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

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Abstract

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 anti-analgesic 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 pro-nociceptive, 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, the present results are consistent with the idea that KOR agonists can be either pro-nociceptive through direct inhibition of off-cells, or anti-analgesic through both postsynaptic inhibition and presynaptic inhibition of glutamate inputs to RVM off-cells.
Introduction.

Kappa- and mu opioid receptors (KOR and MOR) 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; North 1991; 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 midbrain periaqueductal gray 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), it 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; Heinricher et al. 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; Heinricher et al. 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 demonstrated both pre- and post-synaptic 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, pre-synaptic 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 attenuate morphine activation of off-cells and antinociception (Bie and Pan 2003; Heinricher et al. 1999; Heinricher et al. 2001b; Spinella et al. 1996).

The present 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.

**Materials and 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, i.p.) 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, i.v.). 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 at least 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.

**Analgesiometric 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 48-53°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 at least 15 min prior to and 30-60 min following 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 MΩ, FHC, Bowdoinham, ME) was glued parallel to a 30 gauge stainless steel cannula with a separation of 300-600 μm 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 μl 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 was 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 demonstrate 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 three 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 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 greater than 50% reduction in neuronal activity over a 30 min period after the microinjection of drug. Treatment group comparison of the number of neurons demonstrating 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, i.v.) was administered. Off-cell activity and tail flick latencies were recorded for at least 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. (Heinricher et al. 2001a; Heinricher et al. 2001b). Average baseline
values for the off cell pause were compared to the 5-15 min and 20-30 min post-
morphine 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 µg/ml, nor-BNI, Sigma), were dissolved in 45% 2-hydroxypropyl-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, post-fixed 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 (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 at least 30 min post-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 spikes/s 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 spikes/s 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 spikes/s 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 pre-injection versus 3.6 ± 0.3 s post-injection), group data revealed a greater than 50 percent 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 (F1, 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. Out 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 microinjections (see 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 demonstrated a robust reduction in ongoing activity after the RVM U69593 microinjection but before morphine 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 (F2, 20 = 1.75, P = 0.2). Microinjection of U69593 delayed morphine inhibition of the tail flick reflex when compared to 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 (F1, 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 20-30 min post-morphine, the off-cell pause was reduced even in U69593 injected rats (from a pre-morphine control of 63±11% to 86±13% 20-30 min post-morphine, paired t-test, P<0.05). By contrast, off-cell baseline activity remained suppressed during this period (113±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 post-synaptic 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, the present 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 demonstrated (Pan et al. 1990; Pan et al. 1997). Secondary cells are postsynaptically hyperpolarized by MOR but not KOR agonists. In contrast, KOR, but not MOR agonists, directly hyperpolarize approximately 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 of MOR agonists (Heinricher et al. 1992; Heinricher et al. 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 pre-synaptic
mechanisms cannot be ruled out, a pre-synaptic action on glutamatergic terminals seems unlikely given the inability of iontophoresis of the glutamate receptor antagonist kynurenate to inhibit the spontaneous activity of RVM off- or neutral cells under conditions similar to the present 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 pre-synaptic 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).

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 DAMGO 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 the present 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 pre-synaptic inhibition of glutamatergic transmission onto off-cells and direct post-synaptic 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<sub>δ</sub> receptor antagonist bicuculline produces antinociception (Harasawa et al. 2000; Heinricher et al. 1994; Heinricher and Tortorici 1994; Meng and Johansen 2004). In these cases, off-cells are activated by disinhibition, either directly, as is the case with a GABA<sub>δ</sub> 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; Heinricher et al. 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; Heinricher et al. 2001b).

Initially, the anti-mu actions of KOR agonists microinjected into the RVM were proposed to be due to post-synaptic inhibition of primary cells (Pan et al. 1997), which include both off- and neutral cell populations. It now appears that KOR pre-synaptic 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 the present study, KOR effects on morphine activation of off-cells were demonstrated 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 pre-synaptic inhibition of glutamate release is an important
mechanism through which KOR agonists antagonize morphine antinociception (Heinricher et al. 1999; Heinricher et al. 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 anti-analgesic effect of KOR agonists. Selective reduction of the on-cell burst would explain anti-hyperalgesic effects of KOR agonists.

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References.


Figure legends.

Figure 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 six control trials. Although tail flick latencies remained unchanged, the on-cell burst remained attenuated throughout the observation period. Open triangles indicate tail flick occurrence. 1 sec bins. Inset: Histologically verified location of recording site based on center of electrolytic lesion (circle). VII, facial nucleus and ascending fibers of the facial nerve; P, pyramidal tract. Number to the left of section indicates distance caudal to interaural zero. B) Group data demonstrating a reduction in the noxious stimulation evoked activity of on-cells (on-cell burst) following U69593 when compared to activity following the co-injection of U69598 and nor-BNI. Tail flicks were evoked every 3 min for at least 30 min after the injection of drug into the RVM. Open circles, microinjection of U69598 (n=6); closed circles, microinjection of U69598 with nor-BNI (n=7). Values represent mean ± SEM.

Figure 2. Example of a typical U69593 sensitive off-cell (A) and neutral cell (B). Microinjection of U69593 inhibited ongoing activity in only a sub-set of off- and neutral cells. In these U69593 sensitive neurons, inhibition was almost complete for approximately 30 min after completion of the injection. Following initial characterization of the cells, tail flicks were not performed during the baseline or post-drug injection period in these experiments (see Methods). 1 sec bins. Inset: see Fig. 1A inset.
Figure 3. Microinjection of U69593 into the RVM delayed the onset of antinociception produced by intravenous morphine. The reduction in tail flick inhibition following microinjection of U69593 was significant for early time points (n= 8) when compared to animals microinjected with either U69593 plus nor-BNI (n= 10), or 2-hydroxypropyl-[-]-cyclodextrin (HBC) vehicle (n= 5). At later time points, tail flick inhibition was similar in all groups. Squares, U69593; Triangles, U69593 + nor-BNI; Circles, HBC. Values represent mean ± SEM. * p<0.05, ** p<0.01.

Figure 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. Post-morphine ongoing activity increased to 294% of control during the period of analysis (5-15 min post-morphine injection (see Methods)). B) The 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. Open triangles indicate trials in which tail flicks were elicited, whereas closed triangles indicate trials in which the tail flick was blocked. MS, morphine sulfate (1.0 mg/kg, i.v.). 1 sec bins. Inset: see Fig. 1A inset.

Figure 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 pre-treated with RVM microinjections of vehicle (n = 5) or U69593 plus nor-BNI (n =10). By contrast, off-cell ongoing activity did not increase following morphine.
administration in rats microinjected with U69593 (n = 8). ** p<0.01 compared to 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 pre-treated 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 when compared to baseline values (white bars). Values represent mean ± SEM.
Figure 1

A) Firing rate (spikes/s)

B) On-cell burst (% of baseline)

P<0.01
Figure 2

A) Firing rate (spikes/s) over time with U69593 applied for 5 minutes.

B) Similar data presentation as in A, but with different spike counts.

Figure 2
Figure 4

A) Firing rate (spikes/s)

- 3 min
- MS U69593 + nor-BNI

B) Firing rate (spikes/s)

- 3 min
- U69593 MS
A) Ongoing Activity (% Baseline)

- Vehicle
- U69593
- U69593 + nor-BNI

B) Off-Cel Pause (% of Rate Prior to Heat Onset)

- Vehicle
- U69593
- U69593 + nor-BNI

Figure 5