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Glutamate Transporter 1: Target for the Treatment of Alcohol Dependence

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Abstract

Emerging evidence indicates that many aspects of alcohol and drug dependence involve changes in glutamate transmission. A number of studies have reported that drugs of abuse, including alcohol and cocaine, alter glutamate transport. Extracellular glutamate is regulated by a number of glutamate transporters in various brain regions. Of these transporters, glutamate transporter (GLT1) is a key player in the removal of most of the extracellular glutamate. Similar to neurodegenerative disease models, in which there is dysfunction of the glutamatergic excitatory system, the role of GLT1 has been tested in drug dependence models that show dysfunction of glutamate transmission. We and others have recently found that ceftriaxone, an FDA-approved drug known to elevate GLT1 expression, attenuates cue-induced cocaine relapse. Moreover, we recently found that alcohol-preferring rats treated with ceftriaxone showed a significant dose-dependent reduction in alcohol consumption. We also demonstrated that ceftriaxone-induced upregulation of GLT1 expression was associated with increases in glutamate uptake in Huntington's disease mouse model. Importantly, ceftriaxone is currently in clinical trials for the treatment of amyotrophic lateral sclerosis. This review provides information about the potential therapeutic role of GLT1 for the treatment of alcohol abuse and dependence.

Keywords

GLT1; EAAT2; glutamate; alcohol dependence; alcohol addiction; cocaine; GLAST; EAAT1; glutamate transporters; alcohol-preferring rats; glutamate uptake; cystine-glutamate exchanger; basal extracellular glutamate; nucleus accumbens; prefrontal cortex

I. INTRODUCTION

Alcohol dependence has been and continues to be a widespread phenomenon affecting the lives of many people worldwide. A national epidemiological survey reported that nearly 14 million people in the Unites States meet diagnostic criteria for alcohol use disorders [1]. The National Institute on Alcohol Abuse and Alcoholism defines alcohol dependence as a disease characterized by four symptoms – craving, loss of control, physical dependence and tolerance. Nearly 79,000 annual deaths are caused, directly or indirectly, by excessive alcohol consumption (Center for Disease Control and Prevention, 2008).

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CONFLICT OF INTERESTS
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The acute reinforcing effects associated with alcohol are due to its pharmacological interaction with various neurotransmitter systems in the brain's reward and stress circuits. Chronic exposure to alcohol causes changes in neuronal function, thereby precipitating associated symptoms, namely sensitization, tolerance, withdrawal, and dependence [2]. Alcohol tolerance, a common symptom of chronic alcohol consumption, is defined as the reduced response to a given dose of alcohol or the need for greater dose of ethanol to produce a desired level of response. Although the exact mechanism of development of alcohol tolerance remains unknown, studies have indicated the role of the endocannabinoid system in the development of alcohol tolerance [3]. Importantly, administration of N-methyl D-aspartate (NMDA) receptor antagonists induced inhibition of the development of tolerance to alcohol [4]. This demonstrates the implication of glutamatergic system in alcohol tolerance. Alternatively, behavioral sensitization is defined as the gradual enhancement in behavioral response following repeated administration of ethanol. Sensitization is characterized by various neuroadaptations of neurotransmitter systems which promote addictive behavior and hence considered a classified model for drug addiction. For example, studies have shown that dopamine 1 (D1) receptors and mu-opioid receptors play an important role in the development of sensitization to alcohol [5, 6]. In regards to the implication of glutamatergic system in sensitization to alcohol, studies demonstrated that metabotropic glutamate 1 receptor (mGluR1) and NMDA receptor are implicated in the expression of alcohol-induced sensitization [7]. In addition, other studies revealed the existence of links between the alterations of extracellular glutamate levels and alcohol-induced behavioral sensitization [8].

Our focus, in this review, is on the glutamatergic system as a target system for the treatment of alcohol dependence. Glutamate is the major excitatory neurotransmitter in the brain and acts *via* interaction with various glutamatergic receptors, including NMDA receptors. The glutamatergic system has been implicated in the development of acute reinforcing effects of alcohol. Alcohol interferes with the glutamatergic signal transmission by altering the functions of NMDA receptor as well as metabotropic glutamate receptor subtype 5 (mGluR5) [9, 10]. In addition, alcohol is known to inhibit glutamatergic transmission by blocking NMDA receptors [11, 12]. As a result of a compensatory mechanism, chronic alcohol intake has been shown to be associated with upregulation of NMDA receptors [13, 14]. Moreover, alcohol withdrawal increased the extracellular glutamate levels in the striatum along with heighted sensitivity of NMDA receptors in the nucleus accumbens (NAc) [15, 16].

Furthermore, acute alcohol exposure leads to decreased extracellular glutamate levels and reduced glutamatergic transmission in central reward brain regions, including NAc and amygdala [17, 18]. However, following chronic alcohol exposure, glutamate signal transmission was found to be elevated in the amygdala. Although there are no existing compounds targeting glutamatergic system for the treatment of alcoholism, acamprosate, a GABA agonist, has been suggested to act as non-selective antagonist for NMDA receptors and mGluRs, and thus consequently may block excessive alcohol consumption by reducing the excessive glutamate activity [19]. Importantly, we have recently identified in our laboratory that ceftriaxone, a β-lactam antibiotic known to upregulate glutamate transporter 1 (GLT1) termed also as excitatory amino acid transporter 2 (EAAT2) in human [20–23], reduced alcohol intake in alcohol-preferring (P) rat model [24]. The reduction in alcohol intake was associated with upregulation or activation of GLT1. Thus, GLT1 is considered a target for the treatment of alcohol dependence and addiction. In this review article, the neurocircuitry involved in alcohol-drinking behavior implicating glutamatergic system, the role of GLT1 and other glutamate transporters in development of alcohol dependence, and studies evaluating the effects of GLT1 by ceftriaxone on other drugs of abuse are described in detail.

II. NEUROCIRCUITRY INVOLVING GLUTAMATERGIC SYSTEM IN DRUGS OF ABUSE, INCLUDING ALCOHOL

The NAc, located in the ventral striatum (VS), has been well studied for its role in reward mechanism associated with drugs of abuse. It is currently believed that NAc acts as a gateway for limbic structures to reach the motor system [25, 26]. When a novel stimulus, capable of motivating a behavioral response, is encountered, the limbic system is engaged to process new and previously learned information about the stimulus, whereas the prefrontal cortex (PFC) is involved in producing goal oriented behavior [27]. The limbic structures, the basal lateral amygdala (BLA) and the hippocampus, are responsible for emotional processing and making contextual associations, respectively [28, 29]. Moreover, the mesocorticolimbic structures responsible for sensory information processing and action determination relay through the NAc to produce motor actions necessary to execute intended goals. When the NAc is activated through inputs from the PFC and limbic areas, the substantia nigra reticulata and motor thalamus become disinhibited and activate the motor cortex which further projects to the spinal cord to produce movement [30, 31]. Thus, the NAc serves to integrate information contained in the mesocorticolimbic circuit and projects that information to the motor system to produce an appropriate behavioral response as shown in Fig. (1). This behavioral response is a key factor in drugs of abuse-seeking behavior.

Both projections from amygdala and PFC to the NAc and the connections between BLA and PFC are glutamatergic [32]. Glutamatergic neurons arising from PFC also project towards dopaminergic neurons in the VTA. Alternatively, activation of glutamatergic neurons arising from the PFC and amygdala has been directly linked to the development of addiction [32]. The blood flow, as revealed with neuroimaging studies, is increased in brain regions associated with reward mechanism, including PFC and amygdala, with craving to addictive drugs such as alcohol [33, 34]. Moreover, employing the reinstatement model, BLA has been proven to be critical for reinstatement produced by drug-associated cue [35–37]. In addition, NAc receives glutamatergic inputs from amygdala and PFC, which suggests that NAc has a critical role in drug-seeking behavior [38, 39].

It is noteworthy that studies tested compounds targeting NMDA receptor and mGluR5 have revealed the importance of glutamatergic system in alcohol dependence [40, 41]. Furthermore, chronic exposure to alcohol has been associated with a marked increase in extracellular glutamate levels in the NAc [42, 43]. Given the role of glutamatergic system in the development of alcohol dependence, various approaches have been focused on finding compounds to attenuate the elevated level of extracellular glutamate and over activation of glutamatergic system. In this review, we focus on glutamate transporters, in particular GLT1, as target for the treatment of alcohol dependence. It is well known that GLT1 is responsible for the uptake of the majority of extracellular glutamate, which is critical in the induction of alcohol dependence. Thus, manipulating GLT1 level or activity is a key player in determining propensity for alcoholism. Prior to discuss the role of GLT1 in alcohol dependence, it is important to review and discuss the interactive role of glutamatergic system with key neurotransmitter systems in the regulation of alcohol intake.

III. INTERACTION OF GLUTAMATERGIC SYSTEM WITH OTHER NEUROTRANSMITTER SYSTEMS IN THE MODULATION OF ALCOHOL-DRINKING BEHAVIOR

Acute alcohol exposure was first identified to increase the binding capacity of low affinity GABA receptor binding sites in a rat model [44]. This, combined with the dampened

glutamatergic transmission, leads to a net sedative effect. It was further suggested that alcohol acts by facilitating GABA action through $GABA_A$ receptors and increases the chloride influx, thus depressing neuronal excitability [45, 46]. Therefore, alcohol acts to suppress neurotransmission by potentiating GABA receptors and inhibiting NMDA receptors. The effects of chronic alcohol use, however, are associated with a multitude of compensatory actions.

Seizures related to excessive excitation have been observed in subjects going through periods of alcohol withdrawal [47]. Chronic alcohol use has been shown to decrease the cell surface expression of $GABA_A$ receptors and decrease their sensitivity [48, 49]. Repeated alcohol exposure at pharmacologically relevant levels increased NMDA receptor subunits expression, synaptic clustering of these receptors, and receptor functionality [50]. Although acute alcohol exposure seems to suppress neurotransmission, chronic use has been linked to over excitation, possibly due to the compensatory mechanisms associated with repeated administration. Studies have shown that Sprague-Dawley rats withdrawn from chronic alcohol exposure show a significant increase in glutamate output relative to controls when administered with NMDA [51]. These studies demonstrated that administration of NMDA directly into the striatum increased extracellular glutamate in alcohol withdrawn rats as opposed to sucrose controls. These results suggest that the compensatory mechanisms during alcohol withdrawal can lead to an increase in synaptic glutamate release. Moreover, it was found that chronic alcohol exposure increases the density of NMDA receptors in the hippocampus [12]. The hippocampus has been shown to be associated with alcohol withdrawal seizures, and the use of NMDA antagonist, MK801, led to decreased number and intensity of seizures associated with alcohol withdrawal [11–14].

The importance of NAc activation in drug and alcohol dependence has been well studied due to its integrative role in mediating rewarding behavior. The majority of excitatory transmission to the NAc stems from glutamatergic efferents located in the PFC [52], which is composed primarily of glutamatergic pyramidal neurons. Evidence has also shown that dopamine type II (D2) receptors are downregulated in PFC following chronic alcohol consumption [52]. D2 receptor binding sites on pyramidal neurons within the PFC has been shown to have an inhibitory influence, whereas D2 receptors binding sites on GABAergic interneurons have an excitatory influence [31]. D2 receptors are located presynaptically on glutamate efferents projecting from the PFC to other brain regions [31]. Studies have shown that infusion of selective D2 antagonist, sulpiride, into the NAc dose-dependently increased alcohol consumption in P rats [53]. The results obtained from these studies could be attributed to increased glutamate transmission in the NAc by the inability to inhibit pyramidal neuron efferents. The PFC also receives glutamatergic afferents from the dorsomedial thalamus, an area involved in the neurocircuitry of drugs of abuse. Together, these results suggest that during alcohol withdrawal, there is an excessive amount of glutamate within the PFC, causing hyperactivation of pyramidal neurons that further project to the NAc and promote alcohol-seeking behavior. Excessive extracellular glutamate levels are regulated by several glutamate transporters, among them GLT1 is key player in regulating the majority of glutamate uptake [54].

IV. GLUTAMATE TRANSPORTERS

Glutamate is a major excitatory neurotransmitter of the central nervous system (CNS). Excess extracellular glutamate can lead to increased production of reactive oxygen and nitrogen species. Subsequently, this causes an increase in oxidative stress, leading to neuronal death [55]. Therefore, extracellular glutamate levels are tightly controlled *via* series of homeostatic pathways. The extracellular glutamate levels are regulated by a number of glutamate transporters (for review see ref. [54]). Glutamate transporters are classified under

two categories namely, the Excitatory Amino Acid Transporters (EAATs) and the Vesicular Glutamate Transporters (vGLUTs). The EAATs depend on electrochemical gradient of sodium and potassium ions for their actions. EAATs are membrane bound pumps, which are responsible for maintaining a low physiological extracellular glutamate levels [56]. Five subtypes have been identified in humans as well as rodents [57, 58]. Three of these transporters were first identified in rodents as glutamate/aspartate transporter (GLAST), GLT1 and excitatory amino acid carrier type 1 (EAAC1); the human homologues were later identified as EAAT1, EAAT2, and EAAT3, respectively. The other two excitatory amino acid transporters have been classified as EAAT4 and EAAT5. These two transporters have been identified in both rodents and humans. Immunostaining and protein expression studies have demonstrated that EAAT1 is most prominently distributed in the cerebellum and moderately expressed in other brain regions such as the hippocampus and the forebrain. GLT1 distribution, on the other hand, is mostly limited to forebrain, with little expression in the cerebellum.

As opposed to the EAATs, the vesicular glutamate transporters (vGLUTs) do not involve Na^+ electrochemical gradient to accumulate glutamate [59]. Unlike the EAATs that recognize and transport both glutamate and aspartate, the vGLUTs are selective for transporting glutamate [60]. Three types of vesicular glutamate transporters have been identified, namely vGLUT1, vGLUT2 and vGLUT3 [61]. The three isoforms are highly homologous, with up to 90% homology for the membrane spanning region. Alternatively, the N- and C- terminal regions have little homology and give rise to functional differences [62].

Since increased in extracellular glutamate levels has been shown to be closely associated with development of alcohol dependence, the role of glutamate transporters has drawn substantial attention to date. With the inherent potential to regulate the synaptic levels of glutamate, the glutamate transporters, in particular GLT1, represent potential targets for the treatment of alcohol dependence.

V. GLT1 AND ALCOHOL DEPENDENCE

Among various glutamate transporters, GLT1 is expressed in the brain and spinal cord. GLT1 is responsible for the removal of about 90% of extracellular glutamate [54, 63–65]. Dysfunction or reduced expression of GLT1 level results in impaired glutamate reuptake from the extracellular space, which has been implicated in the pathogenesis of various *in vitro* and *in vivo* disease models [66–70]. The mRNA coding for GLT1 has been found as multiple splice variants throughout the brain resulting in variant protein forms [71–73]. Splice variant forms of GLT1 have been found altered in neurodegenerative diseases and neurological disorders [74–77]. Furthermore, study has shown that reduction in extracellular glutamate was a result of increased expression of the glutamate transporters, such as GLT1 and EAAT1 [78].

The increased in extracellular glutamate levels has been observed in postmortem tissues of amyotrophic lateral sclerosis (ALS) patients [79–81]. This increase in extracellular levels of glutamate in ALS patients was accompanied by selective loss of GLT1 [64]. Drugs upregulating the expression of GLT1 therefore represent a viable solution and may help restore normal glutamatergic transmission. For example, Harmine, a naturally found beta-carboline alkaloid, is one of the lead compounds identified during high-throughput screening efforts and has been found to upregulate the GLT1 expression in both *in vitro* and *in vivo* models [82]. Cell based enzyme-linked immunosorbent assay was used in a high-throughput screen of about 140,000 compounds [83]. Few lead compounds were identified from these studies and served a starting point for the development of GLT1 upregulators/activators. In

addition, Thiopyridazine containing compound was shown to increase the levels of GLT1 protein in a dose-dependent manner. Following promising results, a structure-activity relationships study has focused on further improving the scaffold to identify a potent GLT1 activator [84].

Moreover, pharmaceutical modulation of GLT1 has long been studied in several types of neurodegenerative disorders and drug addiction models. Rothstein and colleagues have tested 1,040 FDA-approved drugs and identified that β-lactam antibiotics were some of the most potent stimulators of GLT1 protein expression [21]. These studies revealed that β-lactam antibiotic, ceftriaxone, increased the expression of GLT1 and its functional activity in both the hippocampus and spinal cord treated at a dose of 200 mg/kg administered i.p. once a day for five days. Since these findings, ceftriaxone's positive effects on GLT1 have been well documented in other experiments both *in vitro* [85, 86] and *in vivo* [23, 87, 88]. Recent studies have also shown that ceftriaxone causes an increased expression of GLT1 in the spinal cord [89]. In Huntington's disease mouse model, it was demonstrated that single daily i.p. injections of 200 mg/kg ceftriaxone for five days increased glutamate uptake in striatum, a primary target of cortical glutamatergic inputs [20]. Also, ceftriaxone is neuroprotective in models of ischemic injury and motor neuron degeneration in which glutamate reaches neurotoxic levels [90]. Thus, the ceftriaxone-induced increase in GLT1 expression has a direct effect on glutamate homeostasis.

Although other β-lactam antibiotics and cephalosporins appeared promising in the initial high-throughput screen, only ceftriaxone has been selected to be further studied [21]. This was partly due to the low bioavailability observed with other compounds as well as the lower EC_{50} values as compared to ceftriaxone. This observation puts emphasis on the key substitutions on the compound contributing to its activity. Apart from the pharmacophore, the β-lactam ring (Fig. 2), ceftriaxone differs from the other promising leads in the side chain substitution alpha to the amide bond of the β-lactam ring. Following the initial high-throughput screening, the lead compounds (including the β-lactam antibiotics) were tested in cell lines for their ability to activate the GLT1 promoter fragment. Promising results were obtained with ceftriaxone and amoxicillin while vancomycin did not produce any change in the basal level. Penicillin, although considered active in the initial screen, was unable to elicit a response in *in vivo* models due to the lack of ability of this compound to cross the blood-brain barrier. However, ceftriaxone has been well studied and was found to cross the blood-brain barrier [91–94] and elevate GLT1 expression in the CNS [20, 21, 23, 24]. Thus, ceftriaxone is currently considered a compound that has the potential to regulate GLT1 expression in the CNS.

The role of GLT1 has been tested in drugs of abuse models that show dysfunction of glutamate neurotransmission as a result of chronic exposure to these drugs. For example, activation of GLT1 by an activator drug, MS-153, was effective in a drug abuse mouse model [95]. Thus, administration of MS-153 attenuated conditioned place preference in mice that have been conditioned to morphine, methamphetamine and cocaine [95]. Also, treatment with ceftriaxone attenuated abstinence-induced withdrawal from cocaine in planarians [96]. Importantly, we have found recently that ceftriaxone attenuates cue-induced cocaine relapse in a dose-dependent manner [23]. Rats were trained to self-administer cocaine (0.125 mg per i.v. infusion) in a lever-pressing task in a daily two-hour session for 10–14 days, followed by five days of extinction training. Immediately after each extinction session, rats received ceftriaxone (i.p.) or saline vehicle. On the following day, presentation of the cue (light and tone) previously associated with cocaine self-administration reinstated lever-pressing in rats treated with vehicle, whereas 100 or 200, but not 50 mg/kg ceftriaxone blocked this response [23]. Immunoblotting confirmed that the ceftriaxone-induced blockade to cue-induced relapse to cocaine-seeking behavior to cocaine relapse was

associated with an increase in GLT1 expression in both the PFC and NAc, two forebrain regions in which elevated glutamate transmission appears to drive drug craving. Thus, accelerated removal of extracellular glutamate appears to be a key factor in the ability of ceftriaxone to dampen cocaine craving. In accordance, Kalivas and colleagues found similar effects regarding cocaine relapse [87]. This effect is correlated with an increase in GLT1 expression in PFC and NAc [23, 87].

Related to cocaine-seeking behavior, if an increase in glutamate transmission plays a critical role in alcohol consumption, then up-regulation or activation of GLT1 should attenuate this behavior. We recently tested the hypothesis that GLT1 plays a role in alcohol consumption in male P rats. P rats were selectively bred to determine the neurobiology of chronic alcohol-drinking behavior and the consequences of excessive alcohol intake behaviorally, neurochemically and physiologically. P rats are an established animal model of alcoholism; these rats can consume intoxicating levels of alcohol [97–99]. P rats have been characterized and demonstrate all of the criteria for animal model of alcoholism [100, 101]. P rats are considered a model of alcoholism; these rats drink greater than 4 g of ethanol/kg body weight/day, whereas alcohol non-preferring (NP) rats drink less than 1g/kg/day under similar conditions [102]. When P rats are on an alcohol drinking schedule, their blood alcohol concentrations (BACs) may reach between 50–200 mg% (which corresponds to 0.05–0.20 in clinical terminology) under 24-hour and limited-access conditions [103–105]. It has been shown that P rats develop behavioral tolerance in as little as five weeks of free-choice alcohol consumption [106]. Thus, recent studies from our laboratory used P rats that were given 24-hour concurrent access to 15% and 30% ethanol, water, and food for five weeks [24]. On Week 6, P rats received 25, 50, 100, or 200 mg/kg ceftriaxone (i.p.) or a saline vehicle for five consecutive days. Alcohol consumption was measured daily for 15 days, starting on Day 1 of injections, in order to test both the immediate and long-term effects of ceftriaxone. We revealed a significant reduction in daily alcohol consumption for 15 consecutive days [24]. At higher doses (100 and 200 mg/kg), ceftriaxone-mediated reductions in alcohol intake were correlated with an upregulation of GLT1 expression in the PFC and NAc on Day 8 [24]. The lower doses (25 and 50 mg/kg), did not cause the same increase in GLT1 expression, but did temporarily reduce alcohol consumption as compared to the highest doses. Our findings from this study [24] demonstrated that rats that were administered ceftriaxone showed a reduction in alcohol intake as compared to those that received saline vehicle. The long-lasting effects of ceftriaxone in alcohol intake were found correlated with the upregulation of GLT1 expression in PFC and NAc brain regions. We suggest that upregulation of GLT1 can counteract the increase in basal extracellular glutamate levels that might be caused by chronic alcohol consumption.

Basal extracellular glutamate levels differ between some of drugs of abuse, including cocaine and alcohol. In contrast to animal model with chronic alcohol intake, basal extracellular glutamate levels are decreased in NAc in animals exposed to cocaine [39, 107–109], yet ceftriaxone has similar effects in both models [23, 24, 87]. As reviewed in details by Kalivas that decreased basal extracellular glutamate levels after chronic cocaine self-administration is associated with reduction in cystine-glutamate exchanger (xCT) and reduction in GLT1 levels, but elevation of synaptic glutamate release in NAc occurred during cue-induced relapse to cocaine-seeking behavior [87, 110]. During cue-induced relapse to cocaine-seeking behavior, increased in glutamate release in NAc was found associated with activation of the PFC targeting this brain region [107]. In contrast to chronic alcohol consumption where basal extracellular glutamate is increased, cocaine exposure can lead to decrease in basal extracellular glutamate level. But during reinstatement to cocaine seeking-behavior there is increase in synaptic glutamate release in NAc and decrease in the glutamate uptake [for review see ref. [110]. Although basal extracellular glutamate level is different between cocaine and alcohol models, ceftriaxone was found to restore dysfunction

of the glutamate homeostasis in both models, and consequently attenuates alcohol intake and cue-induced relapse to cocaine-seeking behavior [23, 24, 87]. This was due to the fact that ceftriaxone restored GLT1 and xCT levels in NAc and PFC that were found reduced after cocaine exposure [87] and perhaps in alcohol intake model, in particular with GLT1 [24]. Alternatively, it is unclear whether ceftriaxone can induce upregulation of GLT1 expression in other non-reward brain regions in alcohol and cocaine drug abuse models. Studies are warranted to investigate the effects of ceftriaxone in these other non-reward brain regions and to determine whether this effect may affect the overall brain glutamate homeostasis. It is unclear whether this potential overall effect of ceftriaxone in all brain regions may or may not have negative effects in the neurochemistry of the brain.

The precise cellular mechanism by which ceftriaxone enhances the level of GLT1 remains unknown. It is suggested that there is at least one pathway (which might have direct or indirect interaction) involved in GLT1 expression. Lee and colleagues demonstrated that the canonical NF-κB signaling pathway is necessary for the ceftriaxone-induced increase in GLT1 in human primary fetal astrocytes [85]. Other known GLT1 upregulators, like the epidermal growth factor receptor agonists, have also been shown to be mediated *via* pathway involving the transcription factor NF-κB [111]. Amitriptyline, an antidepressant, has also been found to upregulate the expression of GLT1 and GLAST through NF-κB dependent route in chronic morphine-induced rats [112]. Also, in other *in vitro* studies, the pathway involving mammalian target for rapamycin (mTOR) has also been shown to regulate GLT1 expression and glutamate uptake [113]. In this pathway, mTOR was phosphorylated by Akt, which in turn was found to be responsible for GLT1 expression.

Additionally, ceftriaxone was found to inhibit alcohol intake with lower doses without any change in GLT1 expression thereby suggesting possible additional pharmacological effects of the drug [24]. Other studies have demonstrated that ceftriaxone increased the activity of GLT1, without affecting its expression, in brain regions including hippocampus, striatum, and frontal cortex of an animal stroke model [88]. Furthermore, studies have shown that ceftriaxone increased glutamate uptake in hippocampus region of rats without upregulation of GLT1 expression [114]. A functional increase could involve changes in several mechanisms, involving for example protein phosphorylation such as protein kinase C (PKC) that was found to interfere with cell surface expression of GLT1 [115]. Studies are warranted to investigate other potential mechanisms of action of ceftriaxone in GLT1 function.

It is noteworthy that higher doses of ceftriaxone might cause diarrhea, as reported in humans [116]. However, in rats, ceftriaxone at higher doses (100 and 200 mg/kg) was found to be very tolerable without the occurrence of diarrhea [24]. The dose of 100 mg/kg has also been tested in Huntington's disease mouse model and no severe side effects were observed in this study as well [20]. Moreover, ceftriaxone is currently being evaluated for ALS treatment and no major side effects have been reported from phase I and phase II clinical trials. Phase III of the clinical study is currently underway.

VI. ROLE OF OTHER GLUTAMATE TRANSPORTERS IN ALCOHOL INTAKE

Evidence suggests that chronic alcohol exposure not only leads to functional increases in glutamate output but also to an impaired ability in glutamate transport. Unlike other neurotransmitters, glutamate is not metabolized in the synaptic cleft [117]. The primary means by which it is removed from the synapse is by glutamate transporters, including GLAST and GLT1 (EAAT2) [118]. Studies have shown that alcohol-preferring cAA rats chronically exposed to ethanol (10%, v/v) for 20 months exhibited a reduction in glutamate transport in the cerebral cortex [119]. Recent studies examined the expression levels of

GLAST and GLT1 in post-mortem human alcoholics. A significant decrease in levels of GLAST and GLT1 in the BLA was found in post-mortem alcoholics as compared to non-alcoholic controls [120]. Glutamate uptake *via* these transporters is also altered *via* chronic alcohol exposure, although with varying results *in vitro* [121–123] and *in vivo* [124, 125]. In experiments involving Xenopus oocytes, chronic exposure to high doses of alcohol has been shown to reduce the activity of both EAAT3 and EAAT4 [126, 127]. Also, the expression of EAAT1 and EAAT3 in the parietal cortex was unaltered following chronic alcohol administration in rats, as opposed to EAAT2 (GLT1), which was downregulated in high-dose alcohol fed rats [128] (Table 1).

In postmortem human PFC samples, a marked increase in the expression of EAAT1 was observed in case of alcoholics suggesting a neuroadaptation may occur to reverse the increased extracellular glutamate levels [129] (Table 1). The increased expression of EAAT1 found throughout the PFC of chronic alcoholics indicates that all layers of PFC are affected by increased glutamate levels. Moreover, acute alcohol exposure leads to an increased activity of EAAT3, and this has been linked to some of the alcohol-induced features like sedation, impaired cognition and general anesthesia [130]. Alternatively, chronic exposure has been shown to decrease the activity of EAAT3 *via* a PKC-dependent mechanism. It has been hypothesized that this may be a compensatory mechanism to overcome the inhibitory effects of alcohol on the excitatory glutamatergic transmission in the CNS [127] (Table 1).

Alcohol abuse also changes the expression of vGLUTs in the central reward brain regions, particularly the NAc. We have previously reported that repeated deprivation of alcohol in P rats led to an increased vGLUT2 expression in the NAc shell [131]. However, the expression of vGLUT1 levels, which are part of the cognitive circuit of the brain, remained unchanged. The vGLUT2 expressing glutamate neurons are associated with the motor circuit and this might explain the effects of alcohol intake on motor activity. It is suggested that the increase in number of vGLUT2 carrying glutamate neurons following repeated deprivation of alcohol is associated with a significant change in the ratio of dopamine to glutamate in the NAc shell, which is a key region of the reward circuitry [131] (Table 1).

VII. ROLE OF GLT1 ACTIVATION ON OTHER DRUGS OF ABUSE

Increased glutamate activity produced in response to repeated exposure of morphine has been implicated in the development of physical dependence associated with morphine [132]. Moreover, studies have demonstrated that chronic exposure to morphine is associated with reduced mRNA GLT1 levels in the CNS [133, 134]. Rawls and colleagues have tested the hypothesis that decreased extracellular glutamate levels following administration of β-lactam antibiotic, ceftriaxone, can lead to decreased antinociceptive tolerance caused by chronic exposure to morphine [135, 136]. This study showed that ceftriaxone inhibited the tolerance developed for morphine, after repeated exposure, in a dose-dependent manner. Moreover, inhibition of GLT1 by dihydrokainate (DHK) abolished the effect of ceftriaxone on morphine tolerance demonstrating the key role played by GLT1 in the development of morphine tolerance.

The effect of ceftriaxone was also studied on morphine-induced hyperthermia rat model [137]. Ceftriaxone was shown to inhibit the hyperthermia observed following morphine administration in rats. Upon pretreatment with DL-threo-β-Benzyloxyaspartic acid (TBOA), a non-specific inhibitor of glutamate transporters, the effect of ceftriaxone on morphine-induced hyperthermia was not observed further; this indicates the relationship between glutamate uptake and morphine-induced hyperthermia. Furthermore, the effect of ceftriaxone on kappa-opioid receptor agonist induced hypothermia has also been

investigated [138]. This study demonstrated that subcutaneous administration of U50,488H produced hypothermia in rats. While a single dose of ceftriaxone was found to have no effect on hypothermia observed following acute administration of U50,488H, however, repeated exposure to ceftriaxone was shown to block the tolerance for U50,488H-induced hypothermia.

Repeated administration of ceftriaxone was also found to inhibit the hyperactivity associated with amphetamine administration [139]. Also, ceftriaxone was found to inhibit the amphetamine-induced behavior sensitization in rats. The results were attributed to the fact that ceftriaxone induced increase in GLT1 level leads to increased glutamate uptake thereby preventing the increase in glutamatergic activity at NMDA and AMPA receptors typically observed following amphetamine administration. On the other hand, coadministration of ceftriaxone with nicotine induced inhibition of the development of antinociceptive tolerance [140]. These results have been attributed to the fact that increased GLT1 expression following ceftriaxone administration results in increased glutamate uptake.

VIII. CONCLUSION

Despite of the involvement of multiple neurotransmitters, glutamatergic system seems to play a critical role in the development of alcohol dependence. Alcohol consumption affects the extracellular glutamate level and expression of various glutamate transporters in a complex manner. While acute alcohol exposure may lead to an overall suppression of extracellular glutamate levels, chronic exposure of alcohol has been shown to increase the extracellular glutamate level. The overall effect is the contribution of glutamatergic system towards development of alcohol dependence. Alcohol dependence is associated with a significant decrease in expression of glutamate transporters. Of these transporters, GLT1 is responsible for the uptake of the majority of extracellular glutamate. We suggested that upregulation of GLT1 level in the central reward brain regions would decrease extracellular glutamate and consequently reduce alcohol consumption. We have tested ceftriaxone, a β-lactam antibiotic known to upregulate GLT1, and found that ceftriaxone-induced upregulation of GLT1 in PFC and NAc was associated with reduction in alcohol intake in P rats. It is noteworthy that ceftriaxone-induced upregulation of GLT1 was also found to be associated with the attenuation of relapse to cocaine-seeking behavior. Together, we suggest that GLT1 is a potential target for the treatment of drugs of abuse, including alcohol.

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ABBREVIATIONS

CNS	Central Nervous System
NAc	Nucleus Accumbens
GABA	γ-aminobutyric acid
NMDA	N-methyl D-aspartate receptor
GLT1	Glutamate Transporter 1
VS	Ventral Striatum
PFC	Prefrontal Cortex

BLA	Basal Lateral Amygdala
HP	Hippocampus
VP	Ventral Pallidum
VTA	Ventral Tegmental Area
DMT	Dorsomedial Thalamus
SNr	Substantia Nigra reticulata
MT	Motor Thalamus
MC	Motor Cortex
ALS	Amyotrophic Lateral Sclerosis
DA	Dopamine
D1	Dopamine type I receptors
D2	Dopamine type II receptors
EAAT1	Excitatory Amino Acid Transporter 1
EAAT2	Excitatory Amino Acid Transporter 2
EAAT3	Excitatory Amino Acid Transporter 3
EAAT4	Excitatory Amino Acid Transporter 4
EAAT5	Excitatory Amino Acid Transporter 5
VGLUT1	Vesicular glutamate transporter 1
VGLUT2	Vesicular glutamate transporter 2
VGLUT3	Vesicular glutamate transporter 3

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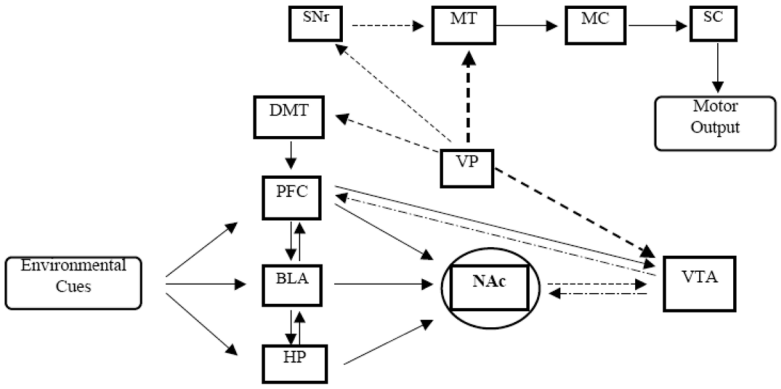

Fig. (1).

Simplified neurocircuitry involving glutamatergic, GABAergic and dopaminergic pathways in drugs of abuse, including alcohol. The nucleus accumbens serves as an integrator of environmental stimuli and as a gateway between the mesocorticolimbic circuit and the motor output pathway. Abbreviations: NAc, nucleus accumbens; PFC, prefrontal cortex; BLA, basal lateral amygdala; HP, hippocampus; DMT, dorsomedial thalamus; VTA, ventral tegmental area; VP, ventral pallidum; SNr, substantial nigra; MT, motor thalamus; MC, motor cortex; SC, spinal cord.

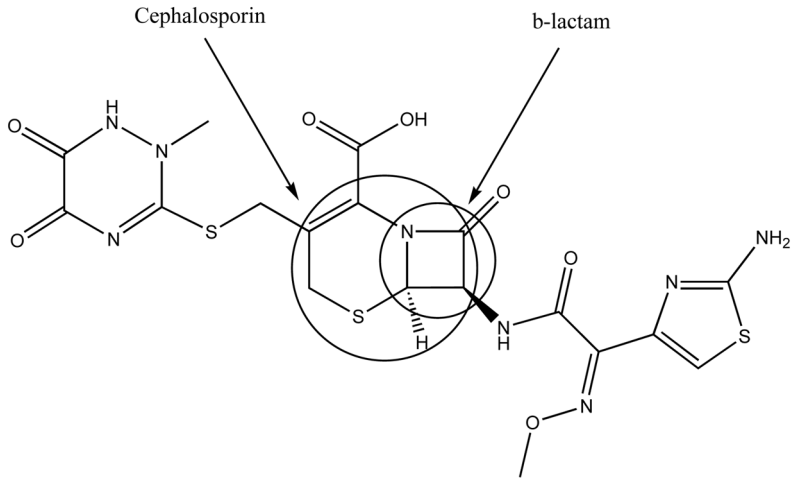

Fig. (2).

Table 1

Different Types of Glutamate Transporters, their Distribution, and the Effects of Alcohol in their Levels of Expression or Activity

Glutamate transporter	Neuronal/Glial	Effects of alcohol on expression/activity	References
1. EAATs			
EAAT1	Glial/low expression on neurons	⇧	[119]
EAAT2 (GLT1)	Glial/low expression in neurons	⇩	[110, 118]
EAAT3 (EAAC1)	Neuronal	Acute ⇧; Chronic ⇩	[117, 120]
EAAT4	Neuronal	⇩	[116–117]
EAAT5	Retina	NA	-
2. vGLUTs			
vGLUT1	Neuronal	Unchanged	[121]
vGLUT2	Neuronal	⇧	[121]
vGLUT3	Neuronal	NA	-

NA: Information not available.