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Species differences in the effects of the κ-opioid receptor antagonist zyklophin

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ABSTRACT

We have shown that dysregulation of the dynorphin/kappa-opioid receptor (DYN/KOR) system contributes to escalated alcohol self-administration in alcohol dependence and that KOR antagonists with extended durations of action selectively reduce escalated alcohol consumption in alcohol-dependent animals. As KOR antagonism has gained widespread attention as a potential therapeutic target to treat alcoholism and multiple neuropsychiatric disorders, we tested the effect of zyklophin (a short-acting KOR antagonist) on escalated alcohol self-administration in rats made alcohol-dependent using intermittent alcohol vapor exposure. Following dependence induction, zyklophin was infused centrally prior to alcohol self-administration sessions and locomotor activity tests during acute withdrawal. Zyklophin did not impact alcohol self-administration or locomotor activity in either exposure condition. To investigate the neurobiological basis of this atypical effect for a KOR antagonist, we utilized a κ-, μ-, and δ-opioid receptor agonist-stimulated GTPγS coupling assay to examine the opioid receptor specificity of zyklophin in the rat brain and mouse brain. In rats, zyklophin did not affect U50488-, DAMGO-, or DADLE-stimulated GTPγS coupling, whereas the prototypical KOR antagonist nor-binaltorphimine (norBNI) attenuated U50488-induced stimulation in the rat brain tissue at concentrations that did not impact μ- and δ-receptor function. To reconcile the discrepancy between the present rat data and published mouse data, comparable GTPγS assays were conducted using mouse brain tissue; zyklophin effects were consistent with KOR antagonism in mice. Moreover, at higher concentrations, zyklophin exhibited agonist properties in rat and mouse brains. These results identify species differences in zyklophin efficacy that, given the rising interest in the development of short-duration KOR antagonists, should provide valuable information for therapeutic development efforts.

Introduction

Alcoholism, a chronic relapsing disorder characterized by continued alcohol use despite numerous adverse consequences, has a prevalence of ~5.3% in the United States, with ~4.4% of the population diagnosed as alcohol-dependent (Lee et al., 2010). An impaired physiological state accompanied by negative affective states and impaired cognitive control are devastating symptoms of alcoholism that promote excessive alcohol consumption and can adversely affect treatment outcome (Dvorak, Lamis, & Malone, 2013; Leeman, Fenton, & Volpicelli, 2007; Walker & Koob, 2008). However, none of the FDA-approved drugs target these symptoms of alcohol dependence and as such there is a pressing need for better therapeutics that could increase treatment compliance and reduce relapse episodes.

Kappa-opioid receptors (KORs) and their endogenous ligand, dynorphin (DYN) (Chavkin, James, & Goldstein, 1982), are present and positioned to modulate numerous neurotransmitters in motivational and emotional neurocircuitry (Sirohi, Bakalkin, & Walker, 2012; Tejeda et al., 2013; Tejeda, Shippenberg, & Henriksson, 2011). Alterations in the DYN/KOR system in motivational, emotional, and decision-making circuitry have been posited to contribute to multiple neuropsychiatric disorders, including alcohol dependence (Barg et al., 1993; Bazov et al., 2013; Hiller, Itzhak, & Simon, 1987; Risser et al., 1996). Recently, it was shown that

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dysregulation of the DYN/KOR system contributes to behavioral deficits that drive an organism to excessively seek and use alcohol in alcohol dependence (Walker & Koob, 2008; Walker, Valdez, McLaughlin, & Bakalkin, 2012), due to the fact that site-specific blockade of the DYN/KOR system alleviates alcohol dependence-induced negative affective states and escalated alcohol consumption (Berger, Williams, McGinnis, & Walker, 2013; Kissler et al., 2014; Nealey, Smith, Davis, Smith, & Walker, 2011; Valdez & Harshberger, 2012; Walker & Koob, 2008).

As a result of such evidence, the KOR has been proposed as a potential therapeutic target to treat addictive and neuropsychiatric disorders such as alcohol dependence and depression (Aldrich & McLaughlin, 2009; Knoll & Carlezon, 2010; Shippenberg, Zapata, & Chefer, 2007; Tejeda et al., 2011; Walker et al., 2012). An extended duration of action, lasting from weeks to months, characterizes classical KOR antagonists (Chartoff et al., 2012; Melief et al., 2011; Metcalf & Coop, 2005; Schlosburg et al., 2013; Walker, Zorrilla, & Koob, 2011; Whitfield et al., 2015), and general opioid-receptor antagonists with an extended duration of action are currently approved for the treatment of alcohol dependence in the USA (Mannelli, Peindl, Masand, & Patkar, 2007). However, an alternate strategy for the treatment of alcohol dependence using a short-acting mixed mu-opioid receptor antagonist/partial KOR agonist (nalmefene; prescribed for use on an as-needed basis) was recently approved for use in the European Union (Mann, Bladström, Torup, Gual, & van den Brink, 2013). However, all currently approved opioid-receptor ligands for the treatment of alcohol dependence modulate the function of multiple opioid receptors. Given the wealth of preclinical data implicating modification of aberrant signaling through KORs as a potential therapeutic target, there have been considerable efforts to develop novel KOR-selective antagonists with a short duration of action.

Zyklophin ([N-benzyl-Tyr^1-cyclo(d-Asp^5,Dap^8)]dynorphin A(1–11)NH_2), a cyclic peptide with a short duration (<12 h) of action, has recently been characterized as a novel KOR antagonist in mouse models (Aldrich, Patkar, & McLaughlin, 2009). In the present study, we evaluated zyklophin efficacy for attenuating excessive alcohol consumption during withdrawal in alcohol-dependent rats. We have previously shown that the long-acting KOR antagonist norBNI selectively attenuated withdrawal-induced escalation of alcohol self-administration in alcohol-dependent rats (Kissler et al., 2014; Kissler & Walker, 2015; Nealey et al., 2011; Walker & Koob, 2008), and it was hypothesized that zyklophin would show selective efficacy in dependent animals for reducing excessive alcohol self-administration during acute withdrawal.

Materials and methods

Animals

Male Wistar rats or C57BL/6J mice ~70 days of age were used in the experiments. Upon arrival, animals were housed in an environmentally controlled vivarium with food and water available *ad libitum* and were gently handled on a daily basis. All work adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and followed Institutional Animal Care and Use Committee guidelines. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to *in vivo* techniques, if available.

Operant alcohol self-administration

Rats were trained to self-administer a 10% alcohol (w/v) solution using a sweetener-fade method (Kissler et al., 2014; Nealey et al., 2011; Samson, 1986; Walker & Koob, 2008). In standard operant chambers (Med Associates, St. Albans, VT), rats pressed a single lever and received 0.1 mL of solution. Following stability (<10% deviation over 3 sessions), the animals were divided into two groups (n = 8/group) that were matched for baseline alcohol self-administration. Half of these animals underwent alcohol self-administration studies and the other half were tested for locomotor activity.

Surgical procedure

Bilateral guide cannulae were implanted in the lateral ventricles using stereotaxic coordinates (from bregma: DV: −3.7; AP: −0.8; and ML: ±1.5; Paxinos & Watson, 2005) under isoflurane gas anesthesia (~2%) and secured to the skull with four jeweler screws and dental acrylic. To preserve patency and reduce risk of infection, obturators were inserted into each guide cannulae. Following surgery, rats were allowed to recover for one week and post-operative care (saline, Flunixin, Baytril, subcutaneous injection) was provided during that time. Following the conclusion of the experiments, cannulae placements were confirmed by injecting 1 μL 0.6% cresyl violet over 1 min, extracting the brain, and confirming intraventricular dye penetration.

Intermittent alcohol-vapor exposure

Following recovery, rats were subjected to alcohol vapor according to an intermittent schedule (14 h on, 10 h off), with controls exposed to air. This procedure reliably induces alcohol dependence-like phenotypes (e.g., escalated self-administration and negative affective-like behavior) as shown previously (Kissler et al., 2014; Walker & Koob, 2008). Blood ethanol concentrations (BECs) were analyzed from samples collected prior to daily vapor termination using the Analox AM1 (Analox Instruments Ltd., Lunenberg, MA). BECs were also assessed prior to any behavioral testing. Target BECs of 175–225 mg% were maintained throughout the experiments.

Drugs

Zyklophin-HCl (J. Aldrich, University of Kansas) was dissolved in artificial cerebral spinal fluid (aCSF) (pH 7.2–7.4), composed of 145-mM NaCl, 2.8-mM KCl, 1.2-mM $MgCl_2$, 1.2-mM $CaCl_2$, 5.4-mM D-glucose, and 0.25-mM ascorbic acid, or assay buffer. NorBNI and U50488 were purchased from Tocris Bioscience (Ellisville, MO). DAMGO and DADLE were purchased from Sigma Chemical Co. (St. Louis, MO). All drugs for GTPγS coupling assay were dissolved in the assay buffer.

Infusions

Following air or intermittent alcohol-vapor exposure, rats self-administered 10% alcohol (w/v) for 30 min twice per week during acute withdrawal (6–8 h after vapor termination) in operant chambers. Upon stability (<10% deviation over 3 days), rats received sham intracerebroventricular (ICV) infusions via insertion of internal cannulae into the guide cannulae for 2 min, followed by a 1.0-h waiting period before self-administration testing. Once stability was again achieved (<10% over 2 sessions), animals were infused with 1.0 μL of aCSF on each side over 1 min with the internal cannulae remaining in place for 1 min to allow for vehicle diffusion. Infusions of aCSF were repeated until stability was achieved again (<10% deviation over 2 sessions). Sham and aCSF infusions were performed to habituate the animal to the infusion process.