[Frontiers in Bioscience 4058-4070, May 1, 2008]

# 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.

**Table 1.** Summary of effects of non selective adenosine antagonists on anxiety

| | Effect | Reference |
|---|---|---|
| **Non selective Antagonists** | | |
| Caffeine | Anxiogenic | 18, 21, 22, 23, 24, 25, 26 |
| Theophylline | Anxiogenic | 18, 23 |
| 8-phenyltheophylline | Anxiogenic | 23 |
| Aminophylline | Anxiogenic | 19 |
| IBMX | Anxiogenic | 27 |
| CGS 15943 | Anxiogenic | 28 |

### 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 adrenocorticotropine (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]triazolol[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

**Table 2.** summary of effects of pharmacological and genetic manipulations of adenosine $a_1$ receptor on anxiety

| | Effect | Reference |
|---|---|---|
| **$A_1$ antagonists** | | |
| DPCPX (CPX) | No effect | 24, 28, 37, 38 |
| | Anxiogenic | 23, 25 |
| CPT | Anxiogenic | 27 |
| FR194921 | Anxiolytic | 39 |
| **$A_1$ agonists** | | |
| I-PIA | No effect | 40 |
| CPA | Anxiolytic | 37 |
| CCPA | No effect | 24 |
| | Anxiolytic | 25, 27 |
| $A_1$ KO mice | | |
| | Anxiogenic | 34, 35 |
| | Mild anxiogenic or no effect | 36 |

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: $A_1$ and $A_{2A}$, which are both G-protein-coupled receptors (30, 31). $A_1$ and $A_{2A}$ 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 $A_1$ receptor

The $A_1$ receptor is widely distributed in the brain. Adenosine $A_1$ 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 $A_1$ receptors to depress synaptic transmission (34).

Adenosine $A_1$ 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 $A_1$ 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 $A_1$ 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 $A_1$ 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 $A_1$ receptor agonist CPA (*N*6-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 $A_1$ selective antagonist DPCPX (37). On the other hand, CPA prevented the anxiogenic-like activity of caffeine (37), but not the anxiogenic effect of DPCPX ($A_1$ antagonist) (23). Thus, a complicated set of effects is shown among studies involving CPA (a selective adenosine $A_1$ agonist) in terms of interactions with $A_1$ 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 $A_1$ 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 $A_1$ antagonists do not show a clear pattern of effects, nevertheless, $A_1$ agonists mostly seem to produce anxiolysis (Table 2).

### 3.2. Studies of the involvement of adenosine $A_{2A}$ receptors

Some authors have suggested that the involvement of adenosine in anxiety is mainly due to $A_1$ activation, while the activation of $A_{2A}$ receptors causes locomotor depression and reduces the effects of $A_1$ receptor activation on anxiety (37).

Nonetheless, $A_{2A}$ 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 $A_{2A}$ 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 $A_{2A}$ receptor gene may confer susceptibility to the development of panic disorders

(42). Some studies suggests that there is a susceptibility locus for panic disorder and agoraphobia, either within the receptor $A_{2A}$ 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 $A_{2A}$ 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 $A_{2A}$ receptor KO mice display more anxiety than WT mice (24, 47). $A_{2A}$ 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 $A_{2A}$ KO mice than in the WT controls (24), and in addition the KO mice voluntarily intake less caffeine solution than WT animals (48). These $A_{2A}$ KO behavioral data are in consonance with other neurohormonal parameters involved in the anxiety/stress response. For instance, adenosine $A_{2A}$ receptors seem to regulate proopiomelanocortin (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 $A_{2A}$ 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 $A_{2A}$ receptor in the regulation of anxiety. The xanthine DMPX (3,7-Dimethyl-Propargylxanthine), an adenosine $A_{2A}$ 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 $A_1$ selective agonist) (37). In the same way, the $A_{2A}$ 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)phenol) (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 $A_{2A}$ agonists are not much more conclusive. Overexpression of human $A_{2A}$ receptors mainly in the cerebral cortex, the hippocampal formation, and the cerebellum of a novel transgenic rat strain, TGR(NSEh $A_{2A}$), 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 $A_{2A}$ 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 $A_{2A}$ 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 $A_1$ 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 $A_{2A}$-selective agonist CGS 21680 (CGS 21680-2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine hydrochloride) has anxiolytic 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 $A_1$ receptor antagonist DPCPX (23).

Overall, although some authors have suggested that there is a preferential involvement of $A_1$ rather than $A_{2A}$ receptors in the modulation of phobic anxiety (27), the absence of any effect of some $A_1$ 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 $A_1$ and $A_{2A}$ 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 $A_1$ and $A_{2A}$ 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 $A_1$ and $A_{2A}$ receptors (52). Thus, in general it seems that the impact of $A_{2A}$ 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 $A_{2A}$ receptor antagonism, are most likely to be responsible for increased anxiety in adenosine $A_{2A}$ 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

**Table 3.** Summary of effects of pharmacological and genetic manipulations of adenosine $A_{2A}$ receptor on anxiety

| | Effect | Reference |
|---|---|---|
| **$A_2$ antagonists** | | |
| DMPX | no effect | 37, 50 |
| ZM241385 | no effect | 24, 38 |
| SCH58261 | no effect | 24, 38 |
| **$A_{2A}$ agonists** | | |
| DPMA | no effect | 37, 38 |
| CGS 21680 | anxiolytic | 24 |
| $A_{2A}$ KO mice | | |
| | Effect | Reference |
| --- | anxiogenic | 24, 47 |
| **Transgenic $A_{2A}$ rats** | | |
| --- | no effect | 51 |

(55). In fact, interactions of adenosine and adenosine receptors with $GABA_A$, NMDA, $CB_1$ 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 $A_1$ and $A_{2A}$ receptors are colocalized with dopamine $D_1$ and $D_2$ receptors, respectively (58, 59). In general, stimulating adenosine receptors decreases the effects of dopamine receptor stimulation. The antagonistic function interaction between adenosine $A_{2A}$ and dopamine $D_2$ receptors is believed, at least in part, to be mediated by heterodimer formation between dopamine $D_2$ and adenosine $A_{2A}$ receptors (60, 61, 62). In a recent study, the combined deletion of $D_1$ and $A_{2A}$ receptors in KO mice resulted in an increased anxiety response (63). Thus, it has been found that $D_1A_{2A}$ 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, $D_1A_{2A}$ receptor KO mice exhibited the same phenotypic profile that mice with a single mutation on $A_{2A}$ receptors, indicating that anxiety-like responses are not modified as a consequence of the double mutation. The authors hypothesized that the similarity in the magnitude of the effect among double ($D_1A_{2A}$ receptors) and single ($A_{2A}$ 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 $A_{2A}$ 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 $A_{2A}$ receptor gene (1976C/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 $A_{2A}$ receptor gene polymorphisms.

### 4.2. Adenosine and GABA interactions

Numerous studies indicate that the $GABA_A$ 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 $GABA_A$ 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 chlordiazepoxide, a benzodiazepine agonist (21).

On the other hand, benzodiazepines also decrease adenosine $A_1$ receptor binding capacity *in vivo* at doses of clinical relevance (69), and a down-regulation of the number of adenosine $A_{2A}$ receptors in rat forebrain was found following chronic treatment with benzodiazepines (70). The role of $A_1$ and $A_{2A}$ 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 $A_{2A}$ receptors facilitates the evoked release of GABA (71), while in the striatum $A_{2A}$ receptor activation leads to an inhibition of evoked GABA release (72). The facilitatory influence of $A_{2A}$ receptors upon GABA release in the septum and hippocampus may be related to the observation that genetically altered mice lacking $A_{2A}$ 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 $A_1$ selective antagonist DPCPX reversed the anxiolytic effects induced by both drugs (75, 76). However, the $A_{2A}$ 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 $A_1$, but not $A_{2A}$ receptors (76). More difficult to explain is the fact that $A_1$ and $A_{2A}$ receptor agonists seem to prevent the anxiolysis induced by selective NMDA antagonists. CPA ($A_1$ receptor selective agonist) is able to prevent anxiolysis by dizocilpine and ifenprodil (75, 76). In a similar manner, the $A_{2A}$ agonist DPMA also reversed the anxiolytic effect induced by dizocilpine (76).

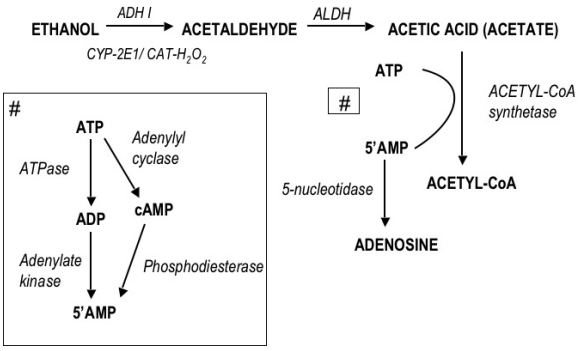


**Figure 1.** Adenosine formation from ethanol oxidation.

Overall, there is a predominant interaction between adenosine $A_1$ receptors and NMDA receptors, as mainly $A_1$ antagonist have an effect compared with $A_{2A}$ 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 $A_1$ 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 $A_{2A}$ receptors and $CB_1$ 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 $A_{2A}$ receptors are involved in the acute behavioral effects of cannabinoids and $CB_1$ receptors agonists (82, 83, 84). Specifically, the involvement of $CB_1$ 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 neurotransmiter systems on anxiety, mice with a double mutation lacking $CB_1$ cannabinoid receptors and adenosine $A_{2A}$ 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 $A_{2A}$ KO mice, or to single $CB_1$ KO mice, the results suggest that both $CB_1$ and $A_{2A}$ 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 $A_1$ receptors seem to be more

involved in this effect (112, 113). There are limited reports implicating the $A_{2A}$ receptor in central responses to ethanol. Adenosine $A_{2A}$ 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 $A_1$ antagonist DPCPX, but not with the $A_{2A}$ 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 $A_1$ receptors also play a role in the anxiogenic effect of ethanol induced hangover, since the selective adenosine $A_1$ receptor agonist CCPA, but not the adenosine $A_{2A}$ 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 $A_1$ 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 $A_1$ receptors seems to reduce the anxiety, since the $A_1$ 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 $A_1$ receptors in regulating the impact of ethanol on anxiety seems to emerge from these results. Such findings suggest that $A_1$ 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 $A_1/A_{2A}$ adenosine antagonists show a clearer anxiogenic effect than either of the individual receptor antagonists alone. Among these two receptors, the $A_1$ subtype seems more clearly involved, since a majority of the animal studies show an anxiogenic profile for the $A_1$ antagonists, while none of the studies so far have found an anxiogenic effect of the $A_{2A}$ antagonists. The $A_1$ 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 $A_1$ and $A_{2A}$ KO mice show an anxiogenic profile. In humans only the Caucasian population appears to have a polymorphism in the adenosine $A_{2A}$ receptors gene that is associated with panic disorder. Thus, the hypothesis of Jain and colleages (37) that the selective agonism of central $A_1$ adenosine receptors induces anxiolytic-like behavior, while the activation of $A_{2A}$ sites causes locomotor depression and reduces the effects of $A_1$ 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 $A_{2A}$ 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 $A_{2A}$ 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 $A_{2A}$ 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, $A_{2A}$ receptor antagonists are being tested in animals and humans for the reversal of parkinsionian 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 $A_1$ 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

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.

## 7. ACKNOWLEDGEMENT

This work was supported by grants to Dr. Mercè Correa (BEST/2007/122) and to Dr. Laura Font (BEST/2007/119) from Generalitat Valenciana, Conselleria d' Empresa, Universitat i Ciència. Spain.

## 8. REFERENCES

1. J.F. Greden: Anxiety or caffeinism: a diagnostic dilemma. *Am J Psychiatry* 131, 1089-1092 (1974)

2. T.W. Uhde, J.P. Boulenger, D.C. Jimerson and R.M. Post: Caffeine: relationship to human anxiety, plasma MHPG and cortisol. *Psychopharmacol Bull* 20, 426-430 (1984)

3. M.S. Bruce: The anxiogenic effects of caffeine. *Postgrad Med J* 66 Suppl, 2:S18-24. (1990)

4. M.A. Lee, P. Flegel, J.F. Greden and O.G. Cameron: Anxiogenic effects of caffeine on panic and depressed patients. *Am J Psychiatry* 145, 632-635 (1988)

5. P.V. Nickell and T.W. Uhde. Dose-response effects of intravenous caffeine in normal volunteers. *Anxiety* 1, 161-168 (1994)

6. J.P. Boulenger, T.W. Uhde, E.A. Wolf and R.M. Post: Increased sensitivity to caffeine in patients with panic disorders: preliminary evidence. Arch Gen Psychiatry, 41 1067-1071 (1984)

7. J. Gea, J. de Pablo, J. Marti, C. Picado, A. Pujol and A. Agusti-Vidal: Anxiety crisis (panic attack) secondary to the administration of theophylline in an oral sustained-release preparation. Rev Clin Esp 183, 280 (1988)

8. D.S. Charney, G.R. Heninger and P.I. Jatlow: Increased anxiogenic effects of caffeine in panic disorders. Arch. Gen. Psychiatry 42, 233-243 (1985)

9. E. DeMet, M.K. Stein, C. Tran, A. Chicz-DeMet, C. Sangdahl and J. Nelson: Caffeine taste test for panic disorder: adenosine receptor supersensitivity. Psychiatry Res 30, 231-242 (1989)

10. E. Klein, J. Zohar, M.F. Geraci, D.L. Murphy and T.W. Uhde: Anxiogenic effects of m-CPP in patients with panic disorder: comparison to caffeine's anxiogenic effects. Biol Psychiatry 30, 973-984 (1991)

11. F. Jenck, J.L. Moreau and J.R. Martin: Dorsal periaqueductal gray-induced aversion as a simulation of panic anxiety: elements of face and predictive validity. *Psychiatry Res* 57, 181-191 (1995)

12. A.S. Lin, T.W. Uhde, S.O. Slate, and U.D. McCann: Effects of intravenous caffeine administered to healthy males during sleep. *Depress Anxiety* 5, 21-28 (1997)

13. M.B. Stein, B. Black, T.M. Brown and T.W. Uhde: Lack of efficacy of the adenosine reuptake inhibitor dipyridamole in the treatment of anxiety disorders. Biol. Psychiatry 33, 647-650 (1993)

14. J.D. Geiger and G.B. Glavin: Adenosine receptor activation in brain reduces stress-induced ulcer formation. Eur J Pharmacol 115, 185-190 (1985)

15. V.S. Westerberg and J.D. Geiger: Central effects of adenosine analogs on stress-induced gastric ulcer formation. Life Sci 41, 2201-2205 (1987)

16. I. Iancu, O.T. Dolberg and J. Zohar: Is caffeine involved in the pathogenesis of combat-stress reaction?. Mil Med 161, 230-232 (1996)

17. J.C. Woodson, T.R. Minor and R.F. Job: Inhibition of adenosine deaminase by erythro-9- 2-hydroxy-3-nonyl adenine EHNA mimics the effect of inescapable shock on escape learning in rats. *Behav. Neurosci* 112, 399-409 (1998)

18. S.K. Kulkarni, K. Singh and M. Bishnoi: Involvement of adenosinergic receptors in anxiety related behaviours. *Indian J Exp Biol* 45, 439-443 (2007)

19.H. Jr. Zangrossi, J.R. Leite and F.G. Graeff: Anxiolytic effect of carbamazepine in the elevated plus-maze: possible role of adenosine. *Psychopharmacology* 106, 85-89 (1992)

20. J. Chen, L. Rinaldo, S.J. Lim, H. Young, R.O. Messing and D.S. Choi: The type 1 equilibrative nucleoside transporter regulates anxiety-like behavior in mice. *Genes Brain Behav* (2007)

21. H.A. Baldwin and S.E. File: Caffeine-induced anxiogenesis: the role of adenosine, benzodiazepine and noradrenergic receptors. *Pharmacol Biochem Behav* 32, 181-186 (1989)

22. S.K. Bhattacharya, K.S. Satyan and A. Chakrabarti: anxiogenic action of caffeine: an experimental study in rats. *J Psychopharmacol* 11, 219-224 (1997)

23. M. Imaizumi, S. Miyazaki and K. Onodera: Effects of xanthine derivatives in a light/dark test in mice and the contribution of adenosine receptors. *Methods Find Exp Clin Pharmacol,* 16, 639-644 (1994)

24. M. El Yacoubi, C. Ledent, M. Parmentier, J. Costentin and J.M. Vaugeois: The anxiogenic-like effect of caffeine in two experimental procedures measuring anxiety in the mouse is not shared by selective A(2A) adenosine receptor antagonists. *Psychopharmacology* 148, 153-163 (2000)

25. R.D. Prediger, L.C. Batista and R.N. Takahashi: Adenosine A1 receptors modulate the anxiolytic-like effect of ethanol in the elevated plus-maze in mice. *Eur J Pharmacol* 499, 147-154 (2004)

26. H. Kayir and I.T. Uzbay: Nicotine antagonizes caffeine- but not pentylenetetrazole-induced anxiogenic effect in mice. *Psychopharmacology* 184, 464-469 (2006)

27. C. Florio, A. Prezioso, A. Papaioannou and R. Vertua: Adenosine A1 receptors modulate anxiety in CD1 mice. *Psychopharmacology* 136, 311-319 (1998)

28. G. Griebel, M. Saffroy-Spittler, R. Misslin, D. Remmy, E. Vogel and J.J. Bourguignon: Comparison of the behavioural effects of an adenosine A1/A2-receptor antagonist, CGS 15943A, and an A1-selective antagonist, DPCPX. *Psychopharmacology* 103, 541-544 (1991)

29. S. Vautrin, Y. Pelloux and J. Costentin: Preference for caffeine appears earlier in non-anxious than in anxious mice. *Neurosci Lett* 386, 94-98 (2005)

30. B.B. Fredholm, M.P. Abbracchio, G. Burnstock, J.W. Daly, T.K. Harden, K.A. Jacobson, P. Leff and M. Williams: Nomenclature and classification of purinoceptors. *Pharmacol Rev* 46, 143-156 (1994)

31. B.B. Fredholm, K. Battig, J. Holmen, A. Nehlig and E.E. Zvartau: Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 51, 83-133 (1999)

32. R.R. Goodman and S.H. Snyder: Kappa opiate receptors localized by autoradiography to deep layers of cerebral cortex: relation to sedative effects. *Proc Natl Acad Sci U S A* 79, 5703-5707 (1982)

33. J. Fastbom, A. Pazos, and J.M. Palacios: The distribution of adenosine A1 receptors and 5'-nucleotidase in the brain of some commonly used experimental animals. *Neuroscience* 22, 813-826 (1987)

34. B. Johansson, L. Halldner, T.V. Dunwiddie, S.A. Masino, W. Poelchen, L. Gimenez-Llort, R.M. Escorihuela, A. Fernandez-Teruel, Z. Wiesenfeld-Hallin, X.J. Xu, A. Hardemark, C. Betsholtz, E. Herlenius and B.B. Fredholm: Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. *Proc Natl Acad Sci U S A* 98, 9407-9412 (2001)

35. L. Gimenez-Llort, A. Fernandez-Teruel, R.M. Escorihuela, B.B. Fredholm, A. Tobena, M. Pekny and B. Johansson: Mice lacking the adenosine A1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate. *Eur J Neurosci* 16, 547-550 (2002)

36. U.E. Lang, F. Lang, K. Richter, V. Vallon, H.P. Lipp, J. Schnermann and D.P. Wolfer: Emotional instability but intact spatial cognition in adenosine receptor 1 knock out mice. *Behav Brain Res* 145, 179-188 (2003)

37. N. Jain, N. Kemp, O. Adeyemo, P. Buchanan and T.W. Stone: Anxiolytic activity of adenosine receptor activation in mice. *Br J Pharmacol* 116, 2127-2133 (1995)

38. R.D. Prediger, G.E. Da Silva, L.C. Batista, A.L. Bittencourt and R.N. Takahashi: Activation of adenosine A1 receptors reduces anxiety-like behavior during acute ethanol withdrawal (hangover) in mice. *Neuropsychopharmacology* 31, 2210-20 (2006)

39. T. Maemoto, M. Tada, T. Mihara, N. Ueyama, H. Matsuoka, K. Harada, T. Yamaji, K. Shirakawa, S. Kuroda, A. Akahane, A. Iwashita, N. Matsuoka and S. Mutoh: Pharmacological characterization of FR194921, a new potent, selective, and orally active antagonist for central adenosine A1 receptors. *J Pharmacol Sci* 96, 42-52 (2004)

40. R.L. Commissaris, T.C. McCloskey, G.M. Damian, B.D. Brown, R.A. Barraco and HJ Altman: Antagonism of the anti-conflict effects of phenobarbital, but not diazepam, by the A-1 adenosine agonist l-PIA. *Psychopharmacology* 102, 283-290 (1990)

41. J.L. Moreau and G. Huber: Central adenosine A(2A) receptors: an overview. *Brain Res Brain Res Rev* 31, 65-82 (1999)

42. J. Deckert, M.M. Nothen, P. Franke, C. Delmo, J. Fritze, M. Knapp, W. Maier, H. Beckmann and P. Propping: Systematic mutation screening and association study of the A1 and $A_{2A}$ adenosine receptor genes in panic disorder suggest a contribution of the A 2A gene to the development of disease *Mol Psychiatry* 3, 81-85 (1998)

43. S.P. Hamilton, S.L. Slager, A.B. De Leon, G.A. Heiman, D.F. Klein, S.E. Hodge, M.M. Weissman, A.J. Fyer and J.A. Knowles: Evidence for genetic linkage between a polymorphism in the adenosine 2A receptor and panic disorder. *Neuropsychopharmacology* 29, 558-565 (2004)

44. K. Yamada, E. Hattori, M. Shimizu, A. Sugaya, H. Shibuya and T. Yoshikawa: Association studies of the cholecystokinin B receptor and A2a adenosine receptor genes in panic disorder. *J Neural Transm* 108: 837-848 (2001)

45. P. Lam, C.J. Hong and S.J. Tsai: Association study of A2a adenosine receptor genetic polymorphism in panic disorder. *Neurosci Lett* 378, 98-101 (2005)

46. K. Alsene, J. Deckert, P. Sand and H. De Wit: Association between $A_{2A}$ receptor gene polymorphisms and caffeine-induced anxiety. Neuropsychopharmacology 28, 1694-1702 (2003)

47. C. Ledent, J.M. Vaugeois, S.N. Schiffmann, T. Pedrazzini, M. El Yacoubi, J.J. Vanderhaeghen, J. Costentin, J.K. Heath, G. Vassart and M. Parmentier: Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. *Nature* 388, 674-678 (1997)

48. M. El Yacoubi, C. Ledent, M. Parmentier, J. Costentin and J.M.Vaugeois: Reduced appetite for caffeine in

adenosine A(2A) receptor knockout mice. *Eur J Pharmacol* 519, 290-301 (2005)

49. S. Jegou, M. El Yacoubi, L. Mounien, C. Ledent, M. Parmentier, J. Costentin, J.M. Vaugeois and H. Vaudry: Adenosine A2A receptor gene disruption provokes marked changes in melanocortin content and pro-opiomelanocortin gene expression. *J Neuroendocrinol* 15, 1171-1177 (2003)

50. A. Thorsell, J. Johnson and M. Heilig: Effect Of The Adenosine $A_{2A}$ Receptor Antagonist 3,7-Dimethyl-Propargylxanthine On Anxiety-Like And Depression-Like Behavior And Alcohol Consumption In Wistar Rats. *Alcohol Clin Exp Res* 31, 1302-1307 (2007)

51. L. Gimenez-Llort, S.N. Schiffmann, T. Shmidt, L. Canela, L. Camon, M. Wassholm, M. Canals, A. Terasmaa, A. Fernandez-Teruel, A. Tobeña, E. Popova, S. Ferre, L. Agnati, F. Ciruela, E. Martinez, J. Scheel-Kruger, C. Lluis, R. Franco, K. Fuxe and M. Bader: Working memory deficits in transgenic rats overexpressing human adenosine $A_{2A}$ receptors in the brain. *Neurobiol Learn Mem* 87, 42-56 (2007)

52. M. Karcz-Kubicha, K. Antoniou, A. Terasmaa, D. Quarta, M. Solinas, Z. Justinova, A. Pezzola, R. Reggio, C.E. Muller, K. Fuxe, S.R. Goldberg, P. Popoli, and S. Ferré: Involvement of adenosine A1 and $A_{2A}$ receptors in the motor effects of caffeine after its acute and chronic administration. *Neuropsychopharmacology* 28:1281-1291 (2003)

53. B.B. Fredholm, A.P. Ijzerman, K.A. Jacobson, K.N. Klotz and J. Linden: International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors *Pharmacol Rev* 53, 527-552 (2001)

54. R.A Cunha: Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. *Neurochem Int* 38, 107-125 (2001)

55. J.A. Ribeiro, A.M. Sebastiao and A. de Mendonca: Participation of adenosine receptors in neuroprotection. *Drug News Perspect* 16, 80-86 (2003)

56. S. Ferre, I. Diamond, S.R. Goldberg, L. Yao, S.M. Hourani, Z.L. Huang, Y. Urade and I. Kitchen. Adenosine A(2A) receptors in ventral striatum, hypothalamus and nociceptive circuitry Implications for drug addiction, sleep and pain. *Prog Neurobiol* (2007)

57. J.L. Short, C. Ledent, E. Borrelli, J. Drago and A.J. Lawrence: Genetic interdependence of adenosine and dopamine receptors: evidence from receptor knockout mice. *Neuroscience* 139, 661-670 (2006)

58. J.S. Fink, D.R. Weaver, S.A. Rivkees, R.A. Peterfreund, A.E. Pollack, E.M. Adler and S.M. Reppert: Molecular cloning of the rat A2 adenosine receptor: selective co-expression with D2 dopamine receptors in rat striatum. *Brain Res Mol Brain Res* 14, 186-195 (1992)

59. S.N. Schiffmann, O. Jacobs and J.J. Vanderhaeghen: Striatal restricted adenosine A2 receptor (RDC8) is expressed by enkephalin but not by substance P neurons: an *in situ* hybridization histochemistry study. *J Neurochem* 57, 1062-1067 (1991)

60. S. Ferre, B.B. Fredholm, M. Morelli, P. Popoli and K. Fuxe Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci* 20, 482-487 (1997)

61. M. Canals, D. Marcellino, F. Fanelli, F. Ciruela, P. de Benedetti, S.R. Goldberg, K. Neve, K. Fuxe, L.F. Agnati, A.S. Woods, S. Ferre, C. Lluis, M. Bouvier and R. Franco: Adenosine A2A-dopamine D2 receptor-receptor heteromerization: qualitative and quantitative assessment by fluorescence and bioluminescence energy transfer. *J Biol Chem* 278, 46741-46749 (2003)

62. S.N. Schiffmann, G. Fisone, R. Moresco, R.A. Cunha and S. Ferre: Adenosine A(2A) receptors and basal ganglia physiology. *Prog Neurobiol* (2007)

63. J.L. Short, C. Ledent, J. Drago and A.J.Lawrence: Receptor crosstalk: characterization of mice deficient in dopamine D1 and adenosine A2A receptors. *Neuropsychopharmacology* 31, 525-534 (2006)

64. C. Hohoff, J.M. McDonald, B.T. Baune, E.H. Cook, J. Deckert and H. de Wit: Interindividual variation in anxiety response to amphetamine: possible role for adenosine A2A receptor gene variants. *Am J Med Genet B Neuropsychiatr Genet* 139, 42-44 (2005)

65. G. Biggio, A. Concas, M.G. Corda, O. Giorgi, E. Sanna and M. Serra: GABAergic and dopaminergic transmission in the rat cerebral cortex: effect of stress, anxiolytic and anxiogenic drugs. *Pharmacol Ther* 48, 121-142 (1990)

66. A.S. Bender, J.W. Phillis, P.H. Wu: Diazepam and flurazepam inhibit adenosine uptake by rat brain synaptosomes. *J Pharm Pharmacol* 32, 293-294 (1980)

67. J.W. Phillis, A.S. Bender and P.H. Wu: Benzodiazepines inhibit adenosine uptake into rat brain synaptosomes. *Brain Res* 195, 494-498 (1980)

68. J.W. Phillis, P.H. Wu and A.S. Bender: Inhibition of adenosine uptake into rat brain synaptosomes by the benzodiazepines. *Gen Pharmacol* 12, 67-70 (1981)

69. G.B. Kaplan, M.M. Cotreau and D.J. Greenblatt: Effects of benzodiazepine administration on A1 adenosine receptor binding in-vivo and ex-vivo. *J Pharm Pharmacol* 44, 700-703 (1992)

70. M. Hawkins, W. Pan, P. Stefanovich and M. Radulovacki: Desensitization of adenosine A2 receptors in the striatum of the rat following chronic treatment with diazepam. *Neuropharmacology* 27, 1131-1140 (1988)

71. R.A. Cunha and J.A. Ribeiro: ATP as a presynaptic modulator. *Life Sci* 68, 119-137 (2000)

72. I.P. Kirk and P.J. Richardson: Further characterization of [3H]-CGS 21680 binding sites in the rat striatum and cortex. *Br J Pharmacol* 114, 537-543 (1995)

73. J.T. Bartrup and T.W. Stone: Inhibition of adenosine responses of rat hippocampal neurones by nifedipine and BAYK 8644. *Brain Res* 525:315-8. (1990)

74. J.T. Bartrup, J.I. Addae, and T.W. Stone: Depression of purine induced inhibition during NMDA receptor mediated activation of hippocampal pyramidal cells--an iontophoretic study. *Brain Res* 564, 323-327 (1991)

75. C.M. Fraser, M.J. Cooke, A. Fisher, I.D. Thompson and T.W. Stone: Interactions between ifenprodil and dizocilpine on mouse behaviour in models of anxiety and working memory. *Eur Neuropsychopharmacol* 6, 311-316 (1996)

76. C.M. Fraser, A. Fisher, M.J. Cooke, I.D. Thompson, and T.W.Stone: The involvement of adenosine receptors in the effect of dizocilpine on mice in the elevated plus-maze. *Eur Neuropsychopharmacol* 7, 267-273 (1997)

77. R. Corradetti, G. Lo Conte, F. Moroni, M.B. Passani, G. Pepeu: Adenosine decreases aspartate and glutamate release from rat hippocampal slices. *Eur J Pharmacol* 104, 19-26 (1984)

78. J. Fastbom and B.B: Fredholm Inhibition of [3H] glutamate release from rat hippocampal slices by L phenylisopropyladenosine. *Acta Physiol Scand* 125, 121-123 (1985)

79. S.P. Burke and J.V. Nadler: Regulation of glutamate and aspartate release from slices of the hippocampal CA1 area: effects of adenosine and baclofen. *J Neurochem* 51, 1541-1551 (1988)

80. L.O. Trussell and M.B. Jackson: Adenosine-activated potassium conductance in cultured striatal neurons. *Proc Natl Acad Sci U S A* 82, 4857-4861 (1985)

81. P. Carriba, O. Ortiz, K. Patkar, Z. Justinova, J. Stroik, A. Themann, C. Muller, A.S. Woods, B.T. Hope, F. Ciruela, V. Casado, E.I. Canela, C. Lluis, S.R. Goldberg, R. Moratalla, R. Franco, S. Ferre: Striatal Adenosine A(2A) and Cannabinoid CB(1) Receptors Form Functional Heteromeric Complexes that Mediate the Motor Effects of Cannabinoids. *Neuropsychopharmacology* (2007)

82. F. Berrendero, A. Castane, C. Ledent, M. Parmentier, R. Maldonado and O. Valverde: Increase of morphine withdrawal in mice lacking A2a receptors and no changes in CB1/A2a double knockout mice. *Eur J Neurosci* 17, 315-324 (2003)

83. A. Bailey, L. Davis, H.M. Lesscher, M.D. Kelly, C. Ledent, SM Hourani and I. Kitchen: Enhanced morphine withdrawal and micro-opioid receptor G-protein coupling in A2A adenosine receptor knockout mice. *J Neurochem* 88, 827-834 (2004)

84. G. Soria, A. Castane, F. Berrendero, C. Ledent, M. Parmentier, R Maldonado and O. Valverde: Adenosine A2A receptors are involved in physical dependence and place conditioning induced by THC. *Eur J Neurosci* 20, 2203-2213 (2004)

85. M. Navarro, E. Hernandez, R.M. Munoz, I. del Arco, M.A. Villanua, M.R. Carrera and F. Rodriguez de Fonseca: Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. *Neuroreport* 8, 491-496 (1997)

86. M. Martin, C. Ledent, M. Parmentier, R. Maldonado and O. Valverde: Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology* 159, 379-387 (2002)

87. E. Fride, R. Suris, J. Weidenfeld and R. Mechoulam: Differential response to acute and repeated stress in cannabinoid CB1 receptor knockout newborn and adult mice. *Behav Pharmacol* 16, 431-440 (2005)

88. M. Correa, J.D. Salamone and C.M.G. Aragon: Central and peripheral effects of ethanol and acetaldehyde on measures of anxiety in rats. *Behavioural Pharmacology* 16, (1): S19 (2005)

89. D.A. Finn and J.C. Crabbe: Exploring alcohol withdrawal syndrome. *Alcohol Health Res World* 21, 149-156 (1997)

90. M. Clark and M.S. Dar: Effect of acute ethanol on release of endogenous adenosine from rat cerebellar synaptosomes. *J Neurochem* 52, 1859-1865 (1989)

91. B.B. Fredholm and A.Wallman-Johansson: Effects of ethanol and acetate on adenosine production in rat hippocampal slices. *Pharmacol Toxicol* 79, 120-123 (1996)

92. L.E. Nagy, I. Diamond, D.J. Casso, C. Franklin and A.S. Gordon: Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. *J Biol Chem* 265, 1946-1951 (1990)

93. L.E. Nagy: Ethanol metabolism and inhibition of nucleoside uptake lead to increased extracellular adenosine in hepatocytes. *Am J Physiol* 262 C1175-C1180 (1992)

94. M. El Yacoubi, C. Ledent, M. Parmentier, J. Costentin and J.M. Vaugeois: Caffeine reduces hypnotic effects of alcohol through adenosine $A_{2A}$ receptor blockade. *Neuropharmacology* 45, 977-985 (2003)

95. F.J. Carmichael, Y. Israel, M.W. Crawford, K. Minhas, V. Saldivia, S. Sandrin, P. Campisi and H. Orrego: Central nervous system effects of acetate: contribution to the central effects of ethanol. *J Pharmacol Exp Ther* 259, 403-408 (1991)

96. Y. Kiselevski, N. Oganesian, S. Zimatkin, A. Szutowicz, S. Angielski, P. Niezabitowski W. Uracz and R.J. Gryglewski: Acetate metabolism in brain mechanisms of adaptation to ethanol. *Med Sci Monit* 9, 178-182 (2003)

97. P. Campisi, F.J.L. Carmichael, M. Crawford, H. Orrego and J.M. Khanna: Role of adenosine in the ethanol-induced potentiation of the effects of general anesthetics in rats. *Europ J Pharmacol* 325, 165-172 (1997)

98. F. Lundquist, N. Tygstrup, K. Winkler, K. Mellemgaard and S. Munck-Petersen: Ethanol metabolism and production of free acetate in the human liver. *J Clin Invest* 41, 955-961 (1962)

99. H. Busch: Studies on the metabolism of acetate-1-C in tissues of tumor-bearing rats. *Cancer Res* 13, 789-794 (1953)

100. C.J. Van den Berg, P. Mela and H.Waelsch: On the contribution of the tricarboxylic acid cycle to the synthesis of glutamate, glutamine and aspartate in brain. *Biochem Biophys Res Commun* 23, 479-484 (1966)

101. S. Berl and T.L. Frigyesi: The turnover of glutamate, glutamine, aspartate and GABA labeled with [1-14C]acetate in caudate nucleus, thalamus and motor cortex (cat). *Brain Res* 12, 444-455 (1969)

102. K.T. Kiviluoma, K.J. Peuhkurinen and I.E. Hassinen: Adenine nucleotide transport and adenosine production in isolated rat heart mitochondria during acetate metabolism. *Biochim Biophys Acta* 974, 274-281 (1989)

103. C.S. Liang and J.M. Lowenstein: Metabolic control of the circulation. Effects of acetate and pyruvate. *J Clin Invest* 62 1029-1038 (1978)

104. J.G. Puig and I.H. Fox: Ethanol-induced activation of adenine nucleotide turnover. Evidence for a role of acetate. *J Clin Invest* 74, 936-941 (1984)

105. M.S. Dar, S.J. Mustafa and W.R. Wooles: Possible role of adenosine in the CNS effects of ethanol. *Life Sci* 33, 1363-1374 (1983)

106. T.V. Dunwiddie, A.S. Basile and M.R. Palmer: Electrophysiological responses to adenosine analogs in rat hippocampus and cerebellum: evidence for mediation by adenosine receptors of the A1 subtype. *Life Sci* 34, 37-47 (1984)

107. M.S. Dar: Central adenosinergic system involvement in ethanol-induced motor incoordination in mice. *J Pharmacol Exp Ther* 255, 1202-1209 (1990)

108. Y. Israel, H. Orrego and F.J. Carmichael: Acetate-mediated effects of ethanol. *Alcohol Clin Exp Res* 18, 144-148 (1994)

109. M. Correa, M.N. Arizzi, A. Betz, S. Mingote and J.D. Salamone: Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Research Bulletin* 62, 197-202 (2003)

110. M.N. Arizzi, M. Correa, A. Betz, A. Wisniecki and J.D. Salamone: Behavioral effects of intraventricular injections of low doses of ethanol, acetaldehyde and acetate in rats: studies with low and high rate operant schedules. *Behavioral Brain Research* 147, 203-210 (2003)

111. W.R. Proctor, R.C. Baker and T.V. Dunwiddie: Differential CNS sensitivity to PIA and theophylline in long-sleep and short-sleep mice. *Alcohol* 2, 387-391 (1985)

112. B.B. Fredholm, N.R. Zahniser, G.R. Weiner, W.R. Proctor and T.V. Dunwiddie: Behavioural sensitivity to PIA in selectively bred mice is related to a number of A1 adenosine receptors but not to cyclic AMP accumulation in brain slices. *Eur J Pharmacol* 111, 133-136 (1985)

113. Z.H. Meng and M.S. Dar: Possible role of striatal adenosine in the modulation of acute ethanol-induced motor incoordination in rats. *Alcohol Clin Exp Res* 19, 892-901 (1995)

114. M. Naassila, C. Ledent and M. Daoust: Low ethanol sensitivity and increased ethanol consumption in mice lacking adenosine $A_{2A}$ receptors. *J Neurosci* 22, 10487-10493 (2002)

115. A. Concas, T. Cuccheddu, S. Floris, M.P. Mascia, and G. Biggio: 2-Chloro-N6-cyclopentyladenosine (CCPA), an adenosine A1 receptor agonist, suppresses ethanol withdrawal syndrome in rats. *Alcohol Alcohol* 29, 261-264 (1994)

116. M.B. Gatch, C.J. Wallis and H. Lal: The effects of adenosine ligands R-PIA and CPT on ethanol withdrawal. *Alcohol* 19, 9-14 (1999)

117. M. Correa, A. Wisniecki, A. Betz, D.R. Dobson, M.F. O'Neill, M.J. O'Neill and J.D.Salamone: The adenosine A2A antagonist KF17837 reverses the locomotor suppression and tremulous jaw movements induced by haloperidol in rats: possible relevance to parkinsonism. *Behavioral Brain Research*. 148, 47-54. (2004)

118. P. Jenner. Istradefylline, a novel adenosine A2A receptor antagonist, for the treatment of Parkinson's disease. *Exp Opin Investig Drugs* 14, 729-38 (2005)

119. K. Ishiwari, L.J. Madson, A.M. Farrar, S. Mingote, J.P. Valenta, M.D. DiGianvittorio, L.E. Frank, M. Correa, J. Hockemeyer, C. Müller, and J.D. Salamone: Injections of the selective adenosine $A_{2A}$ antagonist MSX-3 into the nucleus accumbens core attenuate the locomotor suppression induced by haloperidol in rats. *Behav Brain Res* 178, 190-199 (2007)

120. J.D. Salamone, A. Betz, K. Ishiwari, J. Felsted, L. Madson, B. Mirante, K. Clark, L. Font, S. Korbey, T.N. Sager, J. Hockemeyer and C. Müller: Systemic or

intrastriatal injections of adenosine $A_{2A}$ antagonists reverse the oral tremor induced by antipsychotic agents: studies with rodent models of drug-induced parkinsonism. *Front Biosci* 13, 3594-3605 (2008)

121. A.M. Farrar, M. Pereira, F. Velasco, J. Hockemeyer, C.E. Müller and J.D. Salamone: Adenosine A(2A) receptor antagonism reverses the effects of dopamine receptor antagonism on instrumental output and effort-related choice in the rat: implications for studies of psychomotor slowing. *Psychopharmacology* 191, 579-586 (2007)

122. M. El Yacoubi, J. Costentin and J.M. Vaugeois: Adenosine A2A receptors and depression. *Neurology* 61, Suppl 6:S72-73 (2003)

123. K.A. Jacobson, D.K. von Lubitz, J.W. Daly and B.B. Fredholm: Adenosine receptor ligands: differences with acute versus chronic treatment. *Trends Pharmacol Sci* 17, 108-113 (1996)

124. S.E. File, H.A. Baldwin, A.L. Johnston and L.J. Wilks: Behavioral effects of acute and chronic administration of caffeine in the rat. *Pharmacol Biochem Behav* 30, 809-815 (1988)

**Abbreviations:** HPA: hypothalamic-pituitary-adrenal axis, ACTH: adrenocorticotropine, ENT1: type 1 equilibrative nucleoside transporter, IBMX: 3-isobutyl-1-methylxantine, CGS 15943: 9-Chloro-2-(2-furyl)[1,2,4]triazolol[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, I-PIA: N6-R-phenylisopropyladenosine, CPA: *N*6-cyclopentyladenosine, CCPA: 2-chloro-N6-cyclopentyladenosine, POMC: proopiomelanocortin, DMPX: 3,7-Dimethyl-Propargylxanthine, ZM241385: 4-(2-[7-amino-2- 2-fur-yl 1,2,4 triazolo- 2,3-a 1,3,5 triazin-5-yl-amino] ethyl)phenol), SCH5826: (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5*c*]pyrimidine), DPMA: N6-[2-(3,5-dimethoxyphenyl)-2(2 methylphenyl)-ethyl]adenosine), CGS 21680: (CGS 21680-2-p-(2-carboxyethyl)phenethylamino-5'-N-ethylcarboxamidoadenosine hydrochloride), ATP: adenosine triphosphate, AMP: adenosine monophosphate

**Key Words:** Anxiety, Panic Attack, Post-Traumatic Stress Disorder, Phobic State, Adenosine, Adenosine $A_{2A}$ receptors, adenosine $A_1$ Receptors, Dopamine, Glutamate, GABA, Endocannabinoids, Ethanol, Acetate, Plus Maze, Review

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