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Neuroadaptations in Adenosine Receptor Signaling Following Long-Term Ethanol Exposure and Withdrawal

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Abstract

Ethanol affects the function of neurotransmitter systems, resulting in neuroadaptations that alter neural excitability. Adenosine is one such receptor system that is changed by ethanol exposure. The current review is focused on the A_1 and the A_{2A} receptor subtypes in the context of ethanol-related neuroadaptations and ethanol withdrawal because these subtypes (i) are activated by basal levels of adenosine, (ii) have been most well-studied for their role in neuroprotection and ethanol-related phenomena, and (iii) are the primary site of action for caffeine in the brain, a substance commonly ingested with ethanol. It is clear that alterations in adenosinergic signaling mediate many of the effects of acute ethanol administration, particularly with regard to motor function and sedation. Further, prolonged ethanol exposure has been shown to produce adaptations in the cell surface expression or function of both A_1 and the A_{2A} receptor subtypes, effects that likely promote neuronal excitability during ethanol withdrawal. As a whole, these findings demonstrate a significant role for ethanol-induced adaptations in adenosine receptor signaling that likely influence neuronal function, viability, and relapse to ethanol intake following abstinence.

Keywords

Ethanol Withdrawal; A_1 Receptor; A_{2A} Receptor; Caffeine

Endogenous adenosine is found in all mammalian cells, and it contributes to homeostatic maintenance of cellular metabolism and inhibitory tone in the central nervous system (CNS) (Cunha, 2005; Stiles, 1992; Wardas, 2002). Newby (1984) coined the term “retaliatory metabolite” in reference to the dramatic increase in the extracellular concentration of adenosine observed under conditions of cellular stress, which promotes restoration of basal cellular metabolism. Because of these properties and the ubiquitous nature of adenosine, the adenosine receptor system has been investigated in a variety of disease states. This review discusses ethanol and ethanol withdrawal-related adaptations in adenosinergic signaling and how pharmacological manipulation of adenosine receptors can influence ethanol intoxication behaviors, withdrawal-related behaviors, and neuronal integrity. Beyond preclinical research investigating CNS-related alterations, relevance to human functioning will also be discussed in the context of ethanol and caffeine co-exposure.

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FORMATION AND METABOLISM OF ADENOSINE

Adenosine is continuously formed both intra- and extracellularly under stable cellular conditions (Fredholm et al., 2001). Intracellular adenosine is produced by dephosphorylation of adenosine monophosphate (AMP) by the 5′-nucleotidase cN-I (Borowiec et al., 2006; Schubert et al., 1979; Zimmermann et al., 1998) or by hydrolysis of S-adenosyl-homocysteine (Broch and Ueland, 1980; Turner et al., 2000). Adenosine is synthesized in the extracellular space by metabolism of adenine nucleotides (cAMP, AMP, ATP, ADP) by ectonucleotidases (Dunwiddie et al., 1997). Adenosine triphosphate (ATP) is broken down via hydrolysis, whereas cyclic AMP (cAMP) released from neurons and / or intracellular stores is converted into AMP by extracellular phosphodiesterases, after which AMP can be dephosphorylated into adenosine by ecto-5′-nucleotidase (Brundege et al., 1997). To keep intra- and extracellular levels of adenosine in equilibrium, adenosine that is formed intracellularly may diffuse across the cell membrane via the bidirectional equilibrative nucleoside transporters (ENT1 and ENT2) into the extracellular space (Nagai et al., 2005; Ward et al., 2000). Concentrative nucleoside transporters (CNTs) are also found in the CNS, which equilibrate adenosine levels in relation to the Na^+ gradient (Guillén-Gómez et al., 2004; reviewed in King et al., 2006). Adenosine is metabolized by uptake into the cell from the extracellular space, where it is phosphorylated by adenosine kinase into 5′-AMP, or degraded by adenosine deaminase into adenosine's primary metabolite, inosine (Lloyd and Fredholm, 1995). Additional adenosine metabolites include hypoxanthine, xanthine, and uric acid (for review, see Borowiec et al., 2006).

NEUROPROTECTION BY ENDOGENOUS ADENOSINE

Under basal conditions, adenosine maintains inhibitory tone in the CNS via inhibition of calcium influx (Scholz and Miller, 1991) and also by inhibition of evoked glutamate release by presynaptic reduction in the frequency of miniature excitatory synaptic currents (Corradetti et al., 1984; Scanziani et al., 1992). Newby's (1984) term "retaliatory metabolite" is reflected by several-fold increases in adenosine concentrations or up to approximately 5 to 10 μM in the brain during times of cellular distress (Dux et al., 1990; Latini et al., 1999). Adenosine has been studied for its role as a neuromodulator during times of cellular stress characterized by excitotoxic damage, including ethanol withdrawal, as it is associated with increased extracellular glutamate and adenosine, among other excitatory amino acids and purines (reviewed by Cunha, 2001, 2005; de Mendonca et al., 2000; Hillered et al., 1989; Matsumoto et al., 1992; Wardas, 2002). Administration of specific agonists and antagonists for the adenosine receptor subtypes has demonstrated neuroprotection both in vitro and in vivo (reviewed in de Mendonca et al., 2000; Frenguelli et al., 2003). Further, experimental administration of adenosine reduces ischemic damage, whereas administration of xanthines (adenosine receptor blockers, e.g., caffeine) enhances ischemic damage (reviewed in Rudolphi et al., 1992; Schubert and Kreutzberg, 1993). In in vivo models of excito-toxicity, A_1 agonism and A_{2A} antagonism exhibit similar neuroprotective effects; though, A_1 receptor activation is generally thought to be primarily responsible for adenosine's neuroprotective influence (Ongini and Schubert, 1998).

ADENOSINE A_1 RECEPTOR SIGNALING AND LOCALIZATION

Four adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) have been characterized in mammalian tissue (Fredholm et al., 1994, 2001). The extracellular basal concentration of adenosine ranges from 40 to 460 nM in the brain (Ballarín et al., 1991), resulting in activation of A_1 and A_{2A} receptors (Fredholm et al., 1999). Adenosine has significantly lower affinity for the A_{2B} and A_3 receptor subtypes, requiring markedly elevated adenosine levels for activation (reviewed in Dunwiddie and Masino, 2001). To date, studies

investigating adenosine-related effects and ethanol-related neuropharmacology and behavior have focused on the A_1 and the A_{2A} receptor subtypes, as activation of these receptor subtypes is responsible for maintenance of "basal purinergic tone" (Dunwiddie and Masino, 2001).

Adenosine A_1 receptors are coupled with pertussis toxin-sensitive G-proteins, including G_{i-1}, G_{i-2}, G_{i-3}, G_{o-1}, and G_{o-2} (Fredholm et al., 1994, 1999). Activation of A_1 receptors results in the inhibition of adenylyl cyclase, thereby decreasing the accumulation of cAMP (Akbar et al., 1994; Freund et al., 1994; Peakman and Hill, 1996; Van Calker et al., 1978), increased potassium conductance (Li and Henry, 1992; Segal, 1982), inhibition of P/Q- and N-type calcium channels (Ambrosio et al., 1997; Gundlfinger et al., 2007), and enhancement of phospholipase C, phospholipase A_2, and phospholipase D activity (Akbar et al., 1994; Gerwins and Fredholm, 1995; Rogel et al., 2005, 2006). Consequently, A_1 receptor activation results in neuronal inhibition via postsynaptic hyperpolarization (Li and Henry, 1992; Segal, 1982) and presynaptic inhibition of neurotransmitter release, which occurs primarily at excitatory synapses (Dunwiddie and Haas, 1985; Prince and Stevens, 1992). A_1 receptor activation also affects neuronal excitability by mediating glutamatergic signaling via inhibition of *N*-methyl-D-aspartate (NMDA) receptor-mediated currents (de Mendonca and Ribeiro, 1993; de Mendonca et al., 1995) and inhibition of $mGlu_1$ receptor-mediated inward currents (Tabata et al., 2007).

In the rat CNS, cell surface A_1 receptors are the most abundant adenosine receptor subtype, exhibiting dense expression in the hippocampus, cortex, cerebellum, thalamus, brainstem, spinal cord, and basal ganglia (Reppert et al., 1991; Rivkees et al., 1995). Immunohistochemical and autoradiographic studies reveal dense A_1 receptor labeling on granule cell bodies of the dentate gyrus, dendrites, mossy fibers, pyramidal neurons (Rivkees et al., 1995), and most predominantly, axons (Swanson et al., 1995). In cerebral cortical areas, A_1 receptor expression varies among cell layers. Dense auto-radiographic labeling of the A_1 receptor agonist [^{3}H]N(6)-cyclohexyladenosine ([^{3}H]CHA) has been noted primarily on pyramidal cells in cortical layers I, IV, and VI; though, lower levels are detected in cortical layers II, III, and V (Rivkees et al., 1995). In the cerebellar cortex, the highest density of A_1 receptors is noted on basket cells in the molecular layer (Rivkees et al., 1995). Lower levels of A_1 receptor density, detected with [^{3}H]CHA binding and immunohistochemical localization, have been noted in granule cells in the granule cell layer, with low levels of binding also detected in cerebellar white matter (Fastbom et al., 1987; Goodman and Synder, 1982; Rivkees et al., 1995). Likewise, autoradiography studies with postmortem human brains have found the highest A_1 receptor density in the CA1 hippocampal region (stratum radiatum/pyramidale cell layer) (Fastbom et al., 1986, 1987; Svenningsson et al., 1997).

ADENOSINE A_{2A} RECEPTOR SIGNALING AND LOCALIZATION

In contrast to adenosine A_1 receptors, adenosine A_{2A} receptors are coupled to G_s proteins. Activation of A_{2A} receptors with specific agonists results in the activation of adenylate cyclase and increased concentration of cAMP (Cheng et al., 2002; Furlong et al., 1992; Lupica et al., 1990). Subsequent protein kinase A (PKA) activation by A_{2A} receptor activation initiates or inhibits a number of other signaling molecules (reviewed in Fredholm et al., 2007), including phosphorylation of cAMP response element-binding protein (CREB) in PC-12 cells (Cheng et al., 2002); phosphorylation of dopamine- and cAMP-regulated phosphoprotein (DARPP-32; Svenningsson et al., 1998); and stimulation of adenosine uptake in a protein kinase C (PKC)-dependent manner in hippocampal synaptosomes (Pinto-Duarte et al., 2005). Activation of PKA signaling via A_{2A} receptor activation has also been shown to activate "atypical PKC" in PC12 cells, which has been shown to protect against

apoptosis (Huang et al., 2001). Importantly for control of neural excitiability, A_{2A} receptor agonism has also been shown to decrease NMDA-mediated inward currents in rat neostriatal neurons (Norenberg et al., 1997). A_{2A} receptor interactions with dopamine D_2 receptors have also been well-studied, particularly within the striatum, where they are co-localized on GABAergic enkephalinergic neurons (Fink et al., 1992; Schiffmann et al., 1991). This interaction has been studied for its importance in mediating movement disorders, such as Parkinson's disease (Schwarzschild et al., 2006).

Also in contrast to the ubiquitous expression of A_1 receptors the CNS, A_{2A} receptor expression is predominantly localized in the striatum (Jarvis and Williams, 1989; Jarvis et al., 1989a; reviewed in Svenningsson et al., 1999; Wan et al., 1990). In particular, A_{2A} receptor expression is most dense outside of the active zone of striatal neurons; though, low-level receptor expression is also found in the postsynaptic density (Rebola et al., 2005a). Within striatal neurons, A_{2A} receptor mRNA is found exclusively in GABAergic medium-sized spiny projection neurons (Fink et al., 1992; reviewed in Fredholm et al., 2003; Schiffmann et al., 1991). As compared to the striatum, relatively low levels of A_{2A} receptor binding and A_{2A} receptor mRNA have been noted in the rat cortical tissue, the thalamus, and the hippocampus (Cunha et al., 1994; Jarvis et al., 1989a,b). Among hippocampal neurons, A_{2A} receptor expression is greatest in the presynaptic active zone of glutamatergic neurons (Rebola et al., 2005a,b; Tebano et al., 2005).

Functionally, A_1 and A_{2A} receptors interact and co-localize together and with other neurotransmitter receptors in the CNS. In situ hybridization studies have shown co-expression of A_1 and A_{2A} receptor mRNA in the pyramidal cell layers of the hippocampal CA1 and CA3 regions and the granule cell layer of the dentate gyrus (Cunha et al., 1994). Additionally, immunohistochemical analysis in isolated nerve terminals has shown that as much as 80% of nerve terminals showing reactivity for A_{2A} receptors also showed A_1 immunoreactivy, and approximately 31% of nerve terminals that show A_1 immunoreactivity also showed A_{2A} immunoreactivity. The authors suggest that the apparent imbalance is likely explained by the overall greater density of A_1 receptors in the hippocampus (Rebola et al., 2005b). Importantly, A_{2A} receptor activation attenuates A_1 receptor-mediated inhibition in a PKC-, but not PKA-dependent manner (Cunha et al., 1994; Lopes et al., 1999, 2002; O'Kane and Stone, 1998). Experimentally transfected cells have been used to study actions of A_1 to A_{2A} heteromers, which are believed to fine-tune glutamatergic signaling (Ciruela et al., 2006a,b). Radioligand studies have demonstrated in A_1 to A_{2A} co-transfected cells and rat striatum that activation of A_{2A} receptors decreases A_1 agonist binding affinity, but A_1 receptor activation has no effect on A_{2A} binding characteristics in striatal glutamatergic nerve terminals (Ciruela et al., 2006a,b). A_{2A} receptors also modulate function of dopamine D_2 receptors in the striatum, where they are most abundant. Intramembrane interaction between these 2 receptors occurs, such that activation of A_{2A} receptors inhibits the ability of dopamine or a D_2 receptor agonist to compete for binding with a competitive D_2 antagonist, thus functionally antagonizing the activation of D_2 receptors and resulting in increased extracellular levels of GABA (Ferre et al., 1993; reviewed by Ferré et al., 2008). A_1 and A_{2A} receptors also modulate activity of metabotropic glutamate receptors (mGluRs), modulating mGluR1α and mGluR5 activity, respectively (Ciruela et al., 2001; Nishi et al., 2003; Tebano et al., 2005). Functionally, A_1 receptor agonism significantly reduces $mGlu_1$-potentiated NMDA toxicity; though, pre-exposure to an $mGlu_1$ agonist reduces NMDA toxicity, which is further reduced by the addition of an A_1 receptor agonist (Ciruela et al., 2001).

ADENOSINE AND ETHANOL

In accordance with "retaliatory" actions of adenosine during stressful cellular events such as hypoxia and ischemia, acute ethanol exposure produces dose-dependent increases in extracellular adenosine in rat cerebellar synaptosomes (Clark and Dar, 1989a). In vivo, ethanol infusion directly into the basal forebrain of freely moving rats also results in increased extracellular adenosine (Sharma et al., 2010). Acute ethanol increases extracellular adenosine levels by dose-dependently increasing adenosine release and by reducing adenosine uptake via inhibition of the nitrobenzylthioinosine (NBTI)-sensitive ENT (Clark and Dar, 1989b; Krauss et al., 1993; Nagy et al., 1990). Inhibition of adenosine uptake tolerates with chronic ethanol exposure and results in extracellular adenosine concentrations equivalent to values reported under basal conditions (Nagy et al., 1990). Acute ethanol exposure also results in adenosine-dependent increases in intracellular cAMP levels that is mediated by A_{2A} receptor activation (Nagy et al., 1989); though, A_{2A} receptors become desensitized with prolonged ethanol exposure (Nagy et al., 1989).

ADENOSINE–ETHANOL INTERACTIONS AND ETHANOL DRINKING

In regard to the potential importance of adenosine-ethanol interactions, evidence exists for a role of adenosinergic signaling in behavioral regulation of ethanol intake. A_{2A} receptor antagonismhas been shown to decrease ethanol self-administration in alcohol-preferring rats (Adams et al., 2008) and Long-Evans rats, although this effect may be dependent on the dose of the A_{2A} receptor antagonist that is administered (Arolfo et al., 2004). However, Thorsell and colleagues (2007) showed that although A_{2A} receptor antagonism reduced ethanol self-administration, ethanol preference measured by conditioned place preference was not altered by A_{2A} receptor antagonism. The effect of A_{2A} receptor antagonism on reducing ethanol drinking was similar to the effect of D_2 receptor antagonism on self-administration of ethanol (Arolfo et al., 2004). A_{2A} receptors co-localize with D_2 receptors on striatal neurons and synergistically regulate ethanol-induced increases in PKA signaling to result in greater sensitivity to ethanol, thus providing a potential cellular mechanism to explain how blockade of adenosinergic signaling affects ethanol intake (reviewed by Maillard & Diamond, 2004).

Adenosine interactions with the glutamatergic system have also been implicated in mediating ethanol intake. Nam and colleagues (2011) showed that NMDA receptor-mediated signaling regulates ethanol intake in mice lacking the ENT1 gene ($ENT1^{-/-}$). $ENT1^{-/-}$ mice have greater preference for ethanol compared to wild-type littermates and decreased sensitivity to the acute impairing effects of ethanol (Choi et al., 2004). Although acute ethanol inhibits adenosine re-uptake via ENT1, prolonged ethanol exposure decreases the expression of ENT1 (Nagy et al., 1990), thus impairing presynaptic inhibition of glutamate release by adenosine. This suggests a critical role for adenosinergic signaling in the acute and long-term effects of ethanol, which are mediated, in part, by effects of adenosinergic signaling on glutamatergic tone. A recent review detailed the role of adenosine-glutamate signaling interactions in astrocytes as it relates to ethanol intake and preference (Ruby et al., 2010).

ACUTE ETHANOL ADMINISTRATION AND ADENOSINE RECEPTORS: BEHAVIORAL EFFECTS

Manipulation of adenosinergic signaling has long been observed to alter a variety of behaviors during acute ethanol intoxication and ethanol withdrawal-related behaviors. Mice genetically bred that are more sensitive to the sleep-promoting effects of ethanol (long-sleep mice) are also more sensitive to both the sedative and activating effects of A_1 receptor

agonists and antagonists, respectively, as compared to short-sleep mice (Proctor et al., 1985). This pharmacological interaction suggests a unique interaction between the adenosinergic system and ethanol. Further, acute ethanol promotes decreased wakefulness and increased non–rapid eye movement sleep in rats; though, this effect is reversed by the administration of the A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) into the basal forebrain (Thakkar et al., 2010). Blockade of adenosine re-uptake also prolongs sleep-time and motor incoordination in response to acute ethanol (Dar et al., 1983). Acute ethanol-induced motor incoordination and rapid tolerance to acute ethanol is also alleviated and accentuated by co-administration of an A_1 receptor antagonist and an A_1 receptor agonist, respectively, in male rodents (Batista et al., 2005; Connole et al., 2004; Dar, 1997a). In accord, Connole and colleagues (2004) reported that acute ethanol co-exposure with either caffeine or the A_1 receptor antagonist DPCPX attenuated motor deficits on a test of motor performance, whereas co-administration of ethanol and an A_{2A} receptor antagonist had no effect on motor performance. Importantly, accentuation of ethanol's acute motor impairing effect is mimicked by pertussis toxin and is reversed by intracerebellar co-exposure to a cAMP analog. This provides evidence that the signal transduction mechanisms initiated by A_1 and A_{2A} receptor activation have the expected effects on ethanol intoxication behaviors, and provide a cellular mechanism of ethanol effects on ataxia via adenosine receptors (Dar, 1997b). Anxiolytic responses to acute ethanol are also altered by adenosine receptor ligands, such that nonanxiogenic doses of both caffeine and an A_1 antagonist reverses the anxiolytic effect of acute ethanol, although A_{2A} antagonism does not alter behavior. Further, at doses of drug that do not affect behavior on their own, administration of an A_1 agonist in conjunction with ethanol administration produces an anxiolytic effect (Prediger et al., 2004).

ADENOSINE RECEPTOR SYSTEM NEUROADAPTATIONS FOLLOWING PROLONGED ETHANOL EXPOSURE AND WITHDRAWAL

Apart from the effects of acute ethanol on adenosine levels and behaviors mediated by adenosine receptors, chronic ethanol exposure and withdrawal affect A_1 receptor density and binding characteristics in rats and mice (Concas et al., 1996; Daly et al., 1994; Dar et al., 1983; Jarvis and Becker, 1998). Ethanol dependence induced by long-term intragastric intubation increases A_1 receptor density in rat cerebellar cortical membranes as measured by 2-Chloro-N_6-cyclopentyladenosine ([^{3}H]CCPA) binding at 3, 12, and 24 hours of withdrawal. This adaptation appears short-term, as it is no longer observed 3 to 6 days following the last ethanol administration; though, it is dependent on long-term ethanol exposure, as adaptations were not observed following an acute ethanol administration (Concas et al., 1996). In another model of ethanol dependence, increases in A_1 receptor density following long-term ethanol exposure have been noted depending upon whether ethanol was ingested continuously versus intermittently with multiple periods of withdrawal. Jarvis and Becker (1998) reported increased A_1 receptor density in cerebral cortex of mice with autoradiographic measurement of [^{3}H]CCPA and [^{3}H]CHA at 8 hours of withdrawal following a single withdrawal episode or multiple withdrawal episodes. However, a greater increase in A_1 receptor density was observed in the multiple withdrawal group compared to the single withdrawal and continuous exposure group, suggesting that upregulation of A_1 receptor proteins is potentiated by multiple periods of hyperexcitability produced by removal of ethanol (withdrawal). Although changes were seen in A_1 receptor expression, changes were not observed in A_{2A} receptor B_{max} or K_d values in striatal tissue from mice exposed to this ethanol treatment paradigm. However, in whole brain homogenates from mice, withdrawal from long-term ethanol exposure has been reported to lower the K_d and B_{max} values of ligands for adenosine A_1 receptors in mice at 24 and 48 hours of withdrawal as compared to control-treated mice and ethanol-dependent mice that did not experience

withdrawal, but returned to control levels at 72 hours of withdrawal (Dar et al., 1983). Similarly, Daly and colleagues (1994) reported significant increases in A_1 receptor expression in the cerebral cortex after 7 days of ethanol drinking in male mice, but no change in A_{2A} receptor binding in the striatum. In a model of long-term ethanol drinking (14 days) followed by ethanol withdrawal in male mice, Kaplan and colleagues (1999) did not find changes in expression of A_1 receptors in frontal cortex and cerebellum or changes in the expression of A_{2A} receptors in the striatum, although there was a significant decrease in the expression of NBTI-sensitive ENT1 adenosine transporters in the striatum. In summary, in vivo data suggest changes in the expression of A_1 receptor protein, but not A_{2A} receptor protein; though, A_{2A} receptors become desensitized with prolonged ethanol exposure (Nagy et al., 1989). However, alterations in adenosine A_1 receptor expression may be dependent on the method of ethanol administration; the length of the long-term ethanol exposure and withdrawal periods; and, perhaps, species used, as has been suggested by previous authors (Concas et al., 1996; Kaplan et al., 1999). Despite the disparate results, all the models discussed appear to produce some changes in the adenosine receptor system that would impact basal adenosinergic tone and thus affect neural excitability.

In vitro studies using an organotypic hippocampal cell culture model have shown that female hippocampi are significantly more vulnerable to toxicity than male hippocampi during ethanol withdrawal with exposure to either the A_1 receptor antagonist DPCPX or caffeine. Toxicity produced by A_1 receptor antagonism during ethanol withdrawal was abolished by the NMDA receptor antagonist, APV, suggesting that relief of adenosine's inhibitory influence by A_1 receptor antagonism during ethanol withdrawal made female hippocampal cultures vulnerable to NMDA-mediated cyto-toxicity (Butler et al., 2008, 2009).

ETHANOL WITHDRAWAL IN VIVO AND ADENOSINE RECEPTORS

Studies of behavioral pharmacology using specific adenosine receptor ligands and caffeine provide evidence for adenosine/ethanol interactions and shed light on acute and chronic effects of ethanol that may relate to hyperexcitability and neuronal damage during withdrawal. Anxiety, a predominant characteristic of ethanol withdrawal in both humans and rodents, is attenuated and exacerbated by A_1 receptor agonists and antagonists, respectively. Administration of CCPA, a selective A_1 agonist, produces anxiolytic effects in mice undergoing withdrawal from an acute dose of ethanol, whereas pretreatment with the selective A_1 receptor antagonist DPCPX reverses the anxiolytic effect produced by CCPA administration (Prediger et al., 2006). In rats made dependent by 4 times daily ethanol dosing for 6 days, A_1 agonism with CCPA significantly blocks spontaneous tremors at peak ethanol withdrawal and also block seizures elicited by an audiogenic stimulus (Concas et al., 1996). Microinjections of the selective A_1 agonist 2-chloroadenosine (2-CADO) into the central nucleus of the inferior colliculus has shown a trend toward reduction of clonus during audiogenic seizures in ethanol withdrawing rats (Feng and Faingold, 2000). Gatch and colleagues (1999) reported R-N-phenylisopropyladenosine (R-PIA), an A_1 agonist, did not have an effect on ethanol withdrawal behavior in rats, but 8-cyclopentyltheophylline (CPT), an A_1 antagonist, significantly increased ethanol withdrawal signs. Kaplan and colleagues (1999) reported significant attenuation of ethanol withdrawal behavior in male CD-1 mice after administration of adenosine receptor ligands during withdrawal from a 14-day liquid ethanol diet. Interestingly, both a selective A_1 receptor agonist R-PIA and a selective A_{2A} receptor agonist (2-p-(2-carboxethyl)phenylethyl-amino-5′-*N*-ethylcarboxamidoadenosine; CGS 21680) significantly reduced withdrawal elicited by a brief tail spin at peak ethanol withdrawal (Kaplan et al., 1999).

CAFFEINE AS A MODULATOR OF ADENOSINERGIC SIGNALING

Ethanol and adenosinergic interactions may be of particular importance, as caffeine is the most widely consumed behaviorally active substance in the world (Daly and Fredholm, 1998; Fredholm et al., 1999). Caffeine is a nonselective competitive antagonist at adenosine receptors and binds to all adenosine receptor subtypes; though, caffeine's primary effects are attributed to antagonism of the A_1 and A_{2A} receptor subtypes (Fredholm et al., 1999; Snyder et al., 1981), with dissociation constants for A_1 and A_{2A} receptors of 20 and 8.1 μM, respectively, in the rat brain, and 12 and 2.4 μM, respectively, in the human brain (Fredholm et al., 1999). Caffeine exerts biphasic behavioral effects in humans, such that stimulation occurs with low doses of caffeine, whereas high doses are associated with unpleasant responses to caffeine (e.g., nausea). Biphasic behavioral effects of caffeine are also observed in rodents, with motor stimulation occurring with administration of low doses of caffeine, but motor depressing effects and/or increased incoordination with high doses of caffeine (reviewed in Daly and Fredholm, 1998). At behaviorally relevant doses of caffeine in rodent and humans, adenosine receptor antagonism is the mechanism of caffeine's effects. At higher caffeine concentrations (those that would be toxic to humans, but have been used in vitro), caffeine also reliably produces intracellular calcium release via ryanodine and inositol triphosphate (IP3) receptors (Nagarkatti et al., 2008), phosphodiesterase inhibition, and blockade of $GABA_A$ receptors (reviewed in Fredholm et al., 1999).

Conflicting evidence exists regarding whether caffeine's motor effects are primarily mediated by the A_1 or the A_{2A} receptor. However, Antoniou and colleagues. (2005) conducted a factor analysis to explore a wide spectrum of motor behaviors and indicated the primary importance of A_1 receptor in mediating caffeine's motor effects. The motor-activating effects of caffeine were more closely mirrored by an A_1 receptor antagonist (CPT) than a selective A_{2A} receptor antagonist (MSX-3). Also, CPA, a selective A_1 agonist, was more effective than CGS 21680, a selective A_{2A} agonist, in attenuating the motor-activating effects of caffeine (Antoniou et al., 2005). Acute caffeine effects on motor behavior are found to correlate with in vivo A_1 receptor density (Kaplan et al., 1992, 1993). Additionally, A_{2A} knockout mice are not stimulated by low doses of caffeine, as would be expected if the A_{2A} receptor is the primary mediator of caffeine's motor stimulatory effects (El Yacoubi et al., 2000; Halldner et al., 2004; Ledent et al., 1997). A_1 receptor knockout and heterozygous mice also display biphasic caffeine effects similar to wild-type mice (Halldner et al., 2004). As both receptors are activated by low concentrations of caffeine, this provides a complicated interplay that is further complicated by evidence of A_1 to A_{2A} receptor co-localization (Rebola et al., 2003). It has been suggested that A_1 to A_{2A} heteromers, which to date have only been observed in experimental transfected cells and rat striatum, mediate caffeine's effects (Ferré et al., 2008).

CAFFEINE AND ETHANOL: HUMAN DATA

In recent years, alcoholic beverages containing caffeine have become increasingly available and popular (reviewed in Reissig et al., 2009). However, following a Food and Drug Administration (FDA; 2010) investigation into the safety of caffeinated alcoholic beverages, the FDA sent warnings to some drink manufacturers and has indicated that caffeine is an "unsafe food additive" in combination with alcohol. In a large web-based study of university students in the United States, 24% of current drinkers reported drinking an alcoholic beverage mixed with a caffeinated beverage. Respondents reporting use of caffeine-containing alcoholic beverages reported heavier drinking patterns and greater prevalence of negative alcohol-related consequences (O'Brien et al., 2008).

In laboratory studies, humans report feeling less intoxicated or impaired when caffeine and alcohol are co-administered in the laboratory (Ferreira et al., 2006; Marczinski and Fillmore, 2006); though, laboratory measures of exacerbation or alleviation of ethanol's effects are task dependent. Caffeine has been shown to antagonize alcohol's impairment in a laboratory tasks of inhibitory control and memory performance (Drake et al., 2003; Marczinski and Fillmore, 2003), but worsen performance on a global neuropsychological assessment when compared to predrink assessment scores (Curry and Stasio, 2009). Caffeine is also able to antagonize ethanol's impairment of motor performance and reaction time; though, this is dependent on the dose of caffeine administered, with lower doses of caffeine having no effect on ethanol impairment (Ferreira et al., 2006; Franks et al., 1975). Most interestingly, however, Ferreira and colleagues (2006) showed that although caffeine did not alter alcohol's impairing effect on motor coordination or reaction time, participants perceived less impairment in motor coordination after consumption of an energy drink containing alcohol than after an alcohol drink alone (Ferreira et al., 2006). Also importantly for real-world application, alcohol in combination with caffeine does not fully ameliorate the impairing effects of alcohol on a simulated driving measure (Liguori and Robinson, 2001). A pharmacological interaction between caffeine and alcohol when co-administered has been suggested, given data that show greater tolerance to ethanol compared to either drug alone after co-administration (Fillmore, 2003).

CAFFEINE AND ETHANOL: PRECLINICAL DATA

In mice, intraperitoneal- or intracerebroventricular administered caffeine has a biphasic effect on acute ethanol-induced motor incoordination, such that low doses of caffeine antagonize and high doses of caffeine accentuate incoordination (Dar, 1988). Chronic caffeine also affects the response to ethanol, such that chronically caffeinated mice have shown greater motor incoordination in response to acute ethanol compared to controls (Dar and Wooles, 1986). Conversely, chronic ethanol exposure (7 days in drinking water) has been reported to have no effect on the motor response to a high dose of caffeine (70 mg/kg; Daly et al., 1994). Taken collectively, these data support a CNS interaction between ethanol and the adenosinergic system. The range of behaviors affected by co-exposure to ethanol and caffeine, from inhibitory control to motor incoordination, suggests important mechanistic interactions at the cellular level that deserve further consideration.

Regarding caffeine's effects during ethanol withdrawal, there remains a paucity of research. In vivo, Malec and colleagues (1996) reported that caffeine itself did not worsen audiogenic seizures in rats, but caffeine did reduce the depressing effects of adenosine analogs when it was co-administered with adenosine analogs during ethanol withdrawal. In vitro, caffeine exacerbates hippocampal injury during ethanol withdrawal, though, only in female-derived cultures in the DG and CA1 regions. These results parallel the effects observed with specific A_1 receptor antagonism during ethanol withdrawal, suggesting that caffeine's ability to inhibit the A_1 receptor during ethanol withdrawal mediates the neuronal injury observed (Butler et al., 2008, 2009). Human research regarding caffeine during withdrawal in humans is not presently available.

CONCLUSIONS AND FUTURE DIRECTIONS

Long-term ethanol exposure results in multiple neuroadaptations; many of which make the brain more vulnerable to neuronal excitability and possibly injury during withdrawal. The data described in this review highlight how both acute ethanol exposure and ethanol withdrawal affects adenosinergic systems, and how these neuroadaptations may affect neuronal hyperexcitability, and possibly, neuronal viability. Clinically, neurodegeneration and cognitive impairment are common and are considered major consequences of long-term

alcohol drinking. Adenosine receptor ligands have been identified for their neuroprotective properties during times of neuronal hyperexcitability and thus have the potential to ameliorate neuronal and behavioral excitability during withdrawal that contributes to seizure activity and perhaps greater cognitive impairment with repeated seizure episodes. The increasing prevalence of caffeine and ethanol co-use also supports the importance of understanding how ethanol interacts with the adenosine receptor system and why co-use of these 2 drugs may lead to unsafe and unforeseen consequences. It should also be noted that sex differences have been scarcely studied in models of ethanol exposure and/or withdrawal in regard to adenosine receptor system function, and much work is needed to determine whether neuroadaptations in the female adenosinergic system parallel those changes discussed in the male adenosinergic system in response to long-term ethanol exposure and withdrawal.

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