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Kappa Opioids Inhibit Physiologically Identified Medullary Pain Modulating Neurons and Reduce Morphine Antinociception

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Meng, I. D., J. P. Johansen, I. Harasawa, and H. L. Fields. Kappa opioids inhibit physiologically identified medullary pain modulating neurons and reduce morphine antinociception. *J Neurophysiol* 93: 1138–1144, 2005. First published September 29, 2004; doi:10.1152/jn.00320.2004. Microinjection of kappa opioid receptor (KOR) agonists into the rostral ventromedial medulla (RVM) attenuates mu-opioid receptor mediated antinociception and stress-induced analgesia, yet is also reported to have an analgesic effect. To determine how KOR agonists produce both antinociceptive and antianalgesic actions within the RVM, the KOR agonist U69593 was microinjected directly into the RVM while concurrently monitoring tail flick latencies and RVM neuronal activity. Among RVM neurons recorded *in vivo*, two types show robust changes in activity just prior to the nocifensive tail flick reflex: ON cells burst just prior to a tail flick and their activity is pronociceptive, whereas OFF cells pause just prior to the tail flick and their activity is antinociceptive. Although RVM microinjection of U69593 did not affect tail flick latencies on its own, it did attenuate the ON cell burst, an effect blocked by co-injection of the KOR antagonist, nor-binaltorphimine (nor-BNI). Furthermore, U69593 inhibited ongoing activity in subsets of OFF cells (4/11) and NEUTRAL cells (3/9). Microinjection of U69593 into the RVM also attenuated morphine antinociception and suppressed the excitation of OFF cells. Together with previous *in vivo* and *in vitro* studies, these results are consistent with the idea that KOR agonists can be either pronociceptive through direct inhibition of OFF cells, or antianalgesic through both postsynaptic inhibition and presynaptic inhibition of glutamate inputs to RVM OFF cells.

INTRODUCTION

Kappa and mu opioid receptors (KORs and MORs, respectively) are members of a family of G protein-coupled receptors that have overlapping anatomical distributions and similar pre- and postsynaptic inhibitory actions (Gustein et al. 1998; Mansour et al. 1995; Margolis et al. 2003; Pan et al. 1997). Ligands for the two receptor types can produce distinct and often opposing behavioral effects (Pan 1998; Shippenberg et al. 1993; Spanagel et al. 1992). For example, MOR agonists microinjected into such pain-modulating regions as the mid-brain periaqueductal gray (PAG) or rostroventromedial medulla (RVM) consistently produce robust antinociception (Fields and Basbaum 1999; Yeung et al. 1977). In contrast, although KOR agonists microinjected into the RVM produce antinociception in some tests (Ackley et al. 2001; Tershner et al. 2000), they can also powerfully attenuate MOR or stress elicited antinociception (Foo and Helmstetter 2000; Pan et al. 1997).

Critical to understanding the distinct and sometimes opposing effects of MOR and KOR agonists on nociception is knowing their effect on the different subpopulations of pain-modulating neurons. Three types of RVM neurons have been identified *in vivo* (Fields et al. 1983a): ON cells show a burst of activity beginning just prior to the tail flick withdrawal reflex, OFF cells cease firing prior to the tail flick, and NEUTRAL cells do not consistently change their activity. MOR agonists, at antinociceptive doses, decrease ON cell activity, increase OFF cell activity, and have no effect on NEUTRAL cells (Fields et al. 1983b; Gao et al. 1998; Heinricher et al. 1992, 1994). The increase in OFF cell activity, and the reduction in its tail flick related pause, contributes to the antinociceptive effect of MOR ligands (Heinricher et al. 1994; McGaraughty and Heinricher 2002).

In vitro studies have revealed two distinct populations of RVM neurons: primary and secondary cells. MOR agonists presynaptically inhibit GABAergic inputs to primary cells but have no direct postsynaptic action on them (Pan et al. 1990). In contrast, MOR agonists directly hyperpolarize secondary cells. Based on their response to MOR agonists, secondary cells have been proposed to represent ON cells recorded *in vivo*, whereas primary cells would include both NEUTRAL and OFF cells (Fields et al. 1983b; Gao et al. 1998; Heinricher et al. 1992, 1994).

Although the response of physiologically characterized RVM neurons to KOR agonists has not been tested *in vivo*, studies using whole cell recordings in RVM slices have shown both pre- and postsynaptic effects (Ackley et al. 2001; Bie and Pan 2003; Marinelli et al. 2002; Pan et al. 1997). KOR agonists directly hyperpolarize a subset of the primary cells but have no postsynaptic effect on secondary cells (Ackley et al. 2001; Bie and Pan 2003; Pan et al. 1997; but see Marinelli et al. 2002). KOR agonists also presynaptically inhibit glutamate inputs to both primary and secondary cells (Ackley et al. 2001; Bie and Pan 2003).

Kappa-responsive primary cells have been suggested to represent physiologically characterized OFF cells (Pan et al. 1997). According to this hypothesis, direct postsynaptic inhibition of OFF cells by KOR agonists would account for their ability to attenuate MOR-mediated antinociception. Alternatively, presynaptic inhibition of glutamate inputs to OFF cells could also contribute to the MOR opposing effects of kappa-opioids (Bie and Pan 2003). In support of this hypothesis, microinjection of glutamate receptor antagonists into the RVM

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attenuates morphine activation of OFF cells and antinociception (Bie and Pan 2003; Heinricher et al. 1999, 2001b; Spinella et al. 1996).

This study is the first to examine the effect of a KOR agonist on the activity of physiologically characterized RVM neurons in vivo. A major question addressed was whether the pre- and postsynaptic inhibition of primary cells observed in vitro corresponds to the inhibition of OFF and/or NEUTRAL cells. As a corollary to this issue, to elucidate how KOR agonists act to oppose MOR-mediated antinociception, we investigated the effect of a KOR agonist microinjected into the RVM on activation of OFF cells and antinociception produced by systemic morphine.

METHODS

Experimental animals and surgery

All experiments were performed after the review and approval of the Institutional Animal Care and Use Committee at the University of California, San Francisco. Male Sprague-Dawley rats (325–450 g, Bantin and Kingman, Hayward, CA) were injected with sodium pentobarbital (60–70 mg/kg, ip), and a catheter was inserted into the external jugular vein for administration of anesthetics and, in some experiments, morphine. After placing the rat into a stereotaxic holder, a hole was drilled in the interparietal bone for insertion of an electrode/cannula assembly into the medulla. A pair of needle electrodes was inserted into the sacral longitudinal paraspinous muscles to record the initiation of the tail flick reflex. The ventral surface of tail was blackened, and body temperature was maintained at 37°C with a heating pad. Anesthesia was maintained with a constant, continuous infusion of sodium methohexital (30–50 mg/kg/h, iv). Prior to recording, anesthesia level was adjusted so that tail flicks could be elicited with a consistent latency (3.5–5.0 s) without any signs of discomfort using a feedback controlled projector lamp. Electrophysiological recordings were initiated ≥ 45 min after completion of the surgery. Electrode penetrations were made along midline, 2.0–2.3 mm caudal to interaural zero, and recordings were initiated 8.0–9.5 mm below the surface of cerebellum. Only one neuron was recorded per animal.

Analgesimetric testing

Tail flicks were evoked every 3 min using radiant heat applied 2–6 cm from the distal end of the tail. From a holding temperature of 35°C, the temperature increased linearly to a plateau between 48 and 53°C. To prevent tissue damage, the stimulus was automatically terminated after 10 s in the absence of a tail flick.

Tail flicks were elicited every 3 min for ≥ 15 min before and 30–60 min after drug injection.

Extracellular recording and drug microinjection

An electrode/cannula assembly was constructed for extracellular recordings and drug infusions as previously described (Harasawa et al. 2000; Meng and Johansen 2004). Briefly, a tungsten microelectrode (2–4 M Ω , FHC, Bowdoinham, ME) was glued parallel to a 30-gauge stainless steel cannula with a separation of 300–600 μ m between each tip. The electrode and cannula were inserted in a rostro-caudal alignment with the electrode rostral. The injection cannula was attached to a 50- μ l Hamilton microsyringe by PE-10 tubing, and infusions were conducted over a period of 3–5 min using a syringe pump. Movement of a small air bubble in the PE-10 tubing was monitored to ensure drug delivery.

Action potentials were displayed on a digital oscilloscope and isolated using a window discriminator. Data were acquired through a data acquisition board interfaced with a Macintosh G4 computer

programmed in LabVIEW (National Instruments, Austin, TX) (Budai 1994). ON, OFF, and NEUTRAL cells were categorized according to their pattern of neuronal activity related to the tail flick (Fields et al. 1983a). ON cells were identified by an onset of neuronal activity that occurred just prior to the tail flick (ON cell burst), whereas OFF cells ceased firing prior to the tail flick (OFF cell pause). NEUTRAL cells did not show any change in activity associated with the tail flick or with noxious paw pinch.

Experimental protocols and data analysis

Pilot studies indicated that the KOR selective agonist, U69593, produced only inhibitory effects on RVM neurons recorded in vivo when locally microinjected. The first set of experiments tested the effect of U69593 on the ongoing activity of OFF and NEUTRAL cells and the tail flick-related excitation of ON cells. Once a unit was isolated and characterized, baseline activity was measured for 30 min prior to and 30–60 min following RVM drug microinjection. For ON cells, baseline tail flick latencies were measured every 3 min beginning 15–18 min prior to drug microinjection. A total volume of 200 nl of drug was injected following the baseline period.

Ongoing activity was calculated as the average frequency over a 60-s period prior to heat onset. To determine the effect of drug microinjections on ON cell-evoked activity, the tail flick-related burst was calculated as the average frequency of activity for a 10-s epoch following the heat onset. Pre-drug, baseline tail flick latencies, ongoing activity, and ON cell neuronal responses were averaged for three stimulation trials just before the RVM microinjections.

Data were normalized and experimental groups were compared using a two-way ANOVA for repeated measures with Newman-Keul's test for post hoc comparisons. Inhibition of OFF and NEUTRAL cell ongoing activity was defined as a $>50\%$ reduction in neuronal activity over a 30-min period after the microinjection of drug. Treatment group comparison of the number of neurons showing inhibition versus those showing no inhibition was performed using the nonparametric Fisher's exact probability test.

A second set of experiments determined the effect of U69593 on systemic morphine antinociception and modulation of OFF cell activity. While recording from OFF cells, RVM microinjections of drug (200 nl) were given following at least six stable baseline tail flicks. Five minutes after completion of the RVM microinjection, morphine sulfate (1.0 mg/kg, iv) was administered. OFF cell activity and tail flick latencies were recorded for ≥ 30 min following morphine administration. Tail flick latencies, ongoing activity (average activity for 60 s prior to heat stimulation), and the tail flick-related inhibition of activity (OFF cell pause) were determined before and after treatment. Baseline tail flick latencies and baseline OFF cell activity were calculated as the average of the four tail flick trials directly preceding the RVM microinjection.

The OFF cell pause was calculated by dividing the rate of activity during a 4-s epoch centered around the mean baseline tail flick latency (average latency from baseline data pooled from all experiments ± 2 s) by the average rate of activity during the 10-s period prior to heat onset for each trial and multiplying the result by 100. Using this calculation, a value of 100% indicates no decrease in activity (full morphine effect), whereas 0% indicates a complete cessation of activity during the entire 4-s epoch (i.e., no morphine effect). This type of analysis is similar to that previously performed by Heinricher et al. (2001a,b). Average baseline values for the OFF cell pause were compared with the 5- to 15- and 20- to 30-min postmorphine values. Group comparisons during these time-points for all data were performed using a two-way ANOVA for repeated measures with Newman-Keul's test for post hoc comparisons. Baseline ongoing activity between treatment groups was compared using Student's *t*-test. In all cases, $P < 0.05$ was considered significant.

Drugs

The KOR agonist, U69593 (1.8 mg/ml, Sigma), and antagonist, nor-binaltorphimine (20 μ g/ml, nor-BNI, Sigma), were dissolved in 45% 2-hydroxypropyl- β -cyclodextrin (HBC, Sigma).

Histological verification

Electrolytic lesions were performed at the conclusion of each experiment to mark the recording site. Animals were given an overdose of intravenous sodium pentobarbital and perfused transcardially with saline followed by 10% formalin. The brain was removed, postfixed in 10% formalin followed by 30% sucrose, and cut on a freezing microtome. Sections were stained with cresyl violet (0.1%), and the location of recording sites was determined according to the atlas of Paxinos and Watson (1986).

RESULTS

In the first experiment, tail flick latency and activity of ON, OFF, and NEUTRAL cells were examined following the RVM microinjection of the KOR agonist, U69593. Analysis was performed from 50 rats in which successful injections were made while maintaining stable extracellular recordings for ≥ 30 min after injection. Histological verification of recording sites revealed electrolytic lesions located within the nucleus raphe magnus, the nucleus reticularis gigantocellularis pars alpha, and the nucleus reticularis paragigantocellularis. Comparison of baseline ongoing activity between the U69593 and U69593 plus no-BNI co-injection groups for ON, OFF, and NEUTRAL cells revealed no differences (*t*-test, $P > 0.05$). Baseline ongoing activity for ON cells treated with U69593 or U69593 plus nor-BNI was 4.6 ± 2.6 and 1.2 ± 0.8 spikes/s, respectively; baseline ongoing activity for OFF cells treated with U69593 or U69593 plus nor-BNI was 10.9 ± 2.6 and 9.3 ± 2.3 spikes/s, respectively; baseline ongoing activity for NEUTRAL cells treated with U69593 or U69593 plus nor-BNI was 14.0 ± 2.4 and 12.7 ± 2.0 spikes/s, respectively.

U69593 reduced the ON cell burst for the entire period of observation after RVM injection (Fig. 1A), but did not affect ON cell ongoing activity (data not shown). Although baseline tail flick latencies were not affected by U69593 microinjections (3.3 ± 0.1 s preinjection vs. 3.6 ± 0.3 s postinjection), group data revealed a $>50\%$ mean reduction in the ON cell burst following microinjection of U69593, which was significantly less than the ON cell burst following co-infusion of nor-BNI with U69593 [$F(1,11) = 4.99$, $P < 0.05$; Fig. 1B]. The HBC vehicle has previously been shown to have no effect on ON cell activity or tail flick latencies when microinjected into the RVM (Meng and Johansen 2004).

The consistent attenuation of the ON cell burst was in marked contrast to the variable effect of U69593 on OFF and NEUTRAL cells. OFF and NEUTRAL cells fell into two distinct categories based on the effect of U69593 on ongoing activity: they were either nearly completely inhibited or showed almost no change in activity. Of 11 OFF cells tested with U69593, 4 were almost completely inhibited ($>90\%$ reduction in activity over the 30-min period following injection), while the remaining 7 cells were unaffected ($<50\%$ change in activity following injection, see Fig. 2A for example of inhibition). Similarly, the ongoing activity of 3/9 NEUTRAL cells was reduced following U69593

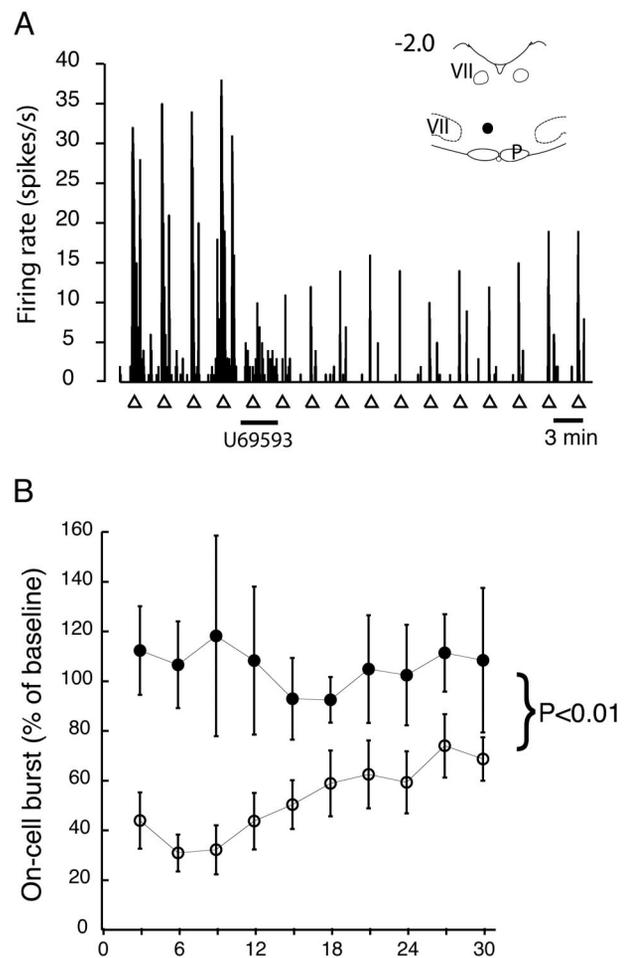


FIG. 1. Microinjection of U69593 attenuates ON cell tail flick-related activity. A: demonstration of ON cell inhibition following the microinjection of U69593. Drug was injected over a 4-min period following 6 control trials. Although tail flick latencies remained unchanged, the ON cell burst remained attenuated throughout the observation period. Δ , tail flick occurrence. 1-s bins. *Inset*: histologically verified location of recording site based on center of electrolytic lesion (\bullet). VII, facial nucleus and ascending fibers of the facial nerve; P, pyramidal tract. Number to the left of section indicates distance caudal to interaural 0. B: group data showing a reduction in the noxious stimulation evoked activity of ON cells (ON cell burst) following U69593 compared with activity following the co-injection of U69593 and nor-binaltorphimine (nor-BNI). Tail flicks were evoked every 3 min for ≥ 30 min after the injection of drug into the rostral ventromedial medulla (RVM). \circ , microinjection of U69593 ($n = 6$); \bullet , microinjection of U69593 with nor-BNI ($n = 7$). Values represent means \pm SE.

microinjections (see Fig. 2B for example of inhibition). Co-infusion of U69593 and nor-BNI was tested in a total of 17 cells (8 OFF, 9 NEUTRAL) and found to produce no reduction in activity in any of the 17 cells. Data from a previous study also found no change in OFF cell activity following microinjection of the HBC vehicle ($n = 6$) (Meng and Johansen 2004). A comparison of the total number of OFF and NEUTRAL cells inhibited ($>50\%$ reduction) by U69593 versus the number of cells inhibited by U69593 plus nor-BNI revealed a significant difference (Fisher's exact probability test, $P < 0.03$).

In the second experiment, we examined the effect of RVM U69593 on the activation of OFF cells by systemic morphine. OFF cells that showed a robust reduction in ongoing activity after the RVM U69593 microinjection but before morphine

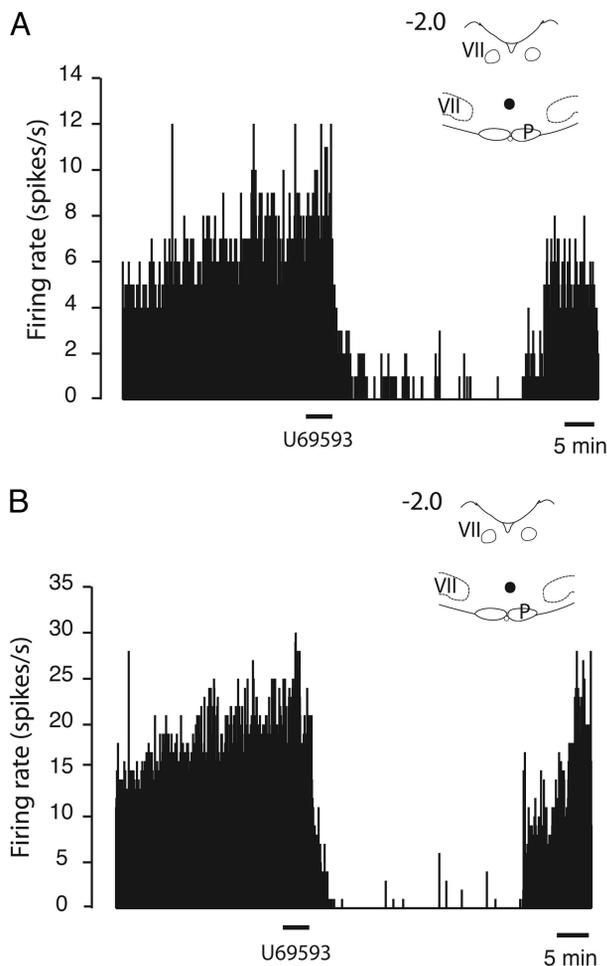


FIG. 2. Example of a typical U69593-sensitive OFF cell (A) and NEUTRAL cell (B). Microinjection of U69593 inhibited ongoing activity in only a subset of OFF and NEUTRAL cells. In these U69593-sensitive neurons, inhibition was almost complete for ~ 30 min after completion of the injection. Following initial characterization of the cells, tail flicks were not performed during the baseline or postdrug injection period in these experiments (see METHODS). 1-s bins. Inset: see Fig. 1A, inset.

administration were excluded from this analysis ($n = 2$), so that effects selective for morphine-induced increases in OFF cell activity could be examined. Baseline ongoing activity for OFF cells treated with U69593 (9.1 ± 2.6 spikes/s, $n = 8$), U69593 plus nor-BNI (7.9 ± 2.0 spikes/s, $n = 10$), and vehicle (15.8 ± 5.1 spikes/s, $n = 5$) were not different [$F(2,20) = 1.75$, $P = 0.2$]. Microinjection of U69593 delayed morphine inhibition of the tail flick reflex compared with RVM vehicle controls and co-administration of U69593 with nor-BNI [$F(2,9) = 3.48$, $P < 0.05$; Fig. 3]. Recovery from the U69593-induced reduction in morphine antinociception was complete by 20–30 min after morphine administration.

Concomitant with the attenuation of behavioral antinociception, RVM microinjections of U69593 reduced the effect of morphine on OFF cell activity (Fig. 4). In U69593-treated rats, OFF cell ongoing activity did not significantly increase after morphine administration and remained significantly lower than in rats microinjected with vehicle or with U69593 plus nor-BNI [$F(2,18) = 9.24$, $P < 0.002$; Fig. 5A]. Furthermore, morphine did not significantly reduce the OFF cell pause following RVM microinjection of U69593, although significant

decreases were apparent in vehicle and U69593 plus nor-BNI treated rats [$F(1,20) = 11.36$, $P < 0.01$; Fig. 5B]. The effect of U69593 on the OFF cell pause showed a similar time course to its effect on behavioral antinociception. At 20- to 30-min postmorphine, the OFF cell pause was reduced even in U69593-injected rats (from a premorphine control of 63 ± 11 to $86 \pm 13\%$ 20- to 30-min postmorphine, paired t -test, $P < 0.05$). In contrast, OFF cell baseline activity remained suppressed during this period ($113 \pm 30\%$ of control). Injection of saline through the catheter did not affect OFF cell activity ($n = 3$, data not shown).

DISCUSSION

In vitro studies using the RVM slice have revealed both pre- and postsynaptic actions of KOR agonists, and results from these experiments have provided a framework for understanding the variable effect of KOR agonists on nociception (Ackley et al. 2001; Bie and Pan 2003; Pan et al. 1997). To provide a link between the synaptic actions of KOR agonists in vitro and the behavioral effects of KOR agonists microinjected into the RVM, this study examined the effect of a KOR selective agonist on physiologically characterized RVM neurons in vivo while measuring nociceptive behaviors.

In the RVM slice, two types of neurons with distinct responses to MOR and KOR agonists have been shown (Pan et al. 1990, 1997). Secondary cells are postsynaptically hyperpolarized by MOR but not KOR agonists. In contrast, KOR, but not MOR, agonists directly hyperpolarize approximately one-half of RVM primary cells. The relationship between primary and secondary cells recorded in vitro and ON, OFF, and NEUTRAL cells characterized in vivo can be inferred based on their response to opiates. Physiologically characterized ON cells correspond to secondary cells, since they are the only cell type inhibited by either RVM microinjection or local iontophoresis

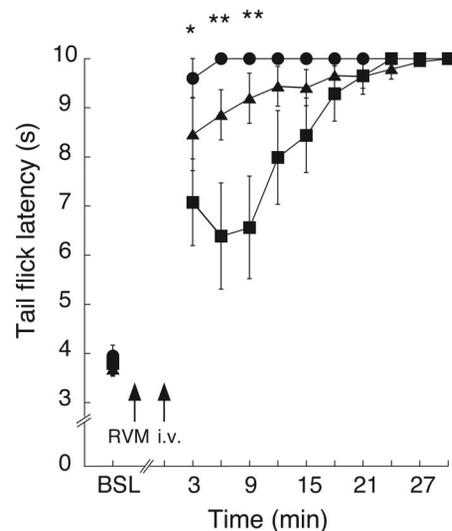


FIG. 3. Microinjection of U69593 into the RVM delayed the onset of antinociception produced by intravenous morphine. Reduction in tail flick inhibition following microinjection of U69593 was significant for early time-points ($n = 8$) compared with animals microinjected with either U69593 plus nor-BNI ($n = 10$), or 2-hydroxypropyl- β -cyclodextrin (HBC) vehicle ($n = 5$). At later time-points, tail flick inhibition was similar in all groups. ■, U69593; ▲, U69593 + nor-BNI; ●, HBC. Values represent means \pm SE. * $P < 0.05$, ** $P < 0.01$.

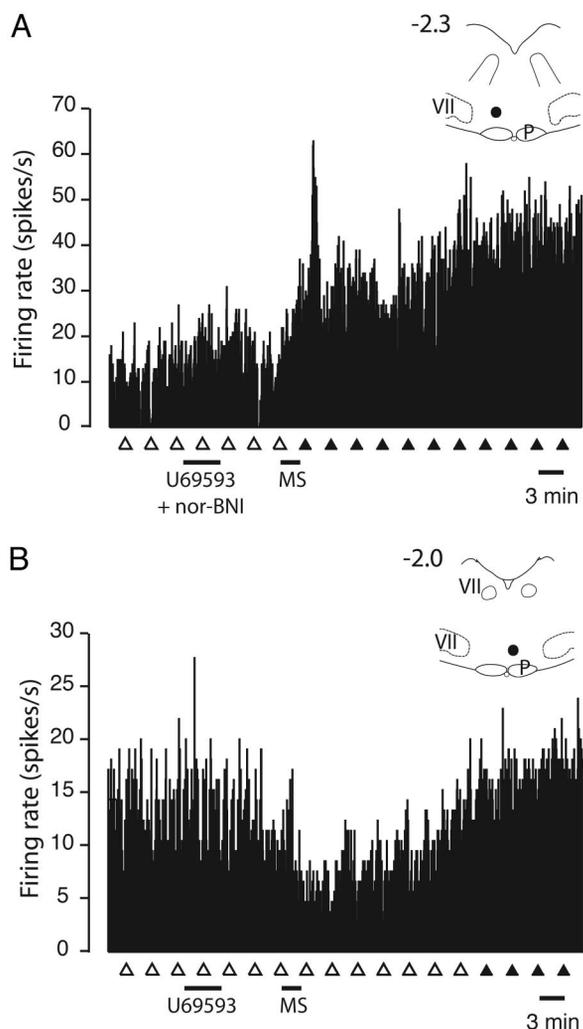


FIG. 4. Example OFF cells in morphine-treated rats. *A*: systemic morphine given after an RVM microinjection of U69593 plus nor-BNI increased OFF cell activity and eliminated the tail flick. Postmorphine ongoing activity increased to 294% of control during the period of analysis (5–15 min after morphine injection; see METHODS). *B*: microinjection of U69593 prevented the increase in OFF cell activity (ongoing activity = 95% of control) and delayed the analgesic response to morphine. The OFF cell pause cannot be identified in these cells because of the scale. Δ , trials in which tail flicks were elicited; \blacktriangle , trials in which the tail flick was blocked. MS, morphine sulfate (1.0 mg/kg, iv). 1-s bins. *Inset*: see Fig. 1A, *inset*.

of MOR agonists (Heinricher et al. 1992, 1994). A corollary is that primary neurons include both OFF and NEUTRAL cells.

KOR inhibition of spontaneous activity of OFF and NEUTRAL cells

Our demonstration that KOR agonists robustly inhibit the baseline activity of a subset of OFF and NEUTRAL cells suggests that the primary cells hyperpolarized by KOR agonists in vitro do not correspond to a single in vivo cell class, but rather include subpopulations of both OFF and NEUTRAL cells. Although inhibition by presynaptic mechanisms cannot be ruled out, a presynaptic action on glutamatergic terminals seems unlikely given the inability of iontophoresis of the glutamate receptor antagonist kynurenat to inhibit the spontaneous activity of RVM OFF or NEUTRAL cells under conditions similar to this study (Heinricher and Roychowdhury 1997).

Attenuation of the ON cell burst

Following microinjections of U69593 into the RVM, the ON cell burst was consistently attenuated. Based on data from in vitro studies, this effect is most likely due to presynaptic inhibition of glutamate release. As mentioned, ON cells are directly inhibited by MOR agonists and likely correspond to secondary cells recorded in vitro (Heinricher et al. 1992). KOR agonists do not directly hyperpolarize mu-responsive secondary cells, but they do inhibit glutamatergic transmission onto secondary cells (Ackley et al. 2001; Bie and Pan 2003; Pan et al. 1997). Furthermore, the ON cell burst is reduced by iontophoresis or microinjection of glutamate receptor antagonists (Heinricher and McGaraughty 1998; Heinricher and Roychowdhury 1997; Heinricher et al. 2001b).

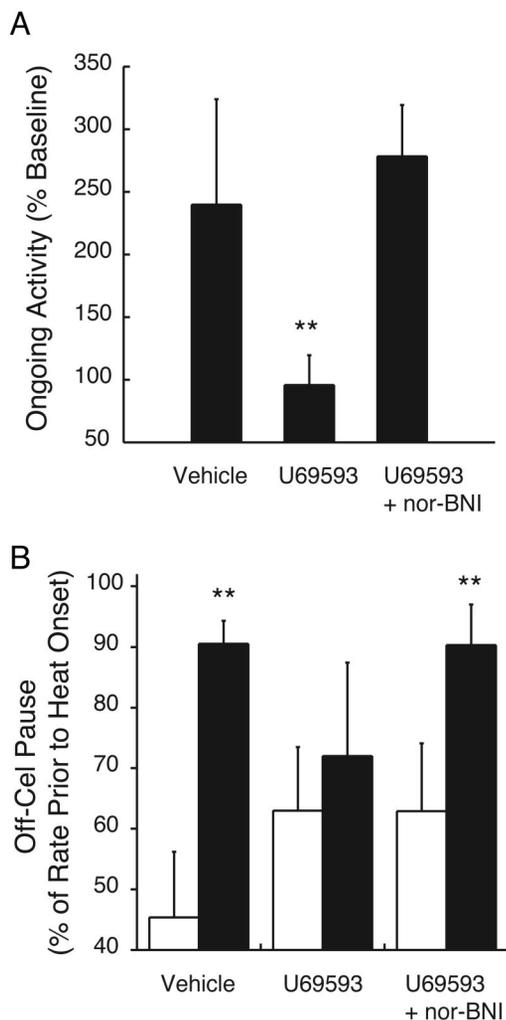


FIG. 5. RVM U69593 prevents the increase in OFF cell activity produced by systemic morphine. *A*: after intravenous morphine (5–15 min), OFF cell ongoing activity increased in rats pretreated with RVM microinjections of vehicle ($n = 5$) or U69593 plus nor-BNI ($n = 10$). In contrast, OFF cell ongoing activity did not increase following morphine administration in rats microinjected with U69593 ($n = 8$). $**P < 0.01$ compared with vehicle and U69593 + nor-BNI treatment groups. *B*: likewise, morphine produced a decrease in the OFF cell pause in rats microinjected with vehicle and U69593 plus nor-BNI. However, morphine did not reduce the off cell pause in rats pretreated with RVM U69593. White bars, baseline values measured prior to morphine and RVM microinjections; black bars, pause measured following morphine and RVM microinjections. $**P < 0.01$ compared with baseline values (white bars). Values represent means \pm SE.

Although microinjection of a KOR agonist into the RVM does not change the tail flick latency (current experiments and Ackley et al. 2001), this result is not surprising since inhibition of ON cells is generally not sufficient to produce antinociception in the tail flick test (Heinricher and McGaraughty 1998). Instead, inhibition of ON cells is more likely to reduce hyperalgesia. Acute, naloxone precipitated opioid abstinence is associated with hyperalgesia as measured by the tail flick reflex (Kim et al. 1990). This state-dependent hyperalgesia requires increased ON cell activity in the RVM (Bederson et al. 1990; Kaplan and Fields 1991). Thus inhibition of ON cells has been proposed to explain the observation that microinjection of U69593 into the RVM reduces withdrawal-induced hyperalgesia (Bie and Pan 2003). This proposal leads to the prediction that other conditions that result from activation of RVM pain-facilitating neurons (presumably ON cells), such as hypersensitivity produced by nerve injury (Gardell et al. 2003; Kovelowski et al. 2000; Porreca et al. 2001), will also be relieved by KOR agonists. These findings suggest an interesting and clinically important line of future research.

KOR reduction of morphine antinociception and OFF cell discharge

Previous studies have reported anti-MOR actions produced by RVM administration of a KOR agonist. Microinjection of U69593 into the RVM attenuated antinociception produced by [D-Ala(2)-MePhe(4)-Gly-ol(5)]enkephalin microinjections into the PAG (Pan et al. 1997), and stress-induced antinociception, which also involves release of endogenous MOR agonists within the RVM, is reduced by the RVM microinjection of a KOR agonist (Foo and Helmstetter 2000). In this study, we found that U69593 microinjected into the RVM consistently attenuates the behavioral antinociception produced by systemic morphine. The mechanisms involved in these kappa anti-mu actions appear to involve both presynaptic inhibition of glutamatergic transmission onto OFF cells and direct postsynaptic hyperpolarization of a subset of OFF cells.

Activation of OFF cells by RVM microinjections of mu- or delta-opioid receptor agonists, cannabinoid receptor agonists, or the GABA_A receptor antagonist bicuculline produces antinociception (Harasawa et al. 2000; Heinricher and Tortorici 1994; Heinricher et al. 1994; Meng and Johansen 2004). In these cases, OFF cells are activated by disinhibition, either directly, as is the case with a GABA_A antagonist, or through presynaptic inhibition of GABA release. In contrast, when antinociception is produced by activation of the PAG-RVM circuit either by local PAG activation or systemic morphine, the release of glutamate in the RVM drives OFF cell activity. Glutamate release in the RVM can be measured after PAG stimulation (Beitz 1990), and microinjection of a glutamate receptor antagonist into the RVM attenuates systemic morphine and PAG stimulation produced antinociception (Aimone and Gebhart 1986; Bie and Pan 2003; Heinricher et al. 1999, 2001b; Spinella et al. 1996). Similar to the present observation for KOR agonists, glutamate receptor antagonists microinjected into the RVM prevent the activation of OFF cells and antinociceptive effect produced by systemic morphine (Heinricher et al. 1999, 2001b).

Initially, the anti-mu actions of KOR agonists microinjected into the RVM were proposed to be due to postsynaptic inhi-

bition of primary cells (Pan et al. 1997), which include both OFF and NEUTRAL cell populations. It now seems that KOR presynaptic inhibition of glutamate release also plays an important role in opposing mu-opiate mediated antinociception, given that the spontaneous activity of only a minority of OFF cells is inhibited by RVM microinjections of U69593, and KOR agonists reduce glutamatergic transmission onto all primary cells (a subset of which are OFF cells). In this study, KOR effects on morphine activation of OFF cells were shown in those OFF cells whose spontaneous activity was not inhibited by U69593. The selective effect of KOR on morphine-evoked activation but not spontaneous activity of OFF cells is critical evidence supporting a presynaptic KOR action in a significant proportion of RVM OFF cells. Additionally, our finding that RVM U69593 prevents the activation of OFF cells by systemic morphine in a manner similar to glutamate receptor antagonists is consistent with the hypothesis that presynaptic inhibition of glutamate release is an important mechanism through which KOR agonists antagonize morphine antinociception (Heinricher et al. 1999, 2001b).

KOR agonists microinjected into the RVM have a wide range of effects on nociceptive thresholds, depending on the type of noxious stimulus or behavioral measure of nociception, the administration of MOR agonists, and the animal's sex (Ackley et al. 2001; Bie and Pan 2003; Tershner et al. 2000). The current experiments have established that KOR agonists reduce the ON cell burst, inhibit spontaneous activity in a subset of NEUTRAL and OFF cells, and reduce both the behavioral antinociceptive effect and the OFF cell activation produced by systemic morphine. Together with previous in vitro neuropharmacology and behavioral studies, the current in vivo recordings of physiologically identified RVM neurons support the hypothesis that, through control of glutamate release, endogenous KOR agonists in the RVM regulate behavioral responses to noxious stimulation in a state-dependent manner. Selective inhibition of MOR activated OFF cells accounts for the antianalgesic effect of KOR agonists. Selective reduction of the ON cell burst would explain antihyperalgesic effects of KOR agonists.

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