Neuropharmacology 118 (2017) 1–12

Contents lists available at ScienceDirect

Neuropharmacology

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

The galanin-3 receptor antagonist, SNAP 37889, suppresses alcohol drinking and morphine self-administration in mice

Karlene J. Scheller [a, c], Spencer J. Williams [b], Andrew J. Lawrence [c], Elvan Djouma [a, *]

[a] Department of Physiology, Anatomy and Microbiology, School of Life Sciences, La Trobe University, Bundoora, Victoria, Australia
[b] Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria, Australia
[c] Florey Institute of Neuroscience & Mental Health, University of Melbourne, Parkville, Victoria, Australia

ARTICLE INFO

Article history:
Received 2 December 2016
Received in revised form
17 February 2017
Accepted 2 March 2017
Available online 6 March 2017

Chemical compounds studied in this article:
SNAP 37889 (PubChem CID: 1471834)
morphine hydrochloride (PubChem CID: 5464110)
ethanol (PubChem CID: 702)
sucrose (PubChem CID: 5988)
saccharin (CID: 5143)

Keywords:
Galanin
Galanin-3 receptor
SNAP 37889
Alcohol
Morphine
Self-administration

ABSTRACT

The neuropeptide, galanin, is widely expressed in the central and peripheral nervous systems and is involved in a range of different functions including nociception, neurogenesis, hormone release, reproduction, cognitive function and appetite. Given the overlap between galanin expression and reward circuitry in the brain, galanin has been targeted for alcohol use disorder (AUD) and opioid dependency. Furthermore, the galanin-3 receptor (GAL_3) specifically regulates emotional states and plays a role in motivation, reward and drug-seeking behaviour. We have previously shown that the GAL_3 antagonist, SNAP 37889, reduces ethanol self-administration and cue-induced re-instatement in alcohol-preferring (iP) rats with no alterations in locomotor activity or anxiety-like behaviour. The aim of this study was to investigate whether SNAP 37889 reduces binge drinking and/or self-administration of morphine in mice. Using the Scheduled High Alcohol Consumption (SHAC) procedure, SNAP 37889 (30 mg/kg) treated mice drank significantly less ethanol, sucrose and saccharin than vehicle treated mice. Using an operant paradigm, SNAP 37889 reduced morphine self-administration but failed to impact cue-induced relapse-like behaviour. SNAP 37889 had no significant effect on locomotor activity, motor co-ordination, anxiety, nor was SNAP 37889 itself positively reinforcing. Liver assays showed that there was no alteration in the rate of hepatic ethanol metabolism between SNAP 37889 and vehicle treated mice suggesting that the reduction in ethanol intake via SNAP 37889 is due to a central effect of GAL_3 signalling. This study implicates the GAL_3 receptor in consummatory drive which may have wider implications for the treatment of different addictions.

1. Introduction

Throughout the world, alcoholic drinks are extensively consumed and while most adults drink at levels of low-risk, there are many that undertake harmful or hazardous drinking. Globally, 3.3 million lives are lost every year from alcohol related issues, accounting for 5.1% of the overall disease burden (Poznyak et al., 2014). Similarly, opioid abuse and dependency is a major problem globally with reports that approximately 32 million adults use opioids and 16 million use opiates; a stable global figure (UNOCD, 2015). The availability of prescription opioids like oxycodone and lack of education about the abuse potential of these drugs has fuelled the increased incidence of addiction. Furthermore, nearly half of young adults who inject heroin reported abusing prescription opioids before starting heroin use (NIDA, 2014). While there are a range of medicines available to treat both AUD (Chick et al., 1992; De Witte et al., 2005; Johnson et al., 2000, 2002; Sinclair, 2001; Tambour and Quertemont, 2007), and opiate dependency (Cornish et al., 1997; Joseph et al., 1999; Walsh et al., 1994) many of the current treatments are inadequate in regard to efficacy, tolerance, side effects and compliance, and the incidence of alcohol and opioid use remains high.

There is abundant literature on the mesocorticolimbic dopamine system and its role in mediating the reinforcing effects of

Abbreviations: iP, alcohol-preferring (rats); AUD, alcohol use disorder; GAL_3, galanin-3 receptor; SHAC, Scheduled High Alcohol Consumption; i.p., intraperitoneal; FR, fixed ratio; PR, progressive ratio; DA, dopamine; NA, noradrenaline; NAc, nucleus accumbens; LC, locus coeruleus.

* Corresponding author. Department of Physiology, Anatomy and Microbiology, School of Life Sciences, La Trobe University, Bundoora, Victoria 3086, Australia.
E-mail addresses: K.Scheller@latrobe.edu.au (K.J. Scheller), sjwill@unimelb.edu.au (S.J. Williams), andrew.lawrence@florey.edu.au (A.J. Lawrence), e.djouma@latrobe.edu.au (E. Djouma).

http://dx.doi.org/10.1016/j.neuropharm.2017.03.004
0028-3908/

ethanol and opioids (Everitt and Robbins, 2005; Kalivas, 2009; Koob and Volkow, 2010). The complex chain of neurobiological events accountable for the development and persistence of drug dependency offer a variety of targets for pharmacotherapies. There is anatomical overlap in expression of neuropeptides, including galanin, with brain regions of the mesolimbic dopamine system and hence galanin has been identified as a novel therapeutic target for drug addiction (Kaplan et al., 1988; Melander et al., 1986a, 1986b; Skofitsch and Jacobowitz, 1986). Galanin is a small highly conserved 29 amino acid neuropeptide (30 amino acids in humans) that is recognized as a promising target to treat alcoholism (Ash et al., 2011; Rada et al., 2004), opioid dependency (Holmes et al., 2012) and mood disorders including depression (Barr et al., 2006; Saar et al., 2013) and anxiety (Lu et al., 2007). Galanin has a broad distribution in both the peripheral and central nervous systems and is co-localised with a number of classical neurotransmitters and other neuropeptides. Due to this wide-ranging expression, galanin is widely implicated in numerous biological processes ranging from learning and memory, nociception and feeding behaviour (Lang et al., 2015).

Galanin mediates its activity by binding to three G-protein coupled receptor subtypes (designated GAL_1-GAL_3). Since GAL_3 mRNA is found mainly in regions of the mesolimbic dopamine system, such as the ventral tegmental area, dorsal raphe nucleus, LC, amygdala, hippocampus, thalamus, hypothalamus, NAc, and pre-frontal cortex (Hawes and Picciotto, 2004; Kolakowski et al., 1998), it was believed that specifically targeting GAL_3 may regulate the motivation to seek drugs (Branchek et al., 2000). Belfer and colleagues found a haplotype association between AUD and galanin in Finnish Caucasians and Plains American Indians, two ethnically remote human populations (Belfer et al., 2006) and further determined that GAL_3, but not the GAL_1 or GAL_2 receptors, contributed to vulnerability to alcoholism (Belfer et al., 2007). While information is somewhat limited on the interface between galanin and opioids, both morphine administration and withdrawal stimulates galanin expression in the LC of mice and the presence of brain galanin decreases signs of withdrawal (Holmes et al., 2012). This effect was proposed to work through GAL_1, as GAL_1 knockout mice experienced more severe withdrawal symptoms than GAL_2 knockout mice when compared to littermate controls (Holmes et al., 2012). To our knowledge, opiates and GAL_3 have not yet been explored, however since GAL_1 and GAL_3 have similar downstream effects, it was postulated that targeting GAL_3 may be beneficial in identifying possible therapeutics for drugs of abuse.

The development of small molecule, blood brain barrier penetrating antagonists selective for the GAL_3 receptor (Swanson et al., 2005) has provided an opportunity to study central galanin physiology. SNAP 37889, and its analog SNAP 398299, are highly selective for GAL_3 over GAL_1 and GAL_2 and bind with high affinity to cells expressing human GAL_3 (Swanson et al., 2005). We have already shown that administration of SNAP 37889 reduces operant responding for alcohol (Ash et al., 2011), diminishes the motivation to consume alcohol and attenuates cue-induced reinstatement of alcohol seeking in iP rats (Ash et al., 2014). The present study therefore aimed to further elucidate the role of GAL_3 in binge drinking and morphine self-administration in mice. We investigated the effect of SNAP 37889 in a binge drinking model by adapting the Scheduled High Alcohol Consumption (SHAC) procedure (Finn et al., 2005; Tanchuck et al., 2011). The SHAC paradigm allows alcohol preferring mice (C57BL/6J) to drink to blood ethanol concentrations that can have quantifiable effects on behaviour and physiology, >1.0 mg ethanol/ml blood (Rhodes et al., 2005) an equivalent effect to what is seen in humans.

Given the high density of GAL_3 in the hypothalamus, this receptor may be related to the modulation of feeding. Indeed, GAL_3 has been shown to play a role in caloric rich ethanol intake, which supports the hypothesis that GAL_3 is involved in modulation of appetite and feeding. We wanted to investigate whether targeting GAL_3 had a generalised effect on feeding related systems or was specific to alcohol. We therefore examined the ability of SNAP 37889 to reduce the intake of two naturally rewarding solutions, sucrose and saccharin. Like ethanol, sucrose has a calorific value, whereas saccharin is non-calorific (Wiet and Beyts, 1992) so it was of interest to compare preference for all solutions. Opioids are generally not administered orally and have no calorific content, hence investigating the potential of this GAL_3 antagonist to decrease use of other drugs of abuse like morphine will help assess the role of the GAL_3 receptor in drug-seeking independent of effects on palatability. To fully characterise the effects of SNAP 37889, various behavioural paradigms were used to assess for differences in motor co-ordination, locomotor activity and anxiety. SNAP 37889 was also assessed for intrinsic rewarding properties using the conditioned place preference paradigm.

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. Male C57BL/6J mice were used in all experiments and were housed in standard mouse boxes with *ad libitum* access to food and water (unless otherwise stated). Mice were maintained on a 12-h light-dark cycle and were group housed, except for the morphine self-administration paradigm and the ethanol, sucrose and saccharin experiments, where mice were housed individually to allow for accurate measurement of daily fluid intake.

A total of 8 cohorts of mice were used for these studies. Cohort 1 ($n = 36$) underwent the SHAC paradigm and received both SNAP 37889 and vehicle, Cohort 2–4 were run using the morphine self-administration protocol. Cohort 2 ($n = 12$) received both SNAP 37889 and vehicle; cohort 3 received either SNAP 37889 ($n = 6$) or vehicle ($n = 6$). Cohort 4 ($n = 26$) underwent both progressive ratio (PR) and relapse receiving either SNAP 37889 ($n = 13$) or vehicle ($n = 13$). During experiments with cohort 4, 2 mice were culled during abstinence due to illness, therefore $n = 12$ received SNAP 37889 and $n = 12$ vehicle during cue induced relapse (the opposite treatment they received during PR testing). Cohort 5 ($n = 20$) underwent the locomotor test and received both SNAP 37889 and vehicle; this same cohort was used 3 weeks later for the light/dark test where half received SNAP 37889 ($n = 10$) and the other half vehicle ($n = 10$). Cohort 6 ($n = 16$) was tested on the rotarod apparatus; half ($n = 8$) received SNAP 37889 and the other half ($n = 8$) received vehicle. Cohort 7 ($n = 20$) was used in the CPP paradigm and received alternating injections of both SNAP 37889 and vehicle. Cohort 8 ($n = 12$) was used for liver assays; half ($n = 6$) were injected with SNAP 37889 and the other half ($n = 6$) were injected with vehicle.

2.2. Drugs

The GAL_3 antagonist, SNAP 37889 (1-phenyl-3-[3-(trifluoromethyl)phenyl]iminoindol-2-one) was synthesised as described in Konkel et al. (2006). The solubilisation of SNAP 37889 at a dose of 30 mg/kg was carried out using recently published methods (Scheller et al., 2014). This dose was employed in all behavioural experiments as it was found to be the most effective at decreasing ethanol consumption in pilot studies (unpublished