Is there a major role for adenosine $a_{2A}$ receptors in anxiety?

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1. ABSTRACT

Clinical investigations, pharmacological studies and models of genetically modified rodents have implicated adenosine in the etiology and modulation of different types of anxiety. Caffeine, a non selective adenosine antagonist, has been involved in many of them. Adenosine seems to interact with other neurotransmitter systems and with some substances like alcohol, which elevate the basal levels of adenosine. A growing body of data describes the role of adenosine $A_1$ and $A_{2A}$ receptors on anxiety. However, a differential role of adenosine receptors is not very clear. $A_1$ receptor antagonists seem to be anxiogenic, but the absence of any effect of some of them and the opposite effects of others does not strongly support this conclusion. Human studies suggest that there is a susceptibility locus for panic disorder and agoraphobia within the receptor $A_{2A}$ gene. On the other hand, pharmacological data do not advocate for a clear implication of the $A_{2A}$ receptor. More research in this area is needed.

2. INTRODUCTION

Several lines of evidence support the involvement of adenosine in anxiety. Data from clinical studies have implicated adenosine in different types of anxiety disorders: phobic or generalized anxiety states, panic attacks and post-traumatic stress disorder. Most of the evidence comes from the use of caffeine and other nonselective adenosine antagonists. However, more recently, the use of selective adenosine receptor agonists and antagonists in animal studies has helped to develop this area of research. The pharmacological work, together with some genetic studies in humans, is trying to approach the study of the neurobiological basis of anxiety from a new perspective with a great potential for new pharmacological treatments. Agents targeting adenosine receptors may offer novel avenues for the development of therapies to manage or treat anxiety/stress related syndromes, and lessen their impact on other pathologies such as addiction.
Thus, there is an extensive preclinical and clinical evidence suggesting that adenosine may also play a role in the pathogenesis of combat-stress traumatic stress effects. Caffeine was demonstrated to be anxiogenic, as well as theophylline, and 8-phenyltheophylline (14,15). Recent studies have shown that adenosine receptor agonists prior to the restraint period significantly reduced ulcer formation and severity, and also lowered plasma corticosterone levels. This protective effect was blocked by the non-specific adenosine antagonists acting as an antagonist at these receptors. It is not yet known which of the various adenosine receptor subtypes is more specifically involved in the regulation of anxiety in these paradigms.

### 2.1. Adenosine involvement in anxiety disorders: Data from non-specific antagonists

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2.1. Adenosine involvement in anxiety disorders: Data from non-specific antagonists

Caffeine (1,3,7-trimethylxanthine), a nonselective adenosine receptor antagonist, exhibits anxiogenic properties in humans and other animals. Caffeine has been reported to be anxiogenic in normal subjects (1,2,3) and in those with anxiety or depressive disorders (4, 5). This xanthine, as well as theophylline, can precipitate panic attacks (6, 7) in patients with susceptibility to this pathology. Indeed, caffeine is occasionally used for diagnostic purposes as a panic precipitating agent (6, 8). In panic disorder patients, caffeine produced greater increases in anxiety, nervousness, fear, nausea, palpitations and restlessness than in healthy subjects, (8) suggesting that some of these patients may have abnormalities in neuronal systems involving adenosine (8, 9, 10). In an animal model of panic attacks (dorsal periaqueductal gray stimulation-induced aversion in rats), caffeine facilitated escape behavior, thus showing anxiogenic properties (11). Furthermore, some of the hormonal parameters that correlate with stress/anxiety are modified by caffeine. Intravenous caffeine increases the hypothalamic-pituitary-adrenal axis (HPA) activity, as shown by increased adrenocorticotropic hormone (ACTH) and cortisol (12). This hormonal upregulation does not occur during the clinical panic attack but does occur with anticipatory anxiety, thus suggesting that the effect of caffeine may be more closely related to chronic anxiety than to the panic attack itself. However, the latter observation, together with the lack of efficacy of the adenosine uptake inhibitor dipyridamole in the treatment of panic disorder, has led to questions about the adenosinergic dysfunction model of panic disorder (13).

Other autonomic and hormonal effects of chronic stress seem to be regulated through adenosine actions. For instance, adenosine has a role in stress-induced gastric pathology. Rats restrained in a cold environment developed a high incidence of gastric ulcers. The administration of adenosine receptor agonists prior to the restraint period significantly reduced ulcer formation and severity, and also lowered plasma corticosterone levels. This protective effect was blocked by the non-specific adenosine antagonists theophylline and 8-phenyltheophylline (14,15). Recent evidence suggests that adenosine may also play a role in traumatic stress effects. Caffeine was demonstrated to be a contributing factor to the pathogenesis of combat-stress reaction (16). In addition, inhibition of brain adenosine deaminase also mimics the effects of inescapable shock, an animal model of helplessness that has been compared to posttraumatic stress disorder (17).

Thus, there is an extensive preclinical and clinical literature suggesting the involvement of the neuromodulator adenosine on anxiety/stress related syndromes. In the following sections we are going to review the preclinical studies that specifically address the involvement of adenosine in the acute anxiety response.

#### 2.2. Role of adenosine in animal models of anxiety

In the study of the neurobiological bases of anxiety, many animal models have been developed. Most of them use rodents as their subjects, and thus use the naturally occurring (phobic anxiety) or learned fears of rodents to assess the ability of genetic or pharmacological manipulations to reverse the natural tendency of the rodents in these paradigms. Most of the models, however, require that the animal displays motor activity, thus introducing a complicating factor that makes the results difficult to interpret; any drug or genetic manipulation that affects motor activity can affect the anxiety scores. For that reason, the use of multiple paradigms to assess the effect of a specific manipulation and the control on independent measures of locomotion is warranted.

Many studies in the adenosine field points to the involvement of adenosine in the regulation of anxiety in rodents. Adenosine administration itself induces anxiolysis (18). Adenosine levels in the brain are regulated, in part, by nucleoside transporters. Papaverine, an inhibitor of neuronal adenosine uptake, causes anxiolytic effects (19). In addition, mice lacking the type 1 equilibrative nucleoside transporter (ENT1) show less anxiety in several anxiety recording paradigms (20). Moreover, pharmacological antagonism of this transporter in the amygdala also reduces anxiety in wild type mice (20). On the contrary, the acute administration of caffeine has demonstrated to have anxiogenic effects in many different animal models of anxiety: plus maze, dark/light box, social interaction test, Vogel test, open field, etc. (18, 21, 22, 23, 24, 25, 26). Other xanthine nonspecific adenosine receptor antagonists, including theophylline and 8-phenyltheophylline (18, 23), aminophylline (19) and IBMX (3-isobutyl-1-methylxantine; 27), as well as CGS 15943 (9-Chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine) (28), a non-xanthine and non-selective A1/A2A adenosine antagonist, also showed anxiogenic properties in some of these paradigms. In addition, preference for caffeine is delayed in some substrains of rodents separated for their response in the anxiety tests. Animals exposed to voluntary consumption of caffeine solutions progressively develop preference for the caffeine solution, but this occurred later in the anxious than in the non-anxious substrains of mice (Table 1)(29).

### 3. ROLE OF THE DIFFERENT ADENOSINE RECEPTORS IN ADENOSINE REGULATION OF ANXIETY

Adenosine in the central nervous system (CNS) acts as a neuromodulator through discrete cell-surface receptors. Adenosine receptors were recognized more than 20 years ago, in part based upon the ability of caffeine to act as an antagonist at these receptors. It is not yet known which of the various adenosine receptor subtypes is more...
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Table 2. Summary of effects of pharmacological and genetic manipulations of adenosine A1 receptor on anxiety

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particularly involved in the anxiogenic properties of caffeine. It is believed that the effects of caffeine, at concentrations reached during habitual caffeine consumption, are a consequence of blockade of tonic activity at two of the four types of adenosine receptors: A1 and A2A, which are both G-protein-coupled receptors (30, 31). A1 and A2A receptors are activated at the low basal adenosine concentrations measured in the resting rat brain (31). It has been proposed that in general, activation of adenosine receptors in the brain reduces anxiety-like behavior in animals and humans, however the data from the antagonists do not show a clear picture of the involvement of the different subtypes on anxiety regulation. A number of adenosine receptor antagonists have been demonstrated to exhibit anxiogenic-like effects in rodent models of phobic anxiety (see references below), but others show no impact in these measures.

3.1. Studies of the involvement of adenosine A1 receptor

The A1 receptor is widely distributed in the brain. Adenosine A1 receptors are present in almost all brain areas, with the highest levels in hippocampus, cerebral and cerebellar cortex, and certain thalamic nuclei. Only moderate levels are found in caudate-putamen and nucleus accumbens (31, 32, 33). Adenosine acts tonically to activate presynaptic and postsynaptic A1 receptors to depress synaptic transmission (34).

Adenosine A1 receptor knock-out (KO) mice have normal levels of locomotion and show more anxiety than the wild-type (WT) mice in several parameters recorded in a broad number of the phobic anxiety tests, including the dark/light box, hole-board, plus maze and open field (34, 35). However other authors find a minor impact of this genotype on some measures of anxiety (emergence test and o-maze) but none in others (dark/light box, novel object exploration and open field) (36). These differences can be due to the strain’s background and to the procedures used for the anxiety tests.

The data obtained from pharmacological manipulations of selective A1 receptor antagonists show some contradictory results. In different studies the same drug has anxiogenic effects while no effect emerges in other studies, and some antagonists have anxiolytic properties. DPCPX (1,3-dipropyl-8-cyclopentylxanthine) decreased the time spent in the light zone of the dark/light test in mice (23) and in a plus maze (25). However, in other studies DPCPX did not affect the anxiety state in the elevated plus maze, dark/light test or open field in mice (24, 28, 37, 38). These differences can be due to the range of doses used (25, 38). CPT (8-cyclopentyl-1,3, dimethylxanthine), had an anxiogenic effect in the elevated plus-maze and the dark/light test (27). Finally, the newly developed adenosine A1 receptor antagonist, FR194921 (2-(1-methyl-4-piperidinyl)-6-(2phenylpyrazolo[1,5-a]pyridin-3-yl)-3(2H)-pyridazinone) showed specific anxiolytic activity in rats in two animal models of anxiety, the social interaction test and the elevated plus maze, with no significant influence on general motor activity (39).

A limited number of selective adenosine A1 agonists have been used for the study of anxiety-related behavior. Early studies showed no significant anti-conflict effects of I-PIA (N6-R-phenylisopropyladenosine) in a punished responding paradigm (40). The selective adenosine A1 receptor agonist CPA (N6-cyclopentyladenosine) had an anxiolytic effect in the elevated plus-maze (37). This anxiolytic effect of CPA was not prevented by the hydrophilic xanthine, 8-(p-sulphophenyl) theophylline, but it was by the A1 selective antagonist DPCPX (37). On the other hand, CPA prevented the anxiogenic-like activity of caffeine (37), but not the anxiogenic effect of DPCPX (A1 antagonist) (23). Thus, a complicated set of effects is shown among studies involving CPA (a selective adenosine A1 agonist) in terms of interactions with A1 antagonists or non specific adenosine receptor antagonists. Moreover, in a more recent study CPA was not found to produce anxiolysis in the elevated plus maze in WT mice in a broad range of doses, however, it did affect locomotion (24). Clearer results have been found with the A1 adenosine agonist, and CPA close analogue, CCPA (2-chloro-N6-cyclopentyladenosine). CCPA had anxiolytic effects in the plus maze and in the dark/light test, with little or no effect on locomotor performance at the same doses (25, 27, 38). In summary, adenosine A1 antagonists do not show a clear pattern of effects, nevertheless, A1 agonists mostly seem to produce anxiolysis (Table 2).

3.2. Studies of the involvement of adenosine A2A receptors

Some authors have suggested that the involvement of adenosine in anxiety is mainly due to A1 activation, while the activation of A2A receptors causes locomotor depression and reduces the effects of A1 receptor activation on anxiety (37).

Nonetheless, A2A receptors are present not only in basal ganglia, but also in extended amygdala and hypothalamus, which are nuclei involved in the regulation of anxiety/stress responses (41). Furthermore, genetic studies in humans have suggested that reduced A2A receptor-mediated neuromodulation may be involved in the development of panic attacks. Systematic mutation screening and association studies of adenosine receptor genes have suggested that the A2A receptor gene may confer susceptibility to the development of panic disorders.
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Some studies suggest that there is a susceptibility locus for panic disorder and agoraphobia, either within the receptor A2A gene or in a nearby region of chromosome 22, in Caucasian population (42, 43), although this has not been found in Asian populations (44, 45). Moreover, an adenosine receptor A2A gene polymorphism that has been associated with panic disorder is also associated with self-reported anxiogenic responses to an acute dose of caffeine (46).

Among the animal studies, the data also show that A2A receptor KO mice display more anxiety than WT mice (24, 47). A2A KO mice exhibited reduced exploratory activity on a novel open-field, spent more time in the closed protected arms of an elevated plus-maze and also in the less threatening dark area of a dark/light box. The chronic administration of caffeine produced slightly less anxiety response in the A2A KO mice than in the WT controls (24), and in addition the KO mice voluntarily intake less caffeine solution than WT animals (48). These A2A KO behavioral data are in consonance with other neurohormonal parameters involved in the anxiety/stress response. For instance, adenosine A2A receptors seem to regulate propiomelanocortin (POMC) gene expression and POMC derived peptides in the anterior lobe of the pituitary, leading to a hyperactivity of the pituitary-adrenocortical axis in the A2A KO mice since ACTH was increased and plasma corticosterone levels were higher than in the WT (49). In the same study, the authors found that although POMC expression was not affected in the perikarya, the melanocyte stimulating hormone (alpha-MSH), which has been implicated in anxiety, is more concentrated in two areas innervated by POMC terminals: the cerebral cortex and amygdala (49).

The pharmacological data however do not point clearly to an involvement of the A2A receptor in the regulation of anxiety. The xanthine DMPX (3,7-Dimethyl-Propargylxanthine), an adenosine A2A receptor antagonist, did not affect anxiety measurements in an elevated plus maze (37, 50), in the peripheral portion of an open field or in a Vogel conflict test in rats (50) even at a dose that increased spontaneous locomotion (50). DMPX also did not modify the anxiolytic-like activity of CPA (an A1 selective agonist) (37). In the same way, the A2A receptor antagonists ZM241385 (4-[2-(7-amino-2, 2-fur-yl)1,2,4 triazolo-2,3-a 1,3,5 triazin-5-yl-amino] ethyl)phenolon) (24, 25) and SCH58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl) pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine), over a wide range of doses, were devoid of acute or chronic effects in the dark/light box or in the plus maze in mice (24).

The data with adenosine A2A agonists are not much more conclusive. Overexpression of human A2A receptors mainly in the cerebral cortex, the hippocampal formation, and the cerebellum of a novel transgenic rat strain, TGR(NSEh A2A), did not affect anxiety-related behavior in an elevated plus maze compared to WT rats (51). However, it might be possible that this lack of effect is mainly due to an A2A receptor overexpression in brain areas that have a minor role in the regulation of anxiety. The anxiolytic-like activity of pharmacological adenosine agonism has been also difficult to demonstrate. The A2A receptor agonist, DPMA (N6-[2-(3,5-dimethoxyphenyl)-2(2-methylphenyl)ethyl]adenosine) had no effect on anxiety behavior but depressed locomotor activity at the highest dose tested (37, 38). This suppression of spontaneous locomotion could explain the DPMA-induced blockade of the anxiolytic properties of A1 agonists, since the administration of DPMA in combination with anxiolytic doses of CPA prevented the anxiolytic-like activity of the latter (37). On the other hand, the A2A-selective agonist CGS 21680 (CGS 21680-2-p-(2-carboxyethyl)phenethylamino-5-N-ethylcarboxamidoadenosine hydrochloride) has anxiogenic effects on mice in a plus maze (24) at the same doses that significantly reduced locomotion. CGS 21680 reduced the anxiogenic effects of theophylline (23), but did not reverse the anxiogenic effect of A1 receptor antagonist DPCPX (23).

Overall, although some authors have suggested that there is a preferential involvement of A1 rather than A2A receptors in the modulation of phobic anxiety (27), the absence of any effect of some A1 antagonists and the opposite effects of others does not strongly support this involvement. More systematic pharmacological studies of the effect of selective agonists and antagonists are warranted. The results available at the present moment also suggest that A1 and A2A receptors in rodents are not activated tonically by endogenous adenosine in order to regulate anxiety (37), since blocking this tonic activity has very little effect. This is important because it raises the problem of explaining the anxiogenic-like profile of caffeine, when it is administered either acutely or chronically. Concerning acute studies, Jain et al. (37) proposed that the anxiogenic-like properties of caffeine could be due to the simultaneous blockade of A1 and A2A receptors, since caffeine does not distinguish between these receptors in the CNS (24). This hypothesis is in agreement with the results about the effects of caffeine on locomotion and on A1 and A2A receptors (52). Thus, in general it seems that the impact of A2A receptors on phobic anxiety measures is very limited, and probably is due to the regulation of motor activity, which is a complicating factor in many of the tests used to assess anxiety in rodents. These results led to the suggestion that adaptive mechanisms, rather than A2A receptor antagonism, are most likely to be responsible for increased anxiety in adenosine A2A receptor KO mice (Table 3)(41).

4. INTERACTIONS OF ADENOSINE WITH OTHER NEUROTRANSMITTER SYSTEMS ON ANXIETY

Adenosine is not primarily released in a transmitter- or hormone-like fashion. It can be formed by breakdown of ATP released by cells either in a regulated fashion or in response to massive trauma. Adenosine is therefore likely to act in concert with several other messengers (transmitters, hormones, growth factors; 53). In this sense, adenosine plays two parallel roles in the CNS, both as a homeostatic modulator and as a modulator at the synaptic level (54). Thus, adenosine modifies the release of neurotransmitters, their post-synaptic responsiveness, and the action of a number of other neurotransmitter systems
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Table 3. Summary of effects of pharmacological and genetic manipulations of adenosine A2A receptor on anxiety

<table>
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<tr>
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<tr>
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</table>

(55). In fact, interactions of adenosine and adenosine receptors with GABA, NMDA, CB1, or dopamine receptors have been described (53, 56). At the behavioral level, although very limited, there is some evidence that these interactions are important in relation to animal models of anxiety. In the present section we will review those data.

4.1. Adenosine and dopamine interactions

Dopamine and adenosine receptors are known to share a considerable overlap in their regional distribution, being especially rich in the basal ganglia (57). Dopamine and adenosine receptors have been demonstrated to exhibit a parallel distribution on certain neuronal populations, and in the cell membrane, adenosine A1 and A2A receptors are colocalized with dopamine D1 and D2 receptors, respectively (58, 59). In general, stimulating adenosine receptors decreases the effects of dopamine receptor stimulation. The antagonistic function interaction between adenosine A2A and dopamine D2 receptors is believed, at least in part, to be mediated by heterodimer formation between dopamine D2 and adenosine A2A receptors (60, 61, 62). In a recent study, the combined deletion of D2 and A2A receptors in KO mice resulted in an increased anxiety response (63). Thus, it has been found that D2A2A receptor KO mice made fewer total transitions and spent less time on the open arms of the elevated plus maze, compared to WT mice. However, although double mutants appeared anxious on the elevated plus maze, D2A2A receptor KO mice exhibited the same phenotypic profile that mice with a double mutation. The authors hypothesized that the similarity in the magnitude of the effect among double (D2A2A receptors) and single (A2A receptors) KO mice may be produced by a ceiling effect in the time that the animals spent in the open arm of the plus maze.

In humans, genetic studies suggest that adenosine A2A receptor gene polymorphisms contribute to interindividual variations in the anxiety response to amphetamine (64). As we have previously mentioned, certain human polymorphism of the adenosine A2A Receptor gene (1966C/T and 2592C/Tins) has been associated with panic disorder (42, 43) and increases in anxiety (46). Self-reported increases in anxiety after caffeine (46) as well as amphetamine (64) administration have been reported to be associated with these A2A receptor gene polymorphisms.

4.2. Adenosine and GABA interactions

Numerous studies indicate that the GABA receptor complex plays a major role in the pharmacology, neurochemistry and physiopathology of stress and anxiety (65). The anxiolytic effect of benzodiazepines, the most widely used family of anxiolytic agents, may be considered a consequence of the activation of the GABA receptors induced by these drugs (65). The existence of interactions between adenosine and benzodiazepines has long been suggested (66). It has been reported that benzodiazepines block adenosine uptake (66, 67) and enhance adenosine release (68). However, the behavioral data are confusing, since caffeine administered to rats had anxiogenic effects in a social interaction test and this effect was antagonized by chloridiazepoxide, a benzodiazepine agonist (21).

On the other hand, benzodiazepines also decrease adenosine A1 receptor binding capacity in vivo at doses of clinical relevance (69), and a down-regulation of the number of adenosine A2A receptors in rat forebrain was found following chronic treatment with benzodiazepines (70). The role of A1 and A2A receptors in nerve terminals that release putative inhibitory neurotransmitters is not completely clarified, and seems to be different in different brain regions. Thus, in the septum and hippocampus, activation of A2A receptors facilitates the evoked release of GABA (71), while in the striatum A2A receptor activation leads to an inhibition of evoked GABA release (72). The facilitatory influence of A2A receptors upon GABA release in the septum and hippocampus may be related to the observation that genetically altered mice lacking A2A receptors show an exaggerated response to anxiogenic stimuli (71).

4.3. Adenosine and glutamate interactions

There are several reports of interactions between the effects of drugs that affect adenosine receptor transmission and those that block the N-methyl-D-aspartate (NMDA) receptor or its associated ion channel (73, 74). Activation of NMDA-receptor-associated ion channels has been proposed to reduce adenosine receptor function, while blockade of these channels enhances adenosine receptor function (73, 74). When co-administered with dizocilpine, (a use-dependent NMDA antagonist) or ifenprodil (a polyamine site NMDA receptor antagonist), the A1 selective antagonist DPCPX reversed the anxiolytic effects induced by both drugs (75, 76). However, the A2A selective antagonist DMPX did not reverse the anxiolytic effect induced by dizocilpine (76). It was concluded that at least part of the anxiolytic and locomotor stimulant properties of dizocilpine may be explained by the release of endogenous adenosine acting at A1, but not A2A receptors (76). More difficult to explain is the fact that A1 and A2A receptor agonists seem to prevent the anxiolysis induced by selective NMDA antagonists. CPA (A1 receptor selective agonist) is able to prevent anxiolysis by dizocilpine and ifenprodil (75, 76). In a similar manner, the A2A agonist DPMA also reversed the anxiolytic effect induced by dizocilpine (76).
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Overall, there is a predominant interaction between adenosine A1 receptors and NMDA receptors, as mainly A1 antagonist have an effect compared with A2A antagonists. Thus, the anxiolytic effects of NMDA antagonists could be the result of enhancing the activity of endogenous adenosine, being possible as it has been proposed a direct interaction between receptors at the membrane level (73, 76). Other authors have proposed that a possible mechanism for this anxiolysis may be explained by the A1 mediated reduction in the release of glutamate (77, 78, 79) or reductions in glutamate-mediated activation of voltage-dependent NMDA receptors (80).

### 4.4 Adenosine and endogenous cannabinoid system interactions

Adenosine A2A receptors and CB1 receptors seem to co-localize and form heteromers in dendritic processes, and possibly nerve terminals, in rat striatum (60, 81). Recent genetic and pharmacological evidence suggests that A2A receptors are involved in the acute behavioral effects of cannabinoids and CB1 receptors agonists (82, 83, 84). Specifically, the involvement of CB1 receptors in behavioral measures of anxiety has been demonstrated in pharmacological and genetic studies suggesting that interfering with this receptor induces anxiogenic effects (85, 86, 87). Regarding the interaction of both neurotransmitter systems on anxiety, mice with a double mutation lacking CB1 cannabinoid receptors and adenosine A2A receptors showed higher anxiety-like responses compared to WT mice with no mutation in two paradigms; plus maze and dark light box (82). In addition, when the double KO mice were compared to single A2A KO mice, or to single CB1 KO mice, the results suggest that both CB1 and A2A receptors control anxiety-like responses in a similar manner and a facilitation of the anxiogenic response is produced by the deletion of both receptors (82).

### 5. ROLE OF ADENOSINE IN THE ANXIOLYTIC EFFECTS OF ETHANOL

The role of adenosine in anxiety is not conclusive, as it has been speculated, because the basal tone of adenosine does not appear to directly regulate this behavior. In the present section we are going to review the impact of adenosine manipulations on ethanol regulation of anxiety, since ethanol elevates adenosine tone and its anxiolytic properties are well known. Acutely, and at moderate doses, ethanol produces anxiolysis in humans and rodents (25, 88). However, after several hours when ethanol levels in blood are not detectable, a high dose of ethanol acutely administered produces a mild withdrawal effect (i.e., hangover) that, among other symptoms, includes increased anxiety (38). Moreover, the withdrawal syndrome after chronic administration or chronic consumption of significant amounts of ethanol is also characterized by an increased anxiety response (89).

Several findings indicate the existence of interactions between adenosine and ethanol. Ethanol can increase adenosine levels by increasing adenosine release (90, 91) and by decreasing adenosine uptake (92) that takes place via a facilitative nucleoside transporter. The presence of an ethanol-sensitive adenosine transporter in cells has been demonstrated (92, 93). An inhibition of this transporter in the presence of ethanol would lead to an increase in extracellular adenosine and could thereby modulate some of the effects of ethanol (91). For example, dipyridamole, an inhibitor of adenosine uptake, increases the sleep time observed following administration of ethanol in mice (94). However, the use of dipyridamole in the treatment of panic attacks in humans has not produced positive results (13). Secondarily, ethanol increases adenosine levels by acting as a precursor through the production of acetate and its metabolism (95). Through oxidative metabolism, ethanol is converted to acetate, and acetate has been demonstrated to increase adenosine levels in the brain (91, 96). Following ethanol ingestion, about 90% is metabolized in the liver to acetaldehyde and acetate (97). Approximately 70% of the acetate generated is released from the liver and is metabolized in extrahepatic tissues (98), including the brain (99, 100, 101). The metabolism of acetate requires adenosine triphosphate (ATP) and coenzyme A and results in the production of acetyl coenzyme A, adenosine monophosphate (AMP) and pyrophosphate. AMP is further metabolized by the enzyme 5-nucleotidase to produce adenosine (102, 103, 104). (Figure 1).

There is evidence that acetate and adenosine may contribute to some behavioral effects of ethanol such as sedation, sleep, and motor suppression or incoordination (90, 95, 97, 105, 106, 107, 108, 109, 110). Nevertheless, very little is known about the impact of acetate on the anxiety response. In the Correa et al. (109) paper, the interior part of the open field was used to assess possible anxiolytic effects of ethanol and ethanol metabolites. Acetate administered in the ventricles reduced locomotion but had no impact on the anxiety index (109). However, in this study the behavior was immediately recorded after the drug was administered thus no time was allowed for acetate to be metabolized to adenosine.

Several reports have also suggested the involvement of brain adenosine receptor regulation in different actions produced by ethanol. It has been shown that mice bred for increased ethanol sensitivity also exhibit increased sensitivity to the behavioral effects of adenosine analogues (111). Adenosine A1 receptors seem to be more
involved in this effect (112, 113). There are limited reports implicating the A2A receptor in central responses to ethanol. Adenosine A2A KO mice, which display an increased anxiogenic phenotype (24, 47), also show less ethanol induced sleep time (94, 114). Although there is almost no evidence of the impact of adenosine uptake inhibitors or acetate-related increases in adenosine on the anxiety induced by ethanol, the role of adenosine receptors in the acute and long term effects of ethanol has started to be explored very recently, thus at present only a handful of studies addresses this issue. Concerning the impact of adenosine modulation on ethanol-induced anxiolysis, pretreatment with caffeine at non-anxiogenic doses significantly reduces the anxiolytic-like effect of acute administration of ethanol to mice in a plus maze (25). In the same study, pretreatment with the A1 antagonist DPCPX, but not with the A2A antagonist ZM241385, reduced the anxiolytic-like effect of ethanol. Moreover, an anxiolytic response was observed by the co-administration of non-anxiolytic doses of the A1 adenosine agonist CCPA and ethanol. Thus, these authors proposed that the activation of adenosine A1 receptors, but not adenosine A2A receptors, mediates the anxiolytic-like effect induced by a moderate dose of ethanol in mice (25). On the other hand, elevated signs of anxiety are observed during withdrawal from chronic as well as acute ethanol exposure, and adenosine receptors can modulate some of the signs of ethanol withdrawal (115). In reference to the role of adenosine receptors, very similar patterns of results were found on anxiety induced by ethanol withdrawal. It has been demonstrated that ethanol hangover-induced anxiety (12-18 h after a high acute dose of ethanol administration) is blocked by the acute administration of nonanxiolytic doses of adenosine (38). In the same study the authors show that A1 receptors also play a role in the anxiogenic effect of ethanol induced hangover, since the selective adenosine A1 receptor agonist CCPA, but not the adenosine A2A receptor agonist DPMA, blocked the anxiogenic response in mice in an elevated plus maze (38). In addition, the anxiolytic effect of CCPA on the anxiety-like behavior of ethanol hangover was reversed by pretreatment with the selective adenosine A1 receptor antagonist DPCPX (38). Nevertheless, the results concerning chronic ethanol administration seem different from the ones observed after acute ethanol administration. In both cases an anxiogenic effect is produced after withdrawal of ethanol (between 12 and 18 hrs), but with chronic ethanol pretreatment blocking the A1 receptors seems to reduce the anxiety, since the A1 receptor antagonist CPT reduced the anxiogenic effects produced by ethanol withdrawal in the elevated plus-maze and in the dark/light test in rats (116).

In summary, a preferential role for adenosine A1 receptors in regulating the impact of ethanol on anxiety seems to emerge from these results. Such findings suggest that A1 adenosinergic compounds may be of use for the treatment of some aspects of ethanol intoxication and withdrawal. Nonetheless, more studies that address the possible therapeutic use of agents targeting adenosine receptors are needed.

6. SUMMARY AND PERSPECTIVE

The well known anxiogenic effect of caffeine at high doses or in individuals with a susceptibility for anxiety disorders has stimulated research questions that at this point have no clear answers. Data from clinical and experimental pharmacological studies suggest that adenosine is involved in the regulation of anxiety, probably through interactions with some other neurotransmitter systems, although there are very few behavioral data that approach this issue. Overall, it seems that non specific A1/A2A adenosine antagonists show a clearer anxiogenic effect than either of the individual receptor antagonists alone. Among these two receptors, the A1 subtype seems more clearly involved, since a majority of the animal studies show an anxiogenic profile for the A1 antagonists, while none of the studies so far have found an anxiogenic effect of the A2A antagonists. The A1 receptor also seems to be more involved in interactions with other neurotransmitter systems like dopamine, glutamate or cannabinoids. The studies with mutant mice do not differentiate between the subtypes of adenosine receptors, as both A1 and A2A KO mice show an anxiogenic profile. In humans only the Caucasian population appears to have a polymorphism in the adenosine A2A receptors gene that is associated with panic disorder. Thus, the hypothesis of Jain and colleagues (37) that the selective agonism of central A1 adenosine receptors induces anxiolytic-like behavior, while the activation of A2A sites causes locomotor depression and reduces the effects of A1 receptor activation on anxiety, seem to be supported by the recent pharmacological data. However, considering the diversity of anxiety disorders, some authors have also speculated that the A2A receptor seems more particularly involved in the development of panic and possibly post-traumatic stress disorders, but they are less important for the control of phobic or generalized anxiety states (41). Thus, in general it seems that the impact of A2A receptors on phobic anxiety measures is very limited and probably it is due to the regulation of the motor activity. This hypothesis would also be in agreement with the fact that A2A receptors are predominantly located in nuclei that regulate not only pure motor output, but also aspects of motivated behavior such as approach. In this regard, the impact of newly developed adenosine compounds for the treatment of different motor and mood disorders should be assessed to determine if the relevant drugs produce any anxiogenic secondary effects. For instance, A2A receptor antagonists are being tested in animals and humans for the reversal of parkinsonian symptoms (117, 118, 119, 120), for reversing anergia and psychomotor slowing in animal models (121), and also for effects in tests related to depression (122). Nonetheless, more studies that address the possible therapeutic use of agents targeting adenosine receptors as anxiolytics are needed. The very limited number of studies on this topic suggests that adenosinergic A1 receptor compounds may be of use for the treatment of some aspects of ethanol intoxication and withdrawal. However, it has been observed that following chronic exposure, tolerance to the acute behavioral effects of various adenosine antagonists develops. Such tolerance has been demonstrated in a variety of behavioral paradigms, including locomotor activity (123). Similarly, tolerance to
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the anxiogenic effects of caffeine has been shown using animal models of anxiety (22, 124). Thus, the separation between therapeutic efficacy and adverse side effects remains a challenge in the discovery and development of novel adenosine-based medicines.

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**Abbreviations:** HPA: hypothalamic-pituitary-adrenal axis, ACTH: adrenocorticotropic, EN1: type 1 equilibrative nucleoside transporter, IBMX: 3-isobutyl-1-methylxantine, CGS 15943: 9-Chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine, CNS: central nervous system, DPCPX (or CPX): 8-cyclopentyl-1,3-dipropylxanthine, CPT: 8-cyclopentyl-1,3, dimethylxanthine, FR194921: 2-(1-methyl-4-piperidinyl)-6-(2 phenylpyrazolo[1,5-a]pyridin-3-yl)-3(2H)-pyridazinone, l-PIA: N6-R-phenylisopropyladenosine, CPA: N6-cyclopentyladenosine, CCPA: 2-chloro-N6-cyclopentyladenosine, POMC: proopiomelanocortin, DMPX: 3,7-Dimethyl-Propargylyxanthine, ZM241385: 4-(2-[7-aminol-2- 2-furyl]1,2,4 triazolo-2,3-a 1,3,5 triazin-5-yl-aminol) ethyl)phenol), SCH5826: (7-(2-phenethyl)5-amino-2-(2-furyl) pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine), DPMA: N6-2-[3,5-dimethoxyphenyl]-3(2 methylphenyl) ethyl]adenosine), CGS 21680: (CGS 21680-2-p-(2-carboxyethyl)phenethylamino-5"-N ethylecarboxymidoadenosine hydrochloride), ATP: adenosine triphosphate, AMP: adenosine monophosphate

**Key Words:** Anxiety, Panic Attack, Post-Traumatic Stress Disorder, Phobic State, Adenosine, Adenosine A2A receptors, adenosine A1 Receptors, Dopamine, Glutamate, GABA, Endocannabinoids, Ethanol, Acetate, Plus Maze, Review

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