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EFFECTS OF AMPICILLIN, CEFAZOLIN AND CEFOPERAZONE TREATMENTS ON GLT-1 EXPRESSIONS IN THE MESOCORTICOLIMBIC SYSTEM AND ETHANOL INTAKE IN ALCOHOL-PREFERRING RATS

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Abstract—Chronic ethanol consumption is known to down-regulate expression of the major glutamate transporter 1 (GLT-1), which increases extracellular glutamate concentrations in subregions of the mesocorticolimbic reward pathway. While β-lactam antibiotics were initially identified as potent upregulators of GLT-1 expression, only ceftriaxone has been extensively studied in various drug addiction models. Therefore, in this study, adult male alcohol-preferring (P) rats exposed chronically to ethanol were treated with other β-lactam antibiotics, ampicillin, cefazolin or cefoperazone (100 mg/kg) once daily for five consecutive days to assess their effects on ethanol consumption. The results demonstrated that each compound significantly reduced ethanol intake compared to the saline-treated control group. Importantly, each compound significantly upregulated both GLT-1 and pAKT expressions in the nucleus accumbens and prefrontal cortex compared to saline-treated control group. In addition, only cefoperazone significantly inhibited hepatic aldehyde dehydrogenase-2 enzyme activity. Moreover, these β-lactams exerted only a transient effect on sucrose drinking, suggesting specificity for chronically inhibiting ethanol reward in adult male P rats. Cerebrospinal fluid concentrations of ampicillin, cefazolin or cefoperazone have been confirmed using high-performance liquid chromatography. These findings demonstrate that multiple β-lactam antibiotics demonstrate efficacy in reducing alcohol consumption and appear to be potential therapeutic compounds for treating alcohol abuse and/or dependence. In addition, these results suggest that pAKT may be an important player in this effect, possibly through increased transcription of GLT-1.

Key words: cefazolin, cefoperazone, ampicillin, glutamate, alcohol intake, EAAT2.

INTRODUCTION

Chronic exposure to drugs of abuse causes dysregulation of glutamatergic neurotransmission, with alterations to glutamatergic projections from the prefrontal cortex (PFC) to the nucleus accumbens (NAc) (Kalivas, 2009; Kalivas and Volkow, 2011; Quintero, 2013). These projections are part of the mesocorticolimbic reward pathway and has been shown to mediate drug-seeking and relapse behaviors (Kalivas, 2009; Kalivas and Volkow, 2011; Quintero, 2013; Wise and Koob, 2014). Furthermore, recent studies have demonstrated that substance abuse is associated with impaired glutamatergic release, leading to an imbalance in glutamatergic homeostasis within the mesocorticolimbic pathway (Parsegian and See, 2014; Shen et al., 2014).

Ethanol, similar to other drugs of abuse, is known to significantly alter extracellular glutamate concentrations in the mesocorticolimbic pathway, which is due, at least partially, to impaired clearance of glutamate from the synapse (Melendez et al., 2005; Ding et al., 2013). These elevated NAc glutamate concentrations following ethanol intake, in turn, appear to promote continued excessive ethanol consumption (Griffin et al., 2014). Consistent with these findings, we have demonstrated a significant down-regulation of the major glutamate transporter, glutamate transporter 1 (GLT-1, its human homolog is excitatory amino acid transporter 2, EAAT2), in the NAc of alcohol-preferring (P) male rats after five weeks of free-choice ethanol exposure compared to their ethanol-naïve counterparts (Sari and Sreemantula, 2012; Sari et al., 2013).

Importantly, upregulation of GLT-1 expression in the mesocorticolimbic pathway is associated with restored glutamate homeostasis and attenuated drug-seeking behavior (Knackstedt et al., 2010; Rasmussen et al., 2011). While ceftriaxone treatment has yielded promising results in reducing drug abuse in cocaine, ethanol, and methamphetamine exposure animal models (Sari et al.,

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Abbreviations: ALDH2, aldehyde dehydrogenase-2; AMP, ampicillin; ANOVA, analysis of variance; CSF, cerebrospinal fluid; CZN, cefazolin; CPZ, cefoperazone; EDTA, ethylenediaminetetraacetic acid; GLM, General Linear Model; GLT-1, glutamate transporter 1; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; NADH, nicotinamide adenine dinucleotide; NAc, nucleus accumbens; NP, alcohol-non-preferring; P, alcohol-preferring; PFC, prefrontal cortex; p-AKT, phosphorylated-AKT; t-AKT, total-AKT.

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2009, 2011; Abulseoud et al., 2012; Rao and Sari, 2014), other β-lactam antibiotics, identified earlier as GLT-1 upregulators (Rothstein et al., 2005), have not been evaluated for their *in vivo* efficacy. Therefore, the aim of this study is to evaluate the effect of other FDA approved β-lactam antibiotics – ampicillin (AMP), cefazolin (CZN) or cefoperazone (CPZ) treatments – on daily ethanol intake in male P rats following five weeks of free-choice ethanol exposure. Since 5 weeks of chronic ethanol exposure results in a consistent reduction of GLT-1 expression in the NAc and PFC (Sari and Sreemantula, 2012; Sari et al., 2013), ethanol-naïve animals were not included in this study.

In order to associate the changes in ethanol consumption following these treatments with changes in glutamatergic activity, GLT-1 expressions in the NAc and PFC were compared between the β-lactam-treated and saline-treated groups. To confirm the previously established pharmacological mechanism of GLT-1 upregulation in these brain regions (Wu et al., 2010), phosphorylation of signaling molecule AKT was also measured in the NAc and PFC of treated vs. control groups. Finally, to determine the CNS bioavailability of drug treatments, the cerebrospinal fluids (CSF) from AMP-, CZN-, and CPZ-treated P rats were analyzed by high-performance liquid chromatography (HPLC). Furthermore, we determined the effects of these β-lactam antibiotics on sucrose intake, a consummatory control for ethanol-drinking behavior. In addition, the N-methyltetrazolethiol side chain present in β-lactams is known to exhibit disulfiram-like effects on ethanol metabolism via inhibition of the enzyme, aldehyde dehydrogenase-2 (ALDH2) (Matsubara et al., 1987). Therefore, liver samples collected from AMP-, CZN-, and CPZ-treated P rats were analyzed for ALDH2 activity, an enzyme accountable for 60% of hepatic acetaldehyde metabolism (Weiner, 1987).

EXPERIMENTAL PROCEDURES

Animals

Adult male P rats were obtained from the Indiana University School of Medicine, Indianapolis, IN and housed in standard plastic tubs with corn-cob bedding in the Department of Laboratory Animal Resources vivarium at The University of Toledo. All animals had *ad lib* access to food and water during the study, and the animal vivaria were maintained at a temperature of 21 °C on a 12-h light/dark cycle (0600 h/1800 h). All of the animal experimental protocols were approved by the Institutional Animal Care and Use Committee of The University of Toledo in accordance with 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 (Institute of Laboratory Animal Resources, Commission on Life Sciences, 1996). P rats, at the age of three months, were single-housed in bedded plastic cages and divided randomly into four different groups: (a) the saline vehicle group that received the saline vehicle (i.p., $n = 6$); (b) the ampicillin group (AMP) that was treated with 100 mg/kg ampicillin (i.p., $n = 8$); (c) the cefazolin group (CZN) that was treated with 100 mg/kg cefazolin (i.p., $n = 8$); and (d) the cefoperazone group (CPZ) that received 100 mg/kg cefoperazone (i.p., $n = 8$). The β-lactams were administered as a solution prepared in 0.9% saline.

Ethanol drinking procedures

At 3 months of age, all male P rats were given concurrent, free-choice continuous access to two ethanol concentrations, 15% and 30%, v/v, as well as food and water for 5 weeks. During week 4, ethanol consumption for each animal was evaluated as g/kg of body weight/day. Water consumption and body weight were also measured three times per week. The amount of ethanol and water consumed were determined to the nearest tenth of a gram by subtraction of the measured bottle weights from their previous day's values.

During the two weeks preceding treatment (weeks 4 and 5), all animals were required to meet the criterion of $\geqslant 4$ g/kg/day of ethanol consumption, a criterion of ethanol consumption based on our previous studies (Sari et al., 2011; Sari and Sreemantula, 2012). The averaged data collected during weeks 4 and 5 served as baseline values for ethanol consumption, water intake, and animal body weight. During week 6, P rats were injected with either saline vehicle, AMP (100 mg/kg, i.p.), CZN (100 mg/kg, i.p.), or CPZ (100 mg/kg, i.p.) once daily for five consecutive days. Ethanol and water consumption, along with body weight, were measured daily for the rest of the study. Previous studies have shown that five-day treatment with ceftriaxone upregulated GLT-1 expression in the mesocorticolimbic pathway (Miller et al., 2008; Sari et al., 2009, 2011), and hence this treatment period was chosen for the present study. P rats were euthanized 24 h after receiving the last β-lactam or saline injection.

Sucrose drinking procedures

The effects of 100 mg/kg, i.p., AMP or CPZ or CZN on sucrose (10% w/v; in water) intake as an appetitive control for drinking-motivated behavior was also examined in a separate group of P rats. For this study, in addition to water and food, all male P rats were provided free-choice, continuous access to a 10% sucrose solution. The animals were divided into four groups: (a) a saline vehicle-treated (control) group (i.p., $n = 6$); (b) an AMP group treated with 100 mg/kg/day of AMP (i.p., $n = 6$); (c) a CZN group treated with 100 mg/kg/day of CZN (i.p., $n = 6$); and (d) a CPZ group treated with 100 mg/kg/day of CPZ (i.p., $n = 5$). These drugs were administered as a solution prepared in 0.9% saline. Starting on Day 11 of this study, daily sucrose intake (ml/kg/day), along with water consumption and body weight, were recorded for all P rats through the end of the study. Average sucrose intake data recorded on Day 11 and Day 12 served as the baseline value for data analysis. All groups were treated from Day 13 through Day 16, once daily, with their respective treatments (100 mg/kg/day, i.p.). Daily sucrose intake was monitored during this treatment period (Day