Neuropharmacology 137 (2018) 86–95

Contents lists available at ScienceDirect

Neuropharmacology

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

Pharmacological inhibition of Receptor Protein Tyrosine Phosphatase β/ζ (PTPRZ1) modulates behavioral responses to ethanol

Rosalía Fernández-Calle [b, 1], Marta Vicente-Rodríguez [b, d, 1], Miryam Pastor [a, 1], Esther Gramage [b], Bruno Di Geronimo [a], José María Zapico [a], Claire Coderch [a], Carmen Pérez-García [b], Amy W. Lasek [c], Beatriz de Pascual-Teresa [a], Ana Ramos [a], Gonzalo Herradón [b, *]

[a] Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28925, Alcorcón, Madrid, Spain
[b] Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28925, Alcorcón, Madrid, Spain
[c] Department of Psychiatry, University of Illinois at Chicago, 1601 West Taylor Street, Chicago, IL 60612, USA
[d] Department of Neuroimaging, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK

ARTICLE INFO

Article history:
Received 2 November 2017
Received in revised form
18 April 2018
Accepted 24 April 2018

Keywords:
ALK
TrkA
Alcohol use disorder
Binge-drinking
Pleiotrophin
Midkine

ABSTRACT

Pleiotrophin (PTN) and Midkine (MK) are neurotrophic factors that are upregulated in the prefrontal cortex after alcohol administration and have been shown to reduce ethanol drinking and reward. PTN and MK are the endogenous inhibitors of Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ (a.k.a. PTPRZ1, RPTPβ, PTPζ), suggesting a potential role for this phosphatase in the regulation of alcohol effects. To determine if RPTPβ/ζ regulates ethanol consumption, we treated mice with recently developed small-molecule inhibitors of RPTPβ/ζ (MY10, MY33-3) before testing them for binge-like drinking using the drinking in the dark protocol. Mice treated with RPTPβ/ζ inhibitors, particularly with MY10, drank less ethanol than controls. MY10 treatment blocked ethanol conditioned place preference, showed limited effects on ethanol-induced ataxia, and potentiated the sedative effects of ethanol. We also tested whether RPTPβ/ζ is involved in ethanol signaling pathways. We found that ethanol treatment of neuroblastoma cells increased phosphorylation of anaplastic lymphoma kinase (ALK) and TrkA, known substrates of RPTPβ/ζ. Treatment of neuroblastoma cells with MY10 or MY33-3 also increased levels of phosphorylated ALK and TrkA. However, concomitant treatment of neuroblastoma cells with ethanol and MY10 or MY33-3 prevented the increase in pTrkA and pALK. These results demonstrate for the first time that ethanol engages TrkA signaling and that RPTPβ/ζ modulates signaling pathways activated by alcohol and behavioral responses to this drug. The data support the hypothesis that RPTPβ/ζ might be a novel target of pharmacotherapy for reducing excessive alcohol consumption.

1. Introduction

Alcohol use disorder (AUD) constitutes a public health crisis. Hazardous use of alcohol is the sixth leading cause of ill health and premature death in high-income countries (Forouzanfar et al., 2016). Approximately 70% of the adults in the WHO European Region drink alcohol and most alcohol is consumed in heavy drinking occasions (60 g of pure alcohol or more on one occasion) which worsen all risks. Binge drinking accounts for over half of the approximately 88,000 deaths yearly that are attributed to alcohol use in the United States, but binge drinkers also put themselves at increased risk for multiple comorbidities (O'Keefe et al., 2007; Petit et al., 2014; Zakhari and Li, 2007). In the United States, recent studies have shown that the prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV AUD has increased significantly (Grant et al., 2017). Thus, new therapeutic approaches to treat AUD are greatly needed.

In an effort to develop new pharmacotherapies to limit alcohol

* Corresponding author. Laboratory of Pharmacology, Faculty of Pharmacy, Universidad San Pablo-CEU, Urb. Montepríncipe, 28925, Alcorcon, Madrid, Spain.
E-mail address: herradon@ceu.es (G. Herradón).
[1] R. F-C, M. V-R and M. P contributed equally.

https://doi.org/10.1016/j.neuropharm.2018.04.027
0028-3908/

consumption and prevent alcoholism, the identification of novel genes and pathways that may predispose individuals to AUD is key. Two genetic factors that have been recently identified as important regulators of alcohol behavioral effects are Pleiotrophin (PTN) and Midkine (MK). PTN and MK are cytokines important in central nervous system (CNS) functions and repair (Herradon and Perez-Garcia, 2014). Both PTN and MK are also upregulated in different brain areas after administration of different drugs of abuse (Herradon and Perez-Garcia, 2014), suggesting PTN and MK signaling may be important in the regulation of drug-induced addictive behaviors. Accordingly, PTN is found upregulated in the nucleus accumbens after a single administration of amphetamine (Le Greves, 2005) and has been shown to contribute to the extinction of amphetamine-seeking behaviors (Gramage et al., 2010a). Importantly, MK expression is higher in the prefrontal cortex (PFC) of human alcoholics and mice selectively bred for high alcohol consumption (Flatscher-Bader and Wilce, 2008; Mulligan et al., 2006) and PTN is upregulated in the PFC of mice injected with a rewarding dose of ethanol (Vicente-Rodriguez et al., 2014a). Both cytokines have been shown to be potent regulators of behavioral effects induced by ethanol (Chen et al., 2017; Vicente-Rodriguez et al., 2014a, 2014b). It has been demonstrated that PTN knockout (PTN−/−) and MK knockout (MK-/) mice are more sensitive to the rewarding effects of alcohol in the conditioned place preference test (Vicente-Rodriguez et al., 2014a, 2014b). In contrast, PTN transgenic overexpression in the mouse brain blocks the rewarding effects of alcohol (Vicente-Rodriguez et al., 2014a). Overall, the data suggest that PTN and MK could be used for the treatment of drug addiction disorders including AUD (Alguacil and Herradon, 2015).

Both PTN and MK bind to the Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ (a.k.a. PTPRZ1, RPTPβ, PTPζ), and inactivate its phosphatase activity (Herradon and Ezquerra, 2009). This leads to an increase in tyrosine phosphorylation of substrates critical for the effects of these cytokines such as Fyn kinase (Pariser et al., 2005) and Anaplastic Lymphoma Kinase (ALK) (Perez-Pinera et al., 2007). We hypothesize that PTN and MK actions on substance use disorders can be reproduced with rationally designed small molecule inhibitors of RPTPβ/ζ (Herradon et al., 2009; Herradon and Perez-Garcia, 2014).

Recently, a new series of blood-brain barrier (BBB) permeable molecules designed to mimic the activity of PTN/MK in the CNS were synthesized (Pastor et al., 2018). These compounds exert their actions by interacting with the intracellular domain of RPTPβ/ζ and inhibiting its tyrosine phosphatase activity. The most potent compounds MY10 and MY33-3 (IC_{50} ~ 0.1 μM) significantly increased the phosphorylation of key tyrosine residues of RPTPβ/ζ substrates involved in neuronal survival and differentiation such as ALK. More interestingly, PTN and MK were previously shown to prevent amphetamine neurotoxicity in vivo and in vitro (Gramage et al., 2010b, 2011) and these RPTPβ/ζ inhibitors have shown similar protective effects against amphetamine-induced toxicity (Pastor et al., 2018).

The purpose of this study was to test inhibitors of RPTPβ/ζ in binge-like drinking, ethanol conditioned place preference, and other behavioral responses to ethanol, and to study the possible interactions of these molecules with ethanol-induced activation of signaling pathways.

2. Materials and methods

2.1. Subjects

Male C57BL/6J mice (8–10 weeks of age) were tested for behavior. Mice were housed under controlled environmental conditions (22 ± 1 °C and a 12-h light/12-h dark cycle) with free access to food and water. All the animals used in this study were maintained in accordance with both the ARRIVE guidelines and the European Union Laboratory Animal Care Rules (86/609/ECC directive) or the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All protocols were approved by the Animal Research Committee of USP-CEU or the Animal Care Committee of the University of Illinois at Chicago and procedures were used to minimize pain and suffering.

2.2. RPTPβ/ζ inhibitors

MY10 and MY33-3 (Fig. 1) were synthesized as previously described (Pastor et al., 2018). MY10 and MY33-3 were administered at a dose of 60 mg/kg in 10% dehydrated ethanol, 20% polysorbate 80, 70% PEG-300 vehicle. Ethanol in the vehicle results in a dose of less than 0.3 g/kg. Pharmacokinetic studies in mice have shown that 1 h after oral administration the brain to plasma ratio is 3:1 (Pastor et al., 2018). Mice were administered compounds or vehicle by oral gavage in a volume of approximately 0.1 ml.

2.3. Drinking in the dark (DID)

Binge-like drinking was measured using the two-bottle DID procedure as previously described using 20% ethanol (Dutton et al., 2017). Mice were individually housed for 2 weeks in the reverse-dark cycle room prior to testing ethanol consumption. Three days before testing ethanol consumption, mice drank water from two tubes made from 10 ml polycarbonate pipettes connected to stainless steel sipper tubes containing ball-bearings to prevent leakage (Rhodes et al., 2005) for one day, in order to acclimate them to the tubes. Three days later mice were given a choice between 20% ethanol and water in the sipper tubes. Fluid consumption was measured every day for 4 days by measuring the volume of fluid in the tubes. The position of the bottles (left or right) was changed every day to control for side preference. On the first 3 days of testing, mice were given access to the ethanol and water tubes 3 h into the dark cycle for a period of 2 h. On the fourth day, mice were given access to ethanol and water tubes for 4 h and the volume consumed was measured at 4 h. All mice were given vehicle (0.1 ml) by oral gavage on days 1 & 2. On the third and fourth days, mice were administered 60 mg/kg MY10, MY33-3 or vehicle (0.1 ml) by oral gavage 1 h before the drinking session in the DID test (n = 12/group). Preference score was calculated as the ratio of the volume of ethanol consumed over the volume of total fluid consumed (Chen et al., 2017; Dutton et al., 2017). For the sucrose consumption test, a separate group of mice were tested exactly as in the ethanol consumption test, except that mice were provided with two tubes containing 2% sucrose in water and water instead of 20% ethanol and water.

Blood samples (20 μl) were collected immediately after the 4-h drinking session on day 4 to measure blood ethanol concentrations (BECs). Blood was collected in heparinized capillary tubes via tail vein puncture. BECs were determined using a nicotinamide adenine dinucleotide-alcohol dehydrogenase enzymatic assay (Zapata et al., 2006).

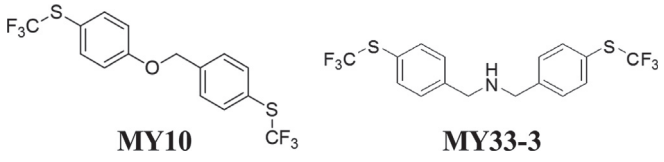

Fig. 1. Structure of the RPTPβ/ζ inhibitors MY10 and MY33-3.