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Research report

Analysis of inflammation-induced depression of home cage wheel running in rats reveals the difference between opioid antinociception and restoration of function

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HIGHLIGHTS

- Home cage wheel running can be used to assess pain and opioid analgesia.
- Low doses of morphine reversed pain-depressed wheel running only.
- High doses of morphine reversed evoked hypersensitivity only.
- All buprenorphine doses depressed wheel running in all rats.
- Wheel running reveals limitations of opioids to restore normal activity.

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ABSTRACT

Opioids are effective at inhibiting responses to noxious stimuli in rodents, but have limited efficacy and many side effects in chronic pain patients. One reason for this disconnect is that nociception is typically assessed using withdrawal from noxious stimuli in animals, whereas chronic pain patients suffer from abnormal pain that disrupts normal activity. We hypothesized that assessment of home cage wheel running in rats would provide a much more clinically relevant method to assess opioid efficacy to restore normal behavior. Intraplantar injection of Complete Freund's Adjuvant (CFA) into the right hindpaw depressed wheel running and caused mechanical allodynia measured with the von Frey test in both male and female rats. Administration of an ED_{50} dose of morphine (3.2 mg/kg) reversed mechanical allodynia, but did not reverse CFA-induced depression of wheel running. In contrast, administration of a low dose of morphine (1.0 mg/kg) restored running for one hour in both sexes, but had no effect on mechanical allodynia. Administration of the atypical opioid buprenorphine had no effect on inflammation-induced depression of wheel running in male or female rats, but attenuated mechanical allodynia in male rats. Administration of buprenorphine and higher doses of morphine depressed wheel running in non-inflamed rats, suggesting that the side effects of opioids interfere with restoration of function. These data indicate that restoration of pain-depressed function requires antinociception in the absence of disruptive side effects. The disruptive side effects of opioids are consistent with the major limitation of opioid use in human pain patients.

1. Introduction

Opioids such as morphine are the most effective treatment for most types of pain [26], but opioid efficacy is limited by unpleasant (e.g., nausea, constipation) and dangerous (e.g., sedation, addiction) side effects [32]. Opioids are very effective at inhibiting responses to noxious stimuli in laboratory animals, but their antinociceptive effects are rarely weighed against their disruptive side effects. The main problem with most analgesic drug development research in animals is that nociception is assessed using withdrawal from a noxious stimulus [20]. This approach fails to address the functional consequences of pain such as disruption of normal activity – the primary problem for chronic pain patients [9,27]. As such, the goal of pain treatments should be to promote and restore normal function, not to completely inhibit nociception as in most animal

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studies [13,16]. Restoration of normal activity requires treatments that attenuate abnormal pain without producing disruptive side effects. Despite their analgesic efficacy, the side effects produced by opioids are a major limitation.

A number of tests of pain-depressed behaviors in rodents have been developed to more closely mimic the functional consequences of pain in humans. Pain-depressed behaviors are defined as behaviors that decrease in rate, frequency, or intensity in response to a noxious stimulus or pain state [23]. Previous animal studies have examined pain-induced depression of feeding [17,30], intracranial self-stimulation [23], attention [12], nesting [24], and wheel running [6]. We have recently shown that home cage wheel running is an objective and sensitive method to assess the functional consequences of inflammatory pain in male and female rats [16]. Continuous assessment of home cage wheel running allows for the duration and magnitude of pain to be assessed in laboratory rats [16] and mimics the reduction in activity in chronic pain patients [5,31]. Thus, home cage wheel running may be an especially effective method to determine whether treatments can restore normal activity by inhibiting pain in the absence of disruptive side effects.

Opioid efficacy has been shown to vary between tests of pain-evoked and pain-depressed behavior [1,2]. Morphine and buprenorphine are two widely used opioids for treating chronic pain [26]. The primary goal of the present study was to test the hypothesis that morphine and buprenorphine will be more effective at inhibiting pain-evoked behavior (von Frey test of mechanical allodynia) than restoring pain-depressed wheel running. Given that opioids produce greater antinociception in male compared to female rats [19], we also hypothesized that morphine and buprenorphine will be more effective at restoring wheel running in male compared to female rats.

2. Materials and methods

2.1. Subjects

Data were collected from 112 adult male (mean weight: 295 g) and 117 female (mean weight: 214 g) Sprague-Dawley rats bred at Washington State University Vancouver (Vancouver, WA, USA). All rats were 50–90 days old at the start of the study and randomly assigned to treatment groups. Prior to experimentation, rats were housed in pairs in a 22–24 °C colony room on a 12/12-h light/dark cycle (lights off at 1800 h). Each rat was moved to an extra tall cage (36 × 24 × 40 cm) with a running wheel. Six to twelve rats were tested at a time in a large sound-attenuating booth (2.1 × 2.2 m; Industrial Acoustics Company, Inc., Bronx, NY, USA). Food and water were available *ad libitum* except for one hour each day when the rat was removed from the cage to assess mechanical allodynia, inject drugs, or induce inflammation. All procedures were approved by the Washington State University Animal Care and Use Committee and conducted in accordance with the International Association for the Study of Pain's Policies on the Use of Animals in Research.

2.2. Running wheel

A Kaytee Run-Around Giant Exercise Wheel (Kaytee Products, Inc., Chilton, WI) with a diameter of 27.9 cm was suspended from the top of the rat's home cage. The floor of the cage was covered with cellulose bedding (BioFresh™, Ferndale, WA, USA). A 0.8 mm thick aluminum plate (5.08 cm × 3.81 cm; K & S Precision Metals, Chicago, IL, USA) was attached to one spoke of the running wheel to interrupt a photobeam projecting across the cage with each rotation. The beam was set 18 cm above the floor of the cage so that only the rotation of the wheel, not the normal activity of the rat, would interrupt the beam. The number of wheel revolutions were summed over 5 min bins for 23 h each day using Multi-Varimex software (Columbus Instruments, Columbus, OH, USA). Wheel running was assessed 23 h/day beginning at 1700 h. The active dark phase of the light cycle began at 1800 h. A full description of the running wheel with video is available in a previous publication [16].

2.3. Opioid effects on allodynia and wheel running

Rats were allowed unrestricted access to the wheel for 23 h/day for 8 days prior to induction of inflammation. Rats that ran less than 400 revolutions on the baseline day were not included in further testing [16]. Rats were removed from their home cage for approximately 50 min each day (1600 h–1650 h) to assess mechanical allodynia using an electronic von Frey anesthesiometer (IITC Inc., ALMEMO® 2450, Woodland Hills, CA). The rat was placed in a Plexiglas chamber (22 cm × 22 cm × 12.8 cm) on an elevated mesh surface and allowed to habituate for approximately 15 min. Baseline von Frey measurements from the right hindpaw were obtained immediately before induction of hindpaw inflammation. The threshold at which a rat withdrew its hindpaw when the von Frey filament was applied to the plantar surface of the hindpaw was recorded in grams. The paw was tested 2 times with approximately one minute separating each trial. Nociceptive sensitivity was defined as the mean of 2 trials/hindpaw.

The number of wheel revolutions that occurred during the 23 h prior to induction of hindpaw inflammation was used as the baseline activity. At the end of the eighth day, the rat was removed from its home cage, briefly anesthetized with isoflurane, and injected with Complete Freund's Adjuvant (CFA; 0.1 mL; Sigma-Aldrich, St. Louis, MO, USA) into the right hindpaw using a 30-gauge needle. Control animals were anesthetized and injected with saline (Hospira Inc, Lake Forest, IL, USA) into the right hindpaw. The rat was returned to its home cage at 1650 h and wheel running was measured for 23 h beginning at 1700 h.

Twenty-three hours later, the rat was removed from its home cage and mechanical allodynia was assessed using the von Frey test. Immediately after, the rat was injected with one of the third log doses of morphine (0.32, 1.0, or 3.2 mg/kg, s c.; n = 6–14 per group), buprenorphine (0.032, 0.1, or 0.32 mg/kg, s c.; n = 6–11 per group), or saline (1 mL/kg; n = 9–14 per group). Mechanical allodynia was assessed again 30 min after opioid administration. The rat was returned to its home cage at approximately 1650 h and wheel running was measured for 23 h beginning at 1700 h.

2.4. Data analysis

All data are expressed as mean ± SEM except where stated. Baseline activity was defined as the total number of wheel revolutions during the 23 h preceding injection of CFA. Given individual differences in wheel running, subsequent wheel running data are presented as a percent change from each rat's baseline value. The percent change in wheel running following CFA or saline administration was analyzed using a 2-way repeated measures ANOVA (CFA/saline x day). Given that the peak time for morphine antinociception is 30–60 min in the rat, a one-way ANOVA was used to compare the effects of opioid dose on wheel running during the hour following administration. CFA and opioid administration did not alter withdrawal thresholds in the uninjected (left) paw so only data obtained from the injected (right) paw are presented. The mean thresholds for the von Frey test were analyzed using a one-way ANOVA on the post-opioid administration data. Statistical significance was defined as a probability of <0.05.