Comparison of Morphine and Kainic Acid Microinjections Into Identical PAG Sites on the Activity of RVM Neurons

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Tortorici, V. and M. M. Morgan. Comparison of morphine and kainic acid microinjections into identical PAG sites on the activity of RVM neurons. J Neurophysiol 88: 1707–1715, 2002; 10.1152/jn.00069.2002. The rostral ventromedial medulla (RVM) modulates nociception through changes in the activity of two classes of neuron, ON- and OFF-cells. The activity of these neurons is regulated, in part, by input from the periaqueductal gray (PAG). The objective of this study was to determine whether PAG-mediated antinociception is associated with excitation of both ON- and OFF-cells in the RVM. Microinjection of morphine into the ventrolateral PAG produced antinociception at 50% of the injection sites. This antinociception was associated with continuous activation of RVM OFF-cells. In contrast, microinjection of kainic acid directly excites PAG output neurons, the changes in ON- and OFF-cell activity associated with microinjection of kainic acid into the ventrolateral PAG were the same as when morphine was injected. Neutral cells show no consistent change in activity associated with nociceptive reflexes and their function remains unclear.

The antinociceptive properties of opioids are mediated, at least in part, by their action on supraspinal structures such as the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). These structures produce antinociception via a descending pathway that runs from the PAG to RVM to spinal and trigeminal dorsal horns (Basbaum and Fields 1984). Microinjection of morphine into the PAG or RVM produces antinociception (Jacquet and Lajtha 1974; Jensen and Yaksh 1986; Morgan and Whitney 2000; Morgan et al. 1998), and inactivation of the RVM disrupts antinociception mediated by the PAG (Prieto et al. 1983; Sandkühler and Gebhart 1984). The RVM is of particular interest because the neural circuitry underlying antinociception has been described. There are three classes of neuron in the RVM: ON-, OFF-, and neutral cells (Fields et al. 1983a; Vanegas et al. 1984b).

INTRODUCTION

The antinociceptive properties of opioids are mediated, at least in part, by their action on supraspinal structures such as the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). These structures produce antinociception via a descending pathway that runs from the PAG to RVM to spinal and trigeminal dorsal horns (Basbaum and Fields 1984). Microinjection of morphine into the PAG or RVM produces antinociception (Jacquet and Lajtha 1974; Jensen and Yaksh 1986; Morgan and Whitney 2000; Morgan et al. 1998), and inactivation of the RVM disrupts antinociception mediated by the PAG (Prieto et al. 1983; Sandkühler and Gebhart 1984).

The RVM is of particular interest because the neural circuitry underlying antinociception has been described. There are three classes of neuron in the RVM: ON-, OFF-, and neutral cells (Fields et al. 1983a; Vanegas et al. 1984b). OFF-cells show an abrupt cessation of activity immediately prior to nociceptive reflexes. More importantly, manipulations that cause OFF-cells to become continuously active (i.e., prevent the OFF-cell pause) invariably produce antinociception (Barbaro et al. 1986; Cheng et al. 1986; Heinricher and Drasner 1991; Heinricher et al. 1989, 1994; Moreau and Fields 1986; Morgan and Fields 1993). In contrast, ON-cells show a burst of activity prior to nociceptive reflexes, and selective activation of ON-cells is associated with facilitation of nociception (Bederson et al. 1990; Heinricher et al. 1989; Kaplan and Fields 1991; Morgan and Fields 1994; Ramirez and Vanegas 1989). Neutral cells show no consistent change in activity associated with nociceptive reflexes and their function remains unclear.

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TABLE 1. Spontaneous activity of RVM neurons following kainic acid microinjection

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<tr>
<th>Kainic Acid Injection</th>
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Values are means ± SE measured over the 30-s interval immediately preceding each tail heating. RVM, rostral ventral medulla.

METH ODS

Male Sprague–Dawley rats (250–350 g, B and K Universal, Kent, WA) were initially anesthetized with pentobarbital sodium (60 mg/kg ip, Abbott Laboratories, North Chicago, IL). Guidelines of the Society for Neuroscience and the International Association for the Study of Pain regarding animal experimentation were followed throughout.

A PE-20 catheter (Intramedic, Sparks, MD) was inserted into an external jugular vein, and the animals were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Two small craniotomies allowed drugs to be injected into the ventrolateral PAG and neurons to be recorded in the RVM. Injections were made through a stainless steel guide cannula (25 gauge, 12 mm length, Small Parts, Miami Lakes, FL) aimed stereotaxically (Paxinos and Watson 1998) at the ventrolateral PAG but remaining 2 mm above the target area. The cannula was attached with dental cement to one screw placed in the skull. Body temperature was maintained at 37°C until the occurrence was monitored and stored for off-line analysis (Datawave C/s) from a holding temperature of 35°C until the latency of the tail on successive tests. Each trial consisted of a linear increase in temperature (1.8°C/min) until the latency of the tail on successive tests. Each trial consisted of a linear increase in temperature (1.8°C/min) until the tail. Heat was applied to areas 3, 4, and 5 cm from the caudal tip of the tail. A thermistor probe in contact with the surface of the tail was continuously monitored on an oscilloscope (TDS 410A, Tektronix, Portland, OR). The electrode was advanced through the medulla in steps of 3 μm using a hydraulic microdrive (TrentWells, Coulterville, CA) until a neuron was identified. Neurons were characterized following the classification system applied to cells of the RVM by Fields et al. (1983). Briefly, OFF-cells showed an abrupt cessation of activity just prior to the occurrence of the tail flick. Neurons that did not display either of these characteristics (i.e., neutral cells) were not studied. Spike waveform, spike time, and time of tail flick occurrence were monitored and stored for off-line analysis (Datawave Systems, Longmont, CO).

After characterization of the cells, three baseline tail flick trials were carried out at 5-min intervals. If the latency of the tail flick was stable (<1 s between each baseline trial), morphine sulfate (5 μg/0.4 μl, Natick, MA) or 0.4 μl of saline (SAL) was microinjected into the ventrolateral PAG using a stainless steel microinjection cannula (31 gauge, 14 mm length, Small Parts). The injection cannula was attached to a 1-μl syringe (Hamilton, Reno, NV) with a length of PE-20 tubing (Intramedic) and extended 2 mm beyond the guide cannula. Drug injections were made by hand over a period of 2 min. Kainic acid (8.5 ng/0.4 μl, Sigma, St. Louis, MO) was injected into the same PAG location 25 min after the morphine injection. For control purposes, kainic acid was injected before morphine in 16 of 40 trials. There was no difference in the data regardless of the order of the injections, therefore these conditions were combined for data analysis (see Tables 1 and 2).

At the end of the experiment, a lesion was made at the recording site (20 μA DC anodal current for 20 s). The injection site was marked with a microinjection of cresyl violet (0.4 μl, Sigma). The animal then received a lethal injection of pentobarbital sodium (120 mg/kg, Abbott Laboratories) and the brain was removed and placed in 10% formalin for 1 wk. Microinjection and lesion sites were located on 50-μm coronal sections using the stereotaxic atlas of Paxinos and Watson (1998).

Tail flick latency and neuronal activity, both spontaneous and tail flick-evoked, were measured at 5-min intervals throughout the experiment.
iment. Spontaneous activity was measured as the mean firing rate over the 30 s immediately preceding tail heating. Evoked activity was measured as the mean firing rate for the 2 s immediately preceding the tail flick reflex. This time period covers the range when the ON-cell burst and OFF-cell pause occur. In cases in which the tail flick reflex was inhibited, mean activity during the last 2 s of the trial was used. All data are presented as means ± SE. Rats were separated into two groups based on whether morphine microinjection inhibited the tail flick reflex. Groups were compared across trials using a two-way ANOVA (groups × trial). Post hoc analysis (Tukey’s HSD) was used where appropriate. P values < 0.05 were considered to be statistically significant.

RESULTS

Data were collected from 40 RVM neurons in 36 rats. Several rats in which microinjection of morphine into the PAG did not produce antinociception were tested again 20- to 25-min later with a more ventral injection and a different RVM neuron. All microinjections were in or immediately adjacent to

### TABLE 2. Reflex-related activity of RVM neurons following kainic acid microinjection

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Values are means ± SE measured over the 2-s interval immediately preceding each tail reflex. RVM, rostral ventral medulla.
the ventrolateral PAG. There was no clear difference in the locations of the morphine injections that inhibited the tail flick and those that did not (Fig. 1, top). In addition, there was no difference in the location of ON- and OFF-cells in the RVM (Fig. 1, bottom).

**Tail flick reflex**

Microinjection of morphine into the PAG produced antinociception at 50% (20/40) of the injection sites. In the analgesic rats, the increase in tail flick latency began within 5 min and reached the cutoff value in all of these rats by 15–20 min. Microinjection of kainic acid inhibited the tail flick reflex in all of the rats in which morphine microinjection produced antinociception and in 17 of 20 rats in which morphine was ineffective. Taken together, microinjection of kainic acid into the PAG produced antinociception 92% of the time (37/40). The onset for kainic acid-induced inhibition of the tail flick reflex was rapid (within 5 min) and short lived (the reflex was evident 10 min after the injection in most rats).

**Spontaneous neural activity**

Microinjection of kainic acid into the PAG inhibited the spontaneous activity of RVM ON-cells similar to that produced by microinjection of morphine. In almost all cases, microinjection of morphine or kainic acid caused a complete inhibition of ongoing ON-cell activity (Fig. 2A). The spontaneous activity of ON-cells following morphine and kainic acid microinjection into the PAG was significantly lower than baseline activity ($F(2,34) = 27.70; P < 0.01$).

Microinjection of morphine and kainic acid caused a significant increase in the spontaneous activity of RVM OFF-cells compared with baseline activity levels on trials in which antinociception was produced ($F(2,38) = 131.20; P < 0.01$). Baseline activity in drug-naive rats was $15.6 \pm 1.9$ Hz preceding morphine administration and $11.9 \pm 0.6$ Hz preceding kainic acid administration. Within 5 min of morphine administration, the spontaneous firing rate of OFF-cells had risen to $28.3 \pm 3.3$ Hz. A peak firing rate of $44.2 \pm 1.8$ Hz occurred 20 min following morphine administration (Fig. 2B). OFF-cells reached a peak firing rate of $58.9 \pm 2.1$ Hz within 5 min of microinjection of kainic acid in these same rats. This increase in ongoing OFF-cell activity following kainic acid administration was significantly greater than following morphine administration (Tukey test, $P < 0.05$). The rapid and short-lived increase in OFF-cell activity following kainic acid microinjection into the PAG corresponded to the time course for tail flick inhibition. Microinjection of kainic acid into the PAG produced a similar increase in OFF-cell activity ($55.7 \pm 5.9$ Hz) in rats in which

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**FIG. 3.** Top: ratemeter record of an ON-cell before and after microinjection of MOR and kainic acid (KA) into the PAG. ON-cells are characterized (bottom left trace) by a burst of activity preceding the TF. In this case, MOR microinjection attenuated the ON-cell burst but had no effect on the TF. In contrast, KA microinjection into the same PAG site inhibited the tail flick reflex (TF) and the ON-cell burst. Bottom: single oscilloscope traces clearly show the change in the ON-cell burst and tail flick reflex (bottom bar, arrow) to noxious radiant heat before and after administration of MOR and KA. Heat onset is indicated by the vertical bar at the beginning of the tail flick trace. Sweep time is 20 s.
morphine administration did not produce antinociception. Moreover, the effect of microinjecting kainic acid into the PAG on the spontaneous activity of RVM ON- and OFF-cells did not vary when the injections preceded or followed morphine administration (Table 1).

Tail flick-related neural activity

ON-cells are defined by a burst of activity immediately prior to the occurrence of the tail flick reflex (see Fig. 3). Microinjection of morphine into the PAG inhibited this burst of activity on trials in which the tail flick reflex was inhibited, but not on trials in which the tail flick reflex was not inhibited (Figs. 3 and 4). The difference in ON-cell activity in these two conditions was statistically significant ($F(1,17) = 16.09$, $P < 0.01$). Microinjection of kainic acid into these same sites inhibited the tail flick reflex in 18 of 19 rats and inhibited the ON-cell burst regardless of whether morphine administration had an effect. For example, Fig. 3 shows a ratemeter record of an ON-cell following an injection of morphine into the PAG that failed to inhibit the tail flick reflex or the ON-cell burst. Subsequent microinjection of kainic acid into the same PAG site inhibited the tail flick reflex and ON-cell burst of activity. The rapid onset and short duration of this inhibition was characteristic of kainic acid microinjections into the PAG.

OFF-cells are defined by a pause in activity immediately prior to the occurrence of the tail flick reflex (Fig. 5). On trials in which microinjection of morphine inhibited the tail flick reflex, this pause in activity was eliminated. In contrast, the OFF-cell pause in activity continued to occur on trials in which the tail flick reflex was not inhibited (Fig. 6). Microinjection of kainic acid inhibited both the tail flick reflex and the OFF-cell pause in 19 of 21 rats. These kainic acid-induced inhibitory effects occurred regardless of the effect of the morphine microinjection (see Figs. 5 and 6). Elimination of the OFF-cell pause caused OFF-cells to be significantly more active following microinjection of morphine or kainic acid compared with baseline activity ($F(2,38) = 95.46$, $P < 0.05$). The effect of microinjection of kainic acid into the PAG on the tail flick-related activity of RVM ON- and OFF-cells did not vary when the injections preceded or followed morphine administration (Table 2).

DISCUSSION

The present data demonstrate that the antinociception produced by morphine or kainic acid administration into the ventrolateral PAG is associated with inhibition of RVM ON-
cells and activation of OFF-cells. Although an excitatory response from PAG output neurons to RVM OFF-cells has been described previously (Cheng et al. 1986; Fang et al. 1989; Vanegas et al. 1984a), the effect of PAG output neurons on RVM ON-cells was unclear. The present findings demonstrate that inhibition, not excitation is the net effect of PAG output neurons on RVM ON-cells.

Previous research showed that activation of the PAG, whether by microinjection of glutamate or electrical stimulation, excited RVM neurons indiscriminately (Behbehani and Fields 1979; Vanegas et al. 1984a). Unfortunately, these studies were inconclusive because either ON- and OFF-cells were not characterized (Behbehani and Fields 1979)–ON- and OFF-cells were first characterized in 1983 (Fields et al. 1983)–or electrical stimulation was used (Vanegas et al. 1984a). Electrical stimulation activates both PAG neurons and fibers of passage, making it impossible to tell whether excitation of ON-cells is mediated by neurons within the PAG. In addition, electrical stimulation of the PAG has been shown to antidromically activate RVM ON-cells (Gao et al. 1997) so previous reports of excitation may not be physiologically relevant. The present study overcame these problems by recording the activity of RVM ON-cells and OFF-cells following microinjection of kainic acid, an excitatory amino acid, into the PAG. When PAG neurons are activated by kainic acid, RVM ON-cells are inhibited and OFF-cells are activated.

Direct comparison of the effect of microinjecting morphine and kainic acid into the same PAG site demonstrated that both drugs affect the activity of RVM neurons in the same way: RVM OFF-cells become continuously active and ON-cells are inhibited. This finding is consistent with many other reports showing that administration of opioids into the PAG inhibits RVM ON-cells and excites OFF-cells (Barbaro et al. 1986; Cheng et al. 1986; Fang et al. 1989; Fields et al. 1983; Morgan et al. 1992). Although excitatory amino acids (EAAs) activate PAG output neurons directly, whereas opioids disinhibit output neurons (Chieng and Christie 1994; Depaulis et al. 1987; Moreau and Fields 1986; Vaughan and Christie 1997; Vaughan et al. 1997), the present findings suggest that EAAs and opioids produce antinociception through the same output neurons. Disinhibition of PAG output neurons by microinjection of the GABA antagonist bicuculline also inhibits ON-cells and increases the activity of OFF-cells (Moreau and Fields 1986). Previous studies comparing the antinociceptive effects of microinjecting morphine and EAAs into the PAG also suggest that both classes of drug produce antinociception through the same mechanism (Jensen and Yaksh 1989). However, a report showing that EAA antagonists in the RVM block the antinociception produced by PAG administration of opioids, but not glutamate suggests there may be subtle differences (van Praag and Frenk 1990), although this difference could be caused by differences in antinociceptive efficacy, not mechanism.

The present data are consistent with anatomical, electrophysiological, and behavioral studies indicating that an excitatory glutaminergic pathway transmits information from the PAG to RVM (Aimone and Gebhart 1986; van Praag and...
Frenk 1990; Wiklund et al. 1988). Analysis of conduction velocities suggests that this excitatory connection running from the PAG to the RVM is monosynaptic (Shah and Dostrovsky 1980; Wiklund et al. 1988). Our data indicate that this direct excitatory connection from the PAG is to RVM off-cells specifically. Activation of PAG output neurons causes RVM on-cells to be inhibited, not excited. It is not known whether this inhibition is the result of a direct or indirect connection. One possibility is that off-cells inhibit on-cells. In contrast, it is unlikely that on-cell inhibition is necessary for off-cell activation. Recent work shows that microinjection of a glutamate antagonist prevents the on-cell burst without preventing the off-cell pause (i.e., the on-cell burst is not necessary for the off-cell pause) (Heinricher and McGaraughty 1998).

An understanding of the relationship between the PAG and RVM is complicated by regional differences within the PAG. Although antinociception can be produced at sites throughout the PAG (Jacquet and Lajtha 1974; Jensen and Yaksh 1986; Morgan and Whitney 2000; Morgan et al. 1998), the characteristics of this antinociception differ between ventrolateral and lateral/dorsal locations in the PAG (Cannon et al. 1982; Fardin et al. 1984; Morgan 1991; Morgan and Liebeskind 1987; Morgan et al. 1988, 1989; Prieto et al. 1983; Thorn et al. 1989; Tortorici et al. 1999). The data reported here derive only from microinjections into the ventrolateral PAG. The previously reported differences between the ventrolateral and lateral/dorsal PAG suggest there are differences in the output of these regions. Whether this difference includes unique connections to RVM on- and off-cells is not known. Thus it is possible that output neurons from the lateral PAG may excite RVM on-cells.

Finally, microinjection of kainic acid was much more likely to produce antinociception than microinjection of morphine. This difference is unlikely to be the result of dose or methodology because our morphine dose was quite high (5 µg/0.4 µl) and our microinjection procedure resulted in a 92% success rate when kainic acid was injected into the same sites. Our relatively low success rate in producing antinociception following morphine microinjections into the PAG (50%) is surprising given previous reports stating that there is no difference in efficacy following microinjections of morphine and EAAs (Jensen and Yaksh 1989). However, several studies report success rates similar to ours (Mohrland and Gebhart 1980; Morgan et al. 1998). In fact, in vitro recordings from PAG neurons revealed that morphine is surprisingly ineffective in
altering the postsynaptic activity of PAG neurons (Vaughan et al. 1997). One possible explanation for this difference in efficacy is that opioids activate PAG output neurons by disinhibition whereas EAAs activate output neurons directly. It should be noted, however, that efficacy could be reduced by anesthetic effects of morphine (see Heinricher et al. 1994).

In summary, our data indicate that the excitatory connection between the ventrolateral PAG and RVM is directed at off-cells specifically. Moreover, direct excitation of this pathway as occurs with microinjection of kainic acid is much more likely to produce antinociception than following microinjection of morphine. These data also highlight the close relationship between changes in the activity of RVM neurons and changes in nociception measured behaviorally.

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REFERENCES


