Adenosine $A_{2A}$ Receptors as Biomarkers of Brain Diseases

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Extracellular adenosine is produced with increased metabolic activity or stress, acting as a paracrine signal of cellular effort. Adenosine receptors are most abundant in the brain, where adenosine acts through inhibitory $A_1$ receptors to decrease activity/noise and through facilitatory $A_{2A}$ receptors ($A_{2A}R$) to promote plastic changes in physiological conditions. By bolstering glutamate excitotoxicity and neuroinflammation, $A_{2A}R$ also contribute to synaptic and neuronal damage, as heralded by the neuroprotection afforded by the genetic or pharmacological blockade of $A_{2A}R$ in animal models of ischemia, traumatic brain injury, convulsions/epilepsy, repeated stress or Alzheimer’s or Parkinson’s diseases. $A_{2A}R$ overfunction is not only necessary for the expression of brain damage but is actually sufficient to trigger brain dysfunction in the absence of brain insults or other disease triggers. Furthermore, $A_{2A}R$ overfunction seems to be an early event in the demise of brain diseases, which involves an increased formation of ATP-derived adenosine and an up-regulation of $A_{2A}R$. This prompts the novel hypothesis that the evaluation of $A_{2A}R$ density in afflicted brain circuits may become an important biomarker of susceptibility and evolution of brain diseases once faithful PET ligands are optimized. Additional relevant biomarkers would be measuring the extracellular ATP and/or adenosine levels with selective dyes, to identify stressed regions in the brain. $A_{2A}R$ display several polymorphisms in humans and preliminary studies have associated different $A_{2A}R$ polymorphisms with altered morphofunctional brain endpoints associated with neuropsychiatric diseases. This further prompts the interest in exploiting $A_{2A}R$ polymorphic analysis as an ancillary biomarker of susceptibility/evolution of brain diseases.

Keywords: adenosine $A_{2A}$ receptors, central nervous system, antagonism, caffeine, biomarkers, polymorphisms

INTRODUCTION

The increased use of intracellular ATP, either because of increased workload or need to cope with stressful conditions, is a main source of increased extracellular levels of adenosine, which generally acts as a paracrine allostatic regulator by locally decreasing metabolism through inhibitory $A_1$ receptors ($A_1R$) and increasing metabolic supply through $A_{2A}R$ (Agostinho et al., 2020). Adenosine receptors are most abundant in the brain, where adenosine fulfills a role as neuromodulator apart from its general paracrine allostatic role: post-synaptic as well as astrocytic integrative activity are major contributors of an adenosine tone acting through inhibitory $A_1$ receptors to decrease activity/noise in excitatory synapses; ATP release, characteristic of increased firing rate conditions associated with synaptic plasticity, is the major source of a second pool of synaptic extracellular adenosine selectively activating facilitatory $A_{2A}$ receptors ($A_{2A}R$) to promote synaptic plastic
changes in physiological conditions (Cunha, 2016). However, ATP is also a general danger signal in the brain (Rodrigues et al., 2015), acting through a variety of ATP/ADP-activated P2 receptors to re-shape the function of astrocytes and microglia to cope with potential threats (Agostinho et al., 2020). Such threats also require adaptive plastic changes in neuronal circuits, which may explain the increased extracellular formation of ATP-derived adenosine by ecto-nucleotidases, with a burst of its rate-limiting step—ecto-5′-nucleotidase or CD73 (Cunha, 2001)—under noxious brain conditions to sustain an overfunction of A2A R that contributes to synaptotoxicity and neurotoxicity in different brain diseases (Cunha, 2016).

**ADENOSINE A2A RECEPTORS IN BRAIN DISEASES**

Upon acute brain injury, probably best exemplified by an ischemic brain stroke, concurrent pharmacological and genetic evidence show that A2A R blockade affords a robust neuroprotection (reviewed in Chen and Pedata, 2008). In parallel, ischemia is accompanied by ATP release (Melani et al., 2005) and up-regulation of CD73 (Braun et al., 1997), thus increasing the formation of extracellular ATP-derived adenosine (Koos et al., 1997; Chu et al., 2014). Likewise, seizure-like activity characteristic of epileptic conditions triggers a neurodegeneration that is critically controlled by pharmacological or genetic A2A R blockade (Canas et al., 2018). Seizure activity also increases ATP release (Wieraszko and Seyfried, 1989) and up-regulates CD73 (e.g., Schoen et al., 1999; Rebola et al., 2003), increasing the contribution of extracellular ATP-derived adenosine formation to overactivate A2A R (reviewed in Tescarollo et al., 2020). A2A R blockade also attenuates brain damage following traumatic brain injury (TBI) (e.g., Li et al., 2009); TBI also bolsters the release of ATP (Faroqi et al., 2021) and CD73 levels (Zheng et al., 2020), although the contribution of extracellular ATP-derived adenosine has not yet been tested in TBI.

Overall, this evidence is compatible with an increase of extracellular adenosine, namely extracellular ATP-derived adenosine, leading to an overactivation of A2A R that contributes for brain dysfunction upon acute brain injury. A similar scenario seems to occur in chronic brain conditions. Thus, the pharmacological or genetic blockade of A2A R affords a consistent neuroprotection in animal models of Alzheimer’s disease (AD) (e.g., Canas et al., 2009; Laurent et al., 2016; Viana da Silva et al., 2016), Parkinson’s disease (PD) (reviewed in Schwarzschild et al., 2006)—where A2A R antagonists were approved by the US-FDA as novel anti-Parkinsoinian drugs (Chen and Cunha, 2020), repeated stress/depression (Batalla et al., 2013; Kaster et al., 2015; Padilla et al., 2018), Machado-Joseph disease (Gonçalves et al., 2013), amyotrophic lateral sclerosis (ALS) (Ng et al., 2015; Rei et al., 2020; Seven et al., 2020), Angelman syndrome (Moreira-de-Sá et al., 2020, 2021), or glaucoma-like disorders (Madeira et al., 2015). Most of these chronic neuropsychiatric conditions are also associated with increased release of ATP, as occurs in animal models of AD (Gonçalves et al., 2019), PD (Carmo et al., 2019; Meng et al., 2019) or as concluded by the anti-depressant effects of P2 receptor antagonists (Ribeiro et al., 2019; but see Cao et al., 2013). Moreover, there is an increased contribution of extracellular ATP-derived adenosine for A2A R overactivation in chronic brain diseases, as best heralded by the observation that CD73 knockout mice phenocopy A2A R knockout mice (Augusto et al., 2013; Carmo et al., 2019; Gonçalves et al., 2019).

A2A R overactivation is not only necessary, but actually sufficient to trigger brain dysfunction, as concluded from the observation that the pharmacological overactivation of A2A R (Pagnussat et al., 2015), the optogenetic activation of A2A R transducing system (Li et al., 2015) or the over-expression of A2A R in the hippocampus (Coelho et al., 2014; Carvalho et al., 2019; Temido-Ferreira et al., 2020) are sufficient to trigger or aggravate brain dysfunction. Notably, A2A R overfunction seems to be an early event in different brain disorders (reviewed in Cunha, 2016), although A2A R antagonists seem to maintain their neuroprotective profile after the establishment of symptoms (e.g., Kaster et al., 2015; Faivre et al., 2018; Orr et al., 2018; Silva et al., 2018).

The tight association between increased release of ATP and its extracellular catabolism to overactivate A2A R as part of the expression of neuronal dysfunction at the onset and throughout the evolution of several brain diseases prompts exploiting this danger signaling pathway as new biomarkers to identify dysfunctional brain circuits in brain diseases. Although the tools are yet to developed, it may be promising to devise soluble sensors to detect altered levels of extracellular ATP to allow an *in vivo* estimate of brain circuits undergoing a particular purinergic pressure and, consequently, are at risk of undergoing dysfunction. An alternative could be the development of PET ligands (not yet available) to assess the density of CD73, which is paramount to link ATP upsurge with the selective overactivation of A2A R; CD73 seems to be consistently up-regulated upon brain stressful conditions and may be a selective biomarker of glia and synapses undergoing adaptive processes (Schoen and Kreutzberg, 1997).

**UP-REGULATION OF ADENOSINE A2A RECEPTORS IN BRAIN DISEASES**

The A2A R overactivation associated with brain dysfunction and disease is not only sustained by an increased bioavailability of the trigger of A2A R—ATP-derived extracellular adenosine—but also involves an up-regulation of A2A R in the afflicted brain areas (reviewed in Cunha, 2016). Indeed, an increased density of cortical A2A R has been reported in animal models of epilepsy (Rebola et al., 2005; Cognato et al., 2010; Canas et al., 2018; Crespo et al., 2018), Rasmussen’s encephalopathy (He et al., 2020), TBI (Zhao et al., 2017), AD (Espinosa et al., 2013; Viana da Silva et al., 2016; Silva et al., 2018), Lyme neuroborreliosis (Smith et al., 2014), ALS (Seven et al., 2020), or chronic stress/depression (Kaster et al., 2015; Machado et al., 2017), as well as in the diseased human brain (Albasanz et al., 2008; Temido-Ferreira et al., 2020). Likewise, A2A R levels are also increased in the cerebellum of Machado-Joseph’s ataxic mice (Gonçalves et al., 2013) and in the
amygdala or fear-conditioned mice (Simões et al., 2016). A2AR up-regulation is in fact an upsurge since it occurs shortly (within hours) after abnormal neuronal function (i.e., convulsions; Canas et al., 2018), but it gradually increases with aggravation of brain dysfunction (Temido-Ferreira et al., 2020). A2AR up-regulation mostly occurs in synapses, in accordance with the involvement of synaptic alterations at the onset of most brain diseases (e.g., Rebola et al., 2005; Kaster et al., 2015; Viana da Silva et al., 2016; Canas et al., 2018), but is also observed in glia cells in the progression of chronic brain diseases (Matos et al., 2012; Orr et al., 2015; Barros-Barbosa et al., 2016; Patodia et al., 2020). It is still unclear if this A2AR up-regulation only involves an increased readout of A2AR mRNAs (Canas et al., 2018) or also involves an overexpression of A2AR mRNA, which has been reported in the dysfunctional or diseased brain (e.g., Costenla et al., 2011; Espinosa et al., 2013; Hu et al., 2016; Dias et al., 2021). In fact, the triggers and mechanisms of this A2AR up-regulation in the diseased brain are essentially unknown. The A2AR gene in both.

### Table 1
Summary of the reported associations between known polymorphic variants of the human adenosine A2A receptor gene (ADORA2A) and different response susceptibility to either pathological threats (A) or distinct physiological responses to external stimuli (B).

| (A) SNP | Type/Position | Risk allele/Genotype | Associated CNS Disorder | References |
|---------|---------------|----------------------|-------------------------|------------|
| rs5751876 | Exon 2 | TT | Huntington's disease (significant variability in age of onset) | Taherzadeh-Fard et al., 2010 |
|         |             | T  | Susceptibility locus for Panic Disorder and Agoraphobia | Deckert et al., 1998; Hamilton et al., 2004; Domschke et al., 2012 |
| rs2298383 | 5' UTR | T  | Prevalent in Gilles de la Tourette syndrome (GTS) patients | Jank et al., 2015 |
| rs2236624 | Intron 4 | CC | Greater risk for CE patients to develop comorbid neurologic disorders | Freitag et al., 2010 |
| rs5996696 | Promoter variant region | C  | Inverse association with the likelihood to develop Parkinson's disease (~49%) | Popat et al., 2011 |
| rs2298383, rs571876, rs35320474, and rs4822492 | 5' UTR, Exon 2, Exon 4, and 3' UTR | C, T, deletion and C, respectively, (Haplotype A) | Predisposition of children to develop Acute Encephalopathy with biphasic seizures and late reduced diffusion (AEDS) | Shinohara et al., 2013 |

| (B) SNP | Type/Position | Risk allele/Genotype | Physiological Response to Stimuli | References |
|---------|---------------|----------------------|----------------------------------|------------|
| rs5751876 | Exon 2 | TT | Significant enhancement of caffeine-induced anxiety | Alsene et al., 2003; Childs et al., 2008 |
|         |             | TT | Associated with an overall lower caffeine intake and a prospective lesser vulnerability to caffeine dependence | Cornelis et al., 2007 |
|         |             | TT | Highest startle magnitudes upon caffeine administration in response to unpleasant pictures (maladaptive emotional processing) | Domschke et al., 2012 |
|         |             | TT | Associated with an ergogenic beneficial effect upon caffeine consumption | Loy et al., 2015 |
|         |             | TT | Higher anxiogenic response susceptibility to caffeine ingestion in usual non-consumers or low consumers (<40 mg per day), but no significant correlation with habitual caffeine intake | Rogers et al., 2010 |
|         |             | C  | Sleep disturbances (and insomnia) triggered by caffeine intake, higher β-activity in non-REM sleep | Rétey et al., 2007 |
|         |             | T  | Lower total sleep time in habitual low caffeine consumers | Erblang et al., 2019 |
|         |             | T  | Increased sleep latency associated with caffeine consumption, lower percentage of N3 sleep stage | Nunes et al., 2017 |
| rs2298383 | 5' UTR | CC | Significant enhancement of caffeine-induced anxiety | Childs et al., 2008 |
|         |             | C  | Lower total sleep time in habitual low caffeine consumers | Erblang et al., 2019 |
| rs4822492 | 3' UTR | CC | Significant enhancement of caffeine-induced anxiety | Childs et al., 2008 |
|         |             | CC | Lower total sleep time in habitual low caffeine consumers | Erblang et al., 2019 |
| rs3761422 | 5' UTR | TT | Greater increase in anxiety upon caffeine ingestion in habitual non-consumers or low consumers (<-40 mg per day) | Rogers et al., 2010 |
|         |             | T  | Lower total sleep time in habitual low caffeine consumers | Erblang et al., 2019 |
rodents and humans has a complex promoter region and can give rise to multiple transcripts (Peterfreund et al., 1996; Lee et al., 2003a; Yu et al., 2004; Kreth et al., 2008; Huin et al., 2019). Although multiple controllers of the A2A receptor (Adenosine A2A receptors) polymorphisms have been proposed, such as methylation patterns of the promoter (Falconi et al., 2019; Micioni Di Bonaventura et al., 2019), transcription factors ZBP-89 and Yin Yang-1 (Buira et al., 2010), microRNAs (e.g., Heyn et al., 2012; Villar-Menéndez et al., 2014; Zhao et al., 2015; Tian et al., 2016), NFK-B (Morello et al., 2006), cAMP-response element-binding protein (Chiang et al., 2005), hypoxia inducible factor-2α (Ahmad et al., 2009; Brown et al., 2011), AP1 transcription factor (Kobayashi and Millhorn, 1999; Lee et al., 2014), or nuclear factor 1 (Lee et al., 2003b), the regulation of the relative expression of these transcripts is largely unknown (Yu et al., 2004; Huin et al., 2019) and little is also known about the relative stability of the different mRNA transcripts. This is certainly an area of research that might open new avenues to design neuroprotective strategies linked to A2A receptor.

The association of A2A receptor overfunction with brain diseases offers another promising opportunity to develop informative biomarkers of the susceptibility and/or evolution of different brain diseases once PET ligands are optimized to detect extra-striatal A2A receptors. In fact, A2A receptors throughout the brain are most abundant in the striatum (reviewed in Svenningsson et al., 1999) and the available PET ligands have been optimized to detect striatal A2A receptors (e.g., Mishina et al., 2011; Ishibashi et al., 2018); however, this population of A2A receptors has a different pharmacology (Orrú et al., 2011; Cunha, 2016), a different adaptive profile (Cunha et al., 1995) and a different role in most brain conditions (Shen et al., 2008, 2013; Yu et al., 2008; Wei et al., 2014). Thus, it is likely that the currently available PET ligands might not be useful to assess modifications of extra-striatal A2A receptors. New cortical A2A receptor-directed PET ligands need to be designed based on the particular properties and interacting partners of cortical A2A receptors (reviewed in Franco et al., 2020) to allow an in vivo detection of A2A receptor upsurge as potential general biomarkers of brain dysfunction (Sun et al., 2020).

A2A receptors are not only located in the brain, but are also present in several peripheral tissues, namely in different blood cells such as leukocytes and platelets (reviewed in Gessi et al., 2000). Based on the association of brain diseases with A2A receptor up-regulation in afflicted brain regions, several studies explored if A2A receptors in blood cells could be biomarkers of brain diseases, such as AD (Arosio et al., 2010, 2016; Merighi et al., 2021), PD (Falconi et al., 2019), or ALS (Vincenzi et al., 2013). However, only the understanding of the mechanisms underlying A2A receptor up-regulation in brain diseases will allow providing a rationale (or lack of thereof) to consider alterations of the density of peripheral A2A receptors as valid readouts of altered A2A receptor density that occurs selectively in afflicted brain circuits in the diseased brain.

**DISCUSSION**

A2A receptor overfunction is necessary and actually sufficient for the expression of neuronal dysfunction upon brain diseases. In particular, A2A receptor overfunction associated with aberrant synaptic plasticity and synaptotoxicity seems to be associated with the onset of symptoms of brain diseases. However, some of these symptoms are comorbidities of other brain diseases, associated with their aggravation, which often involves a spreading of neuroinflammation, also known to be controlled by A2A receptors. Thus, it is also likely that A2A receptor overfunction might be also associated with the evolution of brain diseases. These neuropathological roles of A2A receptors prompts considering the exploitation of this system as candidate biomarkers of the susceptibility and evolution of brain diseases. The development of PET ligands with adequate signal-to-noise ratio and selectivity to detect the relevant extra-striatal A2A receptors may allow a minimally invasive assessment of A2A receptors in different brain regions. This may be complemented by the definition of A2A receptor polymorphisms as an ancillary biomarker for the susceptibility and evolution of brain diseases, which still requires a firm establishment of structural-functional relationships between A2A receptor polymorphisms and brain dysfunction. Finally, the future development of PET-based sensors of extracellular ATP and/or adenosine may well be of additional interest as a biomarker of the status of brain diseases to be used in complement of other available methods.

**AUTHOR CONTRIBUTIONS**

All authors contribute to the organization and writing of the review.

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Conflict of Interest: RC is a scientific consultant for the Institute for Scientific Information on Coffee.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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