Involvement of cAMP-Dependent Protein Kinase in \(\mu\)-Opioid Modulation of NMDA-Mediated Synaptic Currents

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**Xie, Cui-Wei and Darrell V. Lewis.** Involvement of cAMP-dependent protein kinase in \(\mu\)-opioid modulation of NMDA-mediated synaptic currents. *J. Neurophysiol.* 78: 759–766, 1997. We have previously reported dual effects of \(\mu\)-opioids on N-methyl-d-aspartate (NMDA)-receptor-mediated synaptic events in the hippocampal dentate gyrus: an indirect facilitating effect via suppression of GABAergic interneurons (disinhibition) and a direct inhibitory effect in the presence of \(\gamma\)-aminobutyric acid-A (GABA\(_A\)) antagonists. The cellular mechanism underlying the inhibitory effect of \(\mu\)-opioids remains to be determined. In the present study we examine the role of adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) in \(\mu\)-opioid-induced inhibition of NMDA currents in rat hippocampal slices. NMDA-receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) were evoked by stimulating the lateral perforant path and were recorded from dentate granule cells with the use of whole cell voltage-clamp techniques in the presence of the GABA\(_A\) antagonist and a non-NMDA type of glutamate receptor antagonist. Two selective \(\mu\)-agonists, [N-MePhe\(^3\), D-Pro\(^4\)]-morphiceptin and [D-Ala\(^2\), N-MePhe\(^3\), Gly-ol\(^1\)]-enkephalin, induced dose-dependent inhibition of NMDA EPSCs in a concentration range of 0.3–10 \(\mu\)M. This inhibitory effect could be completely reversed by the opioid antagonists naloxone or prevented by a selective \(\mu\)-agonist cyprodime, but was not affected by removal of Mg\(^{2+}\) from the external perfusion medium. Intracellular application of pertussis toxin (PTX) into the granule cell via whole cell recording pipettes completely prevented \(\mu\)-opioid-induced reduction in NMDA currents, suggesting that a postsynaptic mechanism involving PTX-sensitive G proteins might be responsible for the inhibitory action of \(\mu\)-opioids. Further studies were conducted to identify the intracellular messengers that coupled with G proteins and transduced the effect of \(\mu\)-opioids in granule cells. The adenylate cyclase activator forskolin was found to enhance NMDA-receptor-mediated synaptic responses and to reverse the inhibitory effect of \(\mu\)-opioids. Specific PKA activators, also enhanced NMDA EPSCs, whereas the PKA inhibitor Rp-cAMPS reduced NMDA EPSCs and occluded further inhibition of the current by \(\mu\)-opioids. These findings strongly suggest that NMDA receptor function is subject to the modulation by PKA, and that \(\mu\)-opioids can inhibit NMDA currents through suppression of the cAMP cascade in the postsynaptic neuron. Combined with our previous findings, the present results also indicate that \(\mu\)-opioids can modulate NMDA-receptor-mediated synaptic activity in a complex manner. The net effect of \(\mu\)-opioids in the dentate gyrus may depend on the interplay between its inhibitory action, which facilitates NMDA-receptor-mediated responses, and its inhibitory action on the cAMP cascade.

**INTRODUCTION**

Glutamate-gated ion channels mediate excitatory synaptic transmission in the mammalian brain. Evidence has emerged in recent studies that the function of these ion channels can be regulated by intracellular adenosine 3',5'-cyclic monophosphate (cAMP)-dependent protein kinase (PKA). Direct phosphorylation of receptor proteins by PKA has been demonstrated in kainate/\(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-preferring glutamate receptors (Blackstone et al. 1994; Raymond et al. 1993; Wang et al. 1993). Such phosphorylation leads to potentiation of the ionic current through kainate/AMPA receptor channels, as shown in cultured mammalian hippocampal neurons (Green-gard et al. 1991; Rosenmund et al. 1994; Wang et al. 1991) as well as *Xenopus* oocytes (Keller et al. 1992) and human embryonal kidney 293 cells (Raymond et al. 1993; Wang et al. 1993) that express recombinant kainate/AMPA receptors. It remains unclear whether PKA can directly phosphorylate \(N\)-methyl-d-aspartate (NMDA)-type glutamate receptors. Nevertheless, reagents that increase adenylate cyclase and PKA activity can enhance NMDA-receptor-mediated excitatory postsynaptic currents/potentials (EPSC/Ps) in spinal dorsal horn (Yen et al. 1993), amygdala (Huang et al. 1993), and neostriatum (Colwell et al. 1995). Thus NMDA receptor channels seem also subject to modulation through the cAMP cascade. Because NMDA receptors are critically involved in synaptic plasticity, neuronal development, and excitotoxicity, it is of interest to investigate whether neurotransmitters released under those physiological or pathological conditions can regulate NMDA receptor function by altering cAMP-dependent phosphorylation.

Opioids have been known to inhibit adenylate cyclase and reduce cellular levels of cAMP in various neuronal tissues and cell lines (Duman et al. 1988; Johnson and Fleming 1989). This effect is mediated by pertussis-toxin (PTX)-sensitive G proteins (G\(_i\) and G\(_o\)) and is expected to alter the state of cAMP-dependent phosphorylation in the target cells (Nestler 1992). However, the functional consequences of these biochemical changes have not been fully understood. We have previously observed inhibition of NMDA-receptor-mediated EPSCs (NMDA EPSCs) by a \(\mu\)-opioid agonist in hippocampal dentate granule cells (Xie et al. 1992), but it is unclear whether this \(\mu\)-opioid effect is mediated through inhibition of cAMP-dependent phosphorylation. In the present study we examine this possibility in rat hippocampal slices. Our results clearly demonstrate that synaptically activated NMDA currents can be substantially affected by the state of cAMP-dependent phosphorylation in the postsynaptic neurons. Inhibition of PKA by \(\mu\)-opioids may be an
important mechanism for the modulation of NMDA receptor function in the hippocampal dentate gyrus. Some results here have been reported previously in abstract form (Xie and Lewis 1995a).

METHODS

Preparation of hippocampal slices

Young male Sprague-Dawley rats (22–40 days old) were decapitated under halothane anesthesia. Transverse hippocampal slices 500 μm thick were prepared with a Campden Vibroslicer. Slices were maintained in a holding chamber for 1–2 h in artificial cerebrospinal fluid (CSF) continuously bubbled with 95% O₂–5% CO₂. The artificial CSF contained (in mM) 120 NaCl, 25 NaHCO₃, 3.3 KCl, 1.23 NaH₂PO₄, 1.8 CaCl₂, 1.2 MgSO₄, and 10 d-glucose, pH 7.4. Thirty minutes before the experiment was begun, one slice was transferred to a 2-ml submerged recording chamber, where it was continuously perfused with oxygenated, warm (32 ± 1°C, mean ± SE) artificial CSF at a rate of 2–3 ml/min. Unless otherwise indicated, Mg²⁺ was omitted from the perfusion medium during the recording to enhance NMDA-mediated responses.

Lateral perforant path stimulation and extracellular recording

A sharpened monopolar tungsten electrode was placed in the outer third of the dentate molecular layer, the terminal region of the lateral perforant path. Constant-current stimulus pulses (0.1 ms, 30–600 μA, 0.017 Hz) were delivered through the electrode by an isolated Grass stimulator. In some experiments, stimulation-evoked population excitatory postsynaptic potentials (pEPSPs) were recorded extracellularly from granule cell dendrites in the outer molecular layer with the use of a glass micropipette filled with 2 M NaCl (1–8 MΩ).

Whole cell recordings

Stimulation-evoked synaptic currents were recorded from dentate granule cells with the use of whole cell recording techniques as previously described (Xie et al. 1992). The patch pipette had a tip diameter of 1–2 μm and a resistance of 3–6 MΩ. Whole cell currents were recorded with the use of a patch-clamp amplifier (Axopatch-1D) under voltage-clamp conditions. The input resistance of recorded cells, determined by measuring the deflection of holding current during the voltage steps, was in a range of 150–600 MΩ. Measured series resistance was usually 10–20 MΩ and could be compensated up to 80%. Membrane potentials were measured by temporarily switching the amplifier to the current-clamp mode during the recording. Recorded signals were filtered at 2 kHz, displayed on a Nicolet digital oscilloscope, and stored on magnetic disks and video tapes. Data were analyzed off-line. Results were expressed as means ± SE and subject to a one-way analysis of variance and Student’s t-tests. Statistical significance was defined as P < 0.05.

Unless otherwise indicated, the internal solution of the recording pipette (solution A) contained (in mM) 110 KMeSO₄, 10 KCl, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 11 ethylene glycol-bis (β-aminoethyl ether) -N,N’,N’’,N’’’-tetraacetic acid, 1 CaCl₂, 2 MgATP, and 0.3 guanosine 5’-triphosphate (GTP), pH 7.2. A different pipette solution (solution B) was used in some experiments. It contained (in mM) 90 N,N-ethylmaleimide-glutamic acid-N,N-2-aminoethylglycine, 90 methanesulfonate, 40 CsF, 10 HEPES, 2 MgCl₂, 3 lidocaine, N-ethyl bromide quaternary salt (QX314), 2 MgATP, and 0.3 GTP, pH 7.2. Both pipette solutions gave similar results.

Drug application

All drugs except PTX were bath applied after dilution into the artificial CSF from concentrated stock solutions. Forskolin, 6,7-dinitroquinorxaline-2,3-dione (DNQX), and cyprodime were prepared in dimethylsulfoxide (DMSO) as concentrated stock solutions at 40, 100, and 5 mM, respectively, and were diluted to final concentrations before application. The final concentration of DMSO was in a range of 0.02–0.25%. In control experiments, these concentrations of DMSO did not alter the synaptically evoked NMDA responses. To pharmacologically isolate NMDA-receptor-mediated synaptic responses, all experiments were conducted in the presence of 20 μM DNQX and 50 μM bicuculline methiodide to block AMPA-receptor-mediated EPSC/Ps and γ-aminobutyric acid-A (GABAₐ-) receptor-mediated inhibitory postsynaptic currents (IPSCs), respectively. Other drugs were added into this perfusion medium and their effects were determined 10 min after the start of application.

PTX was applied to the granule cell intracellularly through whole cell recording pipettes. Toxin was dissolved in water (125 μg/ml) and activated in 5 mM dithiothreitol at 35°C for 15 min. The activated toxin was diluted to 10 μg/ml with pipette solution and reacted with 20 mM nicotinic acid adenine dinucleotide for 10 min. This solution was further diluted and included in the recording pipette at a final concentration of 1 μg/ml. For control experiments, the vehicle containing dithiothreitol and nicotinic acid adenine dinucleotide was included in the recording pipette.

The μ-agonists [N-MePhe³, D-Pro⁴]-morphiceptin (PL017) and [D-Ala², N-MePhe³, Gly-ol⁵]-enkephalin (DAMGO) were purchased from Peninsula Laboratories. PTX was obtained from List Biological Laboratories. Other drugs were from Research Biochemicals International.

RESULTS

NMDA-receptor-mediated synaptic currents in dentate granule cells

Stimulation-evoked synaptic currents were recorded from dentate granule cells with the use of whole cell voltage-clamp techniques. In the presence of DNQX and bicuculline methiodide, single stimuli to the lateral perforant path terminals in the dentate outer molecular layer evoked a slow inward current in the granule cell. As demonstrated in Fig. 1, this inward current clearly showed characteristics of NMDA EPSCs. In perfusion medium containing 1.2 mM Mg²⁺, the current amplitude was progressively reduced by changing the holding potential in a hyperpolarized direction, with a negative slope conductance between −50 to −100 mV. Removing Mg²⁺ from the perfusion medium profoundly increased the amplitude of this current and mostly eliminated its negative slope conductance. A complete blockade of this current by the NMDA antagonist tetrodotoxin (TTX) (−)-2-amino-5-phosphonovaleric acid (D-APV) further confirmed that it was an NMDA-mediated synaptic current. When the granule cell was held at −50 or −60 mV, the late IPSC was clearly observed following the NMDA EPSC (Fig. 2A). It was an outward current, reversed at around −90 mV, and was sensitive to the GABAₐ antagonist 2-OH-saclofen (400 μM).

Selective μ-agonists inhibit NMDA EPSCs in a Mg²⁺-independent manner

After stable NMDA EPSCs had been observed, the μ-agonist PL017 (3 μM) was bath applied to the slice. As
OPIOID AND PKA MODULATION OF NMDA CURRENTS

FIG. 3. Antagonism of μ-opioid effects by naloxone (NX) or pertussis toxin (PTX). PL017 (10 μM) caused NX-reversible reduction of NMDA EPSCs in control cell (top), but failed to do so in PTX-treated cell (bottom). NX: 10 μM, bath applied. PTX: included in whole cell recording pipette at a final concentration of 1 μg/ml. Note lack of GABA B-mediated late inhibitory postsynaptic current (IPSC) in PTX-treated cell, which was indicator of effective blockade of G proteins.

min after perfusion was started. Furthermore, the μ-opiagonist action seemed not to be dependent on the extracellular magnesium concentration (Fig. 2A). The reduction of NMDA EPSCs produced by 3 μM PL017 was 27 ± 6% (n = 4) in slices perfused with a medium without added Mg2+, not significantly different from that observed in the presence of 1.2 mM Mg2+ (23 ± 5%, n = 5) in another group of slices. The dose-response relationship of the PL017 effect was then established in the nominally Mg2+-free medium. A 12–32% reduction in the amplitude of NMDA EPSCs was observed following applications of 0.3–10 μM PL017 (Fig. 2B).

Another selective μ-opiagonist, DAMGO, caused a dosedependent inhibition of NMDA EPSCs as well. In Mg2+-free medium, 0.3–10 μM DAMGO decreased NMDA EPSCs by 6–34% (Fig. 2B). The effect of DAMGO was also examined at lower concentrations (5–100 nM), and no significant change in NMDA currents was observed (data not shown). Both PL017 and DAMGO caused no significant change in the membrane potential and input resistance of granule cells.

Effect of opioid antagonists

Bath application of 10 μM naloxone caused no significant change in NMDA EPSC amplitudes, but it could effectively prevent the inhibitory effect of subsequently applied PL017. The amplitude of NMDA EPSCs was reduced by only 6 ± 5% (n = 3, P > 0.05) after perfusion of 3 μM PL017 in those naloxone-treated cells. Alternatively, in some experiments naloxone was added into the perfusion medium after PL017 had already induced significant inhibition of NMDA currents. This reversed the inhibition within 5 min (Fig. 3).

A selective μ-agonist, cyprodime, could prevent the inhibitory action of DAMGO completely. When cells were perfused simultaneously with DAMGO and cyprodime for 10 min (both at 3 μM), the reduction in NMDA EPSC amplitudes was only 2 ± 7% (n = 4) as compared with the predrug level.

FIG. 2. Inhibition of NMDA EPSCs by μ-agonists. A: representative recordings from 2 granule cells held at −60 mV and perfused with Mg2+-containing or nominally Mg2+-free medium, respectively. Note similar degree of current inhibition by [N-MePhe1, D-Pro4]-morphiceptin (PL017, 3 μM) in both media. B: dose-response relationship of μ-agonist effects in nominally Mg2+-free medium. Agonist effect was determined 10 min after perfusion was started. Reduction in EPSC amplitudes was calculated as % change relative to baseline level before drug application, and data are expressed as means ± SE. PL017: n = 3–6; [D-Ala2, N-MePhe4, Gly-ol3]-enkephalin (DAMGO): n = 4–8.

FIG. 1. N-methyl-D-aspartate-receptor-mediated excitatory postsynaptic currents (NMDA EPSCs) of dentate granule cells. Current was synaptically evoked by stimulation of lateral perforant path in presence of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) and the γ-aminobutyric acid-A (GABA A) antagonist bicuculline methiodide. A: representative recordings from single granule cell held at −80 mV. Current was inhibited by Mg2+/slices perfused with a medium without added Mg2+, not and completely blocked by the NMDA receptor antagonist D-(−)-2-amino-5-phosphonovaleric acid (D-APV, 40 μM). B: current-voltage curves of significantly different from that observed in the presence of 1.2 mM Mg2+ (23 ± 5%, n = 5) in another group of slices. Note negative slope conductance in Mg2+/containing medium. The dose-response relationship of the PL017 effect was then established in the nominally Mg2+-free medium. A 12 ± 32% reported previously (Xie et al. 1992), the μ-agonist significantly reduced the peak amplitude of NMDA EPSCs. The effect appeared within 5 min and reached a plateau ~10

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Intracellular application of PTX prevents μ-opioid effects

PTX-sensitive G proteins transduce multiple opioid effects in the brain and peripheral neurons. We have examined...
whether these G proteins are also involved in μ-opioid-induced inhibition of NMDA currents. Activated PTX was included in the whole cell recording pipette at a concentration of 1 μg/ml as described in Experimental procedures. The toxin could rapidly diffuse into recorded granule cells after a whole cell configuration was achieved. In those PTX-pretreated cells PL017 induced no significant reduction of NMDA EPSCs (Fig. 3). The current remained at 101 ± 8% of the predrug level after perfusion with 10 μM PL017 for 10 min (n = 6). In contrast, the effect of 10 μM PL017 was unaffected in control cells treated with intracellular vehicle containing no PTX (30 ± 5% of reduction, n = 3).

One indicator we used in the above experiments to determine the efficacy of PTX blockade of μ-opioid agonist effects, we used a different internal solution (solution B) for recording pipettes in some experiments. The solution B contained cesium and QX314, both of which suppress potassium conductance, including GABA<sub>δ</sub>-mediated late IPSCs (Johnston et al. 1980; Nathan et al. 1990). Despite blockade of late IPSCs in these experiments, 3 μM DAMGO reduced NMDA EPSCs by 30 ± 7% (n = 7), which was not significantly different from results observed with recording pipettes containing no cesium and QX314 in another group of slices (26 ± 4%, n = 3). These results suggested that the inhibitory action of μ-opioid agonists and its prevention by PTX were not due to a change in the late IPSCs.

**Forskolin and the PKA activator Sp-cAMPS potentiate NMDA-mediated synaptic responses**

To identify the intracellular messengers that coupled with G proteins and transduced opioid effects in granule cells, the effect of forskolin, an adenylyl cyclase activator, was examined. Forskolin was bath applied to one group of slices at 10, 50, and 100 μM. Perfusion at each concentration lasted for 20 min and was followed by a 30-min washing period for recovery. As demonstrated in Fig. 4A, the cyclase activator induced a dose-dependent increase in the amplitude of NMDA-receptor-mediated pEPSPs. This effect reached peak ~10 min after the perfusion was begun, and could be completely washed out in 20 min. In another group of experiments, PL017 (3 μM) was applied first, inducing a 22 ± 3% reduction in the amplitude of NMDA pEPSP (P < 0.05). Subsequent addition of forskolin (100 μM, 20 min) completely reversed the PL017 effect (Fig. 4B). It should be noted that in these experiments, forskolin only brought NMDA pEPSP amplitudes back to the predrug level, but did not induce a significant increase in the response as shown in Fig. 4A. Obviously, the presence of PL017 had attenuated the facilitation of NMDA currents by activation of the cAMP cascade.

Forskolin reportedly has some nonspecific actions unrelated to adenylyl cyclase. To further confirm the enhancement of NMDA currents by activation of the cAMP cascade, we tested the effect of a specific PKA activator, Sp-cAMPS, on whole cell NMDA EPSCs. Two groups of cells were perfused with Sp-cAMPS at 50 or 100 μM for 10 min. The amplitude of NMDA EPSCs was increased by 22 ± 10% (n = 6) and 29 ± 13% (n = 4), respectively, both being significantly different from the predrug level (P < 0.05). This effect could be washed out in 20 min. Representative recordings are shown in Fig. 4C.

**PKA inhibitor Rp-cAMPS attenuates NMDA EPSCs**

Mimicking the effect of μ-opioid agonists, bath application of Rp-cAMPS (50 and 100 μM) induced a dose-dependent reduction in NMDA EPSP amplitudes in two groups of cells (Fig. 5A). In the third group, PL017 (10 μM) was added into the perfusion medium after Rp-cAMPS (100 μM) had already caused significant reduction of NMDA EPSCs. No further inhibition of the current was induced by the μ-opioid agonist.
in this group (Fig. 5, A and B), suggesting an occlusion of the \( \mu \)-opioid effect by the PKA inhibitor.

**Discussion**

The major findings of this study are as follows. 1) Activation of \( \mu \)-opioid receptors by two selective \( \mu \)-agonists, PL017 and DAMGO, reduced the amplitude of NMDA EPSCs in dentate granule cells. 2) The inhibitory effect of \( \mu \)-opioids on NMDA currents did not depend on the presence of extracellular Mg\(^{2+} \). 3) Intracellular application of PTX into the granule cell postsynaptically blocked \( \mu \)-opioid effects. 4) The adenylate cyclase activator forskolin enhanced NMDA EPSCs and reversed PL017-induced reduction of the current. 5) Sp-cAMPS, a specific PKA activator, increased NMDA EPSCs, whereas the PKA inhibitor Rp-cAMPS attenuated the current and occluded the inhibitory effect of \( \mu \)-opioids.

**Modulation of NMDA currents by \( \mu \)-opioid receptors**

Function of hippocampal NMDA receptors has been extensively studied because of their involvement in different forms of synaptic plasticity, including long-term potentiation (LTP). Opioid peptides and their receptors are abundant in the hippocampus and prove important in the induction of LTP in two major excitatory pathways, the lateral perforant path (Bramham et al. 1988, 1991; Xie and Lewis 1991) and the mossy fiber–CA3 pathway (Derrick et al. 1992; Martin 1983). The lateral perforant path projects from the lateral entorhinal cortex to the outer molecular of the dentate gyrus, providing both glutamatergic (Nadler et al. 1977; White et al. 1977) and enkephalineric input (Gall et al. 1981) to the dendrites of granule cells. Simultaneous activation of \( \mu \delta \)-opioid receptors (Bramham et al. 1991; Xie and Lewis 1991) and NMDA receptors (Colino and Malenka 1993; Hanse and Gustafsson 1992) is required for LTP induction in this pathway. Thus the lateral perforant path–dentate granule cell synapse provides an ideal model for studying the modulation of NMDA receptor function by \( \mu \)– and \( \delta \)-opioids. Use of slice preparation and whole cell recording techniques has made it possible to examine this modulation at both neuronal network and single-synapse levels.

In our previous studies, \( \mu \)-opioids enhanced NMDA-receptor-mediated field potential component and facilitated induction of LTP in the dentate gyrus (Xie and Lewis 1991). This facilitation was primarily caused by suppression of GABA-mediated, inhibitory input to dentate granule cells (disinhibition), and thus depended on the presence of intact GABAAergic transmission (Xie and Lewis 1995b). When slices were pretreated with a GABA\(_A\) antagonist, bicuculline, to occlude this disinhibitory mechanism, the \( \mu \)-agonist PL017 displayed an inhibitory action on NMDA currents evoked in granule cells (Xie et al. 1992). The present study confirms and extends our previous findings, demonstrating a dose-dependent inhibition of NMDA EPSCs by two selective \( \mu \)-agonists in bicuculline-treated slices. This inhibition could be completely antagonized by naloxone and a selective \( \mu \)-antagonist, cyprodime, and therefore was mediated by \( \mu \)-opioid receptors rather than a direct interaction between opioids and NMDA receptors. Taken as a whole, activation of \( \mu \)-receptors appears to have dual effects on NMDA-mediated synaptic transmission in the dentate, a facilitating effect via suppression of GABAAergic interneurons, and an inhibitory effect on granule cells. The net effect of \( \mu \)-opioids thus depends on the interplay between these two actions, and can be substantially affected by the functional state of both synaptic inhibition and NMDA receptor channels.

**Suppression of cAMP-dependent phosphorylation underlies the inhibitory effect of \( \mu \)-opioids on NMDA EPSCs**

Application of PTX effectively blocked \( \mu \)-opioid effects in our experiments, suggesting the involvement of a G-protein-mediated mechanism. PTX-sensitive G proteins (G\(_i\)/G\(_o\)) couple opioid receptors with multiple effectors, including Ca\(^{2+}\) channels (Sternweis and Pang 1990), K\(^+\) channels (Tatsumi et al. 1990), and adenylate cyclase (Johnson and Fleming 1989). Thus several different mechanisms could be responsible for the inhibition of NMDA currents. First, activation of \( \mu \)-receptors reportedly inhibits the release of several neurotransmitters in the hippocampus (Jackisch et al. 1986a,b; Passarelli and Costa 1989). Reduction of Ca\(^{2+}\) influx through N-type calcium channels (Shen and Surprenant 1991) has been attributed to this opioid-induced presynaptic inhibition. It is possible that \( \mu \)-agonists inhibit NMDA currents by reducing glutamate release from the perforant path terminals. However, this presynaptic mechanism has not been supported by our previous study, in which activation of \( \mu \)-receptors selectively inhibits NMDA EPSCs, but not AMPA-receptor-mediated EPSCs, in dentate granule cells (Xie et al. 1992). Furthermore, the effect of PL017 could be postsynaptically blocked by intracellular application of PTX into granule cells in this study, suggesting that the opioid effect observed here is due to an action on post-synaptic granule cells. The second possibility is that \( \mu \)-opioids cause hyperpolarization of granule cell membrane by increasing an inward rectifying potassium conductance (Madison and Nicoll 1988; Williams et al. 1988; Wuarin and Dudek 1990), thus potentiating voltage-sensitive blockade of NMDA channels by Mg\(^{2+}\) (Mayer et al. 1984). If this is
the case, μ-opioid effects should be abolished by removal of Mg^{2+} from the perfusion medium, or should not be seen under membrane-potential-controlled conditions. However, we have observed similar degrees of inhibition of NMDA currents by PL017 in both Mg^{2+}-containing and Mg^{2+}-free media under voltage-clamp conditions. In addition, μ-opioids had no significant effect on granule cell membrane potential in our experiments. These results do not support a major role of granule cell hyperpolarization. Finally, one of the major actions of opioids at the cellular level is inhibition of adenylate cyclase, which may reduce intracellular cAMP level and consequently attenuate PKA-mediated phosphorylation of NMDA channels or closely associated regulatory proteins. In the present study, the adenylate cyclase activator forskolin significantly enhanced NMDA EPSCs and antagonized μ-opioid-induced inhibition. When applied simultaneously, PL017 also attenuated forskolin-induced enhancement of the current. These results are consistent with the hypothesis that μ-opioids inhibit NMDA currents through depression of the cAMP cascade. Furthermore, the specific PKA activator Sp-cAMPS increased the amplitude of NMDA currents, whereas the PKA inhibitor Rp-cAMPS mimicked μ-opioid effects and occluded μ-opioid effects. Taken together, these results strongly suggest that inhibition of PKA may indeed play a key role in the inhibitory effect of μ-opioids.

There are other G-protein-mediated mechanisms that should be taken into consideration, such as involvement of protein phosphatase. Activation of phosphatase 2A has been known to reverse the effect of cAMP on ion channels by selectively dephosphorylating cAMP-dependent sites, thus producing a response similar to that induced by adenylate cyclase inhibitors (Reinhart et al. 1991; White et al. 1991). Can μ-opioids inhibit NMDA channel activity via activation of phosphatase? The effect of PL017 was completely reversed by forskolin in our experiments. This is not in keeping with activation of phosphatase, whose effect is cAMP independent and cannot be reversed by saturating concentrations of cAMP (White et al. 1991). Furthermore, μ-opioids induced no additional inhibition of NMDA EPSCs in the presence of the PKA inhibitor. These results indicate that activation of phosphatase is not significantly involved in the μ-opioid effect.

PKA may play an important role in the modulation of NMDA receptors

NMDA receptors contain multiple consensus sites for phosphorylation by several protein kinases (Hollmann and Heinemann 1994). Protein kinase C and tyrosine kinases have been known to phosphorylate NMDA receptor subunits directly (Lau and Huganir 1995; McBain and Mayer 1994; Moon et al. 1994; Tingley et al. 1993) and substantially potentiate their functions (Chen and Huang 1992; Wang and Salter 1994). In trigeminal neurons such a protein-kinase-C-dependent mechanism has been considered responsible for the long-lasting potentiation of NMDA currents following application of the μ-agonist DAMGO (Chen and Huang 1991). The role of PKA in regulating NMDA receptors, however, is less well understood. Consistent with a few previous reports (Cerne et al. 1993; Colwell and Levine 1995; Huang et al. 1993), our results show that treatments increasing PKA activity enhance NMDA currents, whereas those inhibiting PKA activity depress the current significantly. Thus a basal level of phosphorylation by PKA seems necessary to maintain NMDA currents in dentate granule cells. It should be noted that cAMP and PKA reportedly act at multiple sites in nervous system, either pre- or postsynaptically modulating synaptic transmission (Castellucci et al. 1980; Colwell and Levine 1995; Greengard et al. 1991). Our experiments did not directly determine whether PKA acted at pre- or postsynaptic sites to enhance NMDA currents. Nevertheless, as discussed above, the inhibitory action of μ-agonists was mediated postsynaptically. The opioid action could be occluded by the PKA inhibitor, suggesting a common mechanism for both to inhibit NMDA currents. Therefore it is likely that a postsynaptic site is involved in PKA modulation of NMDA currents, although additional presynaptic actions of PKA cannot be ruled out.

The depression of NMDA currents by μ-opioids through inhibition of PKA suggests that altering PKA activity may be an important means for neurotransmitters that act on G-protein-coupled receptors to regulate NMDA channel activity and control synaptic signaling. Release of endogenous opioids in the hippocampus usually requires high-frequency stimulation of synaptic input (Caudle et al. 1991). During such intensified synaptic activity the concurrent release of opioids with glutamate from the presynaptic terminals could provide a negative feedback mechanism to limit Ca^{2+} influx through NMDA receptor channels on the postsynaptic neuron. Such a mechanism could affect synaptic plasticity, neuronal susceptibility to excitotoxicity, and other NMDA-receptor-dependent processes, and it could become particularly effective under certain pathological conditions when GABA-mediated synaptic inhibition is impaired and μ-opioid effects thus become predominantly inhibitory.

In parallel with our results, a recent study provides evidence that activation of PKA by β-adrenergic receptor agonists overcomes calcineurin-induced inhibition of NMDA EPSCs in cultured neurons from rat hippocampal CA1 region (Raman et al. 1996). Both opioid peptides and norepinephrine (Milusheva et al. 1994) can be released in the hippocampus during synaptic stimulation. They exert opposite effects on adenylate cyclases that are present in a high concentration in hippocampal pyramidal neurons and granule cells (Matsuoka et al. 1992). Therefore those two systems may provide physiological stimulation and inhibition to PKA activity, which in turn modulates NMDA channel function integratively during synaptic activity. Recent findings have suggested that PKA activation is implicated in LTP induction (Frey et al. 1993) and plays a special role in β-adrenergic-receptor-mediated enhancement of LTP in the hippocampus (Thomas et al. 1996). It will be of interest to examine the interplay of these two systems, μ-opioids and norepinephrine/β-adrenergic receptors, in the modulation of NMDA channel currents during LTP induction.

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