A* and *B*). At 50 min after kinase injection, the current was enhanced to 152% ± 3% ($P < 0.01$; n = 8) of the control value obtained immediately after injection of the activated kinase. The oocytes were injected with 50 nl of 2 μM CaM-kinase II, which, assuming an average volume per oocyte of 500 nl, would give ≈200 nM kinase. Injection of the CaM-kinase II buffer solution, identical to the CaM-kinase II injection solution except for omission of the kinase, resulted in an enhancement of only 109% ± 3% (n = 5). The time course of current enhancement after injection of CaM-kinase II (Fig. 2*B*) was rather slow compared to that obtained in the 293 cells or the hippocampal neurons where 2- to 3-fold increases in current were obtained within 10–15 min (Fig. 1 and ref. 18). We initially thought the time course may reflect slow diffusion of the extremely large oligomeric CaM-kinase II (650 kDa) in the oocyte, but injection of an autothiophosphorylated monomeric form (36 kDa) of CaM-kinase II, engineered by truncation at residue 316 (27), gave a similar time course in three experiments (data not shown). It was also possible that the rate of current enhancement was partially suppressed by endogenous protein phosphatases, but coinjection of microcystin (100 μM), a potent protein phosphatase inhibitor (28), did not alter the rate or magnitude of the current enhancement by CaM-kinase II (data not shown).

In these experiments, we used subsaturating concentrations (100 μM) of kainate, so it was possible that the effect of phosphorylation was due to an increased affinity of the GluR1 for kainate. However, a dose–response curve obtained prior to and 50 min after injection of CaM-kinase II gave EC_{50} values of 43 and 42 μM, respectively, with Hill coefficient values of 1.3 in both cases (Fig. 2*C*). Thus, phosphorylation of GluR1 by CaM-kinase II does not alter its affinity for kainate.

Mutagenesis of GluR1 Phosphorylation Site. We next wanted to identify the CaM-kinase II phosphorylation site in GluR1 that was responsible for the current enhancement. Of the multiple potential phosphorylation sites in the putative major intracellular loop of GluR1 (20, 43), Ser-627 conforms to a consensus CaM-kinase II site with an Arg three residues N-terminal of a Ser or Thr (-Arg-Met-Val-Ser-). To test Ser-627 as the regulatory phosphorylation site, it was mutated to Ala by site-specific mutagenesis. When activated CaM-kinase II was injected into oocytes expressing the Ser627Ala mutant GluR1, there was no enhancement of current (Fig. 3*A*). Although the Ser627Ala mutant GluR1 did not respond to CaM-kinase II, it was indistinguishable from wild-type GluR1 in terms of affinity for kainate (data not shown) and its current–voltage relationship (Fig. 3*B*). Both the wild-type GluR1 and the Ser627Ala mutant exhibited strong inward rectification, which is characteristic of expressed homomeric GluR1 (50).

Phosphorylation and Peptide Mapping of GluR1 Synthetic Peptide. The above results establish Ser-627 as the regulatory CaM-kinase II phosphorylation site in GluR1. It should be noted that an analogous phosphorylation site is present in the same putative intracellular loop between transmembrane domains 3 and 4 in all the non-NMDA and one of the NMDA GluR1 ion channels (Fig. 4). Therefore, we wanted to test whether a synthetic peptide containing the sequence around Ser-627 in GluR1 was a substrate for CaM-kinase II. Fig. 5*A* shows that the peptide corresponding to residues 620–638 of GluR1 (see underlined residues in Fig. 4) was phosphorylated

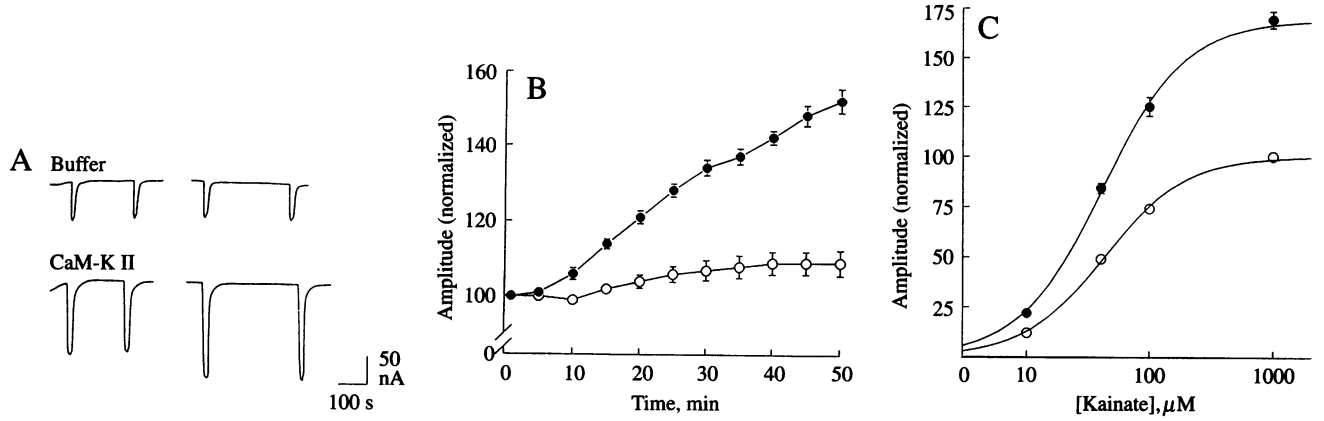

FIG. 2. Enhancement by CaM-kinase II of kainate currents in oocytes expressing wild-type GluR1 (flop). (*A*) Typical current traces in response to a 10-s application of 100μM kainate at 5-min intervals for an oocyte injected with activated CaM-kinase II (50 nl; 2 μM) or the exact same solution lacking CaM-kinase II (buffer). Left traces were obtained 1 and 5 min after injection, whereas the two right traces were made 45 and 50 min after injection. (*B*) Amplitudes of kainate (100 μM) currents are plotted vs. time after injection of activated CaM-kinase II (●; n = 8) or control solution (○; n = 5). Amplitudes were normalized relative to the value obtained 1 min after injection. Data are plotted as means ± SEM. (*C*) Kainate concentration–response curve was obtained before (○) and 50 min after (●) injection of CaM-kinase II (50 nl; 2 μM). Data are normalized to the 1 mM kainate response for control injection and are plotted as means ± SEM with n = 3.

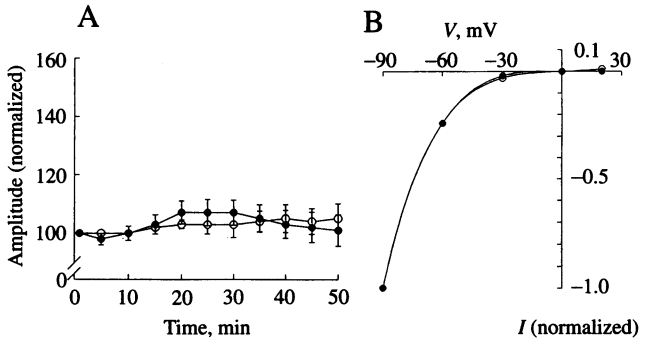

FIG. 3. Effect of Ser627Ala mutagenesis of GluR1. (*A*) CaM-kinase II responsiveness of mutant. Oocytes were expressing the Ser627Ala mutant of GluR1, and other experimental conditions are as in Fig. 2*B*. For CaM-kinase II-injected oocytes (●), $n = 6$; for control injections (○), $n = 3$. (*B*) Current–voltage (I–V) responses. Data were normalized to the value obtained at a holding potential of −90 mV. Both wild-type (○) and Ser627Ala mutant (●) GluR1 data were obtained from three oocytes.

by CaM-kinase II but not by cAMP-dependent protein kinase (PKA) or PKC. We also assayed under identical conditions the abilities of PKA and PKC to phosphorylate their preferred synthetic peptide substrates. The specific activity of PKA with 150 μM kemptide was 9.6 μmol·min^{-1}·mg^{-1} (35) and that for PKC with 150 μM neurogranin 28–43 was 0.1 μmol·min^{-1}·mg^{-1} (36). Thus, both PKA and PKC exhibited good activities under our assay conditions toward their known substrates but not toward GluR1 620–638 peptide. This demonstrated that Ser-627 is a selective phosphorylation site for CaM-kinase II.

We also wanted to test whether Ser-627 is the major phosphorylation site in AMPA receptors in cultured hippocampal neurons stimulated by calcium influx through the NMDA receptor (19). Fig. 5*B* shows that the two-dimensional tryptic ^{32}P-labeled peptide map of GluR1 synthetic peptide 620–638 phosphorylated by CaM-kinase II *in vitro* appears to be the same as the two-dimensional peptide map we obtained for the AMPA receptor ^{32}P-labeled in hippocampal neurons (see figure 6 of ref. 19).

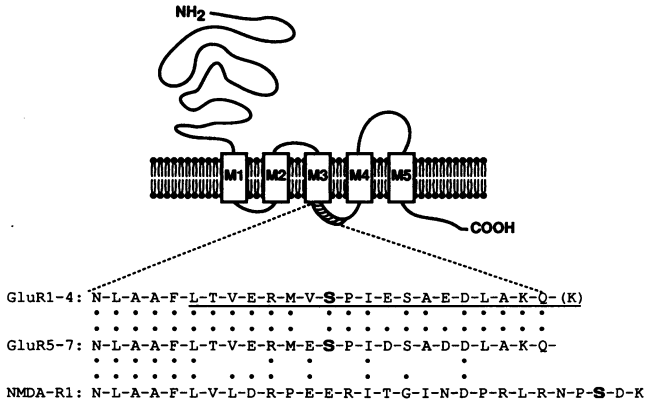

FIG. 4. Transmembrane topology of GluRs and conservation of CaM-kinase II phosphorylation site. Revised membrane topology of non-NMDA (29, 30) and NMDA (31) GluRs is depicted, and sequences of the intracellular loop denoted by the hatched bar are shown for AMPA-type (GluR1–4) and kainate-type (GluR5–7) (32) and NMDA-R1 (33) GluRs with the consensus CaM-kinase II phosphorylation site (Ser-627 in GluR1) in boldface type. Identical residues are indicated by two dots and conservative replacements are indicated by one dot. Underlined residues with the additional C-terminal Lys illustrate sequence of the GluR1 synthetic peptide used for *in vitro* phosphorylation studies.

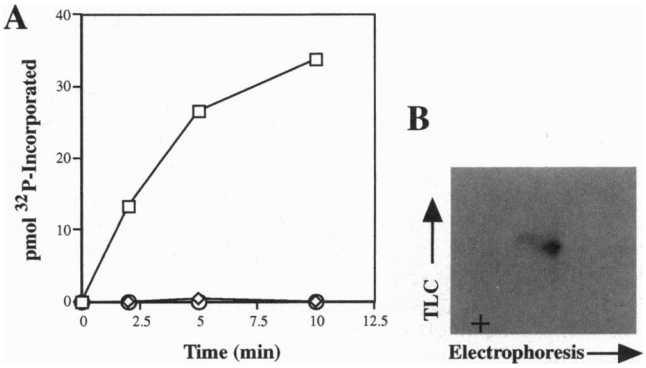

FIG. 5. Phosphorylation by protein kinases and peptide map of GluR1 synthetic peptide 620–638. (*A*) GluR1 synthetic peptide (150 μM) was phosphorylated with 50 nM CaM-kinase II (□), PKC (○), or PKA catalytic subunit (◇) with 10 mM $Mg(OAc)_2$, 0.4 mM [γ-^{32}P]ATP, and 50 mM Hepes (pH 7.5). Additional kinase activators were included as follows: CaM-kinase II, 0.2 mM $CaCl_2$ and 1 μM CaM; PKC, 0.2 mM $CaCl_2$ and mixed micelles containing 0.3% Triton X-100, 8 mol% phosphatidylserine, and 2 mol% diolein. Assays were performed in duplicate at 30°C with aliquots removed at 2, 5, and 10 min and analyzed for ^{32}P incorporation (34). Controls for autophosphorylation of the kinases in the absence of peptide substrate were subtracted. (*B*) GluR1 synthetic peptide (500 μM) was phosphorylated by 50 nM CaM-kinase II for 10 min, and the ^{32}P-peptide was separated from autophosphorylated kinase on an 18% Tricine gel and transferred to nitrocellulose. After autoradiography, the peptide was excised and digested with trypsin, and two-dimensional peptide mapping was performed as described (18). Electrophoresis in the horizonal direction was performed for 50 min at 1 kV, and ascending chromatography in the vertical direction was for 16 hr using 1-butanol, pyridine, acetic acid, and water in the ratio (vol/vol) 0.375:0.25:0.075:0.30. +, Origin.

DISCUSSION

The results of this study demonstrate that CaM-kinase II can enhance kainate current in expressed GluR6 and GluR1 (Figs. 1 and 2). These results are consistent with our previous data showing that CaM-kinase II in isolated synaptosomes and PSDs (18) and in cultured hippocampal neurons (19) is a strong catalyst for phosphorylation of AMPA-type GluRs. Our previous demonstration of kainate current enhancement by CaM-kinase II in cultured hippocampal neurons was essential for establishing the physiological relevance of this regulatory phosphorylation mechanism. This physiological role has been considerably strengthened by the recent report that CaM-kinase II probably mediates a Ca^{2+}-induced enhancement of AMPA receptor-mediated synaptic response in region CA1 of the hippocampus (37). The present results provide additional molecular details by demonstrating phosphorylation-dependent responses in expressed, specific GluRs (i.e., GluR1 as representative of AMPA-type and GluR6 as representative of kainate-type) and by identifying the regulatory phosphorylation site in GluR1 as Ser-627. Since the Ser627Ala mutant no longer responds to CaM-kinase II, this establishes that direct phosphorylation of GluR1 is responsible for this regulatory effect. An artifactual effect of the conservative Ser627Ala mutation (e.g., a change in conformation or overall GluR structure) is extremely unlikely since the mutant had exactly the same kainate responsiveness and I–V relationship as the wild-type channel. Furthermore, the synthetic peptide containing the sequence around Ser-627 was phosphorylated *in vitro* selectively by CaM-kinase II (Fig. 5*A*) and appears to correspond to the site phosphorylated in cultured hippocampal neurons (Fig. 5*B*).

GluR6 does have a separate consensus PKA phosphorylation site (Ser-684), and it is subject to regulatory phosphorylation by PKA (38, 39). The other non-NMDA receptor ion channels do not contain this PKA consensus phosphorylation

site, and we have not observed PKA phosphorylation of AMPA-type GluRs *in vitro* (18) or in cultured hippocampal neurons treated with forskolin (19). Consistent with this lack of phosphorylation of AMPA GluRs by PKA is the observation that the synthetic peptide corresponding to the sequence around Ser-627 was not significantly phosphorylated by PKA or PKC (Fig. 5*A*). Although PKC can phosphorylate AMPA GluRs, its site of phosphorylation appears to be different than the CaM-kinase II site (see figure 1 of ref. 18), and there are conflicting reports as to whether PKC can regulate non-NMDA GluRs (40–42).

The mechanism by which CaM-kinase II phosphorylation of non-NMDA channels enhances their current remains to be determined. The most likely possibility is an alteration of a channel property (e.g., single-channel conductance and/or open time). Another possibility is that phosphorylation of GluRs enhances their assembly or insertion into the membrane. However, an effect of phosphorylation on the rate of GluR desensitization could not account for our observations, since GluR1 does not desensitize to kainate (43).

Phosphorylation of GluR1 and GluR6 by CaM-kinase II may be important physiologically since (*i*) it enhances kainate current, and (*ii*) an analogous phosphorylation site is present in all non-NMDA GluRs (Fig. 4). The hypothesis (5, 44) that phosphorylation of non-NMDA GluRs by CaM-kinase II in the PSD may account in part for the enhanced AMPA component (10) of the excitatory postsynaptic current observed after induction of short-term potentiation or LTP (reviewed in ref. 45) remains to be pursued. Consistent with this hypothesis are the recent reports that induction of LTP results in a rapid and prolonged partial activation of CaM-kinase II (15) and that transfection of region CA1 in hippocampal slices with activated CaM-kinase II potentiates synaptic currents and occludes induction of LTP (46). Furthermore, mutant mice lacking the major isoform of CaM-kinase II are deficient in expressing LTP (47, 48) and another form of hippocampal learning, the Morris water test (49).

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