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Research report

Antinociceptive tolerance to morphine from repeated nociceptive testing in the rat

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Abstract

Repeated morphine administration has been shown to produce tolerance to the antinociceptive effects of morphine. However, the degree to which repeated morphine administration decreases antinociception is exaggerated by repeated behavioral testing, a phenomenon known as behavioral tolerance. An important question is whether behavioral tolerance can be overcome by direct administration of morphine into the ventrolateral periaqueductal gray (vPAG), a key structure contributing to morphine antinociception. Rats were injected with morphine or saline into the vPAG (Experiment 1) or subcutaneously (Experiment 2) followed 20 min later with hotplate testing. The control groups received the same drug administration, but no nociceptive testing. Repeated nociceptive testing or repeated morphine administration produced antinociceptive tolerance regardless of whether morphine was injected into the vPAG or systemically. Administration of a high dose of morphine (20 mg/kg, s.c.) was able to overcome the development of behavioral tolerance, but not pharmacological tolerance revealing separate mechanisms for these two types of tolerance. These data indicate that behavioral tolerance is independent of the route of morphine administration.

Theme: Sensory and motor systems
Topic: Pain: descending modulation

Keywords: Analgesia; Behavioral tolerance; Opiates; Hotplate; Formalin

Repeated morphine administration, either systemic or directly into the ventrolateral periaqueductal gray (vPAG), leads to the development of antinociceptive tolerance [4,8,9,11,12,17,19,20,21]. Tolerance is typically described in terms of changes produced by the direct action of morphine. However, the degree to which repeated morphine administration decreases antinociception may be exaggerated by the development of behavioral tolerance. Behavioral tolerance is the increased sensitivity to a behavioral test that occurs with repeated experience with the test apparatus. Animals that receive repeated systemic morphine administration immediately followed by nociceptive testing demonstrate less antinociception than rats receiving the same amount of morphine and tested only once [1,7,15]. This increased sensitivity to the nociceptive test mimics tolerance to morphine and thus, can amplify the appearance of tolerance to morphine.

The ability of the descending nociceptive modulatory system, including the PAG, RVM, and spinal cord, to inhibit and facilitate nociception makes it well suited to mediate behavioral tolerance. Repeated nociceptive testing produces a lasting change in descending inhibition of spinal dorsal horn neurons suggesting that central structures are involved [2,13]. Direct administration of morphine into the vPAG may provide insight into potential mechanisms of behavioral tolerance.

The purpose of the present study was to determine whether repeated activation of the descending pain modulatory system via microinjections of morphine into the ventrolateral periaqueductal gray (vPAG) paired with repeated nociceptive testing results in behavioral tolerance. Given that behavioral tolerance appears to be mediated supraspinally [2] and repeated microinjections of morphine into the PAG produces tolerance [9,11,17,19,20,21], it is

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hypothesized that behavioral tolerance will contribute to the loss of antinociception produced by repeated morphine microinjections into the vPAG.

1. General methods

1.1. Subjects

A total of 158 male Sprague–Dawley rats (260–430 g) was used. The rats were housed individually following surgery with food and water available ad libitum. Lights were maintained on a reverse 12-h light/dark cycle (off at 7 a.m.). Experiments were conducted in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and with the approval of Washington State University's IACUC. Efforts were made to minimize the number and potential suffering of experimental subjects.

1.2. Surgical procedures

Rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and implanted with a guide cannula (9 mm, 23 gauge) aimed at the vPAG using stereotaxic coordinates (AP: +2.3 mm, ML: +0.6 mm, DV: −5.0 mm from lambda [10]). The guide cannula was affixed to the skull with 2 screws (Small Parts Inc., Miami Lakes, FL) and cranioplastic cement (Dentsply International Inc., Milford, DE).

1.3. Experimental procedures

Rats were handled daily before and after surgery. Three days following guide implantation, each animal received a sham injection in which an 11-mm injector was inserted into the guide cannula but no drug was administered. This procedure habituated the animals to the microinjection procedure and reduced effects resulting from mechanical damage to neurons on the test day.

The day after the sham injection, all rats received a microinjection of morphine to determine whether antinociception could be evoked from the injection site. Microinjections were made using a 31-gauge injection cannula (11 mm) inserted through and extending 2 mm beyond the tip of the guide cannula. Morphine (5 μg/0.4 μl) was injected into the vPAG in 40 s while the rat was awake and gently restrained. The injection cannula remained in place for an additional 20 s to minimize backflow of the drug.

Antinociception was assessed using the hotplate test. The hotplate test measures the latency to lick a hind paw when a rat is placed on a 55 °C surface. Rats were removed from the hotplate if they did not respond within 40 s. Animals that demonstrated antinociception from the morphine pretest were used in the PAG microinjection experiment (Experiment 1). Animals that did not demonstrate antinociception (hotplate latency <12 s) from the morphine pretest were tested with systemic morphine administration (Experiment 2).

1.4. Histology

Following testing, rats were given a lethal concentration of halothane anesthetic. Cannula placement was marked with a microinjection of cresyl violet dye. Brains were removed and placed in a 10% formalin solution. At least 1 week later, brains were sectioned coronally (100 μm) and placed on a slide to identify the injection site.

1.5. Statistical analysis

Data from microinjected animals were analyzed using a repeated measure ANOVA for Trials 1–4, and a two-factor (drug pretreatment × testing paradigm) ANOVA for Trial 5 data. Data from animals receiving systemic administration of morphine were analyzed using a repeated measures ANOVA for Trials 1–4, and a three-factor (drug pretreatment × testing paradigm × morphine dose) ANOVA for Trial 5 data. Specific comparisons were made using independent measure *t* tests. All critical values were set with an alpha level of 0.05.

2. Results

2.1. Experiment 1: morphine microinjections into the vPAG

Rats received two microinjections (one in the morning and one in the afternoon) of either morphine (5 μg/0.4 μl) or saline (0.4 μl) each day for 2 days (Trials 1–4). Half the rats were tested on the hotplate 20 min after each injection then placed back in their home cage. The other half were returned to their cage without testing. On the last trial (Trial 5), all rats received morphine (5 μg/0.4 μl) and were tested on the hotplate test.

Only rats with injection sites within the vPAG were included in the study (7–10 per group). There were no differences in cannula placement between groups (Fig. 1). On Trial 1, microinjection of morphine into the vPAG initially produced antinociception indicated by an increase in hotplate latency compared to the saline-treated animals [$t(13) = 2.24$, $P < 0.05$). There was a gradual decrease in hotplate latencies across trials in both morphine- and saline-pretreated rats [$F(3,36) = 4.26$, $P < 0.05$], indicating the development of behavioral tolerance (Fig. 2).

On the last trial (Trial 5), all rats received a microinjection of morphine (5 μg/0.4 μl) into the vPAG. Animals that were pretreated with morphine or that were repeatedly tested (both morphine- and saline-pretreated rats) demonstrated tolerance to morphine on Trial 5. Surprisingly, animals that received repeated microinjections of saline and were repeatedly tested demonstrated the same amount of tolerance to morphine as animals that received repeated injections of morphine throughout the study. Only saline-pretreated rats that were tested on the last trial alone had an antinociceptive effect to morphine microinjected into the