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SELF ADMINISTRATION OF OXYCODONE BY ADOLESCENT AND ADULT MICE AFFECTS STRIATAL NEUROTRANSMITTER RECEPTOR GENE EXPRESSION

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Abstract—Illicit use of prescription opioid analgesics (e.g., oxycodone) in adolescence is a pressing public health issue. Our goal was to determine whether oxycodone self administration differentially affects striatal neurotransmitter receptor gene expression in the dorsal striatum of adolescent compared to adult C57BL/6J mice. Groups of adolescent mice (4 weeks old, $n = 12$) and of adult mice (11 weeks old, $n = 11$) underwent surgery during which a catheter was implanted into their jugular veins. After recovering from surgery, mice self administered oxycodone (0.25 mg/kg/infusion) 2 h/day for 14 consecutive days or served as yoked saline controls. Mice were sacrificed within 1 h after the last self-administration session and the dorsal striatum was isolated for mRNA analysis. Gene expression was analyzed with real time PCR using a commercially available neurotransmitter receptor PCR array containing 84 genes. We found that adolescent mice self administered less oxycodone than adult mice over the 14 days. Monoamine oxidase A (*Maoa*) and neuropeptide Y receptor 5 mRNA levels were lower in adolescent mice than in adult mice without oxycodone exposure. Oxycodone self administration increased *Maoa* mRNA levels compared to controls in both age groups. There was a positive correlation of the amount of oxycodone self administered in the last session or across 14 sessions with *Maoa* mRNA levels. Gastrin-releasing peptide receptor mRNA showed a significant Drug × Age interaction, with point-wise significance. More genes in the dorsal striatum of adolescents (19) changed in response to oxycodone self administration compared to controls than in adult (4) mice. Overall, this study demonstrates that repeated oxycodone self administration alters neurotransmitter receptors gene expression in the dorsal striatum of adolescent and adult mice.

Key words: oxycodone, adolescent, adult, self administration, neurotransmitter receptor gene expression, dorsal striatum.

INTRODUCTION

Non-medical use of prescription opioids such as oxycodone in adolescents and young adults has increased in recent years (Johnston et al., 2009, 2013). This is particularly worrisome since the adolescent brain is undergoing maturation processes (Spear and Brake, 1983). Exposure to prescription opioids during adolescence may alter the normal developmental processes and increase the likelihood of neurobiological changes which may predispose adolescents to be more susceptible to develop addiction to drugs upon subsequent exposure.

There is accumulating evidence that adolescents show differential response to drugs of abuse compared to adults. For example, adolescent rats showed a significantly higher level of nicotine intake compared with both adult male (Levin et al., 2003) and female (Chen et al., 2007) rats. Periadolescent male but not female rats have higher motor activity in response to morphine than do adult rats (White et al., 2008). However, adolescent rats showed decreased cocaine self administration (SA) (Belluzzi et al., 2005) and cocaine-induced locomotor sensitization (Laviola et al., 1995; Collins and Izenwasser, 2002). Adolescent rats self administered less morphine than adults in 1-h self administration sessions. Also, cue-induced reinstatement was less robust in rats that began morphine self-administration during adolescence vs. adulthood (Doherty et al., 2009). Periadolescent (35-day-old) rats did not exhibit the morphine-induced place preferences found in the adult rats (Bolanos et al., 1996). Our earlier study found that adolescent mice self-administered less oxycodone than adult mice (Zhang et al., 2009).

The differential responses to drugs of abuse in adolescent animals may be due to differential neurobiological alterations induced by drugs of abuse in adolescents. Prescription opioids activate mu opioid receptors (MOP-r) located on the GABAergic interneurons in the ventral tegmental area and the substantia nigra, disinhibiting dopamine neurons and resulting in increases in dopamine release in the ventral and dorsal striata (Johnson and North, 1992). The increases of dopamine levels subsequently alter gene

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Abbreviations: GRP, gastrin-releasing peptide; *Grpr*, gastrin-releasing peptide receptor; ANOVA, analysis of variance; *Maoa*, monoamine oxidase A; MOP-r, mu opioid receptors; *Npy5r*, neuropeptide Y receptor 5.

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expression in the striatum (e.g., Spangler et al., 1993; Angulo and McEwen, 1994; Unterwald, 2001; Unterwald et al., 2001) which may be associated with changes in behavioral effects of MOP-r agonists in rodents, such as increased locomotor activity, development of conditioned place preference and self administration (Kruzich et al., 2003; Schlussman et al., 2008; Zhang et al., 2009; Picetti et al., 2012; Seip et al., 2012).

Studies found that gene expression altered in response to drugs of abuse in adult rodent brains (e.g., Wang et al., 1999; Nestler, 2001; Rodriguez Parkitna et al., 2004). However, there have been few studies on prescription opioid-induced alterations in gene expression in the adolescent brain (Ellgren et al., 2007). Thus, it is essential to determine how exposure to a prescription opioid such as oxycodone affects gene expression in the adolescent brain. In our earlier study, adolescent mice self administered significantly less oxycodone than adult mice (Zhang et al., 2009). We hypothesized that there were differential neurobiological alterations in brain regions associated with reward between adolescent and adult mice as a consequence of oxycodone self administration.

We tested this hypothesis by studying gene expression of neurotransmitter receptors in the dorsal striatum, a brain region closely involved in reward, locomotor regulation and habitual learning (Ito et al., 2002). Since changes in multiple neurotransmitter systems may be involved in the behavioral profile of oxycodone self administration, we chose to use the neurotransmitter receptor array which profiles the expression of 84 genes involved in modulating biological processes through neurotransmitter receptors and five housekeeping genes (Qiagen, Valencia, CA, USA).

To our knowledge, this is one of the few studies comparing the neurobiological changes induced by oxycodone between adolescent and adult subjects (see also Ellgren et al., 2007). The current study should initiate the elucidation of molecular mechanisms underlying the actions of oxycodone, and could shed light on the mechanisms underlying prescription opioid addiction in adolescents versus adults.

Although it is impossible to develop an animal model that exactly matches all the behavioral and neurobiological alterations taking place in human adolescence, the mouse model we have chosen (4-week-old mice) provides a rodent model of a neurological developmental period that bears substantial analogy to human adolescence (Spear, 2000; Adriani et al., 2004). The self-administration paradigm used in this study aims to model drug self administration or abuse in human adolescents.

EXPERIMENTAL PROCEDURES

Subjects

Male adolescent and adult (4 or 11 weeks old on arrival) C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were housed in groups of up to five with free access to food and water in a light-(12:12-h light/dark cycle, light on at 7:00 pm and off at 7:00 am) and temperature-(25 °C) controlled room. The adolescent mice were weaned at 3 weeks of age at the Jackson Laboratory. Animal care and experimental procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996). The experimental protocols used were approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

Self-administration procedure

Catheter implantation. Following acclimation for 7 days, the mice were anesthetized with a combination of xylazine (8.0 mg/kg i.p.) and ketamine (80 mg/kg i.p.). After shaving and application of a 70% alcohol and iodine preparatory solution, incisions were made in the midscapular region and anteromedial to the foreleg. A catheter of approximately 6 cm in length (ID: 0.31 mm, OD: 0.64 mm) (Helix Medical, Inc., CA, USA) was passed subcutaneously from the dorsal to the ventral incision. After exposure of the right jugular vein, a 22-gauge needle was inserted into the vein to guide the catheter into the jugular vein. Once the catheter was inside the vein, the needle was removed and the catheter was inserted to the level of a silicone ball marker 1.1 cm from the end. The catheter was tied to the vein with surgical silk. Physiological saline was then flushed through the catheter to avoid clotting and the catheter then capped with a stopper. Antibiotic ointment was applied to the catheter exit wounds on the animal's back and forearm. Mice were individually housed after the surgery and were allowed 4 days of recovery (due to the limited period of adolescence in the mouse (Spear, 2000; Adriani et al., 2004) before being placed in operant test chambers for the self-administration procedure (Zhang et al., 2006). See Table 1 for details of age and experimental procedure.

Intravenous self-administration chamber. The self-administration chamber, ENV-307W (21.6 cm × 17.8 cm × 12.7 cm, Med. Associates, St. Albans, VT, USA), was located inside a larger sound attenuation chamber (Med. Associates). The front, back and top were constructed of 5.6 mm polycarbonate. Each chamber contained a wall with two small holes (0.9 cm diameter, 4.2 cm apart, 1.5 cm from the floor of the chamber). One hole was defined as active, the other was inactive. When the photocell in the active hole was triggered by a nose-poke, an infusion pump (Med. Associates) delivered an oxycodone infusion of 20 μl/3 s from a 5-ml syringe. The syringe was connected by a swivel via Tygon tubing. The infusion pump and syringe were outside the chamber. During infusion, a cue light above the active hole was illuminated. Each injection was followed by a 20-s ''time-out'' period during which poking responses were recorded but had no programed consequences. All responses at the inactive hole were also recorded. Mice were tested during the dark phase