Neuropharmacology 141 (2018) 11–20

Contents lists available at ScienceDirect

Neuropharmacology

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

Neuropharmacology genetic deletion of the dopamine D3 receptor increases vulnerability to heroin in mice

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HIGHLIGHTS

- Deletion of D3Rs increased heroin self-administration and heroin intake.
- Deletion of D3Rs increased motivation for heroin during PR self-administration.
- Deletion of D3Rs increased basal levels of NAc extracellular DA and decreased DA responses to heroin.
- Deletion of D3Rs increased basal levels of locomotion and decreased locomotor responses to heroin.
- Reduced D3R availability in the brain may facilitate opioid abuse and addiction.

ARTICLE INFO

Keywords:
Heroin
Dopamine
D3 receptors
Reward
Addiction
Self-administration
Reinstatement

ABSTRACT

Despite extensive research, the neurobiological risk factors that convey vulnerability to opioid abuse are still unknown. Recent studies suggest that the dopamine D3 receptor (D3R) is involved in opioid self-administration, but it remains unclear whether altered D3R availability is a risk factor for the development of opioid abuse and addiction. Here we used dopamine D3 receptor-knockout (D3-KO) mice to investigate the role of this receptor in the different phases of opioid addiction. D3-KO mice learned to self-administer heroin faster and took more heroin than wild-type mice during acquisition and maintenance of self-administration. D3R-KO mice also displayed higher motivation to work to obtain heroin reward during self-administration under progressive-ratio reinforcement, as well as elevated heroin-seeking during extinction and reinstatement testing. In addition, deletion of the D3R induced higher baseline levels of extracellular dopamine (DA) in the nucleus accumbens (NAc), higher basal levels of locomotion, and reduced NAc DA and locomotor responses to lower doses of heroin. These findings suggest that the D3R is critically involved in regulatory processes that normally limit opioid intake via DA-related mechanisms. Deletion of D3R augments opioid-taking and opioid-seeking behaviors. Therefore, low D3R availability in the brain may represent a risk factor for the development of opioid abuse and addiction.

1. Introduction

Opioid addiction is a chronic reoccurring disorder characterized by high rates of relapse (Smyth et al., 2010) and risk for overdose (U.S. Department of Health and Human Services, 2017). Heroin, a synthetic opioid, is among the most addictive drugs of abuse (Chartoff and Connery, 2014; Reed et al., 2014), and is associated with massive personal and public health costs. In 2015 alone, heroin use disorder was estimated to cost the U.S. $51.2 billion, or $50,799 per user (Jiang et al., 2017). Rates of heroin abuse and dependence are rising, with diagnoses more than doubling from 2002 to 2013 and fatal overdoses increasing by 533% (Cicero et al., 2017; Kounang, 2017; Lipari and Hughes, 2013). Results from the 2015 National Survey on Drug Use and Health indicate that approximately 5.1 million individuals report using heroin in their lifetime (CBHSQ, 2016). These statistics and others have led many to declare the U.S. in the midst of an “opioid epidemic” (Kolodny et al., 2015).

Despite the rising prevalence of opioid addiction, treatments are limited and the neurobiological risk factors that convey vulnerability to opioid abuse remain unknown (Chartoff and Connery, 2014). The

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https://doi.org/10.1016/j.neuropharm.2018.08.016
Received 16 March 2018; Received in revised form 31 July 2018; Accepted 17 August 2018
Available online 20 August 2018
0028-3908/ Published by Elsevier Ltd.

mesolimbic dopamine (DA) pathway, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc) and prefrontal cortex, is a major neural substrate for drugs of abuse (Pierce and Kumaresan, 2006). Like many other drugs of abuse, opioids such as heroin increase extracellular DA levels in the NAc, where DA binds to post-synaptic receptors to trigger many molecular, physiological and behavioral changes (Pierce and Kumaresan, 2006). There are five DA receptor subtypes, which are classified into D1-like (D1, D5) and D2-like (D2, D3, D4) receptors based on their action on intracellular adenylate cyclase (Beaulieu and Gainetdinov, 2011). The D1 and D2 receptor subtypes have received substantial attention for their roles in substance abuse and addictive disorders (Volkow and Morales, 2015). However, the potential of both receptor subtypes as therapeutic targets is low due to their wide distributions throughout the brain and peripheral tissues and the concern for side effects (Childress and O'Brien, 2000; Desai et al., 1999; Xi and Gardner, 2007). In contrast, D3 receptors are enriched primarily in the mesolimbic DA system, particularly in the VTA and NAc, and therefore represent more attractive pharmacotherapeutic targets for the treatment of drug abuse (Heidbreder, 2008; Heidbreder and Newman, 2010; Sokoloff and Le Foll, 2017; Xi and Gardner, 2007).

Converging evidence in both humans and rodent models supports the involvement of the D3R in drug use disorders. For example, cocaine- and methamphetamine-dependent subjects show increased D3R availability in midbrain regions including the substantia nigra, hypothalamus and amygdala compared to healthy controls (Aleph Prieto, 2017; Boileau et al., 2016; Matuskey et al., 2014). The increase in D3R expression in the substantia nigra and ventral pallidum correlates positively with years of drug use (Matuskey et al., 2014). Genetic deletion of D3R causes escalation in cocaine-taking and cocaine-seeking behavior in mice D3R (Song et al., 2012) (but see Caine et al., 2012). In addition, variations in the D3R gene are also associated with opiate dependence in humans (Duaux et al., 1998), and chronic opioid exposure is associated with a reduction in D3 expression in the amygdala in rats (Rosen et al., 2017). We have recently reported that acute pharmacological blockade of D3R inhibits heroin or oxycodone self-administration in rats and mice in a dose-dependent manner (Boateng et al., 2015; You et al., 2017). However, it is unknown whether altered D3R expression is associated with the *development* of opioid abuse and addiction. In the present study, we used transgenic D3R gene-knockout mice to study whether D3R loss is a susceptibility factor in the development of opioid abuse and addiction by examining animal drug-taking and drug-seeking behaviors during the acquisition of intravenous (i.v.) heroin self-administration, extinction, and reinstatement of drug-seeking. We also examined heroin self-administration maintained by different doses of heroin under fixed-ratio and progressive-ratio reinforcement schedules. Finally, we used *in vivo* microdialysis and a locomotor response paradigm to study whether D3R deletion alters mesolimbic DA response to heroin, and thereby alters heroin-taking and heroin-seeking behaviors.

2. Materials and methods

2.1. Animals

Wild-type (WT) and DA D3R knockout (D3-KO) mice were generated and backcrossed to a C57BL/6J background at the National Institute on Drug Abuse (Baltimore, MD, USA) from three $D3^{+/-}$ breeding pairs purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All mice were genotyped in our laboratory according to the mouse tail D3R-DNA-PCR protocol used by Charles River Laboratories (Wilmington, MA, USA). Experimental WT and D3-KO mice were matched for age (8–14 weeks) and weight (25–35 grams). During experimental testing, mice were housed individually in a climate-controlled animal colony room on a reversed light-dark cycle (lights on at 1900 h, lights off at 700 h). Food and water were available ad libitum. Mice were acclimated to housing conditions and handled once/day for 7 days before the start of experiments. All testing was conducted during the dark phase. All experimental procedures were carried out in accordance with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health.

2.2. Surgery

To enable heroin self-administration, a catheter was implanted into the right external jugular vein under ketamine anesthesia in each experimental subject, using aseptic surgical technique. Briefly, a 6.0-cm length of MicroRenathane tubing (ID 0.012″, OD 0.025″; Braintree Scientific Inc., Braintree, MA, USA) was inserted 1.2 cm into the right jugular vein and anchored to a 24-gauge steel cannula (Plastics One, Roanoke, VA, USA) that was bent at a 100° angle and mounted to the skull with cyanoacrylate glue and dental acrylic. A 2.5-cm extension of flexible tubing was connected to the distal end of the cannula. The mice were allowed 5–7 days for recovery, during which time 0.05 ml of 0.9% saline solution containing 20 IU/ml heparin and 0.33 mg/ml gentamycin was infused daily through the catheter to forestall clotting and infection. Thereafter, 0.05 ml of 0.9% saline solution containing 20 IU/ml heparin was infused immediately prior to and immediately following each daily heroin self-administration session. When needed, i.v. brevital (a barbiturate) was used to test catheter patency between the self-administration sessions. During heroin self-administration sessions, the flexible tubing extension was connected to a perfusion pump (Razel Scientific Instruments, Stamford, CT, USA) via a PE50 tubing connector. Outside self-administration sessions, the free end of the cannula guide was kept sealed at all times.

2.3. Apparatus

Operant test chambers (Model ENV-307A, Medical Associates, Georgia, VT, USA) were used to conduct i.v. heroin self-administration experiments. Briefly, each test chamber contained two levers located 2.5 cm above the floor (one active and one inactive), as well as a speaker and a yellow cue light located ~5 cm above the active lever. A house light mounted on the opposite wall signaled the start of each 3-hour test session and remained illuminated until the session ended. For heroin self-administration sessions, a liquid swivel mounted on a balance arm above the chamber allowed for i.v. drug delivery in freely-moving animals. Depression of the active lever resulted in the activation of an infusion pump; depression of the inactive lever was recorded but had no scheduled consequences. Each heroin infusion was paired with two discrete cues: illumination of the cue light above the active lever, and a cue tone that lasted for the duration of the infusion. Experimental events were controlled by a PC programmed in Medstate Notation and connected to a Medical Associates interface.

2.4. Heroin self-administration under FR1 reinforcement

To determine the effects of D3R deletion on heroin intake, after recovery from surgery mice were placed into operant chambers and allowed to lever-press for i.v. heroin delivered at a rate of 3.57μl/sec under a fixed-ratio (FR)1 reinforcement schedule (each lever press lead to one heroin infusion) for 3 h daily. During the infusion period (4.2 sec), additional responses were recorded but had no consequences. For the first 5 training sessions a 0.1 mg/kg/infusion heroin dose was available for self-administration, for sessions 6 through 10 a 0.05 mg/kg/infusion dose was available, and for sessions 11 through 15 a 0.025 mg/kg/infusion dose was available. Stable self-administration was defined as (i) earning at least 20 infusions per 3-h session, (ii) less than 20% variability in heroin infusions across 2 consecutive sessions, and (iii) an active/inactive lever press ratio exceeding 2:1. Mice that