Casein kinase 1 (CK1) is a highly conserved serine/threonine kinase, present in virtually all cell types, in which it phosphorylates a wide variety of substrates. So far, no role has been found for this ubiquitous protein kinase in the physiology of nerve cells. In the present study, we show that CK1 regulates fast synaptic transmission mediated by glutamate, the major excitatory neurotransmitter in the brain. Through the use of CK1 inhibitors, we present evidence that activation of CK1 decreases NMDA receptor activity in the striatum via a mechanism that involves activation by this kinase of protein phosphatase 1 and/or 2A and resultant increased dephosphorylation of NMDA receptors. Indeed, inhibition of CK1 increases NMDA-mediated EPSCs in medium spiny striatal neurons. This effect is associated with an increased phosphorylation of the NR1 and NR2B subunits of the NMDA receptor and is occluded by the phosphatase inhibitor okadaic acid. The mGluR1, but not mGluR5, subclass of metabotropic glutamate receptors uses CK1 to inhibit NMDA-mediated synaptic currents. These results provide the first evidence for a role of CK1 in the regulation of synaptic transmission in the brain.

Key words: excitatory postsynaptic current; EPSC; NMDA receptor; synaptic transmission; phosphorylation; striatum; metabotropic glutamate receptor

Materials and Methods

Electrophysiology in brain slices

Experiments were approved by the Institutional Animal Care and Use Committee and were performed using methods described previously (Chergui and Lacey, 1999; Chergui et al., 2000). Briefly, coronal brain slices (400 μm thick), containing the striatum and neocortex, were prepared with a microslicer (VT1000S; Leica, Nussloch, Germany) from C57BL/6 mice aged 22–29 d. Slices were incubated at 32°C in oxygenated (95% O₂/5% CO₂) artificial CSF (aCSF) containing the following (in mM): 126 NaCl, 2.5 KCl, 1.2 NaH₂PO₄, 1.3 MgCl₂, 2.4 CaCl₂, 10 glucose, and 26 NaHCO₃, pH 7.4. Slices were transferred to a recording chamber
(Warner Instruments, Hamden, CT) mounted on an upright microscope (Olympus Optical, Melville, NY) and were continuously perfused with oxygenated aCSF at 32°C. Medium spiny neurons in the striatum were visualized within the brain slices using Nomarski-type differential interference contrast optics combined with infrared video microscopy. Whole-cell voltage-clamp recordings of medium-sized neurons in the dorsal striatum were made with patch electrodes filled with a solution containing the following (in mM): 140 CsCl, 2 MgCl₂, 1 CaCl₂, 10 HEPES, 10 EGTA, 2 MgATP, and 0.3 Na₃GTP, pH 7.3. Whole-cell membrane currents were recorded with an Axopatch 200B (Axon Instruments, Foster City, CA) driven by a personal computer. Neurons were voltage clamped at −80 mV. The resting membrane potential of medium spiny striatal neurons measured with a potassium gluconate-based intracellular solution was −84.5 ± 0.5 mV (n = 34), which is in accordance with previously published observations (Calabresi et al., 1991; Jiang and North, 1991). Data were acquired and analyzed with pClamp8 software (Axon Instruments). Numerical values are expressed as means ± SEM, with n indicating the number of medium spiny striatal neurons tested. For EPSCs, data are expressed as percentage of the baseline measured for each cell during the 5 min preceding application of the drug examined. All drugs were applied in the perfusion solution except when noted. EPSCs were evoked every 15 s by electrical stimulation of the slice using a patch electrode filled with aCSF positioned on the slice surface in the vicinity of the recorded neuron. EPSCs mediated by NMDA receptors were recorded in 0.1 mM MgCl₂ to reduce magnesium blockade of the receptor and in the presence of CNQX (10 µM) and bicuculline (10 µM) to inhibit AMPA-EPSCs and GABAA-IPSCs, respectively. EPSCs were mediated by NMDA receptors as demonstrated by their blockade by the NMDA receptor antagonist AP-5 (data not shown). AMPA-EPSCs were recorded in the presence of AP-5 (100 µM) to inhibit NMDA-EPSCs and bicuculline (10 µM). We assessed the stability of our recordings by monitoring the amplitude of NMDA-EPSCs over time with normal intracellular solution in the absence of any bath-applied compound in eight striatal neurons. We found that whole-cell recording did not affect NMDA-EPSC amplitude (see Fig. 2b). The effect of the CK1 inhibitor CK1-7 on NMDA-EPSCs was not associated with a significant change in input resistance of the recorded neurons (60.3 ± 7.2 and 66.1 ± 7.3 MΩ before and during CK1-7, respectively; n = 13). In the experiments using intracellular application of the protein phosphatase inhibitor okadaic acid, this compound was allowed to diffuse from the pipette into the recorded neuron for at least 20 min before bath application of CK1-7 or (S)-3,5-dihydroxyphenylglycine (DHPG). This also allowed NMDA-EPSC amplitude to stabilize because of the effect of okadaic acid (our unpublished observations) (Lu et al., 1999). Whole-cell inward currents were activated with a 2 min bath application of either NMDA or AMPA in the absence of either Ca²⁺ or Mg²⁺. EPSCs were recorded in the presence of tetrodotoxin (0.5 µM), bicuculline (10 µM), and either CNQX (10 µM) or AP-5 (50 µM) for NMDA and AMPA currents, respectively. This method of application of glutamate receptor agonists was chosen, over local application such as microiontophoresis, to stimulate all of the receptors located on the recorded neuron. Bath application of these agonists allowed us to correlate biochemical and electrophysiological data and to compare our results with those published by several other groups who have used this method to activate glutamate receptors. Drugs were obtained from the following sources: CNQX, bicuculline, AP-5, kainic acid, and NMDA were from Sigma (St. Louis, MO); CK1-7 was from UCBiological (Swampscott, MA); IC261 [3-(2,4,6-trimethoxyphenyl)methylidene]-indolin-2-one was from Calbiochem (La Jolla, CA); okadaic acid was from Alexis Biochemicals (San Diego, CA); and (S)-3,3-DHPG, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), and LY367385 [(S)-(+)3,5-carboxy-2-methylbenzeneacetic acid] were from Tocris Cookson (Ballwin, MO). We followed the recommendations of Davies et al. (2000) and Bain et al. (2003) for the use of protein kinase inhibitors in cell-based assays when we designed our experiments to ensure that the concentrations of the inhibitors used (CK1-7 and IC261) are selective for CK1. (1) The concentrations of CK1-7 and IC261 used in our study were less than 10 times higher than the IC50 values found by our group and by others for inhibition of CK1 in vitro (≈10–15 and 1–16 μM, respectively) (Chijiwa et al., 1989; Behrend et al., 2000; Mashhoon et al., 2000; Liu et al., 2001). (2) In a previous study from our laboratory (Liu et al., 2001), we found that 100 μM CK1-7 (the same concentration used in the present study in the same preparation, i.e., brain slices from the striatum) decreases the basal phosphorylation level of DARPP-32 at a site phosphorylated by CK1. (3) CK1-7 and IC261 are two structurally unrelated inhibitors but they produce identical effect on both NMDA receptor activity and phosphorylation.

Anatomical studies

Immunoblot analysis. Frozen mouse cortices or striata were sonicated in 1% SDS, and proteins were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) before incubation with the following antibodies: (1) CK1-7 and IC261 are two structurally unrelated inhibitors but they produce identical effect on both NMDA receptor activity and phosphorylation.

Biochemical studies on NMDA receptor phosphorylation

Striatal brain slices (300 µm) were prepared from adult male C57BL/6 mice as described previously (Svenningsson et al., 1998). For studies of NR1 subunit phosphorylation, slices were incubated in Krebs’ buffer at 30°C under constant oxygenation (95% O₂/5% CO₂) for 60 min, with a change of buffer after 30 min. The slices were thereafter treated with either CK1-7 (100 or 300 µM) or IC261 (50 µM) for 10 min. At the end of the treatment, the slices were rapidly frozen and processed for immunoblot analysis as described above. Immunoblot analysis was performed with a phospho-specific antibody against Ser⁶⁸⁷-NRI (Upstate Biotechnology, Lake Placid, NY). Autoradiograms were quantified using NIH Image 1.62. For studies of NR2 subunit phosphorylation, 32P-labeled slices were used as described previously (Snyder et al., 1998). The slices were incubated for 60 min in phosphate-free Krebs’ buffer containing 2.0 mM of [³²P]orthophosphoric acid. The labeling buffer was then removed, and the tissue sections were incubated in Krebs’ buffer in the absence or presence of either CK1-7 (100 or 300 µM) or IC261 (50 µM) for 10 min. The slices were then immediately sonicated and incubated overnight at 4°C with 5–10 µg of polyclonal antibodies to immunoprecipitate 3²P-NR2A, 3²P-NR2B, and 3²P-NR2C (Calbiochem). Immune complexes were then incubated with protein A-Sepharose, and the beads were subsequently collected, washed, eluted in SDS sample buffer, and resolved by 8% SDS-PAGE. The gels were dried, and [³²P]phosphate incorporation was quantified using a PhosphorImager 400B and ImageQuant software from Molecular Dynamics (Sunnyvale, CA).

Results

CK1 isoforms are expressed in mouse striatum and neocortex

In mammals, there are several different CK1 isoforms known as α, β, γ₁, γ₂, δ, and ε (Gross and Anderson, 1998). The β isoform is only present in bovine brain. Because there is little information on the distribution of the different isoforms of CK1 in the CNS, we examined the expression of CK1α, CK1γ, CK1δ, and CK1ε in the mouse brain with particular emphasis on the striatum and the cerebral cortex, the main source of glutamatergic input to the striatum. Immunoblotting performed with antibodies against each of the CK1 isoforms demonstrated the presence of CK1α, CK1γ, CK1δ, and CK1ε in both the striatum and the neocortex (Fig. 1a). The sizes of the bands identified in our material were similar to those calculated previously for CK1α, CK1γ, and CK1δ, and CK1ε (37.5, 45.0–51.3, 49.1, and 47.3 kDa, respectively) (Graves et al., 1993; Fish et al., 1995; Zhai et al., 1995;
Receptors were pharmacologically isolated. CK1-7 (100 μM) applied in the perfusion solution produced a robust increase in NMDA-EPSC amplitude (percentage of baseline, 184.1 ± 10.6%; n = 21) (Fig. 2a). CK1-7 produced a small increase in the half-decay time of NMDA-EPSCs [20.4 ± 1.4 and 23.9 ± 1.5 ms, before and after CK1-7 (100 μM), respectively; n = 21]. IC261 (50 μM) also increased NMDA-EPSC amplitude (percentage of baseline, 140.2 ± 5.8%; n = 8). Whole-cell currents activated by NMDA, in the presence of tetrodotoxin, were also increased by CK1-7 (Fig. 2c). We additionally examined the effect of CK1-7 in neurons voltage clamped at +20 mV, and we found that this inhibitor also increased NMDA-EPSC amplitude (percentage of baseline, 170.1 ± 22.8%; n = 5). In contrast, AMPA-EPSCs (Fig. 3a) or whole-cell AMPA currents, activated by kainate, an AMPA/kainate receptor agonist (Fig. 3b), were not affected by bath-applied CK1-7 (100 μM). Moreover, direct application of CK1-7 into the recorded neuron by diffusion from the pipette did not affect whole-cell AMPA currents (432.8 ± 94.4 pA in seven control cells and 425.5 ± 102.2 pA in four cells loaded with CK1-7). These data indicate that CK1 specifically downregulates NMDA receptor function in the striatum. A postsynaptic rather than a presynaptic involvement of CK1 inhibition in the increased NMDA receptor function is indicated by both the absence of an effect on AMPA-EPSCs (Fig. 3a) and the observations that the EPSC2/EPSC1 ratio, a measure of presynaptic action,

Figure 1. Expression of CK1 isoforms in the brain. a, Immunoblots performed with antibodies against the CK1 isoforms α, γ, δ, and ε in the striatum (str; left lanes) and the neocortex (cor; right lanes). Molecular weights of 45 and 35 kDa are shown on the left of the blots to indicate relative weight of CK1 isoforms. The arrows indicate the bands that correspond to the expected molecular weight for each of the isoforms. Two clear bands of close molecular weights observed for CK1α and CK1β could correspond to splice variants of these isoforms. The two bands observed for CK1γ could correspond to different isoforms. It is very likely that the CK1 ε band corresponds solely to the CK1 ε splice variant. b, In situ hybridization of mRNAs coding for CK1α, CK1γ, CK1δ, and CK1ε in parasagittal sections of mouse brain. CK1 isoforms are expressed in several brain structures, including striatum (str).

Inhibition of CK1 increases NMDA receptor but not AMPA receptor activity

To assess the possible involvement of CK1 in corticostriatal glutamatergic synaptic transmission, we evaluated the effects of two specific CK1 inhibitors, CK1-7 (Chijiwa et al., 1989) and IC261 (Behrend et al., 2000; Mashhoon et al., 2000), on EPSCs evoked by electrical stimulation of glutamatergic fibers within the striatum. EPSCs mediated by either NMDA receptors or AMPA receptors were pharmacologically isolated. CK1-7 (100 μM) applied in the perfusion solution produced a robust increase in NMDA-EPSC amplitude (percentage of baseline, 184.1 ± 10.6%; n = 21) (Fig. 2a). CK1-7 produced a small increase in the half-decay time of NMDA-EPSCs [20.4 ± 1.4 and 23.9 ± 1.5 ms, before and after CK1-7 (100 μM), respectively; n = 21]. IC261 (50 μM) also increased NMDA-EPSC amplitude (percentage of baseline, 140.2 ± 5.8%; n = 8). Whole-cell currents activated by NMDA, in the presence of tetrodotoxin, were also increased by CK1-7 (Fig. 2c). We additionally examined the effect of CK1-7 in neurons voltage clamped at +20 mV, and we found that this inhibitor also increased NMDA-EPSC amplitude (percentage of baseline, 170.1 ± 22.8%; n = 5). In contrast, AMPA-EPSCs (Fig. 3a) or whole-cell AMPA currents, activated by kainate, an AMPA/kainate receptor agonist (Fig. 3b), were not affected by bath-applied CK1-7 (100 μM).Moreover, direct application of CK1-7 into the recorded neuron by diffusion from the pipette did not affect whole-cell AMPA currents (432.8 ± 94.4 pA in seven control cells and 425.5 ± 102.2 pA in four cells loaded with CK1-7). These data indicate that CK1 specifically downregulates NMDA receptor function in the striatum. A postsynaptic rather than a presynaptic involvement of CK1 inhibition in the increased NMDA receptor function is indicated by both the absence of an effect on AMPA-EPSCs (Fig. 3a) and the observations that the EPSC2/EPSC1 ratio, a measure of presynaptic action,
Inhibition of CK1 increases the phosphorylation level of NR1 and NR2B

Phosphorylation of the NMDA receptor has been shown to regulate its activity (Scannevin and Huganir, 2000). We therefore examined the phosphorylation state of the NMDA receptor subunits NR1, NR2A, NR2B, and NR2C after incubation of striatal brain slices with CK1-7 (100 or 300 μM) or IC261 (50 μM). These inhibitors increased the phosphorylation levels of the two most abundant NMDA receptor subunits in the striatum, NR1 and NR2B (Fig. 4). These subunits are localized to medium spiny projection neurons in which they presumably form native heteromeric functional receptors (Landwehrmeyer et al., 1995; Ghasemzadeh et al., 1996; Go¨tz et al., 1997). Phosphorylation levels of NR2A and NR2C, which are expressed at low levels in medium spiny striatal neurons (Landwehrmeyer et al., 1995; Ghasemzadeh et al., 1996; Go¨tz et al., 1997), were not affected by inhibition of CK1 [percentage of control: NR2A, 100 ± 5% (control), 96 ± 31.5% (CK1-7), 117.5 ± 18% (IC261); NR2C, 100 ± 31.5% (control), 80 ± 13.3% (CK1-7)] (Fig. 4). Thus, our results show a good correlation between the phosphorylation level of functional NMDA receptors and their activity after CK1 inhibition.

Inhibition of PP1/PP2A occludes the effect of CK1 inhibition on NMDA receptor activity

Because inhibition of CK1 increases the phosphorylation of NR1 and NR2B, it seemed likely that this kinase regulates NMDA receptor function via activation of a phosphatase. In light of previous biochemical evidence for interactions between CK1 and the protein phosphatases PP1/PP2A and between PP1/PP2A and NMDA receptors (Agostinis et al., 1987; Marin et al., 1994; Westphal et al., 1999; Chan and Sucher, 2001; Dubois et al., 2002), we hypothesized that the protein phosphatases PP1 and/or PP2A could be intermediary elements between CK1 and NMDA receptors. To examine whether inhibition of protein phosphatases might mediate the effect of CK1-7 on NMDA receptor activity, we applied the PP1/PP2A inhibitor okadaic acid in either the perfusion solution or the intracellular solution and examined the effect of CK1-7 on NMDA-EPSCs. In these neurons, CK1-7 did not increase NMDA-EPSC amplitude [percentage of baseline, 110.1 ± 12.2%, n = 7 in the presence of bath-applied okadaic acid (100 nM)] (Fig. 5a) and 87.5 ± 9.2%, n = 9 neurons loaded with okadaic acid (1 μM) (Fig. 5b) compared with 184.1 ± 10.6, n = 21 in the absence of okadaic acid (Fig. 2a)]. The fact that okadaic acid occluded the effect of CK1-7 on NMDA-EPSCs in-
indicating that increased activity of NMDA receptors after inhibition of CK1 is mediated through inhibition of PP1/PP2A. This is the first demonstration of a physiological interaction between CK1, PP1/PP2A, and an ionotropic receptor. We also found that bath-applied okadaic acid (100 nM, n = 7). Records show superimposed NMDA-EPSCs in a representative neuron before and after CK1-7, in the presence of okadaic acid (1 μM; n = 9). Inset shows superimposed NMDA-EPSCs recorded before and after CK1-7, in a representative neuron. Calibration: 20 pA, 20 ms.

**Figure 5.** Effect of CK1-7 on NMDA-EPSCs is occluded by the PP1/PP2A inhibitor okadaic acid. a. Time course of the effect of CK1-7 (100 μM) on NMDA-EPSC amplitude in neurons pretreated with bath-applied okadaic acid (100 nM, n = 7). Records show superimposed NMDA-EPSCs in a representative neuron before and after CK1-7 in the presence of okadaic acid. Calibration: 20 pA, 20 ms. b. Time course of the effect of CK1-7 (100 μM) in neurons loaded with okadaic acid (1 μM; n = 9). Inset shows superimposed NMDA-EPSCs recorded before and after CK1-7 in a representative neuron. Calibration: 20 pA, 20 ms.

The group I metabotropic glutamate receptor agonist DHPG depresses NMDA-EPSC amplitude via activation of CK1 and PP1/PP2A. Glutamate regulates the physiology of neuronal cells by acting on both metabotropic and ionotropic receptors. The crosstalk between these classes of glutamate receptors is very complicated and incompletely understood. The present observation that CK1 regulates NMDA-mediated synaptic transmission, together with our previous finding that group I mGluRs increase the activity of CK1 (Liu et al., 2001, 2002), raised the possibility that CK1 could act as an intermediary between metabotropic and ionotropic receptors. We therefore tested whether activation of group I mGluRs would produce an effect opposite to that seen during inhibition of CK1, namely a depression of NMDA-EPSCs. Indeed, a group I mGluR agonist, DHPG (50 μM), added to the perfusion solution, rapidly and reversibly depressed NMDA-EPSCs (Figs. 6a; 7b). The observations that DHPG did not affect NMDA-EPSC2/NMDA-EPSC1 [0.83 ± 0.09% before DHPG and 0.86 ± 0.06% after DHPG; n = 6] (Fig. 6a, see EPSCs) or AMPA-EPSCs [100.6 ± 10.1%; n = 3; as observed by others (Calabresi et al., 1999; Battaglia et al., 2001)] suggest a postsynaptic mechanism rather than a downregulation of glutamate release by group I mGluRs.

We next tested the possible involvement of CK1 in the action of DHPG on NMDA-EPSCs. Incubation of slices with CK1-7 abolished the ability of DHPG to depress NMDA-EPSCs (Figs. 6b, 7b). Thus, group I mGluRs selectively downregulate synthetically activated NMDA receptors in the striatum through the activation of CK1. Because we found that CK1 modulates NMDA receptor function through activation of PP1/PP2A, we tested whether mGluR-mediated inhibition of NMDA-EPSCs also involved activation of PP1/PP2A. We found that, in neurons loaded with okadaic acid (1 μM), DHPG did not depress NMDA-EPSC amplitude (percentage of baseline, 115.6 ± 18.2%; n = 7) (Figs. 6d, 7b).

**Figure 7**. The group I metabotropic glutamate receptor agonist DHPG depresses NMDA-EPSC amplitude via activation of CK1 and PP1/PP2A. Glutamate regulates the physiology of neuronal cells by acting on both metabotropic and ionotropic receptors. The crosstalk between these classes of glutamate receptors is very complicated and incompletely understood. The present observation that CK1 regulates NMDA-mediated synaptic transmission, together with our previous finding that group I mGluRs increase the activity of CK1 (Liu et al., 2001, 2002), raised the possibility that CK1 could act as an intermediary between metabotropic and ionotropic receptors. We therefore tested whether activation of group I mGluRs would produce an effect opposite to that seen during inhibition of CK1, namely a depression of NMDA-EPSCs. Indeed, a group I mGluR agonist, DHPG (50 μM), added to the perfusion solution, rapidly and reversibly depressed NMDA-EPSCs (Figs. 6a; 7b). The observations that DHPG did not affect NMDA-EPSC2/NMDA-EPSC1 [0.83 ± 0.09% before DHPG and 0.86 ± 0.06% after DHPG; n = 6] (Fig. 6a, see EPSCs) or AMPA-EPSCs [100.6 ± 10.1%; n = 3; as observed by others (Calabresi et al., 1999; Battaglia et al., 2001)] suggest a postsynaptic mechanism rather than a downregulation of glutamate release by group I mGluRs.

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dependent activation of CK1 and PP1 or PP2A.

EPSCs in a representative neuron before and during DHPG. Calibration: 50 pA, 20 ms.

CK1-7 plus DHPG. Calibration: 20 pA, 20 ms.

EPSCs and blocked inhibition by DHPG. Inset, NMDA-EPSCs before, during CK1-7, and during

Effect of CK1 inhibition on synaptic transmission and NMDA receptor-mediated synaptic transmission in the brain. We found that inhibition of CK1 increases NMDA receptor activity and receptor-mediated synaptic transmission in several brain regions. Future studies using, for example, targeted gene deletions will help identifying the isoform(s) involved in the regulation of NMDA receptor function.

Expression of CK1 isoforms in the brain

Seven CK1 isoforms (α, β, γ1–γ3, δ, and ε), each having several splice variants, have been identified (Gross and Anderson, 1998). The expression of their mRNAs and proteins has been determined using biochemical and histochemical approaches, as well as reverse transcription-PCR analyses. CK1α has four alternately spliced variants (CK1α, CK1αS, CK1αL, and CK1αLS) (Zhang et al., 1996; Green and Bennett, 1998) and is expressed in the human hippocampus (Ghoshal et al., 1999). CK1γ3, which has two splice variants, is abundant in rat brain extracts, but not CK1γ1 or CK1γ2 (Zhai et al., 1995). CK1δ is widely expressed in the human brain, with the highest levels in hippocampus and cerebral cortex (Schwab et al., 2000; Yasojima et al., 2000). There are two variants of CK1δ: the variant 2 has a different exon at the 3’ end, which results in a shorter protein with a different C terminus compared with variant 1. A widespread distribution of CK1ε was found in the rat brain, with the most prominent expression in hippocampus and cerebral cortex (Takano et al., 2004; Takano et al., 2000). CK1ε is expressed in the suprachiasmatic nucleus. The expression of CK1ε2 is restricted to cerebellum.

None of these studies specifically examined the expression of the different CK1 isoforms in the striatum. Our results revealed that all CK1 isoforms are expressed in the mouse striatum and cortex, as well as in several other brain regions such as cerebellum, hippocampus, thalamus, olfactory bulb, and midbrain (Fig. 1). Each of these isoforms could therefore regulate synaptic transmission in several brain regions. Future studies using, for example, targeted gene deletions will help identifying the isoform(s) involved in the regulation of NMDA receptor function.

Figure 7. The group I metabotropic glutamate receptor agonist DHPG depresses NMDA-EPSC amplitude via activation of mGluR1 but not mGluR5. A, Effect of DHPG (50 μM) on NMDA-EPSCs in the presence of the mGluR5 antagonist MPEP (10 μM, n = 6; filled squares) and in the presence of the mGluR1 antagonist LY367385 (100–300 μM; n = 6; combined data, open squares). Records show NMDA-EPSCs in two representative neurons, before and during DHPG in the presence of MPEP (top records) or LY367385 (bottom records). Calibration: 20 pA, 20 ms. B, Summary of the effect of okadaic acid (OA) (1 μM, intracellular), BAPTA (20 mM, intracellular), CK1-7 (100 μM), MPEP (10 μM), and LY367385 (100–300 μM) on DHPG-induced inhibition of NMDA-EPSCs. Data are expressed as percentage of baseline (absence of DHPG). *p < 0.05, **p < 0.001 compared with DHPG alone (unpaired two-tailed t test).

Discussion

Our data indicate that a cascade involving glutamate/mGluR1/CCK and PP2A plays a major role in the regulation of NMDA receptor-mediated synaptic transmission in the brain. We found that inhibition of CK1 increases NMDA receptor activity and phosphorylation levels through inhibition of PP1 or PP2α and that mGluR1 downregulates NMDA-EPSCs through a Ca2+-dependent activation of CK1 and PP1 or PP2α.

Figure 6. The group I metabotropic glutamate receptor agonist DHPG depresses NMDA-EPSC amplitude via activation of CK1 and PP1/PP2α. A, Time course of the effect of DHPG (50 μM) on NMDA-EPSC amplitude (n = 8). Inset shows NMDA-EPSCs recorded in a representative neuron before and during DHPG and no effect of DHPG on EPSC2/EPSC1. Calibration: 20 pA, 20 ms. B, Time course of the effect of DHPG (50 μM) on NMDA-EPSC amplitude in the presence of the mGluR1 antagonist LY367385 (10 μM; n = 6; filled squares) and in the presence of the mGluR1 antagonist LY367385 (100–300 μM; n = 6; combined data, open squares). Records show NMDA-EPSCs in two representative neurons, before and during DHPG in the presence of MPEP (top records) or LY367385 (bottom records). Calibration: 20 pA, 20 ms. B, Summary of the effect of okadaic acid (OA) (1 μM, intracellular), BAPTA (20 mM, intracellular), CK1-7 (100 μM), MPEP (10 μM), and LY367385 (100–300 μM) on DHPG-induced inhibition of NMDA-EPSCs. Data are expressed as percentage of baseline (absence of DHPG). *p < 0.05, **p < 0.001 compared with DHPG alone (unpaired two-tailed t test).

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Effect of CK1 inhibition on synaptic transmission and NMDA receptor activity

Although the CK1 family forms one of the eight major groups of protein kinases in the human and mouse genome (Manning et
Regulation of Synaptic Transmission by CK1

Chergui et al. • Regulation of Synaptic Transmission by CK1

Mg²⁺
tentials and in low and normal concentration of extracellular
are observed at hyperpolarized and depolarized membrane po-
by which CK1 and PP1/PP2A regulate functional properties of the
PP2A regulate NMDA receptor activity in the striatum.
formation on the precise mechanisms by which CK1 and PP1/
brief applications of glutamate agonists to outside-out mem-
Additional studies examining single-channel currents evoked by
tribute to the regulation of NMDA receptor activity by CK1.
CK1 inhibition. Indeed, single-channel and whole-cell studies
these results suggest that activation of the mGluR1/CK1/
Together, these results suggest that activation of the mGluR1/CK1/
PP2α cascade by glutamate depresses the activity of NMDA
receptors located at the synapse and does not modulate extrasyn-
NMDA receptors may regulate, through a CK1 pathway, whole-cell-activated
responses. For example, serotonin acting on 5-HT₂A/C recep-
tors also activates CK1 and modulates NMDA receptor function in
the striatum (our unpublished observations).

In the hippocampus, DHPG induces a long-term depression of glutamatergic synaptic transmission (Bashir, 2003). This finding
in our experiments do not address the precise mechanism
in which CK1 and PP1/PP2α regulate functional properties of the
NMAD receptor, changes in the opening probability or desensi-
tization of this receptor may contribute to the observed increased
amplitude and slight increased decay time of NMDA-EPSCs after
CK1 inhibition. Indeed, single-channel and whole-cell studies
reported that PP1 or PP2α decrease the open probability of the
NMDA receptor channel and that inhibition of PP1/PP2α increases open probability of these channels and NMDA-evoked
currents in cultured hippocampal neurons (Wang et al., 1994; Lu
et al., 1999; Herzig and Neumann, 2000).

We observed that CK1 inhibition slightly increased the half-
decay time of NMDA-EPSCs and increased the phosphorylation
of NR2B but not NR2A. These results agree with the slow deac-
tivation kinetics of NR2B-containing NMDA receptors (Cull-
Candy et al., 2001) and with the study by Lu et al. (1999) showing
that, in hippocampal pyramidal neurons, activation of PKC en-
hances desensitization of NMDA receptors as well as NMDA-
activated whole-cell currents, open probability of the channel,
and amplitude of NMDA-mediated miniature EPSCs. Several
other mechanisms shown previously to influence the shape of
NMDA-EPSCs could also contribute to the regulation of NMDA
receptor function by CK1. These mechanisms may include
changes in the affinity of NMDA receptors for glutamate, in their
sensitivity to magnesium, or in their channel conductance,
changes in the subunit composition of NMDA receptors, in
NMDA receptor desensitization mediated by dephosphorylation
of NR2A by the calcium-dependent protein phosphatase PP2B
(Herstin et al., 1998; Lester and Jahr, 1992; Lieberman and Mody,
1998; Cong et al., 1998; Dingledine et al., 1999; Herzog and
Neumann, 2000; Cull-Candy et al., 2001; Krupp et al., 2002).
However, our observations that the phosphorylation level of
NR2A is not affected by inhibition of CK1, that the concentra-
tions of okadaic acid used in our study (0.1 and 1 μM) do prob-
ably not inhibit PP2α, and that similar effects of CK1 inhibitors
are observed at hyperpolarized and depolarized membrane po-
tentials and in low and normal concentration of extracellular
Mg²⁺ suggest that such mechanisms may not significantly con-
tribute to the regulation of NMDA receptor activity by CK1.
Additional studies examining single-channel currents evoked by
brief applications of glutamate agonists to outside-out mem-
brane patches from striatal neurons should provide valuable
information on the precise mechanisms by which CK1 and PP1/
PP2α regulate NMDA receptor activity in the striatum.

Effect of DHPG on synaptic transmission and NMDA
receptor activity

Previous biochemical studies showed that the group I metabo-
tropic glutamate receptor agonist DHPG stimulates CK1 kinase
activity in a calcium-dependent manner (Liu et al., 2001, 2002).
We found that DHPG had an effect opposite to that produced by
CK1 inhibitors, i.e., a decrease in NMDA-EPSC amplitude. This
depressant effect of DHPG was most probably not attributable
to direct action of this compound on NMDA receptors, as shown
previously (Contractor et al., 1998), because it was dependent
on calcium and on activation of CK1 and PP1/PP2α. In contrast
to its inhibitory effect on synthetically activated NMDA receptors,
DHPG increased whole-cell-activated NMDA currents (data not shown) (Pisani et al., 1997), indicating that synaptic and extra-
synaptic NMDA receptors are regulated by different signaling
pathways by group I mGluRs. Indeed, the decrease in synthetically
mediated NMDA-EPSCs involves CK1 and mGluR1 (present
findings), whereas the increase in whole-cell NMDA currents by
DHPG involves PKC and mGluR5 (Pisani et al., 1997, 2001). These
previous studies by Pisani et al. (1997, 2001) found that
DHPG does not modulate whole-cell-activated NMDA currents
in mGluR5 knock-out mice or after pharmacological blockade of
mGluR5. Given the present data, one could expect DHPG to
reduce, via activation of mGluR1 and CK1, NMDA-activated
whole-cell currents in the absence of functional mGluR5.
Together, these results suggest that activation of the mGluR1/CK1/
PP1-PP2α cascade by glutamate depresses the activity of NMDA
receptors located at the synapse and does not modulate extrasyn-
naptic NMDA receptors. These results also suggest that the con-
tribution of synaptic NMDA receptors to the whole-cell-activated
current may be significantly less than that of extrasynaptic recep-
tors. We found that the activity of extrasynaptic NMDA receptors
(whole-cell-activated NMDA currents) is increased after inhibi-
tion of CK1. These results, combined with the findings of Pisani
et al. (1997, 2001), suggest that activation of receptors other than
mGluRs may regulate, through a CK1 pathway, whole-cell-activated
NMDA responses. For example, serotonin acting on 5-HT₁A/C
receptors also activates CK1 and modulates NMDA receptor function in
the striatum (our unpublished observations).

In conclusion, the present results implicate CK1 as a negative
regulator of glutamate-induced neuronal excitation. Given the
central role of NMDA receptors in several physiological and
pathological processes in the striatum and throughout the CNS,
CK1 may be an important regulatory enzyme in normal states
such as movement- and motivation-related behaviors. Another
important function for CK1 might be to prevent intense activa-
tion of NMDA receptors attributable to excessive glutamergic
activity, which is known to cause neuronal death (Hardingham and Bading, 2003).

References


