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Research Report

Effects of cefazolin and cefoperazone on glutamate transporter 1 isoforms and cystine/glutamate exchanger as well as alcohol drinking behavior in male alcohol-preferring rats

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ABSTRACT

Previously, we have reported that cefazolin and cefoperazone treatments attenuated ethanol consumption, at least in part, through upregulation of GLT-1 expression in male alcohol-preferring (P) rats. In this study, we determined the effects of these compounds on the expression of GLT-1 isoforms (GLT-1a and GLT-1b), cysteine/glutamate exchanger (xCT), which is another glial glutamate transporter co-localized with GLT-1, and glutamate/aspartate transporter (GLAST). We found that cefazolin and cefoperazone treatments decreased ethanol intake and upregulated both GLT-1 isoforms, GLT-1a and GLT-1b, in nucleus accumbens (NAc) and prefrontal cortex (PFC) compared to saline treated group. In addition, cefazolin increased the expression of xCT in NAc and PFC, while cefoperazone upregulated xCT expression only in NAc. However, we did not find any significant differences in GLAST expression between the treated and control groups. Overall, our findings suggest that cefazolin and cefoperazone may be considered as potential compounds for the treatment of ethanol dependence.

1. Introduction

Impairment in synaptic glutamate reuptake has been linked to drug addiction (Shen et al., 2014). Glutamate receptors are known to modulate development of alcohol addiction (Backstrom and Hyytia, 2004; Besheer et al., 2010). A marked increase in the total extracellular glutamate concentration is associated with exposure to ethanol (Ding et al., 2012, 2013; Ward et al., 2009). In addition, studies have demonstrated the effect of ethanol on glutamate transporters (Abulseoud et al., 2014; Alhaddad et al., 2014b). Glutamate transporter 1 (GLT-1, its human homolog is excitatory amino acid transporter-2) regulates majority of total

Abbreviations: GLT-1, glutamate transporter 1; NAc, nucleus accumbens; PFC, prefrontal cortex; CEF, ceftriaxone; P, alcohol-preferring rats; CSF, cerebrospinal fluid; EAAT, excitatory amino-acid transporters

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extracellular glutamate concentration (Jensen et al., 2015; Tanaka et al., 1997) and GLT-1 expression was found to be decreased following chronic exposure to ethanol (Aal-Aaboda et al., 2015; Alhaddad et al., 2014a,b). Therefore, GLT-1 upregulators are rationalized as potential treatment option for treating alcohol addiction. Following the discovery by Rothstein et al. reported β-lactam antibiotics as potent GLT-1 upregulators (Rothstein et al., 2005), we have shown that ceftriaxone, a β-lactam antibiotic, reduced ethanol intake and relapse to ethanol intake presumably by increasing the expression of GLT-1 in the mesocorticolimbic areas of the brain including NAc and PFC (Alhaddad et al., 2014a; Qrunfleh et al., 2013; Rao and Sari, 2014; Rao et al., 2015b). Similarly, cefazolin and cefoperazone at dose of 100 mg/kg/day for 5 consecutive days were found to be effective in reducing ethanol intake in male P rats (Rao et al., 2015a). While GLT-1 upregulation by these compounds has been demonstrated, the effects of cefazolin and cefoperazone on modulating the expression of other transporters regulating extracellular glutamate concentrations have not been investigated.

GLT-1 is known to be expressed as three splice variants – GLT-1a, GLT-1b and GLT-1c – and these isoforms are expressed differentially in the central nervous system (CNS). GLT-1a is expressed in astrocytes as well as neurons, whereas GLT-1b is found to be expressed only in astrocytes (Berger et al., 2005; Holmseth et al., 2009). It has been reported that GLT-1c is localized mainly in the retina (Rauen et al., 2004). However, a difference in ability to transport glutamate amongst these isoforms has not been confirmed (Sullivan et al., 2004). Importantly, the expression of GLT-1 isoforms is known to change differentially depending on the disease model. For example, in motor cortex of patients with amyotrophic lateral sclerosis the expression of GLT-1a is downregulated while GLT-1b expression is upregulated (Maragakis et al., 2004). Previous studies from our laboratory have demonstrated that ceftriaxone-induced GLT-1a and GLT-1b expression in NAc and PFC, and this effect was found associated with a significant decrease in continuous ethanol intake and relapse-like ethanol drinking in male P rats (Alhaddad et al., 2014a; Rao et al., 2015b). In order to investigate differential effects on GLT-1a and GLT-1b expression, in this study, we tested the effects of both cefazolin and cefoperazone on the expression of these isoforms in P rats exposed to free choice ethanol (15% and 30%). We did not test GLT-1c isoform since it is mainly expressed in the retina (Rauen et al., 2004).

While GLT-1 is the major glutamate transporter in brain, the glutamate/aspartate transporter (GLAST, its human homolog is excitatory amino acid transporter-1, EAAT1) is also known to regulate synaptic glutamate homeostasis and is expressed in the cerebellum (Lehre and Danbolt, 1998). Although GLAST is distributed throughout the brain (Schmitt et al., 1997), the most common glutamate transporter in the inner ear and the retina is GLAST (Lehre and Danbolt, 1998; Takumi et al., 1997). In this study, we determined the effects of cefazolin and cefoperazone treatments on GLAST expression in NAc and PFC.

In addition to GLT-1 and GLAST, cystine-glutamate antiporter (xCT) is also known to regulate extracellular glutamate concentrations and plays a crucial role in neuroprotection (For review, see Albrecht et al. (2010)). Importantly, depletion in glutathione content is associated with decreased xCT expression (Bell et al., 2011; Lewerenz et al., 2009). Ceftriaxone treatment was shown neuroprotection by inducing nuclear factor-erythroid 2-related factor (Nrf2)-mediated induction of xCT expression, leading to increase in intercellular glutathione levels (Bell et al., 2011; Lewerenz et al., 2009). A critical role of xCT has also been demonstrated in animal models of drug addiction. For instance, nicotine and cocaine self-administration decreased xCT expression in NAc (Knackstedt et al., 2009, 2010). Similarly, xCT was found to be downregulated in NAc following chronic exposure to free choice ethanol (15% and 30%) as compared to alcohol naïve group (Alhaddad et al., 2014a,b). Interestingly, ceftriaxone treatment was found to upregulate xCT expression in NAc of rats exposed to both free choice ethanol (Alhaddad et al., 2014a; Rao et al., 2015b) and cocaine self-administration (Knackstedt et al., 2010). To explore if treatment with other cephalosporins can modulate xCT expression, in this study, the effect of cefazolin and cefoperazone treatments on xCT expression in NAc and PFC in P rats after chronic exposure to ethanol (15% and 30%) was determined.

2. Results

2.1. *Effect of cefazolin and cefoperazone treatments on ethanol intake and water intake*

Mixed ANOVA demonstrated a significant main effect of day [F (1, 5)=22.74, $p<0.0001$] and a significant day × treatment interaction [F (2, 10)=3.599, $p=0.0005$] of ethanol intake. Moreover, one way ANOVA followed by Dunnett's t-test showed a significant decrease in ethanol intake with cefazolin ($n=6$) and cefoperazone ($n=6$) treated groups as compared to saline treated group ($n=6$) from Day 2 through Day 5 ($p<0.001$) (Fig. 1). In addition, statistical analysis revealed a significant main effect of day [F (1, 5)=2.782, $p=0.0220$] and a significant day × treatment interaction [F (2, 10)=3.206, $p=0.0014$] of water consumption. Dunnett''s t-test followed by one way ANOVA showed a significant increase in water consumption in cefoperazone treated groups starting from Day 2 through Day 5 (except on Day 3) comparing to saline treated group ($p<0.01$). However, cefazolin treatment increased water consumption only on Day 3 ($p<0.01$) (Fig. 1).

2.2. *Effects of cefazolin and cefoperazone treatments on GLT-1a and GLT-1b expression in NAc and PFC*

Independent t-test analyses of immunoblots demonstrated a significant increase in GLT-1a/GAPDH ratio in NAc and PFC with cefazolin- ($p<0.05$) and cefoperazone- ($p<0.05$) treated groups as compared to saline-treated group (Fig. 2). In addition, independent t-test analysis of the immunoblots demonstrated a significant increase in GLT-1b expression in NAc and PFC following treatment with cefazolin ($p<0.05$) and cefoperazone ($p<0.05$) as compared to saline-treated group (Fig. 3).

2.3. *Effects of cefazolin and cefoperazone on xCT and GLAST expression in NAc and PFC*

We further investigate the effect of cefazolin and cefoperazone treatments on xCT and GLAST expression. Quantitative t-test analyses of immunoblots showed a significant