



Research article

Effects of sequential ethanol exposure and repeated high-dose methamphetamine on striatal and hippocampal dopamine, serotonin and glutamate tissue content in Wistar rats

Atiah H. Almalki^{a,b,1}, Sujan C. Das^{a,b}, Fahad S. Alshehri^a, Yusuf S. Althobaiti^{a,2}, Youssef Sari^{a,b,*}

^a University of Toledo, College of Pharmacy and Pharmaceutical Sciences, Department of Pharmacology and Experimental Therapeutics, Toledo, OH, USA

^b University of Toledo, College of Pharmacy and Pharmaceutical Sciences, Department of Medicinal and Biological Chemistry, Toledo, OH, USA

ARTICLE INFO

Keywords:

Ethanol
Methamphetamine
Dopamine
Serotonin
Glutamate
Striatum

ABSTRACT

Alcohol (ethanol) and methamphetamine (METH) co-abuse is a major public health issue. Ethanol or METH exposure has been associated with changes in neurotransmitter levels in several central brain regions. However, little is known about the effect of sequential exposure to ethanol and METH on glutamate, dopamine and serotonin tissue content in striatum and hippocampus. In this study, we investigated the effects of sequential exposure to ethanol and METH on tissue content of these neurotransmitters. Male Wistar rats were orally gavaged with either ethanol (6 g/kg) or water for seven days. Rats were administered with high dose of METH (10 mg/kg, i.p. every 2 h × 4) or saline on Day 8 and euthanized 48 h of last METH or saline i.p. injection. In the striatum, sequential exposure to ethanol and METH increased glutamate tissue content while reducing dopamine and serotonin tissue content as compared to the group exposed to ethanol alone. In the hippocampus, sequential exposure to ethanol and METH decreased serotonin tissue content as compared to the group that was exposed to ethanol alone. However, this study showed that ethanol has no additive effect to METH on tissue content of dopamine and serotonin as compared to METH in the striatum and hippocampus. This study demonstrated that sequential exposure of ethanol and METH has an additive effect on tissue content of certain neurotransmitters in the brain.

1. Introduction

Methamphetamine (METH) is an amphetamine type stimulant that induces drug desire, cognitive impairment and distraction [1,2]. Alcohol (ethanol) addiction is a disorder that is characterized by compulsive ethanol intake and seeking [3,4]. It has been shown that around 75% of amphetamine dependents also consumed ethanol [5]. Several studies from our laboratory and others revealed that glutamatergic, serotonergic and dopaminergic systems in the brain are altered with co-exposure to drugs of abuse, including METH and ethanol [6–10]. Indeed, studies demonstrated that METH exposure (10 mg/kg i.p. every 2 h × 4) increased extracellular glutamate in the striatum [11–13,7]. Furthermore, METH exposure induced damage of dopaminergic terminals leading to lower levels of striatal dopamine [13]. In regards to ethanol, acute exposure has been shown to mediate brain damage, enhance cognitive impairment and increase extracellular glutamate

concentration in the hippocampus [14–17].

Despite the evidence of the high prevalence of ethanol and METH co-abuse, less is known about the effect of sequential exposure of ethanol and METH on the tissue content of neurotransmitters in the striatum and hippocampus. Thus, we hypothesized that sequential exposure to ethanol and METH would lead to synergistic effects in the alteration of tissue content of the studied neurotransmitters. To test this hypothesis, we used male Wistar rats, which received ethanol (6 g/kg) or water through oral gavage for seven days. Rats were administered with high dose of METH (10 mg/kg, i.p. every 2 h × 4) or saline on Day 8 and euthanized 48 h after the last METH or saline injection. In this study, we used ethanol dose of 6 g/kg/day because of its ability to alter neurotransmission and neurogenesis in CNS [18,19]. Moreover, ethanol exposure has been associated with increase in extracellular dopamine and serotonin concentrations in the nucleus accumbens and striatum [20–22]. It has been shown that the hippocampus, brain region

* Corresponding author at: University of Toledo, College of Pharmacy and Pharmaceutical Sciences, Department of Pharmacology and Experimental Therapeutics, Department of Medicinal and Biological Chemistry, Health Science Campus, 3000 Arlington Avenue, HEB 282G Toledo, OH, 43614, USA.

E-mail address: youssef.sari@utoledo.edu (Y. Sari).

¹ Taif University, College of Pharmacy, Department of Medicinal Chemistry, Taif, Saudi Arabia.

² Taif University, College of Pharmacy, Department of Pharmacology, Taif, Saudi Arabia.

<https://doi.org/10.1016/j.neulet.2017.11.043>

Received 9 August 2017; Received in revised form 4 November 2017; Accepted 20 November 2017

Available online 22 November 2017

0304-3940/ © 2017 Elsevier B.V. All rights reserved.

responsible for memory and learning, and the striatum, which controls reward and voluntary movement, were affected by ethanol or METH exposure [23–26]. Therefore, we investigated the effect of repeated high-dose METH exposure, administered to ethanol-exposed rats, on tissue content of several neurotransmitters such as glutamate, dopamine and serotonin in the striatum and hippocampus.

2. Materials and methods

2.1. Subjects

Male Wistar rats weighing 200–300 g were used in this study. Rats were purchased from Envigo RMS, Inc. (Indianapolis, IN). Rats were single-housed and kept in a 12:12 light-dark cycle, controlled temperature at 21 °C, and humidity (30%). Rats were handled and habituated prior to conducting the experiments. Rats had free access to food and water during the experimental procedure; and they were deprived of these for two hours, to make sure that they received the full dose, before oral gavage of either water or ethanol (6 g/kg, made from 40% v/v). Animal housing and experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Toledo. These were in agreement with the guidelines of the Institutional Animal Care and Use Committee of the National Institutes of Health and the Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

(+) – METH hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO). Saline solution (0.9% NaCl) was used to dissolve METH. Ethanol (95%; Decon Labs, Inc.) was diluted in water.

2.3. Experimental design

Four groups of rats were used in this study: (1) Water-saline group was given water through oral gavage route for seven days and received four repeated intraperitoneal (i.p.) injections of saline vehicle on Day 8; (2) Water-METH group was given water through oral gavage route for seven days and received METH (10 mg/kg i.p. every 2 h × 4) on Day 8; (3) Ethanol-saline group received ethanol (6 g/kg, 40% v/v) through oral gavage route for seven days and received four i.p. injections of saline vehicle on Day 8; and (4) Ethanol-METH group received ethanol (6 g/kg) through oral gavage route and METH (10 mg/kg i.p. every 2 h × 4) on Day 8 (i.p.). After 48 h of the last METH or saline i.p. injection, rats were immediately euthanized by CO₂ inhalation and further decapitated. The brains were then extracted and quickly stored in dry ice and then kept at –80 °C. The striatum and hippocampus were dissected out at –20 °C using cryostat machine. Brain Stereotaxic Atlas was used to dissect out the selected brain region [27] as previously described in previous study from our laboratory [28].

2.4. HPLC quantification of dopamine and serotonin

HPLC with electrochemical detection (EC) system was used to detect the tissue content of dopamine and serotonin in the striatum and hippocampus as previously illustrated [9]. Briefly, brain regions were homogenized with pestle in 0.25N perchloric acid. Samples were sonicated for 20 min, and then centrifuged at 14000g for 20 min at 4 °C. The supernatant was filtered through 0.22 µm filters. The filtered supernatant was injected through a C18 column (3.2 × 150 mm, 3 µm particle size, Thermo Scientific). The remaining pellets were re-suspended to measure protein quantity. In this study, the mobile phase consists of 54.3 mM sodium phosphate, 32 mM citric acid, 11% methanol, 0.215 mM octyl sodium sulfate, and 0.22 mM triethylamine. The pH of the mobile phase was 4.4. CoulArray coulometric detector (model 5600A, ESA, Inc.) was used to measure the tissue content of dopamine and serotonin. The data were collected using CoulArray

software. Neurotransmitter concentrations in each sample were analyzed using the area under the curve and compared with external standards in all groups.

2.5. HPLC quantification of glutamate

HPLC with electrochemical detection (EC) system was also used to analyze glutamate tissue content in the striatum and hippocampus as previously illustrated [8,9]. The tissue content of glutamate was determined by HPLC analysis (ESA, Inc) with electrochemical detection. Millipore water was used to lysate brain tissue and centrifuged at 14,000 rpm for 20 min. The supernatant was then filtered using 0.22 µm filter. After the samples were derivatized with O-phthalaldehyde (OPA) and sodium sulfite using a ESA Model 540 autosampler, these samples were then injected through a C18 column (3.0 × 50 mm, 2.5 µm particle size, Waters). In this experiment, the mobile phase was consisted of 0.1 M Na₂HPO₄, 0.1 mM EDTA and 7.5% Methanol (pH 3.0). CoulArray coulometric detection (model 5600A, ESA, Inc.) was used to analyze glutamate tissue contents. The data were collected using CoulArray software. Glutamate tissue content in each sample was analyzed using the area under the curve and compared with external standard in all groups.

2.6. Statistical analysis

Two-way ANOVA was used to analyze tissue content of studied neurotransmitters for comparison between ethanol, ethanol/METH, METH, and water control groups. When significant effects were revealed, Newman-Keuls multiple comparisons test was used to evaluate the significant difference between each drug against its corresponding control group and to assess between concentrations for each drug. Statistical significance was set at $p < 0.05$ and GraphPad Prism was used to assess all data.

3. Results

3.1. Effects of METH administered alone or with ethanol on glutamate tissue content in the striatum and hippocampus

Two-way ANOVA revealed a significant main effect of METH post-treatment in striatum [$F(1, 27) = 17.95, P = 0.0002$] without altering tissue content of glutamate in hippocampus [$F(1, 29) = 0.2253, P = 0.6386$] (Fig. 1). In addition, significant main effect of oral gavage ethanol pretreatment was revealed using two-way ANOVA in striatum [$F(1, 27) = 21.03, P < 0.0001$], but not in hippocampus [$F(1, 29) = 0.3560, P = 0.5554$]. However, no significant interaction between METH post-treatment and ethanol pretreatment was found in both striatum [$F(1, 27) = 0.4771, P = 0.4956$] and hippocampus [$F(1, 29) = 0.2662, P = 0.6098$]. Newman-Keuls multiple comparisons test showed increased glutamate tissue content in the water-METH group as compared to water-saline, ethanol-saline and ethanol-METH groups in the striatum (Fig. 1A). In addition, glutamate tissue content was increased in the ethanol-METH group as compared to the ethanol-saline group (Fig. 1A). However, glutamate tissue content was decreased in the ethanol-saline group as compared to the water-saline group in the striatum (Fig. 1A). No significant changes in glutamate tissue content were found between groups in the hippocampus (Fig. 1B).

3.2. Effects of METH administered alone or with ethanol on dopamine tissue content in the striatum and hippocampus

Two-way ANOVA revealed a significant main effect of METH post-treatment in striatum [$F(1, 28) = 89.15, P = 0.0001$] (Fig. 2A) and hippocampus [$F(1, 27) = 7.775, P = 0.0096$] (Fig. 2B). Significant effect of orally gavaged ethanol pretreatment group was found in both striatum [$F(1, 28) = 35.77, P < 0.0001$] and hippocampus [$F(1, 27)$

Download English Version:

<https://daneshyari.com/en/article/8841853>

Download Persian Version:

<https://daneshyari.com/article/8841853>

[Daneshyari.com](https://daneshyari.com)