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Delta-opioid receptor antagonism leads to excessive ethanol consumption in mice with enhanced activity of the endogenous opioid system

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ABSTRACT

The opioid system modulates the central reinforcing effects of ethanol and participates in the etiology of addiction. However, the pharmacotherapy of ethanol dependence targeted on the opioid system is little effective and varies due to individual patients' sensitivity. In the present study, we used two mouse lines with high (HA) and low (LA) activity of the endogenous opioid system to analyze the effect of opioid receptor blockade on ethanol drinking behavior. We found that LA and HA lines characterized by divergent magnitudes of swim stress-induced analgesia also differ in ethanol intake and preference. Downregulation of the opioid system in LA mice was associated with increased ethanol consumption. Treatment with a non-selective opioid receptor antagonist (naloxone) had no effect on ethanol intake in this line. Surprisingly, in HA mice, the blockage of opioid receptors led to excessive ethanol consumption. Moreover, naloxone selectively induced high levels of anxiety- and depressive-like behaviors in HA mice which was attenuated by ethanol. With the use of specific opioid receptor antagonists we showed that the naloxone-induced increase in ethanol drinking in HA mice is mediated mainly by δ and to a lower extent by μ opioid receptors. The effect of δ-opioid receptor antagonism was abolished in HA mice carrying a C320T transition in the δ-opioid receptor gene (EU446125.1), which impairs this receptor's function. Our results indicate that high activity of the opioid system plays a protective role against ethanol dependence. Therefore, its blockage with opioid receptor antagonists may lead to a profound increase in ethanol consumption.

1. Introduction

Alcohol dependence is a chronic relapsing disorder manifested by compulsive ethanol use and seeking behavior. The progression from recreational alcohol use to addiction involves multiple neurobiological mechanisms. The positive reinforcing effects of drinking are related to the release of neurotransmitters – dopamine, γ-aminobutyric acid (GABA) and opioid peptides in the key limbic regions of the brain reward circuitries (Tupala and Tiihonen, 2004). Despite a well-defined pharmacological mechanism of ethanol effects, the treatment of addiction is limited by weak efficacy and high variability in the therapeutic effect among patients. The individual profile of alcohol dependence is determined by the complex interplay of genetic, psychological and social factors (Miranda et al., 2013). Therefore, both genetic and behavioral classifications of alcoholism might be utilized to develop personalized treatment of alcohol dependence (Leggio et al., 2009). The differences in personality traits, such as the level of anxiety and

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sociability significantly affect individual drinking habits and risk of relapse (Mellos et al., 2010).

The involvement of the opioid system in the development and maintenance of alcohol addiction has been under study for over 30 years (Nutt, 2014). It is widely accepted that endogenous opioids are important for modulation of the reinforcing effects of ethanol (Gianoulakis et al., 1996). Evidence from gene knock-out animals supports the involvement of μ, δ and κ opioid receptors in alcohol intake. Results from transgenic animals indicate that the dysfunction of the δ opioid receptor system leads to an increase (Roberts et al., 2001), while dysfunction of the μ or κ-opioid receptor systems are associated with a decrease in ethanol consumption (Hall et al., 2001; Kovacs et al., 2005).

The usefulness of the opioid receptor antagonists – naltrexone (a pure κ, δ, μ receptor antagonist) and nalmefene (κ opioid receptor partial agonist and a pure δ and μ receptors antagonist) in the management of alcohol abuse disorders is still controversial. There are studies showing effectiveness of the opioid receptor antagonists in decreasing alcohol consumption, both in animal models of alcoholism (Froehlich et al., 1990; Kim et al., 2000; Nielsen et al., 2008) and in patients treated for alcohol dependence (Rosner et al., 2010; Soyka, 2014). However, numerous data indicate that opioid receptor antagonists have limited effects on the reduction of ethanol consumption or in some cases may have an opposite effect (Hill et al., 2010; Juarez and Eliana Bde, 2007). Preliminary evidence suggests that variation in the genes encoding opioid receptors impacts the patients' response to naltrexone used to treat alcohol dependence (Bilbao et al., 2015). A single nucleotide polymorphism (A118G) in the μ opioid receptor gene predicts an unfavorable response to naltrexone in individuals carrying an 'A' allele (Berrettini, 2016).

The genetic component of opioid-related phenotypic traits is supported by our studies conducted on mice selected for high (HA) and low (LA) swim stress-induced analgesia (SSIA) characterized by unique, inherited differences in opioid system activity. HA mice exhibit increased opioid system activity compared to their LA counterparts (Panocka et al., 1986a). This is in agreement with higher binding affinities of exogenous opioid ligands to certain brain regions involved in analgesia and reward observed in HA mice (Kest et al., 1999). As a consequence of extreme differentiation in the activity of the opioid system, these lines differ in basal nociception (Panocka et al., 1986b), morphine efficacy (Panocka et al., 1991) and ethanol-induced analgesia (Mogil et al., 1993). The lines differ also in pro-depressive and pro-nociceptive effects of chronic stress (Ragan et al., 2013) as well as in stress-induced ethanol drinking behavior (Sacharczuk et al., 2008, 2009; 2014). The between-line differences in opioid system activity underlie the discrepancies in the reaction to the environmental changes, as the unpredictable chronic mild stress (CMS). Thus, HA and LA mice serve as a useful model of distinct personality traits and coping styles observed in humans, which can indicate different alcohol drinking behavior as well as distinct response to the pharmacological treatment.

Taking into account the above findings, it was reasonable to test the treatment effects of opioid receptor antagonists on alcohol drinking behavior in HA and LA mice. In this study, we compared the results of opioid receptor blockade on ethanol intake and preference in both lines. Moreover, we also examined naloxone-induced changes in anxiety- and depression-like behavior in the context of alterations in alcohol drinking behavior and δ opioid receptor gene polymorphism. We showed that antagonism of the enhanced opioid system in HA mice leads to increased ethanol drinking which is mediated mainly through δ and to a lower degree by μ opioid receptors. Moreover, we found that suppression of the enhanced opioid system activity increases anxiety- and depressive-like behaviors, which is at least partly responsible for the increased ethanol intake in HA mice. The results strongly suggest that the diversity in the activity of the endogenous opioid system may serve as a predictive marker for the effectiveness of opioid-based therapeutic approaches in alcohol dependence.

2. Materials and methods

2.1. Animals

Experiments were performed on male Swiss-Webster mice from the 85th generation of selective breeding for high (HA) and low (LA) swim stress-induced analgesia. Animals were bred and maintained at the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzebiec according to the protocol described previously (Panocka et al., 1986a,b). Briefly, outbred Swiss-Webster mice of either sex were screened for SSIA magnitude on a hot plate heated to 56 °C, 2 min after the completion of a 3-min swim in 20 °C water. Those displaying the longest (50–60 s) and the shortest (<10 s) post-swim latencies of the hind paw flick or lick response (whichever occurred first) were selected as progenitors of the HA and the LA lines. A similar procedure was repeated in each offspring generation, but only subjects displaying the longest and the shortest post-swim hot plate latencies were mated to maintain the lines (the magnitude of SSIA was expressed as percentage of the maximum possible effect (% MPE) is stable in HA and LA line across all generations). After weaning, mice were housed in groups, 4–5 siblings per cage, at an ambient temperature of 22 ± 2 °C and 55 ± 5% relative humidity and in a 12-h light/dark cycle (lights on at 07:00 a.m.). They were given free access to tap water and pellet food (rodent block chow) provided by LABOFEED H (Kcynia, Poland).

2.2. Ethical note

All the procedures are commonly used and considered ethically acceptable in all European Union countries and North America. They also conform to the NIH Guide for the Care and Use of Laboratory Animals and to the European Communities Council Directive (86/609/EEC) guidelines. All the tests were performed in agreement with the 3R's rule of animal welfare.

2.3. Drugs

Opioid receptor antagonists naloxone hydrochloride (NLX) [(5α)-4,5-Epoxy-3,14-dihydro-17-(2-propenyl)morphinan-6-one hydrochloride] (non-selective antagonist), cyprodime (μ opioid receptors) hydrochloride (Cypr) [17-(Cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one hydrochloride], naltrindole (δ opioid receptors) hydrochloride (NTI) [17 (Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-indolomorphinan hydrochloride] and nor-binaltorphimine (κ opioid receptors) dihydrochloride (nor-BNI) [17,17′ (Dicyclopropylmethyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′tetroldihydrochloride] were purchased from Tocris Bioscience (Bristol, United Kingdom). NLX was standardized to equimolar doses of 1.375, 2.75 13.75 and 27.5 μmol/kg (0.45, 0.9, 4.5 and 9 mg/kg, respectively). Selective antagonists were standardized only to an equimolar dose of 27.5 μmol/kg (Cypr: 9.8 mg/kg; NTI: 11.4 mg/kg; nor-BNI: 18.2 mg/kg). All drugs were dissolved in 0.9% NaCl and administered intraperitoneally in a volume of 0.05 ml/10 g.

2.4. Genotyping of the δ opioid receptor gene

Genotyping was performed on male HA and LA line according to