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RESEARCH ARTICLE

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Glutamate-Glutamine Transfer and Chronic Stress-Induced Sex Differences in Cocaine Responses

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Abstract—Substance use disorders (SUD) often co-occur with other mental disorders such as major depression (MD). Our previous findings revealed sex-dependent changes in extracellular levels of glutamate (Glu) and glutamine (Gln) in the nucleus accumbens (NAc) in Long–Evans rats that were exposed to 21 days of chronic social defeat stress (CSDS), which models MD. The current study investigated the role of a Gln transporter called sodium-coupled neutral amino acid transporter subtype 1/2 (SNAT 1/2), phosphate-activated glutaminase (PAG), and astrocytic glutamate transporter-1 (GLT-1) on CSDS animals exposed to cocaine. Before cocaine exposure, CSDS males already showed decreased levels of SNAT 1/2 in the NAc and prefrontal cortex (PFC) compared to non-CSDS controls. The reduction in SNAT 1/2 levels was associated with an increase in Gln localization in the mitochondrial outer membrane in accumbal glutamatergic nerve terminals projecting from the PFC. CSDS females showed increased GLT-1 levels in the NAc and PFC compared to non-CSDS controls. Both acute and repeated cocaine exposure attenuated locomotor responses in CSDS males but increased those in CSDS females. Cocaine reduced SNAT 1/2 levels in the NAc but increased them in the PFC in CSDS males. Additionally, both PAG and GLT-1 levels were increased in the PFC in CSDS males. On the other hand, cocaine reduced SNAT 1/2 and GLT-1 levels in the NAc and PFC in CSDS females. Our results show that CSDS altered locomotor responses upon cocaine exposure in a sex-dependent manner that may be mediated by molecules associated with the Glu-Gln transfer.

Key words: Chronic social defeat stress, Cocaine, Glutamate transporter-1, Mitochondria, Phosphate-activated glutaminase, Sex difference, Sodium-coupled neutral amino acid transporter 1/2.

INTRODUCTION

Substance use disorders (SUD) often co-occur with other mental illnesses such as major depression (MD). According to The National Survey on Drug Use and Health (NSDUH) and National Institute on Drug Abuse (NIDA), approximately eight million people are currently suffering from both SUD and MD (SUD/MD) (NIDA, 2010; Hedden, 2015). While their symptoms can be treated acutely in ambulatory care settings, the pharmacological options for long-term conditions are lacking (Lima et al., 2003; Hesse, 2004; Ciraulo et al., 2005; Afshar et al., 2012).

Women are more vulnerable to SUD/MD compared to men, as indicated by the higher prevalence and longer hospital stays among female patients (Zilberman et al., 2003; Choi et al., 2015; Heslin et al., 2015). This sex difference is partly explained by the higher prevalence of mental illnesses in women than in men (Karg et al., 2012) and women's higher vulnerability to some stages of drug addiction, such as relapse (Rubonis et al., 1994; Robbins et al., 1999; Kennedy et al., 2013; Hitschfeld et al., 2015). Yet, not much is known about the biological or molecular targets that can explain the sex difference in the manifestation of SUD/MD.

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Abbreviations: CSDS, chronic social defeat stress; CYT, cytosol; DAB, diaminobenzidine; Gln, glutamine; GLT-1, glutamate transporter-1; Glu, glutamate; HRP, horseradish peroxidase; MD, major depression; Mit, mitochondrion; NAc, nucleus accumbens; PAG, phosphate-activated glutaminase; PFC, prefrontal cortex; PM, plasma membrane; PT, pre terminals; SNAT 1/2, sodium-coupled neutral amino acid transporter subtype 1/2; SUD, substance use disorders; T, presynaptic terminals; vGLUT1, vesicular glutamate transporter-1.

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Glutamate (Glu) is an excitatory neurotransmitter that mediates a wide range of behaviors. In the brain, Glu is synthesized from glutamine (Gln) that originates either from the peripheral organs and passes through the blood–brain barrier from circulating blood, or from local glial cells, including astrocytes (Albrecht et al., 2007; Sofroniew and Vinters, 2010; Schousboe et al., 2013). In the latter case, Gln in the astrocytes is synthesized from Glu that enters the cells from the extracellular space through astrocytic membrane-expressing glutamate transporters, such as glutamate transporter-1 (GLT-1) [also as known as excitatory amino acid transporter subtype 2 (EAAT2)]. The synthesized Gln is then released back into the extracellular space and taken up by the adjacent nerve terminal through membrane-expressing Gln transporters, including sodium-coupled neutral amino acid transporters subtype 1/2 (SNAT 1/2). There, Gln is deamidated to Glu by phosphate-activated glutaminase (PAG) located along at the mitochondrial membrane (Bak et al., 2006) (Fig. 1). This metabolic exchange between Glu and Gln involving neurons and astrocytes has been well-studied in an effort to alleviate symptoms of SUD (Brown et al., 2013) and MD (Lener et al., 2017a,b). Besides being the precursor of Glu, Gln is also used for energy production in cells, secondary to glucose. For this reason, Gln is thought to promote cancer cell survival and growth by providing nitrogen and carbon for ATP synthesis in the mitochondria (Wise and Thompson, 2010). However, the role of Gln, particularly in mediating behaviors, remains largely unknown.

Previously, in a Long–Evans rat model of MD, we showed that a 21-day exposure to chronic social defeat stress (CSDS) reduced the number of astrocytes in the nucleus accumbens (NAc) and impaired Glu clearance, resulting in Glu accumulation in the accumbal ECS (Rappeneau et al., 2016). The impairments were only present in females and not in males. Instead, CSDS significantly reduced extracellular Gln levels in males, while their Glu clearance and the number of astrocytes remained unchanged (Rappeneau et al., 2016). Thus, in the current study, we determined the role of transporters and enzymes associated with Glu-Gln transfer, including SNAT 1/2, PAG, and GLT-1, in mediating sex differences in behavioral responses to cocaine.

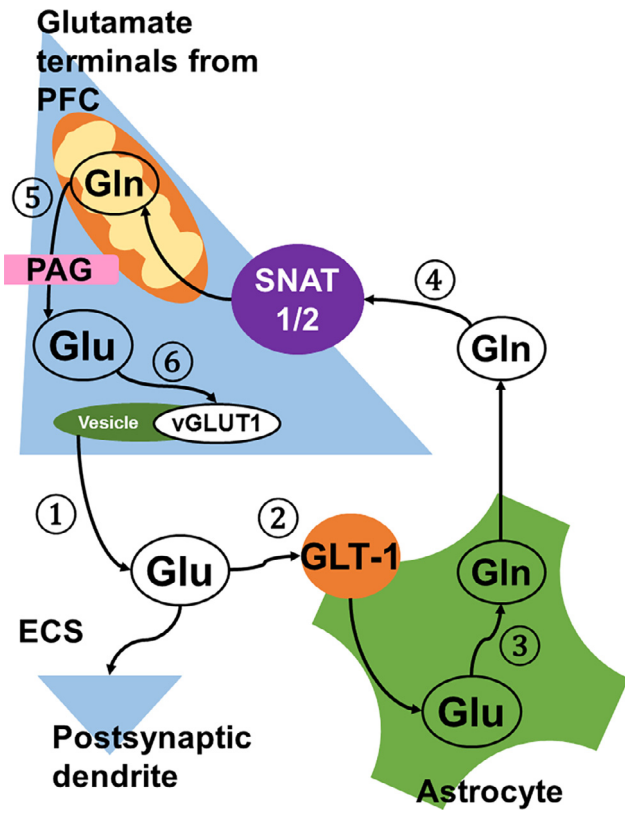

Fig. 1. The glutamate (Glu)-glutamine (Gln) transfer in the NAc. Once released from the presynaptic terminal (①), Glu is taken up to astrocytes through membrane-expressing glutamate transporters, such as the glutamate transporter-1 (GLT-1) (②). Once taken up, Glu is then converted to Gln (③), which is then released back into the ECS. The released Gln is then taken up to the presynaptic terminal through membrane-expressing Gln transporters, including the sodium-coupled neutral amino acid transporter subtype 1/2 (SNAT 1/2) (④). There, Gln is deamidated to Glu by phosphate-activated glutaminase (PAG) along at the mitochondria (⑤). The Glu is then transported into vesicles through the vesicular glutamate transporter, such as vGLUT1 (⑥), until the terminal is activated again, resulting in release of Glu (back to ①). ECS, extracellular space; Glu, glutamate; Gln, glutamine; NAc, nucleus accumbens; PFC, prefrontal cortex; GLT-1, glutamate transporter-1; SNAT 1/2, sodium-coupled neutral amino acid transporter subtype 1/2; PAG, phosphate-activated glutaminase; vGLUT1, vesicular glutamate transporter-1.

EXPERIMENTAL PROCEDURES

Animals

Male (225–250 g, $n = 35$) and female (200–225 g, $n = 28$) Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA) were individually housed in standard rat cages in an environmentally controlled vivarium (21 ± 1 °C; 30–70% humidity; inverted 12-h light–dark cycle) with ad libitum access to food and water. Separate "resident" male and female rats (> 300 g) were paired in guinea pig cages (27 × 51 × 22 cm) located in a separate vivarium. All animal protocols were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Meharry Medical College (National Research Council, 2011).

Drugs and antibodies

All drugs and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) with the following exceptions: cocaine hydrochloride (RTI International, Research Triangle Park, NC, USA through the NIDA Drug Supply Program), sodium pentobarbital (Patterson Vet, Columbus, OH, USA), Bradford reagent (BIORAD, Hercules, CA, USA). Glutaraldehyde, picric acid, and Tris Buffered Saline with Tween (TBST) were purchased from Electron Microscopy Sciences (Hatfield, PA, USA). Antibodies used in this study and their catalog numbers are as follows: GLT-1 (anti-EAAT2, rabbit monoclonal, ab178401, Abcam US, Cambridge, MA, USA); alpha 1 sodium potassium ATPase (anti-Na^+/K^+ ATPase,