Cellular/Molecular

Presynaptic Mechanism for Anti-Analgesic and Anti-Hyperalgesic Actions of κ-Opioid Receptors

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Glutamate neurotransmission plays an important role in the processing of pain and in chronic opioid-induced neural and behavioral plasticity, such as opioid withdrawal and opioid dependence. κ-Opioid receptors also have been implicated in acute opioid modulation of pain and chronic opioid-induced plasticity, both of which are primarily mediated by μ-opioid receptors. Using whole-cell patch clamp recordings in brain slices *in vitro* and system analysis of pain behaviors in rats *in vivo*, this study investigated the functional role of glutamate synaptic transmission and κ-opioid receptors in two behavioral pain conditions: μ-opioid-induced analgesia (decreased pain) and μ-opioid withdrawal-induced hyperalgesia (increased pain). In the nucleus raphe magnus (NRM), a brainstem structure that controls spinal pain transmission, we found that κ-receptor agonists presynaptically inhibited glutamate synaptic currents in both of the two cell types that are thought to respectively inhibit or facilitate spinal pain transmission. In rats, both glutamate receptor antagonists and the κ agonist microinjected into the NRM attenuated μ-opioid-induced analgesia, which is most likely mediated through activation of such pain-inhibiting neurons. However, during opioid abstinence-induced withdrawal, the same doses of glutamate receptor antagonists and the κ agonist administered in the NRM suppressed the withdrawal-induced hyperalgesia, which is thought to be mediated by activation of those pain-facilitating neurons during opioid withdrawal. These results demonstrate that κ-opioid receptors antagonize μ-receptor-induced effects in both analgesic and hyperalgesic states, and suggest inhibition of glutamate synaptic transmission as a presynaptic mechanism for the κ antagonism of these two μ receptor-mediated actions.

Key words: **κ receptors; μ receptors; opioid; glutamate; analgesia; hyperalgesia; pain**

Introduction

Glutamate and other excitatory amino acids (EAAs) are principal excitatory neurotransmitters in neuronal circuits involved in a variety of CNS functions, including pain modulation (Fundytus, 2001). Glutamate and other EAAs can produce potent inhibition of pain when applied locally into such key structures of the endogenous pain-modulating system as the periaqueductal gray (PAG) in the midbrain, and the main projection target of the PAG, the nucleus raphe magnus (NRM) in the medulla (Aimone and Gebhart, 1986; Jacquet, 1988). Systemic application or local administration of glutamate receptor antagonists into the PAG or the NRM blocks morphine-induced analgesia (Aimone and Gebhart, 1986; Jacquet, 1988; Lipa and Kavaliers, 1990; Heinricher et al., 2001), indicating that glutamate-mediated synaptic transmission is required for opioid analgesia. The glutamate neurotransmission system, and in particular, the NMDA receptor, is also critically involved in chronic opioid-induced neural adaptations, such as opioid dependence and withdrawal (Trujillo and Akil, 1991, 1995; Nestler, 1996), and in chronic pain-induced hyperalgesia (increased pain sensitivity) involving the NRM (Coderre et al., 1993; Urban and Gebhart, 1998; Porreca et al., 2002).

Despite the importance of glutamate neurotransmission in these acute and chronic pain states, how it functions in the pain-modulating circuits activated by acute opioids remains unclear, hampering further attempts to understand the role of glutamate synaptic transmission and its plastic changes in chronic opioid-induced or chronic pain-induced behavioral conditions. In our previous studies of the NRM, the main component of the descending pain-inhibiting system (Fields and Basbaum, 1999), we characterized a disinhibition mechanism for the activation of this system by local μ-opioid receptor agonists, without involving glutamate synaptic transmission (Pan et al., 1990, 1997). Nevertheless, other studies have indicated that systemically applied μ-opioids activate glutamate inputs and those neurons thought to inhibit spinal pain transmission in the rostral ventromedial medullar (RVM), which includes the NRM (Spinella et al., 1996; Fields and Basbaum, 1999; Heinricher et al., 2001).

In contrast to the evident role of μ-receptors in opioid analgesia and dependence (Matthes et al., 1996), the function of κ-opioid receptors is less clear. We have shown a μ-opposing effect of κ-receptors through a postsynaptic hyperpolarization in opioid analgesia (Pan et al., 1997), representing an emerging anti-μ function of κ-receptors in many opioid effects in the brain (Pan, 1998). Under normal conditions, κ agonists can have an analgesic action by inhibiting glutamate synaptic currents (Randic et al., 1995; Ackley et al., 2001). Under conditions of chronic opioids or chronic pain, κ-receptors interfere with the development of μ-opioid tolerance and dependence (Takahashi et al.,

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1991; Takemori et al., 1992; Tao et al., 1994), and are implicated in several forms of chronic pain behaviors (Cheng et al., 2002). However, the underlying mechanisms for these κ actions remain unknown.

In the current study of NRM neurons, we used both slice preparations *in vitro* and intact rats *in vivo* to investigate the functional role of glutamate synaptic transmission and its modulation by κ-receptors in both μ-opioid-induced analgesic and μ-opioid withdrawal-induced hyperalgesic states.

Materials and Methods

Brain slice preparations. The brain of a male, neonatal (9–14 d old) Wistar rat was cut in a vibratome in cold (4°C) physiological saline to obtain brainstem slices (200-μm-thick) containing the NRM. A single slice was submerged in a shallow recording chamber and perfused with preheated (35°C) physiological saline (in mM: NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.4; glucose, 11; $NaHCO_3$, 25, saturated with 95% O_2 and 5% CO_2, pH 7.2–7.4). Slices were maintained at ~35°C throughout the recording experiment. Neonatal rats were used for better visualization of neurons in brain slices with an infrared Nomarski microscope. It has been demonstrated that the physiological and pharmacological properties of neurons from these young rats are indistinguishable from those of adult rats (Pan et al., 1997).

Whole-cell recordings and data analyses. Visualized whole-cell patch clamp recordings were made from identified neurons with a glass pipette (resistance 3–5 MΩ) filled with a solution containing (in mM): potassium gluconate, 126; NaCl, 10; $MgCl_2$, 1; EGTA, 11; HEPES, 10; ATP, 2; GTP, 0.25; pH adjusted to 7.3 with KOH; osmolarity 280–290 mOsmol/l. An AxoPatch 1-D amplifier and AxoGraph software (Axon Instruments, Foster City, CA) were used for data acquisition and on-line/off-line data analyses. A seal resistance of ≥2 GΩ and an access resistance of ≤15 MΩ were considered acceptable. Series resistance was optimally compensated. The access resistance was monitored throughout the experiment. Electrical stimuli of constant current (0.25 msec, 0.2–0.4 mA) were used to evoke EPSCs with bipolar stimulating electrodes placed lateral (200–400 μM) to the recording electrode within the NRM. A pair of EPSCs was evoked by two stimuli with an interval of 40 msec. The pair-pulse ratio was determined by the ratio of the second EPSC amplitude over the first one. Spontaneous EPSCs (sEPSCs) or miniature EPSCs (mEPSCs) were obtained in 60 sec epochs in control or in the presence of drugs. The AxoGraph 4.7 was used to detect and measure the amplitude and intervals of the synaptic events and analyze their distribution data. Statistic analyses of sEPSCs and mEPSCs were performed using Statview software with either the Kolmogorov–Smirnov, or the Mann–Whitney *U* test. Other numeral data were statistically analyzed by Student's *t* tests and presented as mean ± SEM.

Cell classification and drug application. All NRM cells recorded were classified into either a primary or secondary cell type according to the criteria described in our previous study (Pan et al., 1990). Briefly, primary cells have a wider action potential, have a more negative resting membrane potential, and are insensitive to μ agonists. Secondary cells have a narrower action potential, often display spontaneous firing, and are hyperpolarized by μ agonists. Cells that could not be clearly classified were not included in the results. Drugs were applied through the perfusing solution.

Behavioral experiments and microinjection. In behavioral experiments, male Wistar rats (250–300 gm) were maintained lightly anesthetized in a stereotaxic apparatus with a constant intravenous infusion of methohexital (10 mg/ml at 0.8 ml/hr). A 26 gauge single-guide cannula was aimed at the ventrolateral PAG [anteroposterior (AP): −7.8; lateral (L): ±0.8; ventral (V): − 6.0] and a second, double-guide cannula was aimed at the NRM (AP: −11.0; L: 0; V: −10.7). Tail-flick latency to a radiant heat stimulus was measured every 2 min. The heat intensity was set to elicit stable baseline latencies with a cutoff time of 12 sec. After six baseline trials, drugs were delivered through a 33 gauge cannula with an infusion pump at a rate of 0.5 μl/min. All cannula placements for both the PAG and the NRM were histologically verified afterward. Drug effects were statistically analyzed by an ANOVA for repeated measures and either the Tukey–Kramer or the Newman–Keuls test of *post hoc* analysis using GB-Stat software. All drugs were purchased from Research Biochemicals International (Natick, MA) or Sigma-Aldrich.

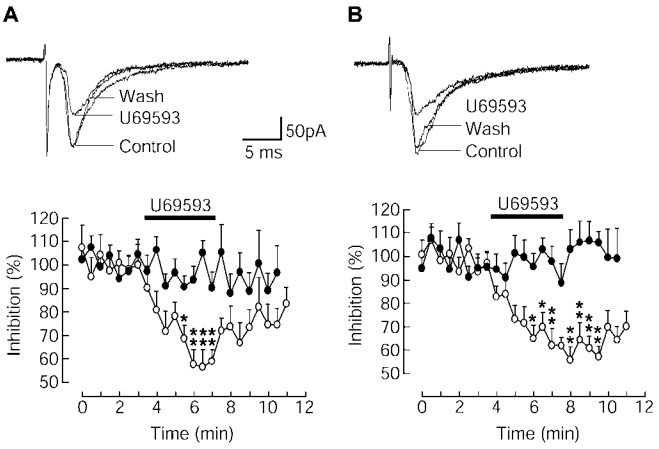

Figure 1. κ-Opioid receptor agonist U69593 inhibits glutamate-mediated EPSCs in brainstem neurons of the NRM. *A*, *B*, Data from primary cells. *B*, Data from secondary cells. Top panels are single EPSCs before (control), during (U69593), and after (wash) application of the κ-receptor agonist U69593 (300 nM). Bottom panels are plots of normalized EPSC amplitudes from pooled neurons in control (open circles, $n = 12$ in *A* and $n = 15$ in *B*) and in the presence of the κ-receptor antagonist nor-BNI (100 nM, filled circles, $n = 11$ in both *A* and *B*). The bar indicates the time of U69593 application. $^*p < 0.05$, $^{**}p < 0.01$ (ANOVA for repeated measures and the Newman–Keuls test of *post hoc* analysis).

Results

κ agonists inhibit glutamate synaptic transmission

NRM cells recorded in brain slices were classified into either a primary cell type lacking μ receptors or a secondary cell type containing μ receptors according to the criteria described in our previous studies (Pan et al., 1990, 1997). Primary cells had an average resting membrane potential of −60.6 ± 0.7 mV and an action potential of 51.5 ± 0.7 mV ($n = 66$). Secondary cells had a less negative resting potential (−54.1 ± 0.5 mV) and an action potential of 49.5 ± 0.7 mV ($n = 85$). EPSCs were evoked through local stimulation in cells under whole-cell voltage clamp with a holding potential of −60 mV. Bicuculline (10 μM) was included in all experiments to block the $GABA_A$ receptor-mediated synaptic transmission present in these cells. The average amplitude of the evoked EPSC (eEPSC) was 114 ± 6 pA in all cells recorded ($n = 151$). The EPSC was completely blocked by the combination of AP-5 (10 μM) and CNQX (10 μM), antagonists of NMDA and non-NMDA receptors, respectively ($n = 8$). Application of the κ-opioid receptor agonist U69593 (300 nM) significantly inhibited the EPSC amplitude in every cell tested. In primary cells ($n = 12$), the average inhibition was 43.3 ± 7.4%, and the EPSC was recovered in minutes after wash of U69593 (Fig. 1*A*). No significant difference was observed in the κ inhibition between primary cells that were hyperpolarized by the κ agonist (44.7 ± 3.9%; $n = 5$) and primary cells that lacked the postsynaptic κ response (42.3 ± 4.5%; $n = 7$). Similar U69593-mediated inhibition of eEPSCs was also found in the μ-sensitive secondary cells with an average inhibition of 44.7 ± 5.7% ($n = 15$) (Fig. 1*B*). U69593 at a lower concentration (10 nM) caused a smaller inhibition in the EPSC amplitude in both cell types (control, 109.0 ± 18.1 pA; U69593, 82.5 ± 16.2 pA; wash, 101.8 ± 17.0 pA; $n = 6$). In the presence of the κ-receptor antagonist nor-BNI (100 nM), the inhibition induced by U69593 (300 nM) was completely blocked in both cell types ($n = 11$ in each type), confirming the action of

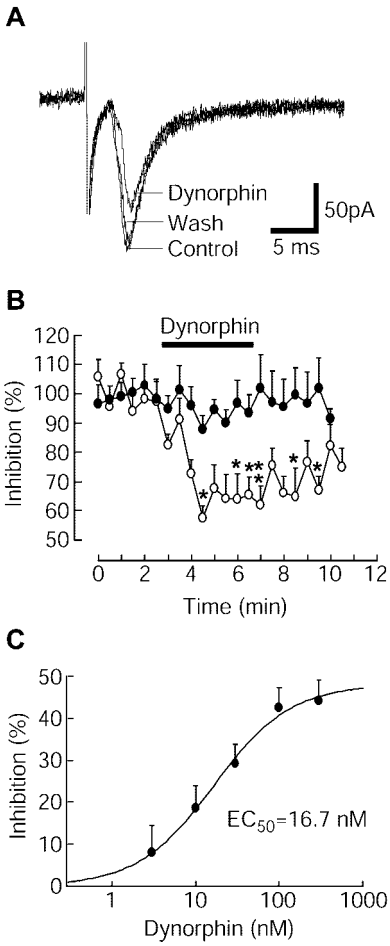

Figure 2. Endogenous κ-receptor agonist dynorphin inhibits glutamate EPSCs. *A*, EPSCs from a secondary cell in control, in dynorphin (300 nM), and after washout of dynorphin. *B*, A plot of normalized amplitudes of pooled EPSCs from secondary cells in control (open circles, $n = 11$) and in nor-BNI (100 nM, filled circles, $n = 10$). *C*, A dose–response plot of EPSC inhibition by dynorphin in secondary cells ($n = 7–8$ cells at each concentration).

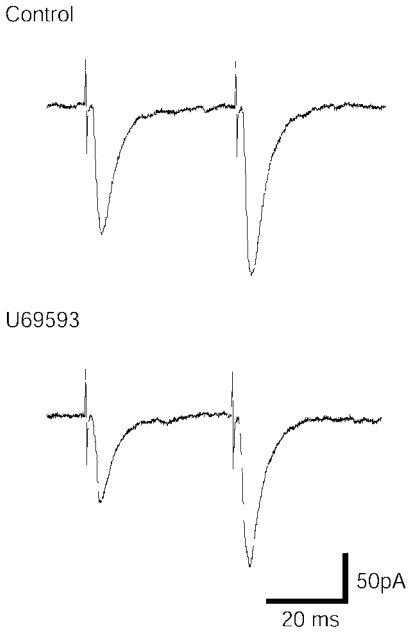

Figure 3. U69593 increases the paired-pulse ratio (PPR) of glutamate EPSCs. Pairs of EPSCs from a primary cell in control and in U69593. Note the increase by U69593 (300 nM) in the PPR (ratio of the second EPSC amplitude over the first).

κ-receptors (Fig. 1). Nor-BNI itself had no effect on the EPSC in all these cells of both types (control vs nor-BNI: 95 ± 13.4 pA vs 97 ± 13.7 pA in primary cells; $n = 11$ and 111 ± 12.6 pA vs 108 ± 17.6 pA in secondary cells, $n = 11$).

The endogenous κ agonist dynorphin also produced a reversible and nor-BNI-sensitive inhibition of the eEPSC in these cells. Dynorphin at 300 nM inhibited the EPSC amplitude by 41.8 ± 5.5% in primary cells ($n = 9$) and by 44.2 ± 4.9% in secondary cells ($n = 11$) (Fig. 2*A*,*B*). The dynorphin effect was dose-dependent with an estimated dose range of 1–300 nM. The EC_{50} estimated from dose–response curves was 14.5 nM in primary cells and 16.7 nM in secondary cells (Fig. 2*C*).

Together, these results suggest that activation of κ-opioid receptors inhibits glutamate synaptic transmission in both primary and secondary cell types.

The κ inhibition is presynaptic

To determine the synaptic site of the κ-receptor action, we first used the paradigm of paired-pulse ratio (PPR), whose change is attributed to an altered transmitter release through a presynaptic mechanism (Manabe et al., 1993). Thus, an increase in the PPR (the ratio of the second EPSC amplitude over the first one) suggests presynaptic inhibition of transmitter release and vice versa. In the present study, primary cells had a PPR of 1.39 ± 0.12 in control ($n = 14$), indicating a synaptic facilitation. The PPR increased to 2.0 ± 0.24 in the presence of U69593 ($p < 0.05$; $n = 14$) (Fig. 3). PPR in secondary cells was similarly increased by U69593 (control, 1.96 ± 0.15; U69593, 2.46 ± 0.21; $p < 0.01$; $n = 23$).

We next examined the κ effect on glutamatergic spontaneous EPSCs (sEPSCs). Primary cells displayed sEPSCs with an average amplitude of 24 pA ($n = 7$) (Fig. 4*A*). U69593 (300 nM) significantly inhibited the frequency of the synaptic events (control, 5.2 ± 1.0 Hz; U69593, 2.3 ± 1.0 Hz; $p < 0.01$; Kolmogorov–Smirnov test, $n = 7$) (Fig. 4*B*), but had no effect on the amplitude (control, 24.3 ± 0.9 pA; U69593, 23.4 ± 1.0 pA; $p > 0.05$; Kolmogorov–Smirnov test, $n = 7$) (Fig. 4*C*). Similar U69593 effects were observed in secondary cells (control vs U69593: 7.2 ± 1.7 Hz vs 4.1 ± 1.2 Hz, $p < 0.01$; 27.0 ± 1.7 pA vs 27.4 ± 2.5 pA; $p > 0.05$; Kolmogorov–Smirnov test, $n = 8$) (Fig. 4*D*,*E*). In additional four cells (two in each type), we tested U69593 effect on eEPSCs and on sEPSCs in the same cell. In all four cells tested, U69593 (300 nM) inhibited the eEPSC with an increase in the PPR (from 1.65 ± 0.34 to 2.35 ± 0.26; $p < 0.05$), and reduced the frequency of sEPSCs (control, 6.34 ± 0.87 Hz; U69593, 4.08 ± 0.91 Hz; $p < 0.05$) with no change in the sEPSC amplitude (control, 22.0 ± 1.3 pA; U69593, 21.5 ± 1.3 pA; $p > 0.05$).

To further verify the κ action site, we then examined the effect of the κ agonist on activity-independent mEPSCs in the presence of TTX (1 μM). In primary cells ($n = 5$), U69593 significantly decreased the frequency of mEPSCs (control, 5.8 ± 0.9 Hz; U69593, 3.6 ± 0.5 Hz; $p < 0.05$), but it did not alter the mEPSC amplitude (control, 24.9 ± 2.0 pA; U69593, 25.2 ± 2.3 pA; $p > 0.05$). As shown in Figure 5, the κ agonist also inhibited the frequency but not the amplitude of mEPSCs in secondary cells (control vs U69593: 10.9 ± 2.0 Hz vs 6.4 ± 1.3 Hz; $p < 0.05$; 24.5 ± 2.4 pA vs 23.7 ± 2.4 pA; $p > 0.05$; $n = 6$).

These findings suggest that activation of κ-receptors on presynaptic sites inhibits glutamate release onto both NRM primary and secondary cell types.

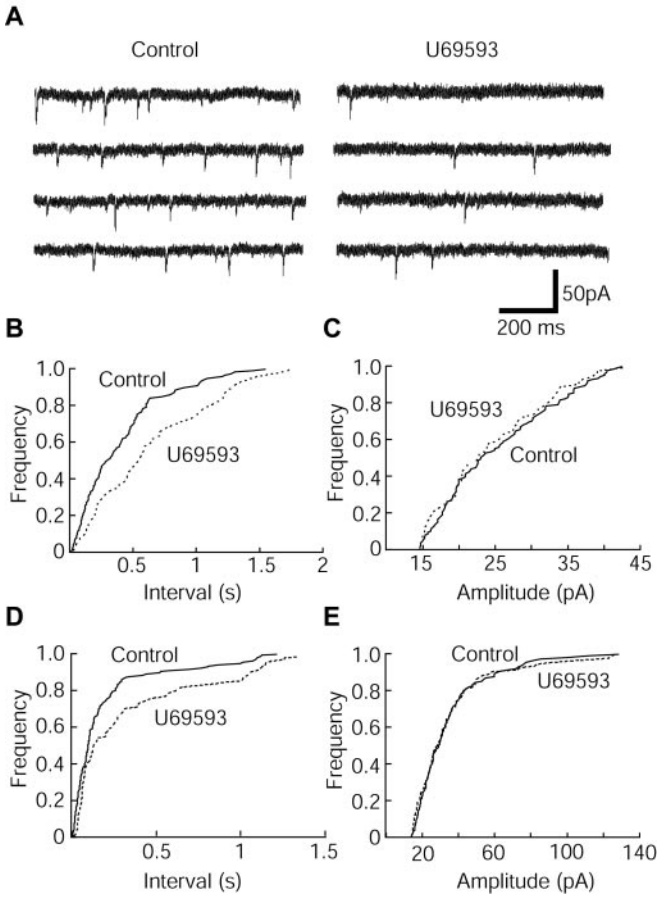

Figure 4. U69593 reduces the frequency of spontaneous glutamate EPSCs. *A*, Current traces showing spontaneous EPSCs from a primary cell in control and in U69593 (300 nM). *B*, *C*, Plots of cumulative distribution of interevent intervals and EPSC amplitudes in control and in U69593 for the cell in *A*. *D*, *E*, Distribution plots for a secondary cell.

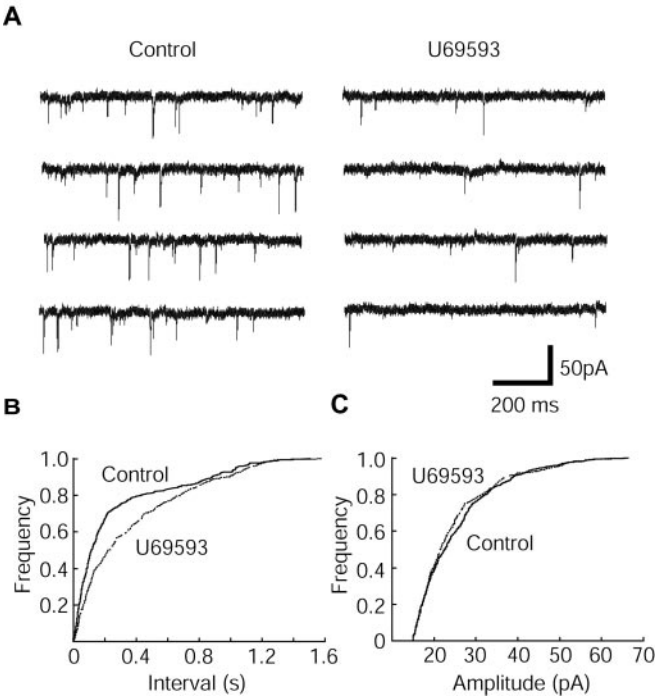

Figure 5. U69593 inhibits the frequency of miniature glutamate EPSCs. Data from a secondary cell. *A*, Current traces showing miniature EPSCs in control and in U69593 (300 nM). *B*, *C*, Plots of cumulative distribution of interevent intervals and miniature EPSC amplitudes in control and in U69593.

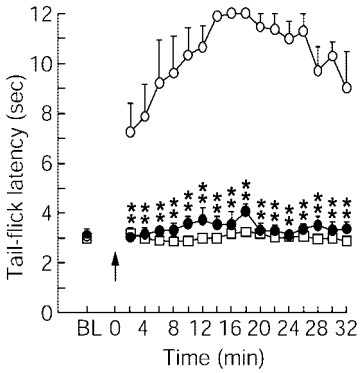

Figure 6. Glutamate receptor antagonists in the NRM block local μ-opioid-induced analgesia. Tail-flick latencies were measured every 2 min before (BL, baseline, average of 6 trials) and after drug microinjections into the NRM and then into the PAG (arrow, time = 0). The cutoff time was 12 sec. Group 1 (open circles): saline in NRM and DAMGO in PAG; group 2 (filled circles): AP-5 + CNQX in NRM and DAMGO in PAG; group 3 (open squares): AP-5 + CNQX in NRM and saline in PAG, $n = 5$ rats in each group, $^{**}p < 0.01$ (ANOVA for repeated measures and the Tukey–Kramer test of *post hoc* analysis).

κ agonist inhibits glutamate-mediated opioid analgesia

We have shown previously that μ-opioids produce analgesia by disinhibiting NRM primary cells and by inhibiting μ-receptor-containing secondary cells in rats (Pan et al., 1990, 1997). To investigate the role of glutamate synaptic transmission in the excitation of primary cells during opioid analgesia, we next performed NRM microinjections in lightly anesthetized rats *in vivo* while monitoring changes in pain threshold with the tail-flick test. Microinjection of the μ-receptor agonist D-Ala^2-*N*-Me-Phe^4-Glycol^5]-enkephalin (DAMGO) (0.05 μg/0.25 μl) into the PAG produced an immediate increase in the tail-flick latency to the cutoff time (12 sec), demonstrating a potent antinociceptive effect ($n = 5$ rats) (Fig. 6). When the glutamate receptor antagonists AP-5 (0.197 μg/1 μl) and CNQX (0.232 μg/1 μl) were microinjected into the NRM just before the DAMGO application in PAG, the DAMGO-induced antinociceptive effect was blocked ($n = 5$ rats). NRM application of the glutamate antagonists alone did not change the pain threshold ($n = 5$ rats) (Fig. 6). These results indicate that during the PAG opioid-induced analgesia, the activation of NRM primary cells is mediated by glutamate synaptic inputs activated through the PAG.

Because κ agonists presynaptically inhibit glutamate synaptic transmission in primary cells, as described above, κ agonists should also reduce the DAMGO-induced antinociception. In fact, we have shown previously that microinjection of the κ agonist U69593 (0.178 μg/1 μl) into the NRM significantly attenuates this PAG DAMGO-induced antinociception and that the U69593 effect can be completely blocked by NRM comicroinjection of nor-BNI (Pan et al., 1997). The same results were obtained in the present study ($n = 2$ rats). These results suggest that activation of presynaptic κ-receptors inhibits the synaptic release of glutamate onto primary cells and thereby decreases the PAG DAMGO-induced analgesia, which requires the activation of NRM primary cells by glutamate inputs.

κ agonist attenuates glutamate-mediated hyperalgesia

NRM secondary cells are directly hyperpolarized by μ-opioids during opioid analgesia and therefore, their glutamate inputs are

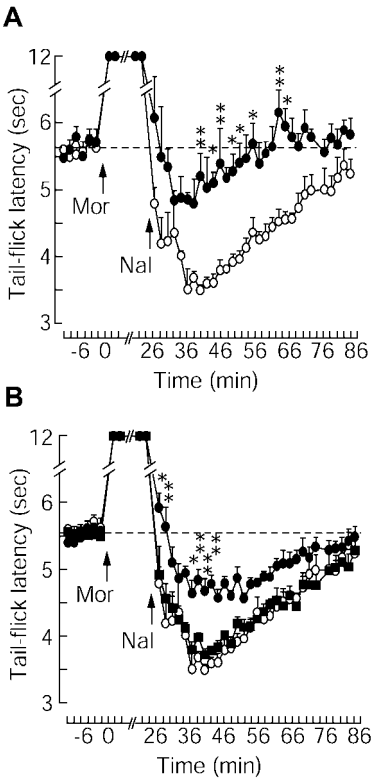

Figure 7. Glutamate receptor antagonists and κ-receptor agonist attenuate opioid abstinence-induced hyperalgesia. After measurements of baseline tail-flick latencies, rats were injected with morphine (2 mg/kg, i.p.) and followed by naloxone (1 mg/kg, i.p.) 26 min later to induce opioid abstinence (withdrawal)-induced hyperalgesia. NRM microinjections were made immediately after the naloxone injection. The dashed line indicates pre-morphine baseline. *A*, Open circles: saline in NRM ($n = 5$ rats). Filled circles: AP-5 + CNQX in NRM ($n = 6$ rats). *B*, Open circles: same as in *A*. Filled circles: U69593 in NRM ($n = 6$ rats). Filled squares: U69593 + nor-BNI in NRM ($n = 5$ rats). $^*p < 0.05$, $^{**}p < 0.01$ (ANOVA for repeated measures and the Tukey–Kramer test of *post hoc* analysis).

unlikely to be activated in a behavioral condition of analgesia (Fields and Basbaum, 1999). However, these cells are activated during opioid abstinence-induced withdrawal, and their activation is implicated in mediating the state of increased pain sensitivity (hyperalgesia) during opioid withdrawal (Kaplan and Fields, 1991; Pan et al., 2000). The following experiments were then conducted to test our hypothesis that glutamate synaptic inputs mediated the activation of secondary cells during opioid withdrawal.

Morphine (2 mg/kg), mainly a μ-receptor agonist, was injected intraperitoneally in rats to induce opioid analgesia. Rats were then injected with naloxone (1 mg/kg, i.p.) to induce acute opioid withdrawal. As shown in Figure 7*A*, systemically applied morphine produced potent antinociception with a marked increase in tail-flick latency. Application of naloxone 26 min later quickly decreased the rat's pain threshold from the cutoff time (12 sec) to levels below the pre-morphine baseline, indicating opioid abstinence-induced hyperalgesia ($n = 5$ rats). When the same doses of AP-5 (0.197 μg/1 μl) and CNQX (0.232 μg/1 μl), instead of saline, were microinjected into the NRM immediately after naloxone injection, this hyperalgesia was significantly attenuated ($n = 6$ rats) (Fig. 7*A*). This demonstrates an important role of glutamate synaptic transmission in the NRM in mediating the hyperalgesic condition following acute opioid withdrawal. Based on above findings that the κ agonist inhibited glutamate EPSCs in secondary cells, we predicted that the κ agonist would also decrease opioid withdrawal-induced hyperalgesia. Indeed, the same dose of κ agonist U69593 (0.178 μg/1 μl) microinjected into the NRM immediately after intraperitoneal injection of naloxone significantly reduced the hyperalgesia ($n = 6$ rats) (Fig. 7*B*). Co-microinjection of U69593 with the κ antagonist nor-BNI (0.367 ng/1 μl) into the NRM completely reversed the U69593 effect, confirming a specific κ-receptor-mediated effect ($n = 5$ rats) (Fig. 7*B*).

Discussion

The present study illustrates that activation of κ-opioid receptors presynaptically inhibits synaptic release of glutamate or other EAAs onto both primary cells and μ-expressing secondary cells in the NRM. Our behavioral data further show that both glutamate receptor antagonists and κ receptor agonists in the NRM antagonize the PAG opioid-induced analgesia or attenuate opioid withdrawal-induced hyperalgesia. These results suggest that the excitatory glutamate synaptic inputs are required to mediate both the activation of NRM primary cells for the μ-opioid analgesia and the activation of secondary cells for opioid withdrawal-induced hyperalgesia. Therefore, presynaptic inhibition of glutamate synaptic transmission by κ-opioid receptors could function as one of the mechanisms for the anti-analgesic and anti-hyperalgesic actions of κ-receptors in the two opposite pain states induced through μ-opioid receptors. Several observations in the current study suggest that κ-opioid receptor agonists inhibit glutamate EPSCs by acting on presynaptic sites in NRM cells. First, U69593 significantly and consistently increased the paired pulse ratio of the eEPSC, indicating a change in presynaptic release rather than a postsynaptic effect. This method often has been used to determine a presynaptic effect (Manabe et al., 1993; Manzoni and Williams, 1999; Hjelmstad and Fields, 2001). Second, the κ agonist reduced the frequency but not the amplitude of sEPSCs, indicating an action on presynaptic sites. Third, although U69593 postsynaptically hyperpolarized a subpopulation of primary cells, the amount of its inhibition of EPSCs was not statistically different in the primary cells hyperpolarized by the κ agonist and in those without the postsynaptic response. It indicates that the κ action on presynaptic sites is dominant for the inhibition of EPSCs in these cells. Last, U69593 inhibited mEPSC frequency, but not its amplitude, suggesting that the κ agonist inhibits glutamate release from terminals. Anatomically, κ-opioid receptor immunoreactivity is present on both processes and cell bodies in the NRM (Kalyuzhny and Wessendorf, 1999).

Mounting evidence indicates that κ receptors can oppose several μ receptor-mediated actions of acute and chronic opioids in the brain, including opioid analgesia (Pan, 1998; Ghozland et al., 2002; Schmidt et al., 2002). Previous research in the RVM or the NRM has shown that μ-sensitive cells are inhibited during opioid analgesia, and it is the activation of RVM off-cells lacking μ receptors (NRM primary cells) that inhibits spinal pain transmission through their spinal projections (Pan et al., 1997; Fields and Basbaum, 1999; Heinricher et al., 2001). The data from this study suggest that the activation of these primary cells is primarily mediated by their excitatory glutamate inputs originating most likely from the PAG (Aimone and Gebhart, 1986). Inhibition of glutamate EPSCs by κ agonists has been reported in the neurons of several brain areas (Wagner et al., 1993; Weisskopf et al., 1993; Randic et al., 1995; Hjelmstad and Fields, 2001). Using the defined NRM circuit for opioid analgesia, the current study suggests that inhibition of glutamate synaptic transmission in primary cells contributes to the anti-analgesic action of κ agonists by reducing glutamate-induced excitation of primary cells. In a previ-

ous study, we described how κ-receptors opposed the μ-opioid analgesia through a postsynaptic hyperpolarization in a subpopulation of NRM primary cells (Pan et al., 1997). Compared with the EC_{50} of 7.4 nM for its postsynaptic effect of membrane hyperpolarization (Pan et al., 1997), dynorphin had a higher EC_{50} value (14.5 nM) at the presynaptic site for EPSC inhibition in primary cells, indicating that dynorphin is more potent at the postsynaptic κ-receptors than at those on glutamatergic terminals. However, while only a subpopulation of primary cells are hyperpolarized by κ agonists, κ inhibition of glutamate EPSCs has been observed in all primary cells tested in the current study. Thus, both of the presynaptic and postsynaptic mechanisms could reduce μ opioid-induced excitation of primary cells and account for the behavioral anti-analgesic action of κ agonists during opioid analgesia.

Hyperalgesia is a common symptom associated with opioid withdrawal (Kaplan and Fields, 1991). Several studies (Kim et al., 1990; Kaplan and Fields, 1991; Pan et al., 2000) have suggested that this sensitized pain state results from the excessive activity of μ-sensitive neurons in the RVM, or NRM secondary cells, which are thought to facilitate spinal pain transmission through their direct spinal projections (Fields and Basbaum, 1999; Ackley et al., 2001). In fact, recent research has provided convincing evidence establishing the importance of the descending pain-facilitating actions of the RVM in several hyperalgesic states of chronic pain, including neuropathic and inflammatory pain states (Zhuo and Gebhart, 1997; Porreca et al., 2002). Interestingly, both μ-receptor-containing neurons (Porreca et al., 2001) and glutamate receptors (Urban and Gebhart, 1999) in the RVM have been implicated in mediating these abnormal pain states. However, what drives the excessive activity of these pain-facilitating neurons in the hyperalgesic states has remained unclear. The present finding that glutamate receptor antagonists in the NRM attenuate the hyperalgesia suggests that glutamate synaptic transmission mediates the excitation of these cells in opioid withdrawal-induced hyperalgesia, and perhaps in the hyperalgesia of those chronic pain states as well.

Although κ agonists can antagonize both analgesia and hyperalgesia under different behavioral conditions, differential κ actions on pain threshold in normal conditions have been reported. Application of κ agonists into the RVM has no effect on pain threshold in male rats (Pan et al., 1997; Tershner et al., 2000), but has an analgesic effect in female rats, as assessed by the tail-flick test (Tershner et al., 2000). A recent study has also shown an analgesic action of κ agonists in the rat RVM with the paw-withdrawal test, but no effect with the tail-flick test, and the analgesic action has been attributed to κ inhibition of glutamate EPSCs in secondary cells (Ackley et al., 2001). Because results of this type of experiment are primarily determined by κ effects on spontaneous and test-elicited activity of neurons and their glutamate synapses in the RVM, factors that alter spontaneous activity or different pain tests could influence the behavioral effects of κ agonists. Such influential experimental conditions include anesthesia levels, animal sex, and analgesia tests. The current study demonstrates the anti-analgesic and anti-hyperalgesic actions of the κ agonist by examining its effect on glutamate synaptic transmission that has been activated differentially in the two NRM cell types under the two behavioral conditions, opioid analgesia and opioid withdrawal-induced hyperalgesia.

In addition to the pain states in neuropathic conditions and tissue inflammation, glutamate synaptic transmission has also been implicated in the effects of chronic opioids. NMDA receptor antagonists block the development of chronic morphine-induced analgesic tolerance and physical dependence (Trujillo and Akil, 1991). Application of κ-receptor agonists also suppresses morphine tolerance and dependence (Takahashi et al., 1991; Takemori et al., 1992; Tao et al., 1994). In view of the important involvement of glutamate receptors in these pain states, κ-receptor-mediated inhibition of glutamate synaptic transmission, as a presynaptic mechanism for regulation of glutamate release, could play an important role in mediating the behavioral states. Although glutamate receptor antagonists could be useful in the clinical treatment of chronic pain and opioid dependence (Trujillo and Akil, 1995; Fundytus, 2001), their therapeutic use may be limited because of the wide distribution and important role of glutamate synapses in many brain functions, such as learning, memory, and cognition. The current results may suggest κ agonists as a potential alternative in the treatment of opioid withdrawal-related pain and other chronic pain problems.

References

Ackley MA, Hurley RW, Virnich DE, Hammond DL (2001) A cellular mechanism for the antinociceptive effect of a kappa opioid receptor agonist. Pain 91:377–388.

Aimone LD, Gebhart GF (1986) Stimulation-produced spinal inhibition from the midbrain in the rat is mediated by an excitatory amino acid neurotransmitter in the medial medulla. J Neurosci 6:1803–1813.

Cheng HY, Pitcher GM, Laviolette SR, Whishaw IQ, Tong KI, Kockeritz LK, Wada T, Joza NA, Crackower M, Goncalves J, Sarosi I, Woodgett JR, Oliveira-dos-Santos AJ, Ikura M, van der Kooy D, Salter MW, Penninger JM (2002) DREAM is a critical transcriptional repressor for pain modulation. Cell 108:31–43.

Coderre TJ, Katz J, Vaccarino AL, Melzack R (1993) Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain 52:259–285.

Fields H, Basbaum A (1999) Central nervous system mechanisms of pain modulation. In: Textbook of pain, 4E Ed (Wall P, Melzack R, eds). London: Churchill Livingstone.

Fundytus ME (2001) Glutamate receptors and nociception: implications for the drug treatment of pain. CNS Drugs 15:29–58.

Ghozland S, Matthes HW, Simonin F, Filliol D, Kieffer BL, Maldonado R (2002) Motivational effects of cannabinoids are mediated by μ-opioid and κ-opioid receptors. J Neurosci 22:1146–1154.

Heinricher MM, Schouten JC, Jobst EE (2001) Activation of brainstem *N*-methyl-D-aspartate receptors is required for the analgesic actions of morphine given systemically. Pain 92:129–138.

Hjelmstad GO, Fields HL (2001) Kappa opioid receptor inhibition of glutamatergic transmission in the nucleus accumbens shell. J Neurophysiol 85:1153–1158.

Jacquet YF (1988) The NMDA receptor: central role in pain inhibition in rat periaqueductal gray. Eur J Pharmacol 154:271–276.

Kalyuzhny AE, Wessendorf MW (1999) Serotonergic and GABAergic neurons in the medial rostral ventral medulla express kappa-opioid receptor immunoreactivity. Neuroscience 90:229–234.

Kaplan H, Fields H (1991) Hyperalgesia during acute opioid abstinence: evidence for a nociceptive facilitating function of the rostral ventromedial medulla. J Neurosci 11:1433–1439.

Kim DH, Fields HL, Barbaro NM (1990) Morphine analgesia and acute physical dependence: rapid onset of two opposing, dose-related processes. Brain Res 516:37–40.

Lipa SM, Kavaliers M (1990) Sex differences in the inhibitory effects of the NMDA antagonist, MK-801, on morphine and stress-induced analgesia. Brain Res Bull 24:627–630.

Manabe T, Wyllie DJ, Perkel DJ, Nicoll RA (1993) Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. J Neurophysiol 70:1451–1459.

Manzoni OJ, Williams JT (1999) Presynaptic regulation of glutamate release in the ventral tegmental area during morphine withdrawal. J Neurosci 19:6629–6636.

Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques

BP, Kieffer BL (1996) Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 383:819–823.

Nestler EJ (1996) Under siege: The brain on opiates. Neuron 16:897–900.

Pan ZZ (1998) mu-opposing actions of the kappa-opioid receptor. Trends Pharmacol Sci 19:94–98.

Pan ZZ, Williams JT, Osborne P (1990) Opioid actions on single raphe magnus neurons from rat and guinea pig *in vitro*. J Physiol (Lond) 427:519–532.

Pan ZZ, Tershner SA, Fields HL (1997) Cellular mechanism for anti-analgesic action of agonists of the κ-opioid receptor. Nature 389:382–385.

Pan ZZ, Hirakawa N, Fields HL (2000) A cellular mechanism for the bidirectional pain-modulating actions of orphanin FQ/nociceptin. Neuron 26:515–522.

Porreca F, Burgess SE, Gardell LR, Vanderah TW, Malan Jr TP, Ossipov MH, Lappi DA, Lai J (2001) Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the μ-opioid receptor. J Neurosci 21:5281–5288.

Porreca F, Ossipov MH, Gebhart GF (2002) Chronic pain and medullary descending facilitation. Trends Neurosci 25:319–325.

Randic M, Cheng G, Kojic L (1995) κ-opioid receptor agonists modulate excitatory transmission in substantia gelatinosa neurons of the rat spinal cord. J Neurosci 15:6809–6826.

Schmidt BL, Tambeli CH, Levine JD, Gear RW (2002) mu/delta Cooperativity and opposing kappa-opioid effects in nucleus accumbens-mediated antinociception in the rat. Eur J Neurosci 15:861–868.

Spinella M, Cooper ML, Bodnar RJ (1996) Excitatory amino acid antagonists in the rostral ventromedial medulla inhibit mesencephalic morphine analgesia in rats. Pain 64:545–552.

Takahashi M, Senda T, Kaneto H (1991) Role of spinal kappa opioid receptors in the blockade of the development of antinociceptive tolerance to morphine. Eur J Pharmacol 200:293–297.

Takemori AE, Loh HH, Lee NM (1992) Suppression by dynorphin A-(1–13) of the expression of opiate withdrawal and tolerance in mice. Eur J Pharmacol 221:223–226.

Tao PL, Hwang CL, Chen CY (1994) U-50, 488 blocks the development of morphine tolerance and dependence at a very low dose in guinea pigs. Eur J Pharmacol 256:281–286.

Tershner SA, Mitchell JM, Fields HL (2000) Brainstem pain modulating circuitry is sexually dimorphic with respect to mu and kappa opioid receptor function. Pain 85:153–159.

Trujillo KA, Akil H (1991) Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251:85–87.

Trujillo KA, Akil H (1995) Excitatory amino acids and drugs of abuse: a role for *N*-methyl-D-aspartate receptors in drug tolerance, sensitization and physical dependence. Drug Alcohol Depend 38:139–154.

Urban MO, Gebhart GF (1998) The glutamate synapse: a target in the pharmacological management of hyperalgesic pain states. Prog Brain Res 116:407–420.

Urban MO, Gebhart GF (1999) Supraspinal contributions to hyperalgesia. Proc Natl Acad Sci USA 96:7687–7692.

Wagner JJ, Terman GW, Chavkin C (1993) Endogenous dynorphins inhibit excitatory neurotransmission and block LTP induction in the hippocampus. Nature 363:451–454.

Weisskopf MG, Zalutsky RA, Nicoll RA (1993) The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. Nature 362:423–427.

Zhuo M, Gebhart GF (1997) Biphasic modulation of spinal nociceptive transmission from the medullary raphe nuclei in the rat. J Neurophysiol 78:746–758.