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Adenosine and Glutamate Signaling in Neuron-Glial interactions: Implications in Alcoholism and Sleep Disorders

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Abstract

Recent studies have demonstrated that the function of glia is not restricted to the support of neuronal function. Especially, astrocytes are essential for neuronal activity in the brain. Astrocytes actively participate in synapse formation and brain information processing by releasing or uptaking gliotransmitters such as glutamate, D-serine, adenosine 5'-triphosphate (ATP) and adenosine. In the central nervous system, adenosine plays an important role in regulating neuronal activity as well as in controlling other neurotransmitter systems such as GABA, glutamate and dopamine. Ethanol increases extracellular adenosine levels, which regulates the ataxic and hypnotic/sedative (somnogenic) effects of ethanol. Adenosine signaling is also involved in the homeostasis of major inhibitory-excitatory neurotransmission (*i.e.* GABA or glutamate) through neuron-glial interactions, which regulates the effect of ethanol and sleep. Adenosine transporters or astrocytic SNARE-mediated transmitter release regulates extracellular or synaptic adenosine levels. Adenosine then exerts its function through several adenosine receptors and regulates glutamate levels in the brain. This review presents novel findings on how neuron-glial interactions, particularly adenosinergic signaling and glutamate uptake activity involving glutamate transporter 1 (GLT1), are implicated in alcoholism and sleep disorders.

Keywords

Adenosine; Glutamate; Alcoholism; Sleep; Signaling; Pharmacology

Introduction

Adenosine has several functions in the central nervous system (CNS) that are critical for proper brain function. As a neuromodulator, one of the main functions of adenosine is to inhibit glutamate release *via* presynaptic A₁ receptors in the nucleus accumbens (NAc) (Harvey and Lacey, 1997). Since adenosine levels are increased in response to acute ethanol exposure, adenosine-regulated inhibition of glutamate release partially accounts for the

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The subject of this mini-review has been presented in a symposium held at the Research Society on Alcoholism (RSA), June 25 to June 29, 2011 (Atlanta, Georgia). Organizer and Chair of the symposium was Doo-Sup Choi. Introducer was Doo-Sup Choi. Speakers were Doo-Sup Choi, Sally R. McIver, Mahesh M. Thakkar, and Youssef Sari. Discussant was Fiona E. Parkinson.

intoxicating effects of ethanol (Dunwiddie, 1985; Dunwiddie and Masino, 2001). In addition, adenosine has an essential role in a wide range of behavioral disorders including some psychiatric and sleep disorders (Asatryan et al., 2011; Cunha et al., 2008; Ruby et al., 2010). Unlike classical neurotransmitters, which are synthesized, stored, and released into the synapse in response to electrochemical stimulation, adenosine concentrations are regulated to a much greater extent by production and transport (Burnstock, 1972; Burnstock, 2006; Burnstock, 2008; Parkinson et al., 2005). This pattern of control allows adenosine levels to change rapidly, which is essential for the fine-tuning of the activity of neighboring neurons. As shown in Fig. 1, adenosine is synthesized extracellularly from ATP that is released by neurons or astrocytes. It is also directly released and taken up by equilibrative nucleoside transporters, which are expressed in both astrocytes and neurons (Asatryan et al., 2011; Haydon et al., 2009; Parkinson et al., 2006).

Adenosine controls neurotransmitter release, modulates neuronal excitability and regulates ion channel function through four subtypes of G protein coupled receptors (GPCRs), A₁, A_{2A}, A_{2B}, and A₃, which all have a distinct affinity for adenosine (Fredholm, 2010; Fredholm et al., 1999; Fredholm et al., 2005a; Fredholm et al., 2005b). A₁ and A_{2A}R have 10-100 nM binding affinities, whereas A_{2B} and A₃R have 1-5 μ M binding affinities. Since normal CNS adenosine levels are 25-250 nM, A₁ and A_{2A}R are the main subtypes thought to be involved in the regulation of anxiety and other psychiatric disorders. Adenosine A₁R are G_i coupled, expressed ubiquitously in the CNS, and mediate the tonic inhibition of neuronal activity. On the other hand, adenosine A_{2A}R are G_s coupled, activate adenylate cyclase, increase levels of cAMP, exert excitatory influences on neurons and are primarily expressed in striatum. A_{2A}R is also known to associate physically with other neurotransmitter receptors, including the adenosine A₁, dopamine D2 and glutamate mGluR5 receptors (Ciruela et al., 2006; Ferre et al., 2010).

Altered adenosine signaling in the brain is implicated in numerous pathophysiologies including alcoholism and sleep disorders. The correlation between alcohol dependency and sleep disorders has been extensively documented in the clinical literature (Colrain et al., 2009). Chronic ethanol use is often associated with sleep disruptions that are reportedly sustained for up to several months after withdrawal. In fact, these sleep impairments are so severe that they are a primary indicator of relapse (Brower and Perron, 2010a). Moreover, the correlation appears to be bidirectional. Recently, a prospective study designed to investigate the link between pre-existing sleep disorders and alcohol dependency reported increased tiredness in childhood as a significant predictor of alcohol misuse in adolescence (Wong et al., 2010).

Furthermore, recent studies demonstrate that deletion of type 1 equilibrative nucleoside transporter (ENT1) causes an alteration in extracellular adenosine and downregulates one of the main astrocytic glutamate transporters (GLT1; termed also excitatory amino acid 2, EAAT2), consequently increasing synaptic glutamate levels (Holmes, 2011; Nam et al., 2011; Wu et al., 2011a). Interestingly, upregulation of GLT1 expression or activity is known to normalize glutamate levels and reduce ethanol self-administration (Sari et al., 2011). Despite these known roles of adenosine and glutamate in mediating ethanol's effects, the cellular sources and subsequent targets of these signaling pathways are not well understood. The role of astrocytes as mediators of synaptic activity is becoming increasingly recognized as a novel contributor to both normal and pathological behavior (Allaman et al., 2011; Araque et al., 1999; Halassa and Haydon, 2010). Recent studies, described below, are providing insight into how identifying these astrocytic signaling pathways may lead to new therapeutic opportunities for treatment of alcohol-related disorders.

Adenosine and Glutamate Signaling Through Neuron-Glial Interaction Regulate Ethanol Drinking in Mice Lacking ENT1

Acute ethanol treatment increases extracellular adenosine in cultured cells by selectively inhibiting ENT1, while chronic ethanol exposure no longer increases extracellular adenosine levels owing to the downregulation of ENT1 gene expression (Nagy et al., 1990). Interestingly, mice lacking ENT1 exhibit reduced ataxic and hypnotic effects of acute ethanol exposure and consume more ethanol compared to wild-type littermates (Choi et al., 2004). Conversely, ENT1 overexpression in neurons increases ethanol intoxication in mice (Parkinson et al., 2009). Additionally, several recent animal studies further illustrate that ENT1 gene expression is inversely correlated with ethanol drinking (Bell et al., 2009; Sharma et al., 2010a; Short et al., 2006). Several genetic variants of ENT1 (*SLC29A1*) are included among a 130 candidate gene-based array for clinical genomic studies of addiction (Hodgkinson et al., 2008) while recent human genetic association studies demonstrate that variants of ENT1 are associated with an ethanol abuse phenotype in women (Gass et al., 2010) and alcohol-dependents with a history of withdrawal seizures (Kim et al., 2011). Therefore, the mechanistic understanding of how ENT1 contributes to alcoholism is important for the development of novel therapeutics.

One of the key neural mechanisms underlying increased ethanol drinking behaviors in ENT1 null mice is attributed to increased glutamate neurotransmission in the NAc (Choi et al., 2004), an essential component of addictive behaviors (Kalivas, 2009). Interestingly, deletion of ENT1 is causally associated with reduced astrocytic GLT1 expression or function (Wu et al., 2010; Wu et al., 2011a), which subsequently causes increased extracellular glutamate levels (Choi et al., 2004; Nam et al., 2011). Choi and colleagues reported that increased resistance to acute ethanol intoxication is related to increased glutamate signaling in ENT1 null mice (Chen et al., 2010; Choi et al., 2004). Interestingly, daily treatment of CGP37849, an NMDA glutamate receptor antagonist, for four days reduced ethanol consumption and preference in ENT1 null mice (Nam et al., 2011). Consistent with this finding, CGP37849 (2-8 mg/kg, *i.p.*) reduced ethanol intake after ethanol deprivation in rats (Vengeliene et al., 2005). Similarly, acamprosate, a clinically used anti-glutamatergic medication (Tempesta et al., 2000), reduced ethanol consumption in ENT1 null mice to a level similar to that of wild-type mice when mice were given a 200 mg/kg dose (*i.p.*) twice a day, for four days in a two-bottle choice drinking experiment (Lee et al., 2011). These results indicate that increased basal glutamate signaling is associated with increased ethanol consumption in ENT1 null mice. Thus, ENT1 null mice are useful to investigate adenosine-mediated glutamate signaling in ethanol use disorders.

Because numerous signaling molecules could mediate increased glutamate signaling through interacting with receptors in the striatum (Lonze and Ginty, 2002), a proteomic technique called iTRAQ was utilized to identify proteome changes in the NAc of ENT1 null mice. With this method, 533 accumbal proteins were detected and quantified using the iTRAQ reporter ions as described (Bantscheff et al., 2008; Han et al., 2008). Among these proteins, five signaling proteins, which were significantly changed in the NAc of ENT1 null mice compared to wild-type mice, were selected. Specifically, neurogranin (Ng) and calmodulin (CaM) were significantly increased in the NAc of ENT1 null mice. Also, GLT1 expression, but not glutamate-aspartate transporter (GLAST, also known as EAAT1), was decreased in the NAc of ENT1 null mice.

Using Western blot analysis, it was shown that Ng protein levels were increased in the NAc of ENT1 null mice compared to wild-type mice. However, phosphorylated Ng (Ser36), an active form of Ng, was significantly decreased in ENT1 null mice compared to wild-type mice. Then pPKC γ (Thr514), an active form of PKC γ that phosphorylates Ser36 of Ng was

examined. As expected, pPKC γ (Thr514) levels were significantly reduced in ENT1 null mice compared to wild-type mice. Interestingly, decreased pNg (Ser36) and pPKC γ (Thr514) or increased total Ng, are known to reduce Ca²⁺-CaM formation due to increased CaM-Ng binding. Thereby, this signaling will lead to decreased CaMK activity including that of CaMK type II (CaMKII), which is highly expressed in dendritic spines (Kennedy, 1998). Finally, pCaMKII (Thr286), an active form of CaMKII, was also found to be reduced.

This decreased PKC γ driven reduction of pNg-pCaMKII was correlated with decreased pCREB (Ser133) levels and activity. To test whether CREB activity is reduced in ENT1 null mice, mice expressing β -galactosidase (lacZ) under the control of seven-repeated CRE sites in an ENT1 null and wild-type background were generated. In accordance, Western blot analysis showed decreased CREB activity in the NAc core region of ENT1 null mice. Interestingly, CREB activity in the NAc shell region was similar between genotypes (Nam et al., 2011). The NAc core region primarily regulates the motivational effects of conditioned stimuli (Everitt and Robbins, 2005), suggesting that reduced CREB activity in the NAc core of ENT1 null mice might contribute to the deficiency of ethanol place aversion and the increase in ethanol drinking (Chen et al., 2010; Choi et al., 2004). These findings indicate that ENT1 contributes to the regulation of glutamate-mediated signaling and CREB activity in the NAc, which are associated with alcohol use disorders (McPherson and Lawrence, 2007; Pandey, 2003).

Chronic Ethanol-Induced Downregulation of Adenosinergic Tone Contributes to Insomnia Observed During Alcohol Withdrawal

Adenosine levels and activation of A₁ receptors (A₁R) are known to inhibit the wake-promoting neurons of the basal forebrain (BF) to promote sleep (Basheer et al., 2004; Porkka-Heiskanen et al., 1997; Radulovacki et al., 1984; Thakkar et al., 2003). Alcohol has a profound impact on sleep (Roehrs and Roth, 2001) and consequently, alcohol-related sleep disorders have a large socio-economic cost. Within the USA, it is estimated that the cost of alcohol-related problems exceeds \$180 billion, out of which more than \$18 billion is associated with alcohol related sleep disorders (Brower, 2001). Acute ethanol intake in non-alcoholics decreases sleep latency (the amount of time to fall asleep) and increases non-rapid eye movement (NREM) sleep quantity as well as quality. However, rapid eye movement (REM) sleep is suppressed during the first half of nocturnal sleep time and is followed by a "REM rebound" (increased REM sleep) during the second half. Alcohol dependent subjects, both during a drinking period and during abstinence, suffer from a multitude of sleep disruptions (Brower et al., 2001; Roehrs and Roth, 2001). During alcohol withdrawal, recovering alcohol-dependent patients commonly experience severe and protracted sleep disruptions manifested by profound insomnia and increased REM sleep along with excessive daytime sleepiness (Allen et al., 1971; Brower et al., 1998; Colrain et al., 2009). Furthermore, subjective and objective indicators of sleep disturbances are predictors of relapse (Brower and Perron, 2010b).

Consistent with clinical studies, animal studies also suggest that ethanol withdrawal is accompanied by insomnia-like symptoms, including increased wakefulness, reduction in total sleep time and reduction in delta activity (Ehlers and Slawecki, 2000; Kubota et al., 2002; Mendelson et al., 1978; Mukherjee et al., 2008; Mukherjee and Simasko, 2009; Veatch, 2006). However, despite strong clinical and preclinical evidence demonstrating insomnia during ethanol withdrawal, very little is known regarding the neural mechanism underlying insomnia during ethanol withdrawal.

Recent studies indicate that the sleep-inducing effects of acute ethanol exposure may be mediated by adenosinergic mechanisms in the wake-promoting BF (Thakkar et al., 2010). Does chronic ethanol exposure impair adenosinergic mechanisms in the BF leading to abnormal sleep? To address this question, Thakkar and colleagues performed a series of experiments to investigate the role of adenosine and the wake-promoting BF in sleep patterns during ethanol withdrawal (Sharma et al., 2010a). To induce ethanol dependency, a chronic binge ethanol protocol was employed (Faingold, 2008; Majchrowicz, 1975). First, the effect of chronic ethanol exposure on sleep-wakefulness was examined. During withdrawal, the ethanol-dependent rats displayed profound insomnia-like symptoms, manifested by significant increases in wakefulness coupled with significant reductions in both NREM and REM phases of sleep. Second, the activation of BF wake-promoting neurons in alcohol dependent rats was examined using c-Fos (a marker of neuronal activation) immunohistochemistry. Alcohol dependency produced a significant increase in the activation of wake-promoting neurons as indicated by a significant increase in the number of cholinergic neurons with c-Fos immunoreactivity (Thakkar et al., 2003). Third, the effects of chronic ethanol exposure on sleep deprivation-induced increases in BF adenosine levels were tested. While control animals displayed significant increases in BF extracellular adenosine during sleep deprivation, the ethanol-dependent rats did not (Sharma et al., 2010b). Finally, the effects of ethanol withdrawal on ENT1 and A₁R expression in the BF were investigated. Since the BF does not express A_{2A}R, only A₁R was examined (Basheer et al., 2004; Porkka-Heiskanen et al., 1997; Radulovacki et al., 1984; Thakkar et al., 2003). It was found that there was a significant reduction in the expression of A₁R and ENT1 in the BF of ethanol dependent rats (Sharma et al., 2010a).

These findings suggest that chronic ethanol induced downregulation of ENT1 and A₁R expression in the BF contributes to reduced adenosinergic inhibition in the BF. Consequently, reduced adenosinergic tone results in increased activation of wake-promoting neurons in the BF, which might contribute to insomnia-like symptoms observed during ethanol withdrawal.

A Role of Astrocytic Modulation of Synaptic Transmission in Sleep Homeostasis and Alcoholism

As mentioned above, alterations in adenosine signaling contribute to sleep disruptions early on during withdrawal (Sharma et al., 2010a; Sharma et al., 2010b). However, neither the cellular source of altered adenosine nor the time course or duration of these changes is known. Whether the pre-existing disruptions in sleep that are known to contribute to alcohol behaviors involve disruptions in astrocytic signaling also remains an open question. Given that gliotransmission provides the source of adenosine signaling that regulates sleep homeostasis, it is possible that it is a key contributor to this phenomenon.

As part of the tripartite synapse, astrocytes are ideally situated to monitor and regulate synaptic transmission via the release of chemical transmitters, including glutamate, D-serine and ATP, which is rapidly hydrolyzed in the extracellular space into adenosine. This process, termed gliotransmission, is becoming increasingly appreciated as a significant contributor to brain function at the cellular, network and behavioral levels. Much of our current understanding of gliotransmission can be credited to the generation of an inducible transgenic mouse line in which SNARE-mediated transmitter release is attenuated specifically in astrocytes (Pascual et al., 2005). For example, recent studies of dnSNARE mice have revealed an essential role of astrocytes in regulating neuronal A₁R activity. Specifically, astrocytes provide a tonic source of adenosine that acts on perisynaptic A₁Rs to modulate both basal and plastic glutamatergic synaptic activity (Pascual et al., 2005). Indeed, in hippocampal slices isolated from dnSNARE mice, there is increased response of

evoked field excitatory postsynaptic potentials (EPSPs), while theta burst stimulation yields long-term potentiation (LTP) of much smaller magnitude than that of littermate controls. These phenotypes can be mimicked by administration of the A₁R antagonist, DPCPX, and rescued by the A₁R agonist, CCPA (Pascual et al., 2005), suggesting that gliotransmission modulates synaptic transmission and plasticity *via* tonic inhibition of A₁R-dependent presynaptic glutamate release.

More recent evidence points to a role for gliotransmission in post-synaptic A₁R-dependent regulation of NMDAR subunit surface expression. In mEPSCs measured from the somatosensory cortex of dnSNARE mice, there is an increased AMPA/NMDA receptor ratio associated with decreased surface expression of the NR2A and NR2B subunits of the NMDA receptor (Deng et al., 2011; Fellin et al., 2009). Furthermore, prolonged incubation of the slice with an A₁R agonist (CCPA) restores surface expression in dnSNARE mice, while the A₁R antagonist (CPT) reduces surface expression in wild-type mice (Deng et al., 2011). In line with these results, decreased phosphorylation of Src kinase and one of its substrates, NR2B, was detected in the cortex of dnSNARE mice. Incubation with the CCPA restored pSrc and pNR2B levels in dnSNARE mice (Deng et al., 2011).

Collectively, these results provide evidence that SNARE-dependent purinergic signaling from astrocytes impacts synaptic transmission *via* activation of A₁R and subsequent regulation of NMDA receptor surface expression. These newly identified roles of astrocytes have significant implications for a relatively unexplored arena of ethanol research. Does gliotransmission contribute to the behavioral response to ethanol and the subsequent maladaptive plasticity that underlies addiction? There are several lines of evidence that suggest this could be the case. First, as described above, ethanol regulates adenosine levels *via* direct blockade of ENT1 (Choi et al., 2004; Nagy et al., 1990). However, whether ENT1 inhibition by ethanol causes a subsequent alteration in astrocytic SNARE-mediated purinergic signaling is not known. Since ENT1 is expressed on astrocytes, there is likely a feedback mechanism involved in adenosine uptake and release pathways. In this way, it is likely that gliotransmitter release of ATP is an additional target of ethanol exposure. The second line of evidence is ethanol's effect on NMDA receptor function and subunit expression. Ethanol blocks NMDARs, causing a decrease in excitatory synaptic transmission. In some brain regions, there is a subsequent compensatory increase in phosphorylation of Fyn, a Src family kinase (SFK) member, and its substrate, the NR2B subunit of NMDA receptors (Miyakawa et al., 1997; Ron, 2004; Yaka et al., 2003). The consequent increase in surface expression of the NR2B subunit causes "rebound potentiation" in excitatory synaptic transmission upon ethanol washout, which is thought to underlie recovery from the intoxicating effects of ethanol. Indeed, mice null for Fyn expression and mice treated with the NR2B antagonist ifenprodil (Miyakawa et al., 1997; Yaka et al., 2003) show increased sensitivity to ethanol. Since gliotransmission regulates synaptic activity *via* tonic regulation of presynaptic A₁Rs and dynamically through post-synaptic regulation of SFK and surface expression of NR2B subunits, it is possible that it contributes to the acute behavioral responses to ethanol outlined above.

Adenosine has been identified as the sleep factor that accumulates with wakefulness and drives the pressure to sleep (Porkka-Heiskanen et al., 1997). Recent studies showed that an astrocytic SNARE-dependent source of adenosine regulates sleep pressure (Halassa et al., 2009). Despite exhibiting a weak sleep phenotype under basal conditions, dnSNARE mice exhibit an attenuated response to sleep deprivation. Specifically, the compensatory "rebound" sleep that normally occurs after sleep deprivation is significantly blunted in dnSNARE mice. In line with this finding, the increase in the power of low frequency slow wave sleep (0.5-1.5Hz), a well established measurement of sleep pressure that is directly related to increased adenosine tone and normally occurs in response to acute sleep

deprivation, is also significantly attenuated in dnSNARE mice. This effect is mimicked in wild-type mice with the administration of the A₁R antagonist CPT (*i.p.*) (Halassa et al., 2009) suggesting that purinergic gliotransmission regulates sleep homeostasis. Given these findings, it is of great interest to determine whether attenuated gliotransmission (via dnSNARE expression) impacts vulnerability to alcohol-induced sleep disruptions. A further understanding of how gliotransmission contributes to alcohol misuse and related sleep disruptions has the potential to guide therapeutic developments selectively targeted to these astrocytic signaling pathways.

Glutamate Transporter 1: A Potential Target for the Treatment of Alcohol Dependence

Given that adenosine powerfully modulates glutamatergic synaptic transmission it is intriguing to ask whether the regulation of the levels of this major excitatory transmitter is altered in alcohol exposure and drug addiction. Indeed there is mounting evidence suggesting that many aspects of neuroplasticity associated with alcohol/drug addiction involve changes in glutamatergic neurotransmission. For example, studies demonstrated that repeated ethanol exposure for seven days (1 g/kg *i.p.* daily) induces a decrease in glutamate uptake in the rat NAc (Melendez et al., 2005), which leads to an increase in extracellular glutamate. In studies investigating the effects of chronic ethanol (10% v/v) exposure for 20 months in ethanol-preferring rats (Schreiber and Freund, 2000), a down-regulation of glutamate transport in the cerebral cortex, relative to naïve rats, was observed. It is noteworthy that extracellular glutamate levels are increased in brain reward regions during ethanol intake (Dahchour et al., 2000; Kapasova and Szumlinski, 2008; Melendez et al., 2005; Moghaddam and Bolinao, 1994; Quertemont et al., 1998; Roberto et al., 2004; Selim and Bradberry, 1996; Szumlinski et al., 2007) and ethanol exposure has been found to alter glutamate transport (Othman et al., 2002; Smith and Weiss, 1999; Smith, 1997). Together, these studies provide evidence that dysfunctional glutamate neurotransmission contributes to the regulation of ethanol intake.

Extracellular glutamate is regulated by a number of glutamate transporters in neurons and glia (Anderson and Swanson, 2000; Gegelashvili and Schousboe, 1997; Seal and Amara, 1999). Of these glutamate transporters, GLT1, a sodium-dependent transporter expressed on astrocytes (Anderson and Swanson, 2000; Rothstein et al., 1994), is responsible for the removal of most of the extracellular glutamate (Danbolt, 2001; Ginsberg et al., 1995; Mitani and Tanaka, 2003; Rothstein, 1995; Rothstein et al., 1995). Thus, targeting the activation of GLT1 might be a key player in regulating glutamate transmission. Interestingly, ceftriaxone, a β -lactam antibiotic known to cross the brain blood barrier (Chandrasekar et al., 1984; Lutsar and Friedland, 2000; Nau et al., 1993; Spector, 1987), has been identified to elevate GLT1 expression (Miller et al., 2008; Rothstein et al., 2005; Sari et al., 2010; Sari et al., 2011; Sari et al., 2009). Therefore, if an increase in glutamate transmission plays a critical role in ethanol consumption, then up-regulation or activation of GLT1 should attenuate this behavior.

Accordingly, a role of GLT1 in ethanol consumption has been tested using male ethanol preferring (P) rats. P rats were selectively bred to investigate the neurobiology of chronic ethanol-drinking behavior and the consequences of excessive ethanol consumption behaviorally, neurochemically and physiologically (Bell et al., 2006; Bell et al., 2005). Treatment with ceftriaxone reduced ethanol intake and the long-lasting effects of ceftriaxone on ethanol intake were correlated with the up-regulation of GLT1 expression in prefrontal cortex and NAc brain regions (Sari et al., 2011). These recent results implicate GLT1 as a potential therapeutic target for the treatment of alcohol dependence.

Conclusions

It is clear that interactions between adenosine and glutamate signaling pathways are involved in the acute and chronic effects of ethanol. While the effects of adenosine and glutamate are largely on neurons, astrocytes also play a fundamental role through the release of the gliotransmitter ATP and its byproduct, adenosine, and via uptake of glutamate.

Adenosine is an endogenous sleep-promoting agent. It has been clearly demonstrated that acute treatments with ethanol promote sleep by inhibiting wake-promoting neurons in basal forebrain. These hypnotic effects are mimicked or inhibited by adenosine A₁ receptor agonists or antagonists, respectively. In contrast to the acute effects, however, chronic ethanol consumption is associated with decreased sleep and insomnia in human alcohol dependent patients. It has now been shown that ethanol withdrawal activates the wake promoting neurons that are inhibited by acute ethanol (Sharma et al., 2010a). These changes are paralleled by decreased ENT1 and A₁R expression. In brain regions rich in dopamine signaling and therefore relevant to reward and addiction, such as the NAc, ENT1 null mice show decreased adenosine tone. This is associated with an increase in extracellular glutamate and a decrease in GLT1 expression. These changes were associated with decreases in activated neurogranin, PKC γ , calcium-calmodulin kinase II, and CREB (Nam et al., 2011). Normalizing glutamate signaling with an NMDA receptor antagonist reduced ethanol consumption. Furthermore, increasing expression of GLT1 with the antibiotic ceftriaxone was also able to decrease ethanol consumption in ethanol preferring rats with long-term heavy consumption of ethanol (Sari et al., 2011).

Future Directions

Adenosine and glutamate signaling are interrelated, with adenosine decreasing glutamate neurotransmission but glutamate, and receptor agonists, increasing cellular release of adenosine. From the above studies, it is clear that this interrelationship is involved in the actions of acute and chronic ethanol as well as ethanol withdrawal. Interestingly, acute ethanol has been found to directly inhibit both ENT1 (Allen-Gipson et al., 2009; Nagy et al., 1990) and NMDA receptors (Wu et al., 2011b), whereas acute tolerance to ethanol and chronic ethanol exposure have more complex effects on these proteins, including a lack of inhibition, activation and altered expression (Coe et al., 1996; Wu et al., 2011b). In regards to ENT1, a detailed analysis of its inhibition by ethanol has not been performed. This inhibition has been clearly documented in only a few cell types and it has not been followed up with chimeric proteins or site directed mutagenesis or similar methods to ascertain the precise site and mechanism by which ethanol inhibits ENT1. Considering that many diverse molecules have been reported to inhibit ENT1, better characterization of the interaction between ethanol and ENT1 is warranted.

As an inhibitor of ENT1, acute exposure to ethanol has been found to increase adenosine levels and promote adenosine receptor signaling. Other ENT1 inhibitors, such as nitrobenzylthioinosine and dipyridamole, similarly increase adenosine receptor signaling and mice over-expressing ENT1 in neurons show reduced adenosine tone relative to wild-type mice (Zhang et al., 2011). However, ENT1 null mice also show decreased adenosine levels in the striatum and reduced adenosine signaling (Kim et al., 2011; Nam et al., 2011). Therefore, it is important to characterize further the regulation of adenosine levels by ENT1 across brain regions and the dynamic regulation, both direct and indirect, by neuronal activity and signal transduction pathways.

From the above discussion of the role of adenosine in sleep, one might suppose there is good rationale for development of adenosine A₁R agonists as sleep promoting agents for the insomnia associated with alcoholism and recovery from alcoholism. While these compounds

are sleep-promoting, their expected effects to decrease cardiac contractility have diminished enthusiasm for their drug development. Other strategies could include adenosine kinase inhibitors, which were in clinical development as anticonvulsants; however, toxicity concerns would need to be overcome. The data with ceftriaxone are encouraging since the expression of GLT1 was restored to baseline levels in ethanol consuming rats; thus, the risk of excessive glutamate uptake may be low.

Overall, despite the mounting evidence that astrocytes modulate synaptic activity, little is known regarding how astrocyte function directly impacts behavior, especially in the context of alcoholism. A more clear understanding of how astrocyte-dependent purinergic signaling and GLT1-regulated glutamate levels contributes to ethanol behaviors may provide a unique potential to impact the development of therapies for treatment of ethanol abuse and dependence.

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Figure 1.

Schematic drawing of adenosine-regulated glutamate signaling in neuron-glia interactions. 1. The SNARE protein in astrocytes promotes ATP release into the extracellular region, which can then be converted to adenosine through extracellular nucleotidase activity. 2. Adenosine levels are also regulated by ethanol-sensitive ENT1, which is a bidirectional nucleoside transporter. 3. In presynaptic neurons, adenosine is known to inhibit glutamate release, especially through adenosine A₁R activity. 4. In postsynaptic neurons, adenosine A₁R activity increases GIRK channel activity, which decreases NMDA glutamate receptor activity. 5. Astrocytic GLT1 uptakes synaptic glutamate, which is then converted to Gln. 6. Activation of NMDA receptors increases neuronal activity through calcium (Ca²⁺) influx. 7. Repeated NMDA receptor activation alters gene expression and leads to neuroadaptation. SNARE, SNAP [Soluble NSF (N-ethylmaleimide factor) Attachment Protein] receptor; Ado, adenosine; ENT1, type 1 equilibrative nucleoside transporter; A₁R, adenosine receptor; Glu, glutamate; Gln, glutamine; GIRK, G-protein coupled inward rectifying K⁺ channel; NMDA, N-methyl-D-aspartate; GLT1, glutamate transporter 1.

A Acute EtOH

B Chronic EtOH

Figure 2.

Simplified illustration showing the effect of ethanol on adenosine signaling and its implication in alcoholism and sleep disorders. **(A)** Acute ethanol exposure increases extracellular adenosine by inhibiting uptake activity of ENT1. Increased adenosine activates A₁R, which promotes sleep in the BF. Activation of presynaptic A₁R in the NAc inhibits glutamate release and decreases NMDA receptor function contributing to ethanol intoxication, which includes ataxia and altered locomotion. **(B)** On the other hand, chronic ethanol exposure reduces ENT1 expression and bidirectional adenosine transporting activity, which leads to reduced extracellular adenosine levels. Decreased presynaptic A₁R activation increases glutamate release and activation of NMDA receptors in the NAc. Also, decreased GLT1 expression and function contribute to increased glutamate levels and increased NMDA receptor activity in the NAc. Together, increased NMDA receptor activity could lead to increased ethanol drinking. In the BF, ethanol withdrawal after chronic ethanol exposure decreases ENT1 expression and transport activity similar to chronic exposure alone. Consequently, reduced A₁R activation appears to be causally related to ethanol withdrawal-induced insomnia. EtOH, ethanol; ENT1, type 1 equilibrative nucleoside transporter; Ado, adenosine; A₁R, adenosine receptor; Glu, glutamate; NMDA, N-methyl-D-aspartate; GLT1, glutamate transporter 1; NAc, nucleus accumbens; BF, basal forebrain.