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Research paper

Leptin status alters buprenorphine-induced antinociception in obese mice with dysfunctional leptin receptors

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ABSTRACT

Buprenorphine is an opiate used for pain management and to treat opiate addiction. The cytokine leptin can modulate nociception, but the extent to which buprenorphine-induced antinociception varies as a function of leptin signaling has not been characterized. Four congenic mouse lines with phenotypes that include differences in body weight and leptin status were used to test the hypothesis that the antinociceptive effects of buprenorphine vary as function of sex and leptin signaling. Each mouse line was comprised of males (n = 12) and females (n = 12) for a total of 96 animals. Groups included C57BL/6J (B6) mice (wild type), B6 mice with diet-induced obesity (DIO), obese B6.Cg-Lep^{ob}/J (ob/ob) mice lacking leptin, and obese B6.BKS(D)-Lepr^{db}/J (db/db) mice with dysfunctional leptin receptors. The dependent measure was tail flick latency (TFL) in seconds for mouse-initiated tail removal from a warm water bath. Independent variables were intraperitoneal administration of saline (control) or buprenorphine (0.3 mg/kg). Within every mouse line, buprenorphine significantly increased TFL relative to saline. Compared to the other mouse lines, db/db mice with dysfunctional leptin receptors had a significantly longer TFL after saline and after buprenorphine. TFL did not vary significantly by body weight or sex. The results provide novel support for the interpretation that acute thermal nociception is associated with altered leptin signaling.

1. Introduction

Buprenorphine is a mu opiate receptor agonist and kappa opiate receptor antagonist used for pain management [6] and for treating opiate use disorder [13,14]. Most data regarding buprenorphine have been derived from studies of normal weight males. In contrast, morphine is known to cause sex-specific differences in control of human breathing [10]. Closed claims analyses reveal that being female and obese are the greatest risk factors for opiate-related adverse events [18]. Obesity is co-morbid with increased reports of pain in humans [2,24] and with altered nociception in mice [29,34,37].

Obesity is a proinflammatory disorder with the potential to modulate nociception via multiple tissue systems [9]. Adipocytes secrete leptin, a proinflammatory cytokine that also functions to signal satiety [3]. Nociceptive processing is altered in obese mice [26,27,33] and previously we have shown that leptin replacement can restore thermal nociception in obese, leptin-deficient mice [34,37]. We are aware of no comparable data from mice determining whether the antinociceptive effects of buprenorphine are altered by obesity and/or sex. The current study used four congenic lines of mice with normal or dysfunctional leptinergic transmission. These four lines of mice made it possible to test the hypothesis that the antinociceptive effects of buprenorphine vary as a function of sex and leptin signaling. Preliminary results have been presented [20].

2. Materials and methods

2.1. Animals

All procedures using animals were approved by the University of Tennessee Institutional Animal Care and Use Committee and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (The National Academies Press, 8th Ed., Washington, D.C., 2011). Adult male and female mice age 6–8 weeks were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The congenic mouse lines included: C57BL/6J (B6) mice (wild type), B6 mice fed a 60% fat diet (D12492, Research Diets, Inc., New Brunswick, NJ, USA) to create diet-induced obesity (DIO), obese B6Cg-Lep^{ob}/J (ob/ob) mice that do not produce leptin, and obese B6.BKS(D)-Lepr^{db}/J (db/db) mice that produce leptin and have dysfunctional leptin

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Table 1
Tail flick latency in seconds (mean ± SD) did not differ significantly between males and females for each of four congenic mouse lines. Values are based on 12 male and 12 female mice per group. B6 = C57BL/6J (wild-type mice); DIO = B6 mice with Diet-Induced Obesity (mice are obese); ob/ob = B6.Cg-Lep ^{ob}/J (mice are obese and leptin deficient); db/db = B6.BKS (D)-Lepr ^{db}/J (mice are obese and lack normal leptin receptors).

Treatment	Sex	B6	DIO	ob/ob	db/db
Saline	Males	3.11 ± 0.67	2.94 ± 0.59	3.27 ± 0.51	5.04 ± 0.58
	Females	3.40 ± 0.85	3.23 ± 0.39	2.99 ± 0.62	4.85 ± 1.16
Buprenorphine	Males	5.09 ± 1.33	4.75 ± 0.90	4.48 ± 0.71	5.83 ± 1.73
	Females	5.05 ± 0.72	4.32 ± 0.44	4.52 ± 1.22	6.33 ± 1.97

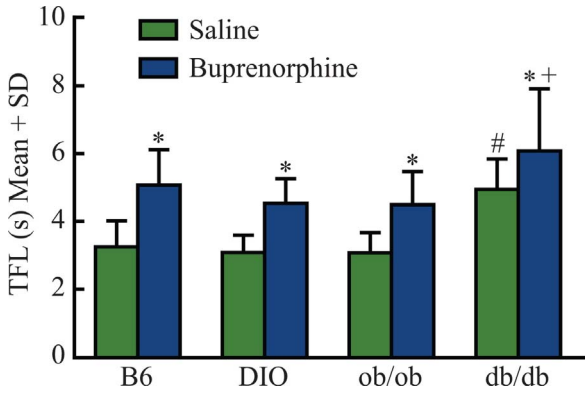

Fig. 1. Mean and standard deviation (SD) tail flick latency (TFL) in seconds among four congenic lines of mice. In all four lines of mice buprenorphine significantly (*) increased TFL. Mice (db/db) with dysfunctional leptin receptors displayed a significantly longer TFL than the other three congenic lines after saline (#) and after buprenorphine (+) administration.

receptors. None of these mice are transgenic animals. The ob/ob and db/db mice were discovered as spontaneous mutations in the B6 line. Quality control protocols at The Jackson Laboratory regularly confirm genetics and leptin status of mouse lines [21] via End Point Genotyping to insure the presence of the ob/ob and the db/db mutations. Additionally, genome scans confirm that these congenic lines are on a B6 background.

Each mouse line included 12 males and 12 females for a total sample size of 96. Mice were housed in a temperature-controlled environment and provided with *ad libitum* access to food and water. B6, ob/ob, and db/db mice were fed Teklad 22/5 rodent chow containing 5.5% fat.

2.2. Nociceptive testing and drug administration

The dependent measure was time in seconds for mouse-initiated tail removal from a water bath maintained between 48° and 50 °C. These measures of tail flick latency (TFL) provide an established index of acute thermal nociception [8,25]. For one week prior to quantifying TFL, mice were conditioned for 2 min to being placed in an acrylic tube with their tails extending outside the tube. During data collection, the distal third of the tail was lowered into the water bath. A timer was started when the tail was immersed in the water. The timer was stopped when the mouse flicked its tail out of the water. Each of three TFL measures was separated by a 10-s interval of no stimulation. A cutoff time of 15 s was implemented to prevent tissue damage, and this 15-s interval served as the maximum possible TFL value. Injection volume was 0.3 mL. The 0.3 mg/kg dose of buprenorphine reliably produces antinociception in mice [19,28] and buprenorphine can have an antinociceptive effect on mouse TFL for as long as 270 min after administration [15]. Based on these previous findings, TFL measures were collected 120 min after intraperitoneal (i.p.) administration of saline (vehicle control) or buprenorphine (0.3 mg/kg). Studies of acute thermal nociception often compare pre-injection (baseline) and post-injection (treatment) measures in order to normalize the data as percent maximum possible effect. As cautioned previously [1], normalization using percent maximum possible effect is misleading for nociceptive studies involving subjects that are known to differ by strain or line. Therefore, it was not appropriate in the present study to normalize the TFL measures.

2.3. Data analysis

Statistical analyses were performed using Prism 7.0b. To circumvent the potential confound of inflated degrees of freedom, all statistical analyses were performed using TFL values expressed as a mean for each mouse. The data from each of the four lines of mice were confirmed via D'Agostino and Pearson tests to satisfy the assumption of a normal distribution. The hypothesis that the antinociceptive effects of buprenorphine varied as a function of sex and leptin signaling was evaluated using two-way analysis of variance (ANOVA) followed by *post hoc* multiple comparisons tests. The magnitudes of the treatment effects within each mouse line were quantified with Cohen's *d* statistic. For each of the four mouse lines and for each drug condition, regression coefficients (r^2) quantified the amount of variance in TFL that was accounted for by body weight.

3. Results

Comparisons of nociception after saline or buprenorphine administration revealed no significant differences in TFL between males and females (Table 1). Subsequent analyses thus combined TFL measures from male and female mice within each congenic line (Fig. 1). The increase in TFL caused by buprenorphine for each mouse line was B6 = 55.8%, DIO = 47.0%, ob/ob = 43.8 %, and db/db = 23%. Two-way ANOVA showed statistically significant differences in TFL as a function of mouse line ($F = 32.09$; $df = 3,184$; $P < 0.0001$) and buprenorphine ($F = 102.3$; $df = 1,184$; $P < 0.0001$). Post-hoc analyses indicated that db/db mice had significantly ($P < 0.0001$) longer TFLs than B6, DIO, and ob/ob mice after administration of saline or buprenorphine.

Cohen's *d* statistic provided an additional metric for phenotyping the magnitude of the treatment effect. All Cohen's *d* values were large, indicating non-overlap in the TFL distributions after buprenorphine relative to saline: DIO ($d = 2.5$), B6 ($d = 2.0$), ob/ob ($d = 1.6$), and db/db ($d = 0.88$). Expressed as percent of non-overlap, the Cohen's *d* values revealed non-overlapping distributions ranging from > 98% percent non-overlap for B6 and DIO mice to 73% for ob/ob, and 52% for db/db mice. Thus, the significant differences in TFL as a function of mouse line (Fig. 1) were not an artifact of inflated degrees of freedom associated with large sample sizes.

ANOVA confirmed that body weight varied significantly ($F = 142$; $df = 3,92$; $P < 0.0001$) as a function of congenic line. Body weights (mean ± standard deviation in g) at the time of TFL testing were B6 female (19.8 ± 1.2), B6 male (27.4 ± 1.9); DIO female (30.7 ± 2.9), DIO male (43.5 ± 5.4); ob/ob female (48.4 ± 2.9), ob/ob male (46.4 ± 2.3); db/db female (49.4 ± 2.0), db/db male (48.0 ± 1.9). Post hoc analyses showed that body weights of DIO, ob/ob, and db/db mice were significantly ($P < 0.0001$) greater than body weight of control (B6) mice. Our assessments of higher-level