Psychoneuroendocrinology (2011) 36, 728—736

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/psyneuen

Social influences on plasma testosterone levels in morphine withdrawn adolescent mice and their drug-naïve cage-mates

Rebecca S. Hofford, Paul J. Wellman, Shoshana Eitan *

Behavioral and Cellular Neuroscience, Department of Psychology, Texas A&M University, 4235 TAMU, College Station, TX 77843, USA

Received 14 March 2010; received in revised form 17 August 2010; accepted 12 October 2010

KEYWORDS
Opioid;
Age-dependent;
Peer-influences;
Corticosterone;
Drug addiction;
Pain management

Summary Opioid administration in males results in opioid-induced androgen deficiency which persists throughout the treatment. In adults, this quickly reverses once opioid administration is suspended. However, less is known about the duration of the effect following drug discontinuation in adolescents. Given the significant implications to sexual maturation in adolescent males, this study examined plasma testosterone levels in both morphine withdrawn mice and their drug-naïve (saline-injected) cage-mates as compared to drug-naïve mice housed physically and visually separate from the morphine-treated mice ('saline only'). Consistent with the literature, plasma testosterone levels in morphine withdrawn adults were reduced on withdrawal day 1 (WD1) and returned to baseline levels by WD9. No significant effects were observed in their saline cage-mates. In the adolescents, no significant differences were observed on WD1 between the morphine withdrawn mice, their saline cage-mates, and the saline only mice — all of which had significantly lower plasma testosterone levels than adults. By WD9, testosterone levels in the saline only adolescent mice had reached adult levels. Notably, plasma testosterone levels were reduced in both the morphine withdrawn adolescent mice and their saline cage-mates, as compared to saline only mice. The effect was not a drug effect per se, given that reduced plasma testosterone levels were not observed in individually housed morphine withdrawn mice. Moreover, our results also suggest that these social effects are not solely explained by stress. These results have numerous implications to the short term and long term health of both adolescents requiring pain management and of adolescent drug addicts.

1. Introduction

Opiates are the most effective and commonly used analgesics in treating moderate-to-severe pain. Moreover, nonmedical use of opioid prescription pain relievers (such as hydrocodone, oxycodone, and morphine) is the second most common form of illicit drug use in the US after marijuana (SAMHSA, 2009). Gonadal hormones are known to play a role in mediating the effects of opioids. This includes opioid antinociception (i.e. analgesia), and the development of tolerance, dependence, and withdrawal symptoms (Verdi and Ahmadiani, 2007; Nayebi and Rezazadeh, 2008; Sadeghi et al., 2009). Thus, the effects of opioids on testosterone levels have numerous impli-

* Corresponding author. Tel.: +1 979 845 2508; fax: +1 979 845 4727.
E-mail address: seitan@psych.tamu.edu (S. Eitan).

0306-4530/$ — see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.psyneuen.2010.10.008

cations for the short and long term health of patients requiring pain management and for drug addicts.

The endogenous opioid system is suggested to regulate the activity of the hypothalamic–pituitary–gonadal axis (Cicero, 1980; Gerendai, 1991). As a result, opioid treatment in males causes opioid-induced androgen deficiency, i.e. a significant decrease in plasma testosterone levels (Rasheed and Tareen, 1995; Abs et al., 2000; Aloisi et al., 2009). This effect is observed in humans as well as in experimental animals, including rodents (Gabriel et al., 1986; Budziszewska et al., 1999). This opioid effect is dramatic – a single administration can cause a robust decrease in testosterone levels comparable to castration (Aloisi et al., 2005). Moreover, tolerance does not develop to this opioid-mediated effect, thus this reduction lasts for the entire duration of opioid administration (Aloisi et al., 2005).

The effect of opiates on the suppression of testosterone levels is suggested to be mediated through effects on both the hypothalamus and the testes (Cicero, 1980; Gerendai, 1991). Opiates are suggested to affect the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (Cicero et al., 1979). This in turn results in a decreased release of luteinizing hormone (LH) from the anterior pituitary gland, which is important for the stimulation of Leydig cells to produce testosterone. Moreover, opiates were also demonstrated to increase the sensitivity of the hypothalamus to the negative feedback effects of testosterone resulting in a marked suppression in LH release (Gabriel et al., 1986). Alternatively, modulation of the sensitivity of the hypothalamus–pituitary axis by opiates was also suggested to result from a decreased sensitivity of the pituitary to GnRH (Kalra et al., 1988). In addition to their effects on the hypothalamus, opioids were demonstrated to inhibit gonadal function through specific opioid receptors within the testes (Adams et al., 1993). This was demonstrated to be mediated through suppression of testicular steroidogenesis, which results in decreases in both testicular interstitial fluid formation and plasma testosterone levels.

Similar to adults, opioids also decrease gonadal hormone levels in adolescents (Cicero et al., 1989; Yilmaz et al., 1999). Moreover, the endogenous opioid system is involved in mediating the onset of puberty and sexual maturation (Bhanot and Wilkinson, 1983; Sirinathsinghji et al., 1985; Donham et al., 1986; Cicero et al., 1988, 1989). As a result, morphine exposure during adolescence results in marked disruptions in reproductive endocrine parameters in male rodents (Cicero et al., 1989) and in long term consequences on both reproductive fertility and the development of the offspring (Cicero et al., 1991). This is in contrast to adults where morphine exposure was demonstrated to cause only a relatively minor and transient reduction in hypothalamic–pituitary–gonadal axis activity (Cicero et al., 1989). Although the opioid system was demonstrated to play a role in gonadal hormone regulation in both adults and adolescents, differences were noted in the mechanisms underlying the interactions between the opioid and gonadal systems across ages. For example, unlike adults, in pubertal boys the opioid system does not modulate the sensitivity of the hypothalamus–pituitary axis to the negative feedback of testosterone (Kletter et al., 1991, 1997). Similarly, in immature animals, the regulation of the opioid system on the LH pathway appears to be underdeveloped (Trudeau et al., 1988).

In adults, the opioid effect on testosterone quickly reverses once opioid administration ceases (Aloisi et al., 2009). However, less is known about the effects of opioids on testosterone levels following the cessation of drug administration in adolescents. Thus, this study examines the effect of morphine on plasma testosterone levels in adolescent and adult mice following the cessation of morphine administration. Three experimental groups were examined for each of these age groups: (1) group-housed morphine withdrawn mice, (2) drug-naïve mice housed together with the morphine withdrawn mice, referred to as 'saline cage-mates', and (3) drug-naïve mice that were group-housed physically and visually isolated from the morphine withdrawn mice, referred to as 'saline only'. Additionally, individually housed morphine withdrawn and saline-treated adolescent mice were also tested. Plasma testosterone levels were examined 1 and 9 days following the last dose of morphine, i.e. withdrawal days (WD) 1 and 9. Different mice were used for each day.

Note that isolation (i.e. individual housing) affects adolescent and adult rodents differently. In adult rodents, isolation is a known stressor and thus was suggested as an animal model for depression (Guidotti et al., 2001). However, individual housing (isolation) does not seem to activate the HPA axis in male adolescent rodents the same way as in adults. In contrast to adults, individually housed male adolescent rodents did not exhibit a higher state of stress and anxiety as compared to group-housed adolescents (Weintraub et al., 2010). Thus, the differences between the individually and group-housed adolescent mice represent the effect of social exposure status, given that stress levels during the adolescence period is not expected to differ between those two housing conditions. However, given that isolation in adults is a stressor, we did not examine individually housed adults.

2. Methods

2.1. Animals

All procedures were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the TAMU Institutional Animal Care and Use Committee. Male C57BL/6 mice, purchased from Harlan Lab. (Houston, TX), were housed with food and water *ad lib*. In this study both group-housed (n = 10–18 per group) and individually housed (n = 16–29 per group) mice were examined. For the group-housed mice, 4 mice were housed per cage. For each age there were two housing conditions. In the first group-housing condition, morphine-treated mice were housed together with saline-injected mice (i.e. 2 mice receiving morphine and 2 mice receiving saline were housed in each cage). We refer to these treatment groups as 'morphine withdrawn' and 'saline cage-mates', respectively. In the other group-housing condition, saline-injected mice were housed physically and visually separated from the mice receiving morphine (i.e. all mice in the cage received saline). We refer to this saline-injected group as 'saline only'. The different experimental groups are summarized in Table 1. All mice were acclimated to the temperature-controlled (21 ± 2 °C) vivarium with a 12 h/12 h light/dark cycle (light on at 07:00 h) for one week prior to treatment.

Adult and adolescent mice were examined in this study. The age choice of the adolescent mice was based on studies by Spear and colleagues (reviewed in Spear, 2000) which