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Research report

Synergistic blockade of alcohol escalation drinking in mice by a combination of novel kappa opioid receptor agonist Mesyl Salvinorin B and naltrexone

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ABSTRACT

Mesyl Salvinorin B (MSB) is a potent selective kappa opioid receptor (KOP-r) agonist that has potential for development as an anti-psychostimulant agent with fewer side-effects (e.g., sedation, depression and dysphoria) than classic KOP-r agonists. However, no such study has been done on alcohol. We investigated whether MSB alone or in combination with naltrexone (mu-opioid receptor antagonist) altered voluntary alcohol drinking in both male and female mice. Mice, subjected to 3 weeks of chronic escalation drinking (CED) in a two-bottle choice paradigm with 24-h access every other day, developed rapid escalation of alcohol intake and high preference. We found that single, acute administration of MSB dose-dependently reduced alcohol intake and preference in mice after 3-week CED. The effect was specific to alcohol, as shown by the lack of any effect of MSB on sucrose or saccharin intake. We also used the drinking-in-the-dark (DID) model with limited access (4 h/day) to evaluate the pharmacological effect of MSB after 3 weeks of DID. However, MSB had no effect on alcohol drinking after 3-week DID. Upon investigation of potential synergistic effects between naltrexone and MSB, we found that acute administration of a combination of MSB and naltrexone reduced alcohol intake profoundly after 3-week CED at doses lower than those individual effective doses. Repeated administrations of this combination showed less tolerance development than repeated MSB alone. Our study suggests that the novel KOP-r agonist MSB both alone and in combination with naltrexone shows potential in alcoholism treatment models.

1. Introduction

Alcohol consumption affects multiple neurobiological systems including the endogenous opioid systems. Specifically, activation of the kappa opioid receptor (KOP-r) system has been implicated in the negative reinforcing aspects of alcohol, opiate, and psychostimulant addictions (Herz, 1997; Koob and Kreek, 2007). In rats and mice, acute administration of KOP-r agonists attenuates alcohol drinking (Sandi et al., 1988; Lindholm et al., 2001; Henderson-Redmond and Czachowski, 2014), increases alcohol drinking (Anderson et al., 2016; Rose et al., 2016), and alters alcohol-induced conditioned place preference (Logrip et al., 2009; Sperling et al., 2010), while the selective KOP-r antagonist nor-binaltorphimine (nor-BNI) increases alcohol drinking in Lewis rats with relatively high intake (Mitchell et al., 2005). Recently KOP-r antagonists have been reported to attenuate stress-induced alcohol-seeking behavior in mice and rats (Sperling et al., 2010; Deehan et al., 2012; Funk et al., 2014) and to reduce alcohol consumption in alcohol "dependent" rats (Walker and Koob, 2008). These findings provide support for the importance of the KOP-r systems in the processes of alcohol consumption and addiction.

Alcohol may activate the endogenous dynorphin neurons involved in neuronal structures related to alcohol's reinforcing and motivational behaviors. Microdialysis studies have found that acute alcohol administration increases the extracellular dynorphin A1-8 concentrations in the nucleus accumbens (NAc) and the central nucleus of the amygdala (CeA), the two brain regions known to be involved in the regulation of alcohol consumption (Marinelli et al., 2006; Lam and Gianoulakis, 2011). In support of this concept, dynorphin mRNA and peptide levels in the CeA have been found to be increased after acute withdrawal from multiple alcohol "binge" administrations in Sprague-Dawley rats (D'Addario et al., 2013), voluntary alcohol drinking in Sardinian alcohol-preferring rats (Zhou et al., 2013a) or in alcohol-dependent Wistar rats (Kissler

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et al., 2014). This enhanced dynorphin/KOP-r expression and/or activity in the CeA may be involved in the homeostatic adaptations of the brain after chronic alcohol exposure and in the negative affective state during withdrawal.

It has been found that activation of KOP-r by the novel neoclerodane diterpene salvinorin A (Sal A) and classic agonists such as U69,593 and U50,488H have anti-psychostimulant (including cocaine and amphetamine) effects in preclinical models of drug addiction (Negus et al., 1997; Schenk et al., 1999; Morani et al., 2009). However, most "classic" KOP-r agonists produce significant sedation and dysphoria, and those side effects limit their clinical use potential. Mesyl Salvinorin B (MSB) is a potent and selective KOP-r agonist, with a longer duration of action in mice than the structurally similar Sal A. As a novel KOP-r agonist with few side effects, MSB has been developed as a potential anti-cocaine compound, as acute administration of MSB significantly attenuates cocaine seeking in a rat self-administration model (Simonson et al., 2015). However, there is no report on the effect of MSB, Sal A or its analogues on alcohol related behaviors in rodent models. In this study, therefore, we investigated whether MSB alters voluntary alcohol drinking in mice to explore its potential for development as a therapeutic agent for alcoholism.

In the present study, we evaluated the pharmacological effect of MSB in both male and female mice using both chronic escalation drinking (CED) and drinking-in-the-dark (DID) models. In the CED model, the mice had access to alcohol drinking for 3 weeks in a two-bottle choice paradigm with voluntary alcohol drinking every other day, and the mice exposed to alcohol rapidly developed high alcohol consumption (15–25 g/kg/day). Thus, the CED constitutes an appropriate animal model for studying escalation of alcohol drinking (Hwa et al., 2011). We further determined the pharmacological effect of MSB in the DID model, which allows for limited access (4 h/day) to a single alcohol bottle after the beginning of the dark period (Rhodes et al., 2005), and the DID appears to be more suited to model "binge" drinking to the point of intoxication.

Numerous pharmacological studies provide consistent evidence that the opioid antagonist naltrexone (NTN) decreases alcohol reward, consumption, craving, and relapse episodes in human alcoholics (O'Malley et al., 1992; Volpicelli et al., 1992). Preclinical investigations have consistently found that NTN decreases alcohol intake and alcohol's reinforcing and motivational properties (Zhou and Kreek, 2014). In select experiments, therefore, we used the well-known MOP-r antagonist NTN as a reference compound to compare its effects on mouse alcohol drinking behaviors with those of the KOP-r agonist MSB.

Finally, an investigation into the combination of these two compounds could be particularly intriguing, given that these drugs have distinctly different mechanisms of actions (MOP-r antagonism and full KOP-r agonism). Therefore, we specifically tested a combination of MSB and NTN using doses of each compound low enough that no effect on alcohol consumption was seen with either drug alone. These doses were also chosen to minimize potential side effects of both drugs and avoid tolerance to the KOP-r agonist after repeated administration, in order to maximize its effect on alcohol drinking. We hypothesized that this combination would be more effective in reducing alcohol escalation drinking than either drug alone.

2. Results

2.1. Single, acute administration of MSB alone dose-dependently reduced alcohol (but not sucrose or saccharin) intake and preference after CED

2.1.1. CED model and blood ethanol concentration (BEC) levels

Both male and female mice exposed to the 2-bottle "15% alcohol *vs.* water" choice regimen every other day rapidly acquired alcohol drinking behavior, and daily alcohol intake subsequently rose to levels averaging approximately 15–20 g/kg/day in males and 20–25 g/kg/day in females after 3 weeks with 15% alcohol, with a high preference ratio above 0.8–0.9 in both sexes (Table 1). In a separate experiment, mice had alcohol intake of 5.3 ± 0.8 g/kg in males (n = 10) and 8.1 ± 1.3 g/kg in females (n = 11) after 4 h of alcohol access following 3 weeks of CED. Blood samples were taken at the 4-h time point (2 males and 1 female did not show reliable alcohol intake and were excluded from the BEC measurement), and this 3-week voluntary CED exposure was found to give rise to BECs of 0.50 ± 0.08 mg/ml in males (n = 8) and 0.63 ± 0.07 mg/ml in females (n = 10). These BEC values were within the range required to produce specific psycho-pharmacological effects (e.g., Rhodes et al., 2005; Hwa et al., 2011).

2.1.2. Single, acute MSB dose-dependently reduced 15% alcohol intake and preference

After 0.3 or 1 mg/kg MSB, there was no significant effect on alcohol intake, water intake or alcohol preference ratio in males or females. At 3 mg/kg, MSB significantly reduced alcohol intake in males [2-way ANOVA, $F(1,13) = 5.5$, $p < 0.05$] at 4 h [*post hoc* test $p < 0.01$] (Fig 1A1, left) and in females [2-way ANOVA, $F(1,26) = 11.2$, $p < 0.005$] at 4 h [*post hoc* test $p < 0.01$] (Fig 1A1, right). In comparison to the vehicle controls, 3 mg/kg MSB reduced mean alcohol intake by 46% in males and 68% in females. This was associated with a compensatory increase in water intake in males [2-way ANOVA, $F(1,13) = 5.4$, $p < 0.05$] at 4 h [*post hoc* test $p < 0.01$] (Fig 1B1, left) and in females [an apparent but not statistically significant increase at 4 h] (Fig 1B1, right), resulting in virtually unchanged total fluid intake in both sexes (Table 2). At this dose, MSB also significantly reduced preference ratio in males [2-way ANOVA, $F(1,13) = 7.8$, $p < 0.05$] at 4 h [*post hoc* test $p < 0.01$] and in females [2-way ANOVA, $F(1,26) = 7.2$, $p < 0.05$] at 4 h [*post hoc* test $p < 0.01$] (Fig 1C1).

The full-dose response of single, acute MSB administration (0, 0.3, 1 and 3 mg/kg) in terms of 15% alcohol intake and preference

Table 1
Alcohol intake and alcohol preference in a 3-week CED model in male and female mice. The mice were exposed to the 2-bottle "alcohol (15%) *vs.* water" choice regimen every other day for 3 weeks. Data are presented after 4, 8 and 24 h of alcohol access in session 1 on the first day and in session 10 after 3 weeks of CED. Session difference: $^{*}p < 0.05$, $^{**}p < 0.01$ vs. the same sex at the same hour in session 1; and Sex difference: $^{\&\&}p < 0.01$ vs. the male at the same hour in the same session (see the statistical analysis in Supporting Information section).

		Male (n = 10)		Female (n = 11)	
		Session 1	Session 10	Session 1	Session 10
Intake g/kg	0-4 h	1.34 ± 0.65	5.48 ± 0.56**	1.92 ± 0.23	8.17 ± 1.15**
	5-8 h	4.22 ± 0.51	5.00 ± 1.03	5.34 ± 0.97	3.36 ± 0.73
	9-24 h	5.83 ± 1.02	8.73 ± 0.93	10.9 ± 2.00$^{\&\&}$	14.1 ± 1.47$^{\&\&}$
Preference	0-4 h	0.46 ± 0.08	0.88 ± 0.03**	0.44 ± 0.07	0.86 ± 0.05**
	5-8 h	0.43 ± 0.07	0.58 ± 0.05	0.60 ± 0.04	0.50 ± 0.07
	9-24 h	0.55 ± 0.06	0.64 ± 0.06	0.52 ± 0.08	0.72 ± 0.05*