Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-d-aspartate receptor

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ABSTRACT The molecular basis of long-term potentiation (LTP), a long-lasting change in synaptic transmission, is of fundamental interest because of its implication in learning. Usually LTP depends on Ca\(^{2+}\) influx through postsynaptic N-methyl-d-aspartate (NMDA)-type glutamate receptors and subsequent activation of Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII). For a molecular understanding of LTP it is crucial to know how CaMKII is localized to its postsynaptic targets because protein kinases often are targeted to their substrates by adapter proteins. Here we show that CaMKII directly binds to the NMDA receptor subunits NR1 and NR2B. Moreover, activation of CaMKIIα by stimulation of NMDA receptors in forebrain slices increases this association. This interaction places CaMKII not only proximal to a major source of Ca\(^{2+}\) influx but also close to α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptors, which become phosphorylated upon stimulation of NMDA receptors in these forebrain slices. Identification of the postsynaptic adapter for CaMKII fills a critical gap in the understanding of LTP because CaMKII-mediated phosphorylation of AMPA receptors is an important step during LTP.

Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) mediates a variety of different cellular responses to Ca\(^{2+}\) influx (1, 2). An important source of Ca\(^{2+}\) influx into neurons is the N-methyl-d-aspartate (NMDA)-type glutamate receptor, which is activated by the excitatory neurotransmitter glutamate (2). NMDA- and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-type glutamate receptors are clustered at postsynaptic sites opposing presynaptic neurotransmitter release sites (3, 4). Brief trains of presynaptic high-frequency stimulation efficiently activate NMDA receptors (5), resulting in postsynaptic Ca\(^{2+}\) influx and long-term potentiation (LTP). LTP is a long-lasting increase in neurotransmission thought to represent the physiological correlate of learning and memory (5, 6). The induction of NMDA receptor-dependent LTP requires activation of CaMKII in the postsynaptic neuron (6, 7). CaMKII is enriched at postsynaptic densities (8, 9), where it is well placed for activation by Ca\(^{2+}\) influx through NMDA receptors and subsequent phosphorylation of neighboring AMPA receptors, an event contributing to LTP (7, 10). A kinase anchoring proteins, usually called AKAPs, place cyclic AMP-dependent protein kinase (PKA) next to selected substrates such as AMPA receptors (11), and receptors for activated C kinase (RACKs) are important for subcellular localization of different protein kinase C (PKC) isoforms (12). Crucial information about the subcellular targeting of CaMKII is lacking. NMDA receptors would be ideal postsynaptic adapter sites for CaMKII, where it would have full access to Ca\(^{2+}\) influx through these receptors. Cortical NMDA receptors consist of one or two NR1 and two or three NR2A and 2B subunits whose C termini are intracellular (13–16). We show that CaMKII is directly associated with NR1 and NR2B.

EXPERIMENTAL PROCEDURES

Materials. Tetrodotoxin, microcystin-LR, KN62, KN93, and GF109203X were purchased from Calbiochem, MK-801 and CPP were purchased from Research Biochemicals, and enhanced chemiluminescence detection kits were purchased from Amersham. [γ-\(^{32}\)P]ATP (111 TBq/mmol) was obtained from New England Nuclear, protein A-Sepharose was from Sigma, recombinant CaMKII was from A. R. Means (Duke University, Durham, NC), and the AC3-I peptide as well as anti-CaMKII antibodies were from H. Schulman (Stanford University, CA).

Immunoprecipitation and Immunoblotting. Crude rat brain membranes were prepared as described (17) and extracted with deoxycholate (1%) at 0–4°C for solubilization of whole NMDA receptor complexes or with 1% SDS at 50°C, followed by dilution with Triton X-100, to obtain dissociated NMDA receptor subunits, (for details see refs. 17 and 18). Immunoprecipitations and immunoblots were performed with the NR1-, NR2A-, NR2B-, GluR1-, and GluR2/3-specific antibodies αNR1, αNR2A, αNR2B, αGluR1, and αGluR2/3, respectively, and with monoclonal mouse antibodies against CaMKIIα and β (anti-CaMKIIα and anti-CaMKIIβ, respectively) as described (17, 18). SDS treatment of either membrane fractions or immunoprecipitated glutamate receptor complexes at 50°C dissociates the receptor complexes and subsequently allows specific immunoprecipitation of individual subunits after neutralization of SDS by adding an excess of Triton X-100 (17, 18). The specificities of the antibodies have been carefully characterized (17–19). Chromatographically purified nonspecific mouse or rabbit IgG antibodies (Zymed) were used for control immunoprecipitations to test for non-specific antibody interactions.

CaMKII Assays. Deoxycholate-extracted NMDA receptor complexes, SDS-dissociated NMDA receptor subunits, or Triton X-100-solubilized AMPA receptor complexes were immunoprecipitated and incubated for 30 min at 32°C in 50 μl phosphorylation buffer (50 mM Hepes-NaOH, pH 7.4/10 mM MgCl\(_2\)) containing 1 μM microcystin-LR, 50 μM unlabeled ATP, and 0.2 μM [γ-\(^{32}\)P]ATP (111 TBq/mmol; DuPont/NEN) (for more details see refs. 17 and 20). To activate CaMKII, 1 mM calmodulin and 0.5 mM CaCl\(_2\) were included and, in some experiments, exogenous recombinant CaMKIIα.

This paper was submitted directly (Track II) to the Proceedings office. Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CaMKII, Ca\(^{2+}\)/calmodulin-dependent protein kinase II; CPP, (–)-3-(2-carboxypropylazine-4-yl)-1-phosphonic acid; GST, glutathione S-transferase; LTP, long-term potentiation; NMDA, N-methyl-d-aspartate; PKA, cyclic AMP-dependent protein kinase; PKC, protein kinase C.

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Unsolubilized material was sedimented by ultracentrifugation with inhibitors pepstatin A (10 μM), leupeptin (10 μM), aprotinin (10 μg/ml), and Pefabloc (0.2 mM; Boehringer Mannheim) as well as the phosphatase inhibitors n-pitrophenyl phosphate (1 mM) and microcystin-LR (2 μM) were present in all buffers.

Stimulation of Cortical Rat Brain Slices. Acute hippocampal slices (0.4 mm) were prepared from 3-week-old Sprague-Dawley rats using a vibratome and pretreated as described (22). After drug treatment, slices were homogenized in 0.4 ml ice-cold 320 mM sucrose/10 mM Tris-Cl, pH 7.4/10 mM EDTA/10 mM EGTA by triturating with an insulin syringe. Membranes were collected by ultracentrifugation (50,000 rpm for 30 min, 70.1 Ti-rotor, 4°C) and solubilized in 1% deoxycholate/50 mM Tris-Cl, pH 8.0/10 mM EDTA/10 mM EGTA (17). Unsolubilized material was sedimented by ultracentrifugation as before. Supernatants were used immediately for immunoprecipitation (17) using a mixture of anti-CaMKIα and anti-CaMKIβ antibodies. For immunoprecipitation of AMPA receptors, slices were solubilized directly in 1% Triton X-100/0.1% SDS/50 mM Tris-Cl, pH 7.4/20 mM EDTA/10 mM EGTA and cleared by ultracentrifugation. The protease inhibitors pepstatin A (1 μM), leupeptin (10 μg/ml), aprotinin (10 μg/ml), and Pefabloc (0.2 mM; Boehringer Mannheim) as well as the phosphatase inhibitors n-pitrophenyl phosphate (1 mM) and microcystin-LR (2 μM) were present in all buffers.

Association of CaMKII with Glutathione S-Transferase (GST) Fusion Proteins. The recombinant GST fusion protein GST-NR2B839–1482 was expressed from pGEX, a modified pGEX vector (23), in Escherichia coli in parallel with nonchimeric GST or nonrelevant GST fusion proteins carrying the full-length sequence of cyclin G2 (24) or the SH3 domain of Src or Abl (25) for control, as described (18). GST-NR2B839–1482 contains the C-terminal 644 aa of NR2B (residue 839–1482) fused to the C terminus of GST. Plasmids encoding GST-NR2B839–1346, GST-NR2B839–1120, and GST-NR2B1120–1482 fusion proteins were produced by digesting the GST-NR2B839–1482 plasmid with BamHI/SacI, BamHI/Eco47 III, or Eco47 III/Hind III, respectively. The corresponding fragments were treated with T4 DNA polymerase to obtain blunt ends, gel-purified, re-ligated, and transformed into E. coli (Novablaue; Novagen). All constructs were confirmed by DNA sequence analysis. E. coli BL21 (Novagen) was used as host for protein expression.

RESULTS AND DISCUSSION

NMDA receptor complexes were solubilized with deoxycholate, immunoprecipitated from rat forebrain, and incubated with [γ-32P]ATP. Two prominent phosphorylated polypeptides were detectable under conditions that activate CaMKII (Fig. 1, lane 1). The endogenous kinase activity required Ca2+ and calmodulin (Fig. 1, lanes 4–8) and was blocked by the CaMKII-selective inhibitor KN93 (Fig. 1, lane 10) but not by a PKA-specific inhibitory PKI peptide (26) or by the bisindolylmaleimide GF109203X, which inhibits multiple PKC isoforms (Fig. 1, lanes 11–13). The efficacy of the two inhibitors for PKA and PKC was confirmed in parallel experiments (M. A. Davare and J.W.H., unpublished results) using the Ca2+ channel α1C subunit as a substrate for purified PKA and PKC, respectively (20). These data indicate that CaMKII is part of the NMDA receptor complex.

The specificity of the coimmunoprecipitation of the CaMKII activity with NMDA receptors was confirmed with control antibodies (Fig. 1, lane 2). The negative results obtained with the control precipitations exclude the possibility that the phosphorylated polypeptides bound nonspecifically to the immunoprecipitating antibodies or the resin. Because CaMKII is a highly abundant neuronal protein, it is possible that the observed interaction with NMDA receptors may be a result of nonspecific binding of CaMKII to this receptor. Therefore, we carefully evaluated whether CaMKII would also associate with the AMPA receptor complex under our conditions. Like NMDA receptors, AMPA receptors are heteromers formed by GluRI–4 subunits that are homologous to the NMDA receptor subunits (14). However, the phosphorylated polypeptides are not part of or associated with AMPA receptor complexes (Fig. 1, lane 3), further suggesting that the coprecipitation of CaMKII with NMDA receptors is highly specific.
Accordingly, CaMKII does not bind to any membrane protein in an indiscriminate fashion.

To evaluate whether the larger $^{32}$P-labeled polypeptide was identical to NR2A or 2B, which migrate with an apparent $M_r$ of about 200 kDa in our SDS/PAGE system (17), NMDA receptors were phosphorylated by the endogenous kinase and dissociated with SDS before NR2A and 2B were separately reprecipitated (17). $^{32}$P-labeled polypeptides of about 200 kDa were detectable in NR2A and 2B precipitations (Fig. 1, lanes 14–16), demonstrating that both NR2A and NR2B were phosphorylated by the endogenous kinase.

CaMKII is a multimeric complex formed by homologous $\alpha$ and $\beta$ subunits ($M_r = 50$ and 60 kDa, respectively) (1). Both subunits undergo rapid autophosphorylation in the presence of Ca$^{2+}$ and calmodulin, allowing their detection by autoradiography (1). Accordingly, the signal at 55 kDa probably is autophosphorylated CaMKII$\alpha$. A phosphorylated polypeptide of 65 kDa also was observed often, suggesting that the $\beta$ subunit is present in the NMDA receptor complex as well (data not shown). However, the 65-kDa signal was very weak, probably because in the forebrain the $\beta$ subunit is much less prevalent than the $\alpha$ subunit (1).

To further test whether the endogenous kinase is CaMKII, NR2A and NR2B were phosphorylated either by endogenous kinase or after dissociation with SDS by recombinant exogenous CaMKII$\alpha$ and analyzed by two-dimensional phosphopeptide mapping. The pattern of the major phosphopeptides derived from NR2A (A.S.L. and J.W.H., data not shown) or NR2B (Fig. 1B) were very similar upon phosphorylation by endogenous or exogenous kinase. These results provide additional evidence for the hypothesis that the endogenous kinase is CaMKII.

CaMKII complexes were immunoprecipitated from forebrain deoxycholate extracts with anti-CaMKII$\alpha$ and anti-CaMKII$\beta$ antibodies, which were mixed for higher efficiency. Subsequent immunoblotting with antibodies against NR1, 2A, and 2B subunits demonstrated their presence in CaMKII precipitates but not in control precipitates (Fig. 2A, lanes 1–3), corroborating that CaMKII is associated with NMDA receptor complexes.

Deoxycholate-solubilized NMDA receptor complexes contain not only various receptor subunits but also structural proteins of the PSD-95 family (18). To investigate whether CaMKII directly interacts with receptor subunits, NMDA receptor complexes were treated with SDS under conditions that completely separate the NMDA receptor subunits from each other and from members of the PSD-95 family (refs. 17 and 18; A.S.L. and J.W.H., unpublished results). Subsequently, NR1, 2A, and 2B subunits were immunoprecipitated individually. Recombinant CaMKII$\alpha$ was preincubated in [y-$^{32}$P]ATP-containing phosphorylation buffer for radioactive labeling by autophosphorylation before the immunocomplexes were added to this mixture. CaMKII$\alpha$ stably bound to NR1 and NR2B, but not NR2A (Fig. 3A, lanes 2, 4, 5). In similar experiments, CaMKII$\beta$ did not coprecipitate with AMPA receptor GluR1 subunits (A.S.L. and J.W.H., data not shown). The finding that CaMKII$\alpha$ did not form stable complexes with NR2A or GluR1, which possess high degrees of sequence similarities with NR1 and 2B, demonstrates the specificity of the binding of CaMKII$\alpha$ to NR1 and 2B. Using increasing amounts of CaMKII$\alpha$, we observed that binding of CaMKII$\alpha$ to NR1 as well as to NR2B was saturable (Fig. 3B). NR2A and 2B, but not NR1 (Fig. 3A, lanes 4, 5, and 2, respectively; see also Fig. 1), are phosphorylated under these conditions; however, $^{32}$P-labeled NR2A and 2B were absent in NR1 precipitates, further indicating that NR1 and NR2 subunits were efficiently dissociated during the SDS extraction and that they did not reassociate during the immunoprecipitation. When no recombinant CaMKII was added during these experiments, no phosphorylated polypeptide was detectable, arguing that the endogenous CaMKII had been removed completely during dissociation with SDS (Fig. 3A, lanes 8–10).

The synthetic peptide AC3-I is derived from the autoinhibitory domain spanning residues 278–290 of CaMKII$\alpha$ and inhibits CaMKII by competitively binding to the catalytic site.

**Fig. 2.** Coimmunoprecipitation of NMDA receptors with CaMKII. (A) To test whether NMDA receptors are associated with CaMKII in vivo, crude membrane fractions were prepared from total rat forebrain and NMDA receptors were solubilized with deoxycholate. After immunoprecipitation with antibodies against NR1, a mixture of the antibodies against CaMKII$\alpha$ and $\beta$ (19) or with control mouse IgG, immunoblotting was performed (lanes 1–3) with antibodies against NR2A (Left Top), NR2B (Left Middle), and NR1 (Left Bottom). To measure the effect of NMDA receptor-mediated Ca$^{2+}$ influx on CaMKII association with NMDA receptors in intact neurons, acute cortical slices were prepared and treated under control conditions (vehicle) or with 200 $\mu$M NMDA for 5 min in the absence or presence of 50 $\mu$M MK801 or 50 $\mu$M KN62. Crude membrane fractions were prepared and solubilized with deoxycholate. Immunoprecipitations were performed with a mixture of the antibodies against CaMKII$\alpha$ and $\beta$ (19) before immunoblotting with anti-NR1 (Right Lower) or a mixture of antibodies against NR2A and NR2B (Right Upper) (lanes 4–7). Similar results were obtained in two other experiments; the results of all three cortical slice experiments were quantified by densitometry of the immunoblotting signals (33) and are summarized in B. Bars = SEM.
 Approximately 2 phosphorylation. Twenty microliters of glutathione Sepharose loaded with after SDS and autophosphorylated CaMKII complexes were incubated with the whole phosphorylation mixture. The phosphorylation conditions with CaMKII was added (together with 20 μg blue) of GST or GST fusion proteins (as indicated at the bottom) then binding of CaMKII recombinant CaMKII and endogenous kinase because no phosphorylation was detectable if inhibitor AC3-I (20 μM) was added after autophosphorylation of CaMKII bound to three different GST fusion proteins carrying residues 839–1482, 839–1346, and 839–1120 equally well but not to GST alone (Fig. 3C Lower, lanes 1–4). Accordingly, residues 839–1120 of NR2B contain a CaMKII-association site. A recent report showed that CaMKII can bind to the NR2B region formed by residues 1260–1309 (28). Serine-1303, which is present in this sequence, is the major phosphorylation site for CaMKII in the C-terminal domain of NR2B in vitro (29). Because of the low K_M value of serine-1303 for CaMKII (about 50 nM), CaMKII binding to residues 1260–1309 might have been mediated by serine-1303 binding to the catalytic site. Our shortest fusion protein (839–1120) does not carry this phosphorylation site, nor is it phosphorylated by CaMKII under our conditions (Fig. 3C Upper, lanes 1–4), suggesting that binding of CaMKII to this sequence is not mediated by an interaction between the catalytic site and a substrate site. Furthermore, AC3-1 did not affect binding to any of our three GST-NR2B fusion proteins, corroborating that the binding was not due to catalytic site interactions (Fig. 3C Lower, lanes 5–7). Of note, AC3-1 did inhibit phosphorylation of GST-NR2B839–1482 and 839–1346 (Fig. 3C Upper, lanes 5 and 6), confirming its efficacy as a catalytic site inhibitor under our conditions. To test whether residues surrounding the serine-1303 phosphorylation site also may contribute to CaMKII binding (28), we made a GST fusion protein containing residues 1120–1482. Interestingly, this fusion protein specifically bound CaMKII as efficiently as 839–1120 (Fig. 3C Lower, lanes 9–13). This interaction was also insensitive to AC3-1 (Fig. 3C, lane 14), although AC3-1 blocked phosphorylation of GST-NR2B1120–1482. These results suggest that CaMKII binding to 1120–1482 is not mediated by an interaction between the catalytic site of CaMKII and its phosphorylation site at serine-1303. Accordingly, NR2B residues 1260–1309 (28) may contain a second association site for CaMKII close to the serine-1303 phosphorylation site.

Fig. 3. CaMKIIα binding to NR1 and NR2B. (A and B) Crude membrane fractions were extracted and NMDA receptor subunits were dissociated with SDS before immunoprecipitation of individual subunits as indicated on the bottom (A) or top (B). Control immunoprecipitations were performed with nonspecific murine (A, lane 1) or rabbit IgG (A, lanes 7 and 10). Recombinant CaMKIIα was precipitated under phosphorylation conditions in the presence of [γ-32P]ATP for labeling by autophosphorylation, and immunocomplexes were incubated with the whole phosphorylation mixture. The signal at 55 kDa (A, lanes 2, 3, 5, and 6) reflects binding of recombinant and autophosphorylated CaMKIIα rather than the presence of the endogenous kinase because no phosphorylation was detectable if recombinant CaMKIIα was omitted (A, lanes 8–10). Phosphorylation of NR2B (A, lane 6) but not association of CaMKIIα with NR1 (A, lane 3) or NR2B (A, lane 6) was inhibited if the competitive catalytic site inhibitor AC3-I (20 μM) was added after autophosphorylation of CaMKIIα (27). Similar results were obtained in two other experiments. Binding of CaMKIIα to NR1 and NR2B was saturable (B; the amounts of CaMKIIα associated with NR1 or NR2B were quantified after SDS/PAGE by using PhosphorImager analysis; values are means ± SEM; n = 3). (C) CaMKIIα was precipitated under phosphorylation conditions with [γ-32P]ATP for labeling by autophosphorylation. Twenty microliters of glutathione Sepharose loaded with approximately 2 μg (as confirmed by staining with Coomassie brilliant blue) of GST or GST fusion proteins (as indicated at the bottom) then was added (together with 20 μM AC3-1 when indicated) before samples were washed and analyzed by SDS/PAGE and autoradiography. GST-NR2B839–1482, 839–1346, and 1120–1482, but not 839–1120 or GST alone, were phosphorylated by CaMKII in an AC3-I-sensitive manner (Upper). Autophosphorylated CaMKIIα bound equally well to all four GST-NR2B fusion proteins but not to GST alone or to nonrelevant GST fusion proteins with the Src or Abl SH3 domain or cyclin G2 (Lower).
Autophosphorylation of CaMKII makes the kinase Ca$^{2+}$-independent (1) and is important for its binding to residues 1260–1309 (28). To investigate whether binding to 839–1120 can occur independently of autophosphorylation of CaMKII, we incubated GST-NR2B839–1120 with recombinant CaMKIIa in the absence of ATP and Ca$^{2+}$/calmodulin. After washing of the complex we detected a strong, specific signal for CaMKIIa by immunoblotting with anti-CaMKIIa (A.S.L. and J.W.H., unpublished data). Therefore, association with our binding site does not require autophosphorylation of CaMKII. Accordingly, nonphosphorylated CaMKIIa can also bind to GST-NR2B839–1482 (A.S.L. and J.W.H., unpublished data). However, because CaMKIIa has to be autophosphorylated for association with 1260–1309 (28), autophosphorylation increases CaMKIIa binding to GST-NR2B839–1482 (A.S.L. and J.W.H., unpublished data) and also to a 190-kDa protein in the postsynaptic density, which is most likely NR2B (30).

NMDA receptor-mediated Ca$^{2+}$ influx and induction of LTP stimulates autophosphorylation of CaMKII (10, 31, 32). Therefore, Ca$^{2+}$ influx may help to recruit CaMKII to the postsynaptic site by increasing its association with the NMDA receptor. Incubation of rat brain slices with 200 μM of NMDA in the presence of the Na$^{+}$ channel blocker tetrodotoxin induces NMDA receptor-mediated Ca$^{2+}$ influx and activation of Ca$^{2+}$-dependent events at postsynaptic sites without damaging the physiological integrity of the slices (33). Following this protocol, cortical slices were incubated with and without NMDA and crude membrane fractions were prepared and solubilized with deoxycholate. After immunoprecipitation with a mixture of antibodies against CaMKII and β, NR1 and 2B were detected by immunoblotting. A strong increase in NR1 and 2B immureactivity present in the immunoprecipitated CaMKII complexes is obvious upon stimulation with NMDA (Fig. 2A, lanes 4 and 5, and B). Pretreatment with the NMDA receptor antagonist MK801 or the CaMKII inhibitor KN93 blocked this NMDA-induced effect (Fig. 2, lanes 7 and 8). Thus, NMDA receptor-mediated Ca$^{2+}$ influx and subsequent autophosphorylation of CaMKII, which can be inhibited with KN62, strongly increased the association of CaMKII with the NMDA receptor in intact neurons.

Phosphorylation of the AMPA receptor GluR1 subunit by CaMKII plays a central role in LTP that is dependent on Ca$^{2+}$ influx through NMDA receptors (7, 10). As demonstrated in the previous paragraph, NMDA receptor-mediated Ca$^{2+}$ influx, which causes autophosphorylation and thereby activation of CaMKII, also results in a strong increase of CaMKII recruitment to postsynaptic sites by its association with NMDA receptors. Because NMDA receptors and AMPA receptors are colocalized, this association may coordinate phosphorylation of AMPA receptors by CaMKII. Therefore, we investigated whether NMDA receptor activation in cortical slices causes CaMKII-dependent phosphorylation of AMPA receptors. All cortical slices were treated in the presence of the bisindolylmaleimide GF109203X to block PKC because serine-831 in the GluR1 subunit of the AMPA receptor interacts with NR1 and NR2B. Upon NMDA receptor-mediated Ca$^{2+}$ influx and subsequent autophosphorylation, binding of CaMKII to NMDA receptors and, thereby, recruitment of CaMKII to the postsynaptic site are increased. This interaction places CaMKII at an ideal location for stimulation by Ca$^{2+}$ influx through NMDA receptors. It also brings CaMKII in close proximity to AMPA receptors that are phosphorylated and subsequently up-regulated in their activity by CaMKII upon NMDA receptor-mediated Ca$^{2+}$ influx, especially during LTP (7, 10). Accordingly, NMDA receptors not only are sources for Ca$^{2+}$ but also serve as the postsynaptic adapter sites for CaMKII. In summary, our findings demonstrate that CaMKII directly interacts with NR1 and NR2B. Upon NMDA receptor-mediated Ca$^{2+}$ influx and subsequent autophosphorylation, binding of CaMKII to NMDA receptors and, thereby, recruitment of CaMKII to the postsynaptic site are increased. This interaction places CaMKII at an ideal location for stimulation by Ca$^{2+}$ influx through NMDA receptors. It also brings CaMKII in close proximity to AMPA receptors that are phosphorylated and subsequently up-regulated in their activity by CaMKII upon NMDA receptor-mediated Ca$^{2+}$ influx, especially during LTP (7, 10). Accordingly, NMDA receptors not only are sources for Ca$^{2+}$ but also serve as the postsynaptic adapter sites for CaMKII. In summary, our findings demonstrate that CaMKII directly interacts with NR1 and NR2B. Upon NMDA receptor-mediated Ca$^{2+}$ influx and subsequent autophosphorylation, binding of CaMKII to NMDA receptors and, thereby, recruitment of CaMKII to the postsynaptic site are increased. This interaction places CaMKII at an ideal location for stimulation by Ca$^{2+}$ influx through NMDA receptors. It also brings CaMKII in close proximity to AMPA receptors that are phosphorylated and subsequently up-regulated in their activity by CaMKII upon NMDA receptor-mediated Ca$^{2+}$ influx, especially during LTP (7, 10). Accordingly, NMDA receptors not only are sources for Ca$^{2+}$ but also serve as the postsynaptic adapter sites for CaMKII.
and NMDA receptor subunits by producing reagents that disrupt that interaction after injection into intact neurons.

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