Neuroscience Letters 673 (2018) 19–23

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Effects of mesyl salvinorin B alone and in combination with naltrexone on alcohol deprivation effect in male and female mice

Yan Zhou[a,*], Rachel Crowley[b], Thomas Prisinzano[b], Mary Jeanne Kreek[a]

[a] Laboratory of the Biology of Addictive Diseases, The Rockefeller University, NY, USA
[b] Department of Medicinal Chemistry, University of Kansas School of Pharmacy, Lawrence, KS, USA

ARTICLE INFO

Keywords:
Alcohol deprivation effect
Combined therapy
KOP-r
Mesyl salvinorin B
Naltrexone
Relapse

ABSTRACT

Alcohol relapse plays a major role in alcohol dependence and is an important focus for the treatment of alcoholism. The alcohol deprivation effect (ADE) is a widely used paradigm in rodents to model the relapse episodes that occur in human alcoholics. Mesyl Salvinorin B (MSB) is a potent and selective kappa opioid receptor (KOP-r) full agonist, with fewer side effects (*e.g.*, sedation or anhedonia) than classic KOP-r full agonists and a longer duration of action in mice than the structurally similar salvinorin A. We have recently found that MSB prevents cocaine seeking in a rat self-administration model and reduces excessive alcohol drinking in a mouse escalation model *via* a KOP-r-mediated mechanism. Here, we further investigated whether MSB alone (0.3–3 mg/kg) or in combination with naltrexone (mu-opioid receptor antagonist at 1 mg/kg) altered alcohol "relapse" drinking using a mouse ADE paradigm. Both male and female mice, exposed to 3-week intermittent access alcohol drinking in a two-bottle choice paradigm with 24-h access every other day, developed excessive alcohol intake and then displayed pronounced ADE after 1-week abstinence. Acute administration of MSB prevented the ADE at 3 mg/kg in both male and female mice. Upon investigation of potential synergistic effects between naltrexone and MSB, we found that acute administration of a combination of MSB (0.3 mg/kg) and naltrexone (1 mg/kg) reduced the ADE at doses lower than those individual effective doses, with no sex difference. Our study suggests that the KOP-r full agonist MSB both alone and in combination with naltrexone shows potential in alcohol "relapse" treatment models.

1. Introduction

Activation of the kappa-opioid receptor (KOP-r) by the natural product salvinorin A (Sal A) has anti-addictive effects (including cocaine and amphetamine) in preclinical models of drug addiction [1,2]. However, Sal A has a very short half-life, which limits its potential for clinical use. Mesyl Salvinorin B (MSB), an analogue of Sal A, is a potent KOP-r full agonist and has improved pharmacokinetic properties with fewer side effects (sedation in the locomotor activity test or anhedonia in the sucrose preference test) compared to the natural product Sal A or other "classic" KOP-r agonists [3,4]. Recently, it has been found that acute administration of MSB significantly attenuates cocaine seeking in a rat self-administration model [3] and reduces alcohol drinking in a mouse escalation model in a dose-dependent manner [4]. This suggests that MSB may have potential utility in treating drug abuse. To date, no study has investigated the effects of MSB in alcohol "relapse" in rodent models.

After a period of imposed abstinence, the phenomenon of a transient increase in alcohol consumption observed in both humans and rodents has been termed the alcohol deprivation effect (ADE) [5,6]. ADE has been demonstrated as an appropriate animal model for studying alcohol "relapse" drinking included in this study. While the ADE is widely studied in rats [7,8], studies on the ADE in C57BL/6J mice have not been established after excessive alcohol drinking. Based on the above rat models, we recently developed a simple behavioral protocol that rapidly and reliably induced ADE in C57BL/6 mice [9]. In this model, after mice have access to intermittent access alcohol drinking for 3 weeks, both male and female mice display excessive alcohol consumption (15–25 g/kg/day) [4,10]. After they experience 1 week of imposed abstinence, mice show a pronounced ADE when alcohol is presented again (a significant increase in alcohol intake after 4 h of alcohol access), modeling relapse drinking that occur in human alcoholics. Based on our recent findings as mentioned above, we hypothesized that MSB would prevent alcohol "relapse" drinking in the mouse ADE model. In this study, therefore, we determined the pharmacological effects of MSB on ADE in both male and female mice, to explore its potential for development as an anti-relapse agent for alcoholism.

In human alcoholics and rodent models, pharmacological studies

* Corresponding author at: Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY, 10065, USA.
E-mail address: zhouya@rockefeller.edu (Y. Zhou).

https://doi.org/10.1016/j.neulet.2018.02.056
Received 15 September 2017; Received in revised form 23 February 2018; Accepted 25 February 2018
Available online 26 February 2018
0304-3940/

provide consistent evidence that the mu-opioid receptor (MOP-r) antagonist naltrexone (NTN) decreases alcohol relapse episodes [11], and relapse-like drinking in an ADE model [8]. In the present study, therefore, we used the well-known NTN as a reference compound to compare its effects on mouse ADE with those of MSB. Another particularly interesting question is whether the proper combination of these two drugs could be more effective in reducing alcohol "relapse" drinking than either drug alone, given that the two compounds have different mechanisms of actions (KOP-r agonism for MSB and MOP-r antagonism for NTN). Therefore, we specifically tested combinations of MSB and NTN using doses of each drug that, when given alone, had no effect on ADE.

2. Material and methdods

2.1. Animals

Male and female adult C57BL/6J mice (8 weeks of age) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA) and housed in a temperature-controlled room (21 °C). Mice were placed on a 12-h reverse light-dark cycle (lights off at 7:00 am) upon arrival, and acclimated for a week prior to testing. Mice were individually housed in ventilated cages fitted with steel lids and filter tops and given *ad libitum* access to food and water. Animal care and experimental procedures were conducted according to *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources Commission on Life Sciences 1996), and were approved by the Institutional Animal Care and Use Committee of the Rockefeller University.

2.2. Materials

Mesyl Salvinorin B (MSB) was synthesized from Sal A as described previously [12], and dissolved in 1% DMSO. Ethanol solutions were prepared from 190 proof absolute ethyl alcohol (Pharmco-AAPER, Brookfield, CT, USA) and dissolved in tap water. Naltrexone hydrochloride was purchased from Sigma-Aldrich and dissolved in physiological saline.

2.3. Procedures

2.3.1. Chronic intermittent access alcohol drinking

In C57BL/6J mice, this model of excessive alcohol drinking is widely used by many laboratories [*e.g.*, 9,10]. During alcohol drinking in their home cages for 3 weeks, mice had access to food and water at all times. This chronic intermittent access model (two-bottle choice paradigm with chronic alcohol exposure every other day) was described in detail in earlier reports (Table S1). Starting at 10:00 am (3 h after lights off), both the alcohol (15%) solution and water sipper tubes were placed on their home cages. The position of the tubes on left or right side of the cage was randomly set to avoid the development of side preference. The alcohol tubes were filled with fresh alcohol solution, and placed for 24 h before being replaced with the water tubes. Alcohol and water intake values were recorded after 4, 8 and 24 h of alcohol access in the drinking days, and these data were used to calculate consumed alcohol intake (g/kg) and relative preference for alcohol (alcohol intake/total fluid intake).

2.3.2. Alcohol deprivation effect (ADE) after 3-week intermittent access alcohol drinking

This model of alcohol "relapse" drinking in C57BL/6J mice has recently been developed by our laboratory [9], based on the rat models by others [7,8]. Briefly, mice experienced chronic intermittent access alcohol drinking (see the above Section 1) for 3 weeks (Table S1). In the baseline session on day 21 in week 3, 30% (but not 15%) alcohol and water intake values were recorded at 4, 8 and 24 h. Then, alcohol tubes were taken away for 7 days. After 1-week abstinence, alcohol (30%) tubes were presented to the mice again at 10:00am (3 h after the dark cycle) on day 28 (week 5) and the alcohol and water intakes were recorded at 4, 8 and 24 h in the ADE session.

2.3.3. Acute administration MSB, NTN or their combination in the ADE model (Table S1)

Mice were randomly assigned as the vehicle- and drug- treated groups in each sex with similar alcohol intake in the baseline session. An experimenter, blinded to the treatments given to the experimental groups, injected the drug and vehicle. The mice in control groups received one vehicle injection before the ADE test on day 28; and the mice in test groups received one drug injection (MSB or NTN) or two drug injections (MSB followed by NTN) before the ADE test on day 28. Then, the alcohol tube was presented after the drug or vehicle injection, and alcohol and water intakes were recorded after alcohol access.

The MSB doses were based on our recent publication [4]: the mice in test groups received one MSB injection (0.3, 1 or 3 mg/kg, i.p.), and the mice in control groups received one vehicle injection (1% DMSO). The NTN doses were also based on the above publication [4]: the mice in test groups received one NTN injection (0.5, 1 or 3 mg/kg, i.p.) and the mice in control groups received one saline injection. The MSB + NTN dose chosen was based on the above two experiments with each compound alone: the mice in test groups received the first i.p. injection of MSB (0.3 mg/kg) followed by the second i.p. injection of NTN (0.5 or 1 mg/kg) 20 min later; and the mice in control groups received one vehicle followed by saline.

2.4. Data analysis

We performed power analyses to determine the number of animals required to provide statistical significances, based on the levels of differences seen previously [9], and predicted that these studies require 9–15 males and 9–10 females per group. As similar effects on the ADE with no significant sex differences were seen after the individual compounds and their combinations, data of each sex were analyzed and presented separately. Alcohol intake differences across the different groups were analyzed using two-way ANOVA for treatment (vehicle *vs* drug doses) and for sessions (baseline *vs* ADE) in each sex, with testing our *a priori* hypothesis that there were effects of ADE, NTN, MSB or their combinations, based on the published findings [7,8] and our new hypothesis. This was followed by Newman-Keuls *post-hoc* tests. The accepted level of significance for all tests was $p < 0.05$. All statistical analyses were performed using *Statistica* (version 5.5, StatSoft Inc, Tulsa, OK).

3. Results

3.1. Effect of MSB on ADE in both male and female mice

In this experiment, we tested the effect of MSB at 0.3, 1 or 3 mg/kg on alcohol intake. In the males at 4 h (Fig. 1A), two-way ANOVA revealed a significant effect of MSB treatment [$F(1,84) = 3.5$, $p < 0.05$], Session [$F(1,84) = 11$, $p < 0.01$], and a significant interaction between Session and Treatment [$F(1,84) = 2.8$, $p < 0.05$]. *Post hoc* analysis showed that: (1) the males had more intake in the ADE session on day 28 than the baseline on day 21 [$p < 0.05$]; and (2) the 3 mg/kg MSB-treated males had less intake than the vehicle-treated ones in the ADE session [$p < 0.01$]. In the females at 4 h (Fig. 1B), two-way ANOVA showed a significant effect of MSB treatment [$F(1,72) = 2.8$, $p < 0.05$], Session [$F(1,72) = 7.1$, $p < 0.01$], and a marginally significant interaction between Session and Treatment [$F(1,72) = 2.7$, $p = 0.05$]. *Post hoc* analysis further showed that: (1) the females had more intake in the ADE session than the baseline [$p < 0.05$]; and (2) the 3 mg/kg MSB-treated females had less intake than the vehicle ones in the ADE session on day 28 [$p < 0.01$]. However, MSB at either 0.3 or 1 mg/kg did not significantly reduce ADE in either males or females.