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Research Report

Intermittent dosing prolongs tolerance to the antinociceptive effect of morphine microinjection into the periaqueductal gray

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Abstract

Tolerance to the antinociceptive effect of microinjecting morphine into the ventrolateral periaqueductal gray (vPAG) develops with repeated administration. The objective of the present experiment was to determine whether the magnitude and duration of tolerance differ depending on the interval between injections. Rats were injected with morphine or saline into the vPAG either once daily for 4 days or twice daily for 2 days. All rats were injected with morphine into the vPAG for the fifth injection to determine whether tolerance had developed. Morphine microinjection produced tolerance in both morphine-pretreated groups regardless of inter-dose interval. One and two weeks later, microinjection of morphine produced an increase in hot plate latency in all groups except rats pretreated with daily morphine microinjections. That is, tolerance was evident 2 weeks following the induction of tolerance produced by daily, but not twice daily injections of morphine. Although a long inter-dose interval has been shown to prolong the duration of tolerance after systemic morphine administration, this is the first report showing a similar effect with direct administration of morphine into the brain. Given that associative learning underlies prolonged tolerance with systemic morphine administration, the present data suggest that associative mechanisms of tolerance are also engaged when morphine administration is restricted to the PAG.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Opioid; Analgesia; Associative tolerance; Pain; Nociception

1. Introduction

Microinjection of morphine into the ventrolateral periaqueductal gray (vPAG) of the rat produces antinociception [8,18,28]. This antinociception decreases with repeated microinjections as expected with the development of tolerance [9,20,23,26,27]. A near complete loss of antinociception is evident with as few as four injections of morphine into the vPAG. Moreover, tolerance develops whether morphine is injected once [20] or twice a day [26] or administered continuously into the vPAG [13].

Previous research in which morphine was administered systemically shows that the duration of tolerance is longer when associative cues, such as a distinct environment, predict morphine administration [24]. Associative tolerance can persist for up to 30 days following the induction of morphine tolerance [24]. Although associative tolerance requires cues that predict drug administration, other factors such as morphine dose and inter-dose interval influence conditioning. Associative tolerance to systemically administered morphine is more likely with low doses and long inter-dose intervals [3,4,24,25]. In contrast, non-associative tolerance is more likely with high doses and short inter-dose intervals.

Tolerance has been shown to persist for 1 week following repeated microinjections of morphine into the PAG [23]. Although a long inter-dose interval prolongs the duration of

Abbreviation: vPAG, ventrolateral periaqueductal gray

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tolerance with systemic morphine administration, the effect of inter-dose interval on tolerance to direct administration of morphine into the brain is not known. An intracranial microinjection restricts the direct effect of morphine to relatively few neurons but exposes these neurons to high concentrations followed by periods of no exposure until the next injection. The relative duration that neurons are exposed to morphine will vary with the inter-dose interval even when the absolute duration of exposure is held constant. For example, the relative duration of morphine exposure is 50% less when injections are administered once a day compared to twice a day.

The relative duration of neuronal exposure to morphine could influence the development of tolerance. Thus, the present experiment examined whether inter-dose interval influences the magnitude and duration of tolerance when morphine is administered directly into the vPAG. It is hypothesized that tolerance to direct administration of morphine into the vPAG will be greater and last longer when a short inter-dose interval is used because the relative duration of morphine exposure will be greater.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (235–375 g) were anesthetized with pentobarbital (60 mg/kg, i.p.) and implanted with a guide cannula (23 gauge; 9 mm long) aimed at the ventrolateral PAG using stereotaxic techniques (AP: +1.7 mm, ML: ±0.6 mm, DV: −5.0 mm from lambda). The guide cannula was attached to two screws in the skull by dental cement. At the end of the surgery, a stylet was inserted to plug the guide cannula, and the rat was treated with the antibiotic cefazolin (15 mg/0.15 ml, i.m.).

Following surgery, rats were housed individually in a room maintained on a reverse light/dark schedule (lights off at 7:00 AM). All injections and testing were conducted during the dark phase of the cycle. Food and water were available at all times except during testing. Rats were handled daily before and after surgery. Testing began at least 7 days following surgery. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Microinjections

Drugs were administered through a 31-gauge injection cannula inserted into and extending 2 mm beyond the tip of the guide cannula. Injections were administered in a volume of 0.4 μl, while the rat was gently restrained by hand. Drugs were administered at a rate of 0.1 μl/10 s. The injection cannula remained in place an additional 20 s to minimize backflow of the drug up the cannula track. Following the injection, the stylet was replaced, and the rat was returned to its home cage.

2.3. Behavioral tests

Nociception was assessed using the hot plate and formalin tests. The hot plate test consisted of measuring the latency for a rat to lick a hind paw when placed on a hot plate (52 °C). The rat was removed from the hot plate if no response occurred within 40 s.

The formalin test consisted of injecting 50 μl of formalin (2%) into the plantar surface of the hind paw. Immediately after the injection, the rat was placed in a Plexiglas chamber (32 × 32 cm) for 20 min to assess pain behavior. Nociception was rated each minute during the first 5 min using the following criteria: 0 = normal use of paw; 1 = paw favored but touching the ground; 2 = paw elevated off floor; 3 = paw elevated and licked. The average score for the 5-min period was used for data analysis.

2.4. Procedure

Two days before initiation of testing, rats were taken into the test room, and the injection cannula was inserted through the guide cannula, but no drug was injected. This process habituates the rats to the injection procedure and reduces confounds on the test day from mechanical damage to neurons caused by insertion of the injection cannula. Six hours after the practice injection, rats were given a morphine pretest to determine whether the injection site supported antinociception. Morphine (5 μg/0.4 μl) was injected into the vPAG, and a single hot plate test was conducted 30 min later. Only rats with a hot plate latency greater than 20 s were included in the study.

The tolerance induction procedure was initiated at least 24 h after the pretest. Rats were injected with morphine (5 μg/0.4 μl) or saline (0.4 μl) into the vPAG either once a day for 4 days (10:00 AM) or twice a day for 2 days (10:00 AM and 3:30 PM). Thus, all four groups received the same number of injections, but the interval between injections varied (Table 1). One day later, both morphine and saline-pretreated rats were injected with morphine to assess the development of tolerance. Nociception was assessed using the hot plate test following the first (Trial 1) and fifth injections (Trial 5). No testing was conducted on Trials 2–4

Table 1
Injection schedule to induce tolerance in the four groups

	Day 1	Day 2	Day 3	Day 4	Day 5
AM	Groups 1 and 2	Groups 1 and 2	Groups 1 and 2	Groups 1 and 2	Morphine
			Groups 3 and 4	Groups 3 and 4	Morphine
PM			Groups 3 and 4	Groups 3 and 4	

Group 1: daily saline injections.
Group 2: daily morphine injections.
Group 3: twice daily saline injections.
Group 4: twice daily morphine injections.