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Differential effects of methadone and buprenorphine on the response of D2/D3 dopamine receptors in adolescent mice☆

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ABSTRACT

Background: There is a lack of studies that examine the effects of opioid maintenance drugs on the developing adolescent brain, limiting the ability of physicians to conduct a science-based risk assessment on the appropriateness of these treatments for that age group. Our recent observations indicate higher potential risks in repeated exposure to morphine during adolescence, specifically to the D2/D3 dopamine receptors' signaling. Disturbances in dopaminergic signaling could have broader implications for long-term mental health. Thus, this study examined whether buprenorphine and methadone differentially alter the responses of the D2/D3 dopamine receptors in adolescents.
Methods: Adolescent mice were orally administered buprenorphine (0.1–0.4 mg/kg), methadone (25–100 mg/kg), or saline once daily for 6 days. Two hours or three days later, the mice were tested for their locomotor response to 10 mg/kg quinpirole, a D2/D3 dopamine receptor agonist.
Results: Buprenorphine-treated adolescent mice did not significantly differ from control drug-naïve animals in their response to quinpirole. However, an enhanced response was observed in methadone-treated adolescent animals. This enhanced locomotion was significantly higher two hours following the final dose of methadone, as compared to three days afterwards.
Conclusions: This study suggests that exposure to various opioids carries differential probabilities of altering the highly sensitive neurochemistry of adolescent brains. Methadone exposure disturbs the D2-like receptor's response, indicating a potential risk in administering methadone to adolescents (either for the treatment of opioid dependency/abuse or for pain management). In contrast, buprenorphine appears to have a significantly lower effect on the behavioral sensitivity of D2/D3 dopamine receptors in adolescents.

1. Introduction

Nonmedical use of opioids is the second most common form of illicit drug use in the United States after marijuana (SAMHSA, 2009). In 2008, one in five teens abused a prescription pain medication (PATS, 2009), and this high prevalence has continued over the last few years (Johnston et al., 2011). Thus, there is growing need to improve our knowledge on the consequences of adolescents' opioid use, as well as on the treatment options available for them. Our recent observations indicate that adolescent mice exposed to morphine subsequently exhibited a supersensitive response to a D2/D3 dopamine receptor agonist (Hofford et al., 2012). This effect of morphine was extremely pronounced in adolescents, but was barely observed in adults. These findings suggest that opioid use during adolescence results in markedly robust disturbances of the dopaminergic signaling as compared to use during adulthood.

Different opioids are known to have unique molecular profiles and to differentially modulate the activity of various opioid receptors (Zhang et al., 1998; Patel et al., 2002; Arttamangkul et al., 2008). Thus, different opioids are likely to differentially modulate brain neurochemistry. However, there is a lack of studies examining the differential effects of opioids on the developing brains of adolescents. Specifically, buprenorphine and methadone are two drugs approved for maintenance treatment of opioid addiction in adults, and also recently in adolescents (Kleber et al., 2006). For adolescents, a few clinical studies demonstrated improved retention in treatment programs using these drugs (Bell and Mutch, 2006). However, the advantages and risks associated with using these

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drugs in this age group are controversial (Kleber et al., 2006; Simkin and Grenoble, 2010). Various authorities thus instated restrictions on the use of pharmacological maintenance treatments, especially methadone, for treating opioid-dependent adolescents (Fiellin, 2008; Hillier, 2011). Additionally, methadone and buprenorphine are also used for pain management in children and adolescents (Anghelescu et al., 2011; Michel et al., 2011). Methadone is also recreationally used (misused/abused) by adolescents (Johnston et al., 2011). However, there are currently very limited studies that directly examine the differential effects of these opioids on the developing brains of adolescents, limiting the ability of physicians to conduct a science-based risk assessment on the effects of using these treatments in youths.

Given the mental health implications of an altered dopaminergic system, this study examined the effects of orally administering buprenorphine (0.1, 0.2 and 0.4 mg/kg) and methadone (25, 50 and 100 mg/kg) for 6 consecutive days on altering the locomotor responses to quinpirole, a D2/D3 dopamine receptor agonist. Additionally, in order to determine the equivalence between the doses used in this study and doses used for treating humans, plasma levels of buprenorphine and methadone were measured following these treatment regimens.

2. Methods and materials

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 Texas A&M Institutional Animal Care and Use Committee. Adolescent male C57BL/6 mice, purchased from Harlan Lab (Houston, TX), were housed 4 per cage with food and water ad lib. They were acclimated to the temperature-controlled (21 ± 2 °C) vivarium with a 12 h/12 h light/dark cycle (light on at 07:00) for approximately one week prior to treatment.

The choice for the age of the adolescent mice was based on studies by Spear and colleagues (reviewed in Spear, 2000). Accordingly, mice were purchased at postnatal day 22 (PND 22). They were acclimated to the vivarium until PND 28, when methadone, buprenorphine, or saline injections began, and behavioral testing was performed on PND 33 or 36. Thus, in this study, mice were injected during what is considered the late phase of their prepubescent period, and were tested during their mid-adolescence/periadolescent period. The different experimental groups are summarized in Table 1 of the Supplementary materials.

2.2. Methadone and buprenorphine treatment regimen

Adolescent mice (n = 12–29 per group) were administered buprenorphine (0.1, 0.2, or 0.4 mg/kg, 10 ml/kg), methadone (25, 50, or 100 mg/kg, 10 ml/kg) or saline (10 ml/kg) once daily (8 a.m.) for six days via gavage. Drugs were purchased from Sigma–Aldrich Chemicals (St. Louis, MO). These doses were selected to represent plasma levels generated by the therapeutic doses used for maintenance treatment in humans (Leavitt, 2003; Bell and Mutch, 2006; Moody et al., 2011). The selection was based on the existing literature on the pharmacokinetics of these drugs in mice (Middaugh et al., 1983; Kalliokoski et al., 2011). In addition, plasma levels of buprenorphine and methadone were examined to compare with the reported plasma levels in human opioid addicts receiving buprenorphine or methadone for maintenance treatments.

2.3. Locomotion testing

The assessment of activity was made in a set of 8 automated optical beam activity monitors (Model RXYZCM-16; Accuscan Instruments, Columbus, OH, USA) as described in detail in (Wellman et al., 2009). Briefly, each monitor is housed within a 40 cm × 40 cm × 30.5 cm acrylic cage. Activity monitors and cages were located in a sound-proof room with a 40 dB white noise generator continuously operating. A multiplexor-analyzer simultaneously tracks the interruption of beams from optical beam activity monitors. The multiplexor-analyzer updates the animal's position in the acrylic cage every 10 ms using a 100% real-time conversion system. The general activity is obtained from the computerized integration of the data using total distance traveled scores (in cm; Sanberg et al., 1987).

Activity was recorded two hours or three days following the final treatment dose of methadone, buprenorphine, or saline. Mice were placed in the testing room 30 min prior to the test. Baseline activity was recorded for 30 min. The mice were then injected with quinpirole (10 mg/kg, 10 ml/kg, i.p.) or vehicle and recorded for 120 min. Different mice were used on each testing day. The data for the vehicle tests are presented in the Supplementary materials. The apparatus was cleaned thoroughly with ethanol followed by water and completely dried between tests.

The choice of quinpirole to assess the effects of buprenorphine and methadone on the behavioral sensitivity of the D2/D3 dopamine receptors was based on previous studies in both rats (Piepponen et al., 1996; Druhan et al., 2000) and mice (Hofford et al., 2012) that demonstrated locomotor hypersensitivity to quinpirole following morphine administration. Moreover, in our previous study (Hofford et al., 2012) a range of quinpirole doses (starting at 0.01 mg/kg) were tested. Morphine did not alter the suppressive response of quinpirole (i.e., the response at the presynaptic D2 receptors) for any of the doses examined. However, morphine did alter the subsequent activating effect of quinpirole (i.e., the response at the postsynaptic receptors). Studies focused on the presynaptic receptors specifically used lower doses of quinpirole as to only activate the presynaptic receptors. These lower doses would not be suitable here since they do not activate the postsynaptic receptors. Therefore, in this study we used a dose of quinpirole that is established to affect the postsynaptic receptors.

It is important to note that the dose used is well within the range found in the literature. In mice, quinpirole doses up to 20 mg/kg were used by many studies and was considered to have specific effects on the D2-like dopamine receptors (Marsteller et al., 2009). Quinpirole specificity for up to 32 mg/kg was also established by the lack of effect in CBA/J mice that are deficient in the expression of dopamine receptors (Shannon et al., 1991).

2.4. Plasma levels of buprenorphine and methadone

Mice (n = 8–9 per group) were administered 0.2 mg/kg buprenorphine or 50 mg/kg methadone for six days, as described above. Two, six or 24 h after the final treatment dose they were anesthetized with pentobarbital (100 mg/kg, i.p.) and their blood collected via intra-cardiac puncture. Plasma was separated via centrifugation (15 min, 1000 × g, 4 °C) and stored at −80 °C. Buprenorphine and methadone levels in the plasma were determined using ELISA Kits (Neogen Corporation, St. Joseph, MI).

2.5. Data analyses

For each mouse, the scores for the total distance traveled (in cm) during the 120 min post-vehicle or post-quinpirole were normalized to the total distance traveled (in cm) during the 30 min baseline locomotion using the formula: [total distance traveled post-vehicle or post-quinpirole/baseline total distance traveled] × 100. Then, data for the between-subject factors of treatment was analyzed for the normalized total distance traveled scores (% from baseline) during the 120 min post-vehicle or post-quinpirole using the Univariate Analysis of Variance (ANOVA, SPSS Statistics 18, Somers, NY). Additional temporal analyses were also computed for between-group factors of treatment (buprenorphine, methadone, or saline) and for the within-group factor of time (1–120 min post-injection period summed in 5 min intervals). For this analysis, each animal's the score for the last 5 min interval prior to vehicle or quinpirole injections (i.e., baseline) was used to normalize the data. Post hoc contrasts between each treatment group were computed using Bonferroni's post hoc procedure. Differences with p-values of less than 0.05 were deemed statistically significant. Results are presented as mean ± SEM. The data for the vehicle tests are presented in the Supplementary Materials.

3. Results

3.1. Experiment I: the effects of buprenorphine and methadone on the response to quinpirole three days following the final treatment

3.1.1. Total distance traveled. The scores for the total distance traveled (% from baseline) during the 120 min post-quinpirole are presented in Fig. 1 Analysis revealed significant differences in the locomotor response to quinpirole between animals treated with the various drugs ($F(6, 117) = 4.38$, $p < 0.0001$). Post hoc comparison revealed no differences in quinpirole-induced suppression of activity level between saline-injected mice and mice treated with 0.1, 0.2 and 0.4 mg/kg buprenorphine. In contrast, significant differences were observed between saline-injected mice and mice treated with methadone ($p < 0.05$). Specifically, significantly less suppression of activity by quinpirole was observed in methadone-treated animals as compared to the drug-naïve animals.

3.1.2. Temporal analysis. Additional temporal analyses were computed using the distance traveled scores during each 5 min interval of the 120 min post-quinpirole injection. The results for the buprenorphine-treated animals are presented in Fig. 2A. Analysis revealed a main effect of time ($F(23, 1633) = 13.37$, $p < 0.0001$),