Potential therapeutic role of glutamate transporter 1 for the treatment of alcohol dependence

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Abstract

Introduction

Evidence has demonstrated that deficits in glutamate transmission impair neurocircuits involved in drug abuse or drug-seeking behaviour and affect many aspects of neuroplasticity associated with alcohol and drug addiction. Alcohol-seeking behaviour is promoted by increased glutamate transmission in key regions of the mesocorticolimbic reward circuit, including the nucleus accumbens and prefrontal cortex. Glutamate transmission or glutamate uptake is regulated by a number of glutamate transporters in the brain regions. Among these glutamate transporters, glutamate transporter 1 (GLT1; its human homolog is the excitatory amino acid transporter 2, EAAT2) regulates the removal of majority of the extracellular glutamate. The role of GLT1 has been tested in alcohol and other drugs of abuse models with dysfunction in glutamate transmission. We recently reported that treatment of alcohol-preferring rats with compounds ceftriaxone and GPI-1046, known to upregulate GLT1 levels, showed reduction in alcohol intake and attenuation of relapse-like ethanol-drinking behaviour. Furthermore, we demonstrated that upregulation of GLT1 was associated in part with attenuation of cue-induced cocaine relapse. Together, we suggest that GLT1 is considered as a potential therapeutic target for the treatment of drug dependence, including alcohol. The aim of this critical review was to discuss the potential therapeutic role of GLT1 for the treatment of alcohol dependence.

Conclusion

Dysfunction of glutamate transmission has been suggested to impair neurocircuits involved in alcohol dependence, which affect neuroplasticity that is associated with ethanol intake.

Introduction

Alcohol abuse and dependence continue to be significant public health problems. A better understanding of their neurobiology will facilitate the development of interventions targeting prevention and/or treatment of these major health issues. Evidence has suggested that several neurotransmitters are involved in the development of drug abuse and dependence, including alcohol.

While it is established that dopaminergic neurotransmission plays an important role in alcohol addiction, increasing evidence suggests that many aspects of neuroplasticity in drug addiction involve changes in glutamatergic neurotransmission as well. Neuroadaptations of the glutamatergic system play a key role in alcohol tolerance, dependence and withdrawal. The selective effects of alcohol include inhibition of glutamatergic neurotransmission by alteration of N-methyl-D-aspartate (NMDA) receptors. One of the effects of chronic alcohol exposure is the upregulation of NMDA receptors that are part of the compensatory mechanism, which results from chronic inhibition of glutamatergic neurotransmission. In addition, the effects of alcohol withdrawal are associated with increased extracellular glutamate levels in the striatum of alcohol dependent rats and enhanced NMDA sensitivity in the nucleus accumbens (NAc). Importantly, drugs that have the potential to target NMDA receptors and metabotropic glutamate receptor subtype 5 (mGlur5) have indicated the important role of the glutamatergic system in alcohol dependence and seeking-behaviour. In addition, a marked increase in the levels of extracellular glutamate was found in the NAc of animals exposed chronically to ethanol.

Extracellular glutamate levels and glutamate neurotransmission are regulated by several glutamate transporters in the brain. Among these glutamate transporters, glutamate transporter 1 (GLT1, termed also as excitatory amino acid transporter 2, EAAT2) is a key player in the removal of the majority of extracellular glutamate. Similar to disease models in which there is dysfunction of the glutamatergic excitatory system, the role of GLT1 has been tested in drug abuse models that show dysfunction of glutamate transmission. It is noteworthy that the activation of GLT1 by MS-153, a cerebroprotective agent, was effective in reducing the induction of conditioned place preference to methamphetamine, cocaine and morphine. Moreover, we have recently found that ceftriaxone, a β-lactam antibiotic, known to upregulate GLT1 levels, attenuates cue-induced cocaine relapse in a dose-dependent manner in a rat model. In accordance with this data, Knackstedt et al. have found similar effects with ceftriaxone in cocaine relapse-like behaviour. Focusing on the role of GLT1 in alcohol-drinking behaviour, we have previously reported that male alcohol-preferring (P) rats treated
with ceftriaxone for five days, at different doses, showed a significant dose-dependent reduction in ethanol intake compared to saline-treated rats. This reduction in ethanol intake was associated, in part, with the upregulation of GLT1 levels in the NAc and prefrontal cortex (PFC). Moreover, we have recently reported that chronic alcohol consumption can lead to downregulation of GLT1 levels in the NAc. These findings suggest that GLT1 upregulation or activation has potential for the treatment of alcohol dependence and other drugs of abuse. In this critical review, we have discussed the neurocircuitry involving the glutamatergic system in alcohol dependence as well other drugs of abuse. We have further discussed the importance of upregulation of GLT1 in chronic ethanol consumption and relapse-like alcohol drinking behaviour.

**Discussion**

The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institutions in which they were performed. Animal care was conducted in accordance with the institution guidelines.

**Neurocircuitry of the glutamatergic system in alcohol dependence and other drugs of abuse**

Although the neurocircuitry of the glutamatergic system in the modulation of alcohol dependence and drugs of abuse is not fully defined, it is suggested that this system within the PFC and the NAc plays a critical role in drug reinforcement. These brain regions receive substantial input from the midbrain dopaminergic neurons and all major drugs of abuse, including alcohol, and increase forebrain dopamine transmission. It is important to note that the NAc is suggested as a gateway for limbic structures targeting the motor system for the development of drug dependency, including alcohol.

Among the glutamatergic projections are those occurring from the PFC to the NAc and reciprocal glutamatergic connections occurring between the PFC and the amygdala. Moreover, glutamatergic projections from the PFC to the NAc are critical in the expression of addictive behaviours. In addition, activation of the glutamatergic neurons of the PFC, targeting the amygdala, was found to be directly involved in the development of drug addiction. It is noteworthy that both the PFC and NAc receive glutamatergic projections from the amygdala and hippocampus, and this neurocircuit was suggested to be implicated in the initiation of drug-seeking behaviour. There are also glutamatergic projections from the PFC to dopaminergic neurons in the ventral tegmental area (VTA). The importance of the glutamatergic projections from the PFC to the NAc and the VTA have been observed in neuroimaging studies performed during craving periods in several different paradigms, for commonly abused drugs such as alcohol, cocaine, methamphetamine, heroin and nicotine. Alternatively, the basal lateral amygdala is considered as a critical brain region for reinstatement to cue-drug-seeking behaviour.

**Role of GLT1 in alcohol-drinking behaviour in the chronic alcohol consumption paradigm**

GLT1 is an excitatory amino acid transporter, such that its modulation depends on the electrochemical gradient of sodium and potassium ions. The transporter is a membrane-bound pump that regulates the extracellular glutamate levels. Increased levels of extracellular glutamate were revealed in post mortem brains that suffered from amyotrophic lateral sclerosis (ALS). These increased levels of extracellular glutamate were associated, in part, with the downregulation of GLT1 in post mortem brains with ALS. GLT1 activity is important in maintaining normal excitatory synaptic neurotransmission, and its dysfunction is implicated in different neurological disorders, including Huntington’s disease (HD), stroke, ALS, brain tumours and epilepsy. Although there is no evidence related to any deficit in GLT1 levels in post mortem drug addicts, preclinical studies from our laboratory and other studies have shown downregulation of GLT1 and increased extracellular glutamate levels which were associated with alcohol dependence, relapse-like alcohol-drinking behaviour and relapse to cue-cocaine-seeking behaviour. Thus, the importance of identifying and developing drugs that upregulate GLT1 would have therapeutic benefits for the treatment of drug dependency, including alcohol, and also for the treatment of certain neurodegenerative diseases.

Rothstein et al. screened 1,040 Food and Drug Administration-approved drugs and nutritional supplements and discovered that many β-lactam antibiotics are potent stimulators or upregulators of GLT1 levels, which might be mediated through increased transcription of the GLT1 gene. In addition, this later study demonstrated that several β-lactam antibiotics, including ceftriaxone, were the most active in increasing GLT1 levels in the brain. Furthermore, ceftriaxone treatment delayed neuronal loss and increased animal survival. This neuroprotective effect was associated, in part, with upregulation of GLT1 levels in the brain. We have recently shown that ceftriaxone treatment increased GLT1 levels in the striatum, which consequently decreased the extracellular levels of glutamate in a HD R6/2 mouse model. In addition, we also demonstrated that ceftriaxone reversed the deficit in GLT1 levels observed in the PFC and striatum of R6/2 mice. It is noteworthy that ceftriaxone-induced upregulation of GLT1 levels may have a direct action on glutamate homeostasis.

Studies focused on the effects of upregulation or activation of GLT1...
in animal models of drugs of abuse, including alcohol, have shown promising findings with the implication of this transporter for potential therapeutic targets in the treatment of these disorders. We hypothesised that if an increase in extracellular glutamate is a key player in alcohol dependence, then upregulation of GLT1 levels would increase glutamate uptake, thus reducing ethanol intake. We tested this hypothesis in our established animal model for alcoholism, P rats, and found that ceftriaxone-induced GLT1 upregulation in the PFC and NAc reduced, in part, the ethanol intake. Ceftriaxone was found to have a long lasting effect on ethanol intake at higher doses. Moreover, we have also recently examined the effects of neuroimmunophilin GPI-1046 (3-(3-pyridyl)-1-propyl (2S)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinedicarboxylate), in our established P rats. GPI-1046 is a derived compound from the FK506 immunophilin ligand (tacrolimus). We reported that GPI-1046 treatment reduced ethanol intake in a dose-dependent manner in male P rats. This attenuation of ethanol was associated, in part, with upregulation of GLT1 levels in the PFC and NAc. We postulated here that elevation of GLT1 levels in these brain regions may counteract the increase in the levels of extracellular glutamate caused by chronic ethanol intake.

Recent studies from our laboratory revealed that GLT1 levels were significantly downregulated in the NAc core but not in the PFC of male P rats that consumed ethanol chronically. In accordance with these results, findings by Knackstedt et al. also revealed a significant reduction in GLT1 levels in the NAc core but not the PFC in rats self-administered with cocaine. As we have reviewed recently, it is not clear as to how both ethanol and cocaine had similar effects with regard to the GLT1 levels in the PFC and NAc. It is important to note that cocaine and alcohol may act mechanistically different, but both may share the same neurocircuity. The PFC receives and projects glutamatergic inputs and outputs, respectively to and from other brain regions, including the central reward brain regions. Alternatively, the NAc receives only glutamatergic inputs from the PFC and amygdala, which may explain the differences in the levels of GLT1 in the NAc in chronic alcohol exposure and cocaine self-administration animal models.

Role of GLT1 in the relapse-like alcohol-drinking behaviour paradigm

Relapse behaviour is a persistent problem for individuals recovering from alcoholism. Although there are a number of criteria for relapse-like behaviour, our criteria are based on the definition that a return to levels of consumed alcohol equal to or greater than those observed prior to abstinence is considered relapse-like to ethanol-drinking behaviour. With regard to selectively bred high alcohol-consuming lines of rats including P rats, the alcohol deprivation effect (ADE) has been considered the primary model for assessing relapse-like behaviour. The ADE has been used to assess relapse-like behaviour in P rats. Thus, we have used P rats as a model for relapse-like ethanol-drinking behaviour. We found that ceftriaxone treatment attenuated relapse-like ethanol-drinking behaviour in P rats. This attenuation of relapse-like behaviour was associated with upregulation of GLT1 levels in the PFC and NAc core. In our recent study, we focused on the NAc core since findings have demonstrated that the increase in glutamate release was more pronounced in the NAc core than in the NAc shell in rats developing behavioural sensitisation. Furthermore, glutamate release was increased in the PFC-NAc pathways, mainly in the NAc core; this increase in glutamate transmission was observed with reinstatement of cocaine-seeking behaviour. Moreover, we have examined the effect of upregulation of GLT1 levels with ceftriaxone using the cocaine-seeking behaviour paradigm. We found that ceftriaxone treatment attenuated cue-induced relapse to cocaine self-administration, which was associated with upregulation of GLT1 levels in the PFC and NAc. In accordance with these results, studies found similar findings showing that ceftriaxone treatment attenuated cue to relapse to cocaine-seeking behaviour; this attenuation was associated with upregulation of GLT1 levels in the NAc and PFC.

Evidence demonstrated that extracellular glutamate levels might be different between drugs of abuse, including alcohol and cocaine. For example, basal extracellular levels are lower in the NAc of rats self-administered with cocaine as compared to rats chronically exposed to alcohol. It is important to note that ceftriaxone treatment has similar effects between these two models of cocaine self-administration and alcohol intake, including the relapse paradigm. Kalivas et al. suggested recently that a decrease in the levels of basal extracellular glutamate in the cocaine-self-administration chronic paradigm might be associated with a decrease in the down-regulation of GLT1 and cystine-glutamate exchanger (xCT) levels in the NAc. However, increased glutamate release at the synapse was found in the NAc during cue-induced relapse to cocaine-seeking behaviour. This increase in glutamate release found in the NAc was associated with activation of the PFC. It is important to note that basal extracellular glutamate levels are different between cocaine-seeking behaviour and alcohol intake models, and yet, ceftriaxone was found to attenuate alcohol intake, relapse-like ethanol-drinking behaviour and cue-induced relapse to cocaine-seeking behaviour. This is probably associated with the effects of ceftriaxone in restoring GLT1 or xCT levels, which are found downregulated after cocaine or alcohol intake in...
the NAc and PFC\textsuperscript{17,18}. xCT has been shown to play a key role in glutamate homeostasis (Figure 1). For example, findings reported that administration of N-acetylcysteine restored the levels of extracellular glutamate and attenuated cocaine-induced seeking behaviour through mGluR2/3 receptors\textsuperscript{39}. Thus, GLT1, xCT and mGluR2/3 receptors are critical in regulating drug-seeking behaviour, including alcohol, through modulation of glutamate homeostasis (Figure 1).

**Conclusion**

It is concluded here that dysfunction of glutamate transmission has been suggested to impair neurocircuits involved in alcohol dependence, which affect the neuroplasticity associated with ethanol intake. Alcohol-seeking behaviour is promoted by increased glutamate transmission in key regions of the mesocorticolimbic reward circuit, including the PFC and NAc. GLT1 is a key player in the regulation of the majority of glutamate uptake and consequently, modulating ethanol consumption. We have identified two compounds, ceftriaxone and GPI-1046 known to upregulate GLT1 levels in the central reward brain regions for the attenuation of ethanol intake. Ceftriaxone was also effective in the attenuation of cue-induced relapse to cocaine. Ceftriaxone and GPI-1046 treatments inverted the effect of ethanol consumption via the upregulation of GLT1 levels in the NAc and PFC. These findings indicate that GLT1 is a potential therapeutic target for the treatment of alcohol dependence.

**Abbreviations list**

ADE, alcohol deprivation effect; ALS, amyotrophic lateral sclerosis; GLT1, glutamate transporter 1; HD, Huntington’s disease; mGluR5, metabotropic glutamate receptor subtype 5; NAc, nucleus accumbens; VTA, ventral tegmental area; xCT, cystine-glutamate exchanger.

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