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Opioid Selective Antinociception Following Microinjection Into the Periaqueductal Gray of the Rat

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Abstract: Morphine and fentanyl produce antinociception in part by binding to mu-opioid receptors in the periaqueductal gray (PAG). The present study tested the hypothesis that the PAG also contributes to the antinociceptive effects of other commonly used opioids (oxycodone, methadone, and buprenorphine). Microinjection of high doses of oxycodone (32–188 μg/.4 μL) into the ventrolateral PAG of the rat produced a dose-dependent increase in hot plate latency. This antinociception was evident within 5 minutes and nearly gone by 30 minutes. In contrast, no antinociception was evident following microinjection of methadone or buprenorphine into the ventrolateral PAG despite use of a wide range of doses and test times. Antinociception was evident following subsequent microinjection of morphine into the same injection sites or following systemic administration of buprenorphine, demonstrating that the injections sites and drugs could support antinociception. Antinociception to systemic, but not PAG, administration of buprenorphine occurred in both male and female rats. These and previous data demonstrate that the mu-opioid receptor signaling pathway for antinociception in the PAG is selectively activated by some commonly used opioids (eg, morphine, fentanyl, and oxycodone) but not others (eg, methadone or buprenorphine). The fact that methadone and buprenorphine produce antinociception following systemic administration demonstrates that mu-opioid receptor signaling varies depending on location in the nervous system.

***Perspective:** This study demonstrates that the PAG contributes to the antinociceptive effects of some commonly used opioids (morphine, fentanyl, and oxycodone) but not others (methadone or buprenorphine). Such functional selectivity in PAG-mediated opioid antinociception helps explain why the analgesic profile of opioids is so variable.*

***Key words:** Functional selectivity, analgesia, oxycodone, methadone, buprenorphine.*

Five of the most commonly used opioids to treat pain are morphine, fentanyl, oxycodone, methadone, and buprenorphine.[44] All of these drugs produce analgesia by binding to mu-opioid receptors (MOPrs),[24] but they vary with regard to the receptor domain to which they bind[13] and the efficacy with which they activate distinct signaling pathways. These differences include the recruitment of G-protein subtypes,[14,48] activation of ATP-sensitive K^+ channels,[45] and ability to induce MOPr internalization.[29,31] The coupling of MOPrs to different signaling molecules caused by agonist-induced changes in the conformation of the receptor could render some neural structures sensitive to the analgesic effects of some opioids but not others.

The periaqueductal gray (PAG) is a midbrain structure that plays a particularly important role in morphine antinociception. Microinjection of morphine into the rat PAG produces antinociception,[19,36,51] and blocking morphine actions in the PAG attenuates antinociception to systemically administered morphine.[22,53] Microinjection of a wide range of other MOPr agonists (eg, [D-Ala^2,N-MePhe^4,Gly-ol]-enkephalin [DAMGO], fentanyl, dermorphin, ß-endorphin, morphine-6ß-glucuronide, [D-Ser2,Leu5]enkephalin-Thr6) into the PAG has been shown to produce antinociception.[5,7,27,42,50] These data suggest that activation of MOPrs in the ventrolateral PAG by other commonly used opioids will produce antinociception.

However, several studies suggest that the PAG may not contribute to the antinociceptive effects of oxycodone, methadone, or buprenorphine. Oxycodone and

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buprenorphine show limited guanosine 5′-O-[gamma-thio]triphosphate (GTPγS) binding in the PAG compared to their activity in other structures (eg, striatum, cingulated cortex) or compared to morphine in the PAG.[23,49] Inhibition of cell activity in the PAG does not disrupt the antinociceptive effect of systemically administered buprenorphine,[3,40] and microinjection of methadone into the PAG of the cat does not produce antinociception.[39]

These findings suggest that the involvement of the PAG in antinociception depends on the opioid. Specifically, we hypothesized that oxycodone, methadone, and buprenorphine, unlike morphine and fentanyl, would have little or no effect on nociception when microinjected into the ventrolateral PAG. We tested this hypothesis by examining both the time course and dose response for antinociception following microinjection of oxycodone, methadone, and buprenorphine into the ventrolateral PAG of the rat. In addition, control groups were included to show that the lack of antinociception to some opioids was not caused by ineffective microinjection sites, an inability of the drug to produce antinociception in our test procedure, or a difference in sensitivity between male and female rats.[4,5,7,19,25,36]

Method

Subjects

Experiments were conducted on 48 male and 11 female Sprague Dawley rats purchased from Harlan Laboratories (Livermore, CA) or bred at Washington State University Vancouver. Rats were maintained in a temperature-controlled room on a reverse light cycle (lights off at 7:00 AM) so that behavioral testing could be conducted during the active dark phase. Following surgery, rats were housed individually and allowed to recover in their home environment for at least 1 week prior to testing. Rats were handled daily before and after surgery. Food and water were available at all times, except during testing. All procedures were approved by the Washington State University Animal Care and Use committee and conducted in accordance with the guidelines for animal use described by the International Association for the Study of Pain. Efforts, such as using a within-subjects design, were made to minimize the number of subjects. No rat was tested in more than 3 sessions, so different control conditions were used in different groups of rats.

Rats were anesthetized with pentobarbital (60 mg/kg, intraperitoneal) and implanted with a guide cannula (23 gauge, 9 mm long) aimed at the right ventrolateral PAG using stereotaxic techniques (anteroposterior: +1.7 mm; mediolateral: ± .6 mm; dorsoventral: −4.6 mm [males] or −4.5 mm [females] from lambda). The guide cannula was affixed to 2 screws in the skull with dental cement. A stylet (9 mm) was inserted into the guide cannula to maintain patency. Surgery was completed within 30 minutes, and the rat was allowed to recover under a heat lamp until awake. Male and female rats had a mean weight on the first day of testing of 302 ± 5.5 g and 244 ± 4.7 g, respectively.

Microinjections and Behavioral Testing

The 3 opioids oxycodone hydrochloride, (±)-methadone hydrochloride, and buprenorphine hydrochloride were microinjected into the ventrolateral PAG through a 31-gauge injection cannula that extended 2 mm beyond the tip of the guide cannula. Oxycodone and methadone were purchased from Sigma-Aldrich (St. Louis, MO), and buprenorphine was a gift from Purdue Pharma (Cranbury, NJ). To prevent confounds caused by mechanical stimulation of neurons on the test day, 24 hours prior to the start of behavioral testing, rats received a sham injection in which the injector was inserted through the guide cannula without drug administration. Each drug was administered in a volume of .4 μL at a rate of .1 μL/10 seconds. The injection cannula remained in place an additional 20 seconds to minimize backflow up the cannula track. Immediately after each microinjection, the stylet was replaced and the rat was returned to its home cage until nociceptive testing.

Nociception was assessed by measuring the latency for the rat to lick a hind paw when placed on a 52.5°C hot plate. The rat was removed from the plate if no response occurred within 50 seconds. Only 1 rat had a baseline hot plate latency greater than 25 seconds, and this rat was not included in data analysis for that experiment.

Antinociception was assessed by microinjecting cumulative doses of oxycodone, methadone, or buprenorphine into the ventrolateral PAG. Rats were placed on the hot plate for assessment of baseline nociception and then given repeated injections into the ventrolateral PAG. Injections occurred every 20 minutes with an increasing dose of the opioid. Nociception was assessed 15 minutes after each injection. Given that buprenorphine has a relatively long duration of action following systemic administration,[2] it was injected every 30 minutes and the hot plate test was conducted 25 minutes following each injection. We have used this cumulative dosing procedure previously to produce clear antinociceptive dose-response curves to morphine and fentanyl microinjections into the ventrolateral PAG.[4,5,34]

Histology

Rats were exposed to a lethal dose of halothane immediately following the last test. The brain was removed and placed in formalin (10%). At least 48 hours later, the brain was sectioned coronally (100 μm) to determine the location of the cannula tip. Only placements located within or immediately adjacent to the ventrolateral PAG[43] were included in data analysis. The buprenorphine injection sites are shown in Fig 1. The locations of the other drug injections are indistinguishable from those shown.

Data Analysis

Dose-response curves were generated when possible. Differences in the half maximal antinociceptive effect (D_{50}) between groups were analyzed using analysis of variance (GraphPad Prism; GraphPad Software Inc, La Jolla, CA). Time course data were analyzed using a 2-way analysis of variance (Group × Trial), and