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Original article

Thermal sensitivity as a measure of spontaneous morphine withdrawal in mice ☆

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ABSTRACT

Introduction: Opioid withdrawal syndrome is a critical component of opioid abuse and consists of a wide array of symptoms including increases in pain sensitivity (hyperalgesia). A reliable preclinical model of hyperalgesia during opioid withdrawal is needed to evaluate possible interventions to alleviate withdrawal. The following study describes a method for assessing increases in thermal sensitivity on the hotplate in a mouse model of spontaneous morphine withdrawal. **Methods:** C57BL/6J mice received 5.5 days of 30, 56, or 100 mg/kg morphine or saline (s.c., twice daily). In Experiment I, thermal sensitivity data were collected at baseline and at 8, 24, 32, 48 h and 1 week following the final injection. Thermal sensitivity was assessed by examining latency to respond on a hotplate across a range of temperatures (50, 52, 54, and 56 °C). In Experiment II, 0.01 mg/kg buprenorphine was administered 30 min prior to each testing session during the withdrawal period. In Experiment III, jumping during a 30 min period was assessed at baseline and at 0, 8, 24, 32, and 48 h following the final morphine injection. **Results:** During the withdrawal period, thermal sensitivity increased significantly in all morphine-treated mice as compared to saline-treated mice. Thermal sensitivity was greater in mice treated with 56 mg/kg morphine compared to 30 mg/kg and peaked earlier than in mice treated with 100 mg/kg (32 h v 1 wk). The increase in thermal sensitivity following 56 mg/kg morphine was attenuated by a dose of buprenorphine that did not produce antinociception alone (i.e., 0.01 mg/kg). In general, the results of the jumping experiment paralleled those obtained in Experiment I. **Discussion:** Response latency on the hotplate is a reliable and sensitive measure of spontaneous morphine withdrawal in mice, making it an ideal behavior for assessing the potential of medications and environmental interventions to alleviate opioid withdrawal.

1. Introduction

The opioid withdrawal syndrome consists of a constellation of symptoms that appear following the termination of a prolonged period of opioid administration. The presence or desire to avoid these symptoms may even contribute to continued drug taking (Le Moal & Koob, 2007). As such, withdrawal is a critical component of opioid abuse. One of the many symptoms that make up the Clinical Opiate Withdrawal Scale or COWS (Tompkins et al., 2009) is an increase in pain or sensitivity to pain. An increase in pain sensitivity or hyperalgesia during spontaneous withdrawal occurs in pain patients in experimental settings (Lipman & Blumenkopf, 1989) and is reported in case studies, as well (Devulder, Bohyn, Castille, De Laat, & Rolly, 1996). Healthy human subjects show hyperalgesia during both spontaneous (Angst, Koppert, Pahl, Clark, & Schmeiz, 2003) and antagonist precipitated withdrawal (Compton, Athanasos, & Elashoff, 2003; Sun, 1998).

The development of pharmacological and environmental interventions to mitigate hyperalgesia during opioid withdrawal requires reliable preclinical models of this symptom of withdrawal. In 1973, Tilson et al. reported that sensitivity to electric foot shock increases following the cessation of morphine in rats. Since then a modest number of papers have described hyperalgesia in animal models of opioid withdrawal. In rats, hyperalgesia occurs during both precipitated as well as spontaneous morphine withdrawal and is observed with multiple pain assays: hotplate, tail flick, and shock discrimination (Devillers, Boisserie, Laulin, Larcher, & Simonnet, 1995; Dunbar & Pulai, 1998; Grilly & Gowans, 1986; Jin et al., 2012; Li, Angst, & Clark, 2001; Tilson, Rech, & Stolman, 1973). Hyperalgesia in rats also occurs during withdrawal from fentanyl (Laulin et al., 2002) and heroin (Devillers et al., 1995; Laulin, Larcher, Célèrier, Le Moal, & Simonnet, 1998). Beyond rodents, withdrawal hypersensitivity is seen in both dogs (Martin, Gilbert, Jasinski, & Martin, 1987) and cats (Johnson & Duggan, 1981).

Traditionally, opioid withdrawal in mice is measured by the presence of behavioral signs such as jumping, wet dog shakes, piloerection, diarrhea, and ptosis (e.g. Kest et al., 2002; Papaleo & Contarino, 2006). To the best of our knowledge only two studies from laboratories other than our own employ a hyperalgesia model for examining opioid

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withdrawal in mice. These studies examine only a single time point during spontaneous withdrawal (Rubovitch, Pick, & Sarne, 2009) or employ a precipitated, rather than a spontaneous, withdrawal procedure (Crain & Shen, 2007).

The current study describes a new method for assessing hyperalgesia in a mouse model of spontaneous morphine withdrawal. We hypothesize that thermal sensitivity on a hotplate will increase during spontaneous withdrawal from a range of morphine does. Further, we hypothesize that buprenorphine treatment during the withdrawal period will attenuate the increase in sensitivity. Buprenorphine, a low efficacy mu agonist, was selected because it is commonly used in agonist replacement therapy for opioid dependence (e.g. Connock et al., 2007; Kraus et al., 2011), and used to suppress spontaneous opioid withdrawal symptoms during the induction phase of treatment (Strain, Harrison, & Bigelow, 2011).

2. Methods

2.1. Animals

All experiments were conducted in male C57BL/6J mice (Jackson Labs, Raleigh, NC), 10 weeks of age upon delivery. Male C57BL/6J mice were selected to allow comparison with other data collected in our laboratory regarding morphine's pharmacological effects as well as the extensive literature on the behavioral effects of opioids in C57BL/6 mice. Additionally, in comparison to other inbred strains, C57BL/6J mice are known to be highly sensitive across many behavioral assays. Specifically, they exhibit high sensitivity in measures of acute nociception (Mogil, Chesler, Wilson, Juraska, & Sternberg, 2000), naloxone precipitated morphine withdrawal (Kest et al., 2002) and morphine self-administration (Elmer, Pieper, Hamilton, & Wise, 2010).

Mice were individually housed in polycarbonate cages (floor area = 335 cm^2) with continuous access to food and water throughout the study. The colony room was maintained on a 12-h, reverse, light/dark cycle (lights off at 7:00 am) and all behavioral testing was conducted during the dark cycle, between 9:00 am and 7:00 pm. Mice were habituated to handling and the colony room environment for two weeks prior to any experimental manipulation. Mice were also exposed to the testing environment for at least two days prior to initiation of an experiment and for 1 h prior to all behavioral testing. Although a criterion was set such that mice <20 g or those that lost >20% of initial body weight would be removed from the study, it was not necessary to remove any mice from the study. Animal protocols were approved by the Institutional Animal Care and Use Committee, and the methods were in accord with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council, 2011).

2.2. Experimental procedures

2.2.1. Thermal sensitivity

Thermal sensitivity was assessed using a hot plate analgesia meter (25.3×25.3 cm), Columbus Instruments, Columbus, OH. During each 1-h hot plate testing period, a temperature-effect curve was determined for each mouse. Sensitivity was evaluated by recording the latency to lick or flutter the hind paw(s), or to jump from the hot plate surface at each of four temperatures presented in the following order: 50, 54, 52, 56 °C with 15-min intervals between temperatures. Response latency was measured to the nearest 0.1 s. To prevent tissue damage, a predetermined cutoff time of 20 s was defined as the maximal trial duration. Immediately following the termination of a trial, whether due to a mouse's response or elapsed cutoff time, mice were removed from the hot plate surface. Parameters were selected based on prior work in our laboratory regarding responses on the hot plate (e.g. Balter & Dykstra, 2012; Fischer, Zimmerman, Picker, & Dykstra, 2008).

2.2.2. Jumping

To measure jumping, mice were removed from their home cages and placed in a 4 L beaker in the center of a Med Associates Inc. activity chamber. Vertical beam breaks, monitored by a computer, were used to count the number of jumps that occurred in a 30-min period.

2.2.3. Pharmacological procedure

During the saline/morphine administration period, doses of saline, 30 mg/kg, 56 mg/kg or 100 mg/kg of morphine were administered daily for 5.5 days, with injections occurring at 10:00 am and 8:00 pm daily (11 injections total). Morphine sulfate and buprenorphine hydrochloride, provided by the National Institute on Drug Abuse (Bethesda, MD, USA), were both dissolved in 0.9% saline to yield all concentrations. Doses were injected subcutaneously at a volume of 0.1 ml/10 g.

2.3. Experimental design

2.3.1. Experiment 1: thermal sensitivity following saline, 30, 56, or 100 mg/kg of morphine

On day one, thermal sensitivity was assessed in all four groups of mice (n = 8) at 10:00 am (baseline 1) and at 6:00 pm (baseline 2). A 2-way repeated measures ANOVA revealed no difference between baseline 1 and baseline 2; therefore, baselines were averaged for all analyses and figures. At 10:00 am on day two 30, 56, 100 mg/kg morphine or saline administration began as described above and continued for 5.5 days. Following the last dose of morphine on day seven, thermal sensitivity was assessed six more times: immediately after the final injection (10:00 am on day 7), at 8 h (6:00 pm on day 7), at 24 h (10:00 am on day 8), at 32 h (6:00 pm on day 8), at 48 h (10:00 am on day 9) and at 1 week (10:00 am on day 14). This period (days 7–14) was designated as the withdrawal period.

2.3.2. Experiment 2: buprenorphine and thermal sensitivity

In order to select a dose of buprenorphine that did not produce antinociception on its own, a cumulative dose effect curve (0.01 to 0.32 mg/kg) was obtained for buprenorphine at each of the four temperatures tested during the thermal sensitivity assessment (50, 52, 54 and 56 ± 0.1 °C). Baseline response latencies on the hot plate were determined twice prior to the beginning of the buprenorphine dose effect curve and spaced 30 min apart. Data from these baselines were averaged to yield one baseline value. Following baseline determination, responding on the hot plate was examined over multiple cycles, and doses of buprenorphine were spaced 30 min apart. Drugs were administered at the start of each cycle and latency on the hot place was determined during the last minute of the cycle. Drug doses were increased cumulatively, with the dose increasing in one-half log unit increments prior to each cycle (0.01, 0.03, 0.1, 0.32 mg/kg). Buprenorphine effects were expressed as a percentage of the maximal possible effect (% MPE) using the following formula:

$$\%\text{MPE} = \frac{[\text{Postdrug latency} - \text{baseline latency}]}{[\text{cutoff time } (20\,\text{s}) - \text{baseline latency}]}$$

During the withdrawal experiment, on day one thermal sensitivity was assessed in two groups of mice (n = 8) at 10:00 am (baseline 1) and 6:00 pm (baseline 2). A 2-way repeated measures ANOVA revealed no difference between baseline 1 and baseline 2; therefore, baselines were averaged for all analyses and figures. At 10:00 am on day two 56 mg/kg morphine administration began for all mice as described above and continued for 5.5 days. Following the last dose of morphine on day seven, thermal sensitivity was assessed five more times: immediately after the final injection (10:00 am on day 7), at 8 h (6:00 pm on day 7), at 24 h (10:00 am on day 8), at 32 h (6:00 pm on day 8), and at 48 h (10:00 am on day 9). A dose of 0.01 mg/kg buprenorphine or saline was administered subcutaneously 30 min prior to each testing