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Dissertação

Mestrado Integrado em Medicina

POTENTIAL OF ADENOSINE-SYSTEM-BASED THERAPIES IN THE TREATMENT OF NEUROIMMUNE DISORDERS

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Contents

Abstract

One of the grand challenges in the 21st century is to unravel the molecular elements associated to the dynamic crosstalk between immune and neuronal cells in neurologic diseases, and then use that knowledge to design effective neuroimmune-mediated therapeutic strategies. In light of the critical ties between the nervous and immune systems, adenosine pathways may be a key element in the control of neuroimmune homeostasies since adenosine receptors (ARs) play an important role in the modulation of neuronal activity and inflammatory/immune responses. Indeed, the omnipresence of ARs in neurons, glia and immune cells makes adenosine a key regulator of many facets of brain function and, therefore, a valuable target under brain pathological conditions. Disorders under the umbrella of neuroimmunology are not just the prototypic immune-mediated central (CNS) and peripheral nervous system diseases, such as Multiple Sclerosis and *Myasthenia Gravis*. It is worth noting that virtually all of the major neurologic conditions (including Alzheimer's disease, cerebrovascular disease, epilepsy, Parkinson's, and CNS infection) are now recognized to have immune/inflammatory components. In the presence of inflammation and immune dysfunction, the adenosine system acts as a sensor, which through dynamic modification in the expression of ecto-enzymes and ARs, adapts neuronal tissue metabolism status and contributes to mechanisms deputed to neuroprotection. In keeping with these concepts it is becoming increasingly appreciated that drugs targeted on adenosine pathways can exert beneficial effects in neuroimmune disorders. This review aims to discuss the role of adenosine in the pathophysiology of neurodegenerative disorders in which the immune system plays a key role, such as Multiple Sclerosis, Alzheimer's, Parkinson's and Huntington's disease, as well as to highlight the mechanisms through which pharmacological modulation of the adenosine pathway may have potential application in their therapeutic management.

Keywords: Adenosine; Adenosine Receptors; Neurodegenerative disorders; Neuroimmune disorders; Adenosine-based therapies.

Resumo

Um dos grandes desafios no século XXI é a descoberta dos elementos moleculares envolvidos na interação dinâmica entre as células imunológicas e neuronais em doenças neurológicas e aplicar esse conhecimento no desenvolvimento de estratégias terapêuticas eficazes com mediação neuro-imunológica. Tendo em conta as conexões entre os sistemas nervoso e imunológico, o sistema adenosinérgico parece ser um elemento chave no controlo da homeostasia neuro-imunológica uma vez que os recetores de adenosina têm um papel importante na modulação tanto da atividade neuronal como das respostas inflamatórias/imunológicas. De fato, a omnipresença destes recetores nos neurónios, glia e em células do sistema imunológico faz da adenosina um regulador importante de vários aspetos da função cerebral e, portanto, um alvo terapêutico potencial nas patologias cerebrais. A área da neuroimunologia não abrange apenas as doenças de mediação imunológica prototípicas que afetam o sistema nervoso central e periférico, tais como a Esclerose Múltipla e a *Miastenia Gravis*. Na verdade, virtualmente todas as doenças neurológicas (tais como a doença cerebrovascular, infeção cerebral, doenças de Alzheimer e Parkinson e epilepsia) têm hoje uma componente inflamatória/imunológica reconhecida. Na presença de inflamação e disfunção imunológica, o sistema adenosinérgico atua como um sensor que através de modificações dinâmicas na expressão de ectoenzimas e dos seus recetores consegue adaptar o metabolismo dos neurónios e contribuir com efeitos neuroprotetores. Posto isto, tem vindo a crescer o interesse sobre os efeitos benéficos do controlo das vias da adenosina em doenças neuro-imunológicas. Este artigo de revisão tem objetivo de discutir o papel da adenosina na fisiopatologia de doenças neurodegenerativas em que o sistema imunológico tem um papel preponderante, tais como a Esclerose Múltipla e as doenças de Alzheimer, Parkinson e Huntington, assim como esclarecer os mecanismos pelos quais a modulação farmacológica das vias da adenosina poderá ter um lugar importante no seu tratamento.

Palavras-Chave: Adenosina; Receptores de Adenosina; Doenças Neurodegenerativas; Doenças Neuroimunes; Terapias baseadas na adenosina.

Abbreviation List:

AD – Alzheimer's Disease
ADA – Adenosine Deaminase
AK – Adenosine Kinase
AMP – Adenosine 5'-monophosphate
APP – Amyloid Precursor Protein
AR – Adenosine Receptor
ATP – Adenosine 5'-triphosphate
BDNF – Brain-derived neurotrophic factor
CB – Cannabinoid System
CCL2 – Chemokine (C-C motif) ligand 2
CD39 – Ecto-apyrase
CD73 – Ecto-'5-nucleotidase
CNS – Central Nervous System
COX - Ciclooxygenase
DR – Dopamine Receptor
EAE –Experimental Autoimmune Encephalomyelitis
ENT – Equilibrative nucleoside transporter
FOXP3 – Forkhead Box P3 protein
GABA – γ-Aminobutyric acid
GLT – Glutamate transporter
Gpi – Globus Pallidus, internal segment
HD – Huntington's Disease
IFN-γ – Interferon gamma
IL – Interleukin
iNOS – Inducible nitric oxide synthase
L-DOPA – 3,4-Dihydroxy-L-phenylalanine
MAO-B – Monoamine Oxidase B
mGlu – Metabotropic glutamate receptor
MHC – Major histocompatibility complex
MS – Multiple Sclerosis
MSN – Medium spiny neuron
NFT – Neurofibrillary Tangles
NGF – Nerve growth factor
NMDA – N-Methyl-D-aspartate
NO – Nitric Oxide
PD – Parkinson's Disease
SNc – Substantia Nigra pars compacta
SNr – Substantia Nigra pars reticulata
TGF-β – Transforming Growth Factor-beta
TNF-α – Tumor necrosis factor alpha
Trk receptors – Tropomyosin-related kinase receptors
UPS – Ubiquitin Proteossome

Introduction

Adenosine has emerged as an extracellular signalling molecule able to coordinate homeostasis in all cells with effects on tissue protection and repair (Linden 2005). However, in the nervous system, adenosine also exerts a rather specific neuromodulatory role, controlling synaptic transmission and synaptic plasticity (Gomes 2011) as well as coordinating neural networks (Sperlagh and Vizi 2011). The double action of this system led to the consideration of adenosine as a therapeutic target in the management of neurologic disorders.

Part of the role of adenosine seems to be related to the regulation of neuroinflammatory processes and modulation of immune responses, especially in pathological settings, offering neuroprotection potential and a means of control of neurodegenerative and neuroimmune conditions (Stone *et al.* 2009).

The combined effects of adenosine on neuronal viability and immune responses propelled extensive research in this field and a large body of evidence encouraging adenosine-system-based therapies has emerged.

Objectives

This review article aims to summarize the current evidence on the adenosine-mediated mechanisms in the pathophysiology of neuroimmune disorders, focusing on neurodegenerative and autoimmune diseases and to highlight therapeutical rationale and future potential of the adenosine system.

A short overview of the adenosine role in the nervous system function and in the brain immune system signaling will ensue, followed by clinical implications in particular neurologic disorders.

Adenosine in the Brain

There are four known subtypes of ARs – A_1, A_{2A}, A_{2B} and A_3 ARs (Fredholm *et al.* 2011). All of them are metabotropic G-protein-coupled receptors, each of which has a unique pharmacological profile, tissue distribution and effector coupling (Jacobson and Gao 2006). Traditionally, A_1 and A_3 ARs would negatively couple, while A_{2A} and A_{2B} ARs would positively couple to adenylate cyclase. However, since these receptors have shown to couple with different G proteins or different transducing systems in different cell types, localizations, and with varying degrees of activation, they are now considered pleiotropic receptors (Cunha 2005) (see Table 1).

With the observations of higher densities and more widespread distribution of these receptors and through the use of many available pharmacological tools and of receptor knockout mice strains (Wei *et al.* 2011 for review), the A_1 and A_{2A} ARs, have particularly shown impact on neuromodulation.

A_1 ARs are most abundant in brain cortex, cerebellum, hippocampus and dorsal horn of the spinal cord but they have a widespread distribution in the brain (Ribeiro *et al.* 2003). The A_{2A} ARs are predominantly located in the striatopallidal medium spiny γ-aminobutyric acid (GABAergic) neurons (MSN) and olfactory bulb, but their expression in several other brain regions is widely recognized, though in lower levels than in the striatum (Sebastião and Ribeiro 2009a).

Electrophysiological and biochemical studies designed to understand the role of adenosine in neuronal circuits concluded that adenosine inhibits neuronal excitability and synaptic transmission (Dunwiddie and Massino 2001). This is achieved through tonic pre-synaptic inhibition of neurotransmitter release by A_1 AR activation (Thompson 1992), primarily in excitatory synapses. In addition, A_1 ARs also act post-synaptically, influencing responsiveness to excitatory stimuli by a control of N-type calcium channels and N-methyl-D-aspartate (NMDA) receptors (de Mendonça *et al.* 1995; Klishin *et al.* 1995) and non-synaptically, inhibiting potassium conductance and leading to neuronal hyperpolarization (Greene and Haas 1991). On the contrary, extra-striatal pre-synaptic A_{2A} ARs facilitate the release of most neurotransmitter types, including GABA (Cunha and Ribeiro 2000). Indeed, the observation of co-localization of A_1 and A_{2A} ARs in hippocampal glutamatergic nerve terminals (Rebola *et al.* 2005) and of functional interaction between the two receptor subtypes (Lopes *et al.* 1999) led to the proposition that a A_1-A_{2A} ARs heteromer provides a synaptic adenosine concentration-dependent switch, namely producing opposing effects on glutamate release (Ferre *et al.* 2007).

Thus, A_1 ARs seem to be important at lower concentrations of adenosine, breaking excessive synaptic activation and providing neuroprotection in physiological and pathological contexts while A_{2A} ARs are stimulated with higher extracellular adenosine levels, particularly in stressful conditions, antagonizing the activation of A_1 AR, mediating excitotoxicity and fostering synaptic plasticity mechanisms (Cunha 2005). However, other factors such as the topographical arrangement of ectoenzymes, transporters and receptors as well as the neuronal firing frequency may also influence the A_1 versus A_{2A} AR-mediated actions at each synapse where both receptors co-localize (Sebastião and Ribeiro 2009c) (see Table 1).

Besides these direct actions of adenosine on the neurosecretory mechanisms, tuning neurotransmitter release, ARs (mostly of the A_{2A} subtype), show ability to oligomerize and functionally interact with other receptors, transporters, and intracellular second messenger systems, fine-tuning the action of several neurotransmitters and neuromodulators for example through cannabinoid (CB)1, metabotropic glutamate (mGlu)5, NMDA and dopamine D_2 receptors (D_2R) (Ribeiro and Sebastião 2010). In particular, A_{2A} ARs, can also interact with receptors for neurotrophic factors, namely through the transactivation of tropomyosin-related kinase (Trk)B receptors of brain-derived neurotrophic factor (BDNF) and the facilitation of BDNF actions on synapses (Sebastiao *et al.* 2011 for review) which may have several implications for neurodegenerative disorders.

The sources of extracellular adenosine are either release through an equilibrative bi-directional nucleoside transporter (ENT) or as a result of cell damage or nucleotidase-mediated hydrolysis of extracellular adenine nucleotides, which have their own signaling properties mediated by purinergic adenosine 5'-triphosphate (ATP) receptors (Burnstock 2012 for review). Moreover, ectonucleotidases like ecto-apyrase (CD39) and ecto-5'-nucleotidase (CD73) are upregulated upon increased neuronal firing and noxious brain conditions such as hypoxia, ischemia and cell poisoning (Fontella *et al.* 2004), probably in response to increases in ATP release. This elicits large increases in the concentration of extracellular adenosine, convening a valuable therapeutical target while governing purinergic signaling under pathological conditions (Yegutkin 2008 for review). Adenosine itself is metabolized by adenosine kinase (AK) and by adenosine deaminase (ADA) to adenosine 5'-monophosphate (AMP) and inosine, respectively. Notwithstanding, the precise location of the sites of formation and removal of extracellular adenosine and its sites of action need to be pinpointed to clarify the relation between extracellular adenosine gradients and the differential activation of each adenosine receptor subtype (Fredholm 2005).

Adenosine in Neuroglia / Neuroinflammation

Research and publications in this field have generally focused on the role of purinergic signaling in neurons. However, the past few years brought new insights into our understanding of the tripartite synapse and gliotransmission in neurological diseases (Halassa *et al.* 2009). Glial cells, originally regarded as a mere structural support for neurons, are now known to be active and responsive to environmental changes, able to detect and influence neuronal function and to communicate with other glial and vascular cells (Fields and Burnstock 2006). The purinergic system has emerged as the most compelling pathway for this intercellular crosstalk and this gains special relevance in pathological conditions, where this interplay could be modulated and afford neuroprotection. Another pronounced body of evidence similarly points towards the influences of the adenosinergic system in the chronic neuroinflammation hallmark of neurodegenerative conditions.

Astrocytes have several important physiological properties related to nervous system homeostasis (Parpura *et al.* 2012 for review). The observations that ATP is a major gliotransmitter *in vivo* (Halassa and Haydon 2010) and that the well-known tonic A_1 AR-mediated presynaptic inhibition of excitatory synaptic transmission is conveyed by adenosine derived from an astrocytic source *in vitro* and *in vivo* (Pascual *et al.* 2005; Halassa *et al.* 2009) demonstrate a potential of the purinergic system in the glial modulation of neuronal function. In addition, this interaction is bidirectional, with astrocytes detecting and responding to neuronal firing (Fellin *et al.* 2006).

All four adenosine receptors are known to be present in astrocytes (Dare *et al.* 2007). A_1Rs reduce astrocyte proliferation (Ciccarelli *et al.* 1994) and inhibit apoptosis (D'Alimonte *et al.* 2007) whereas occupancy of A_{2A} and A_3 ARs in primary cultures induces reactive astrogliosis (Brambrilla *et al.* 2003). Paradoxically, excessive levels of adenosine acting on the A_3 AR induces apoptosis in astrocytes (Appel *et al.* 2001; Di Iorio *et al.* 2002), which may be interpreted as a concentration-dependent mechanism to limit excessive proliferation and glial-mediated injury. Furthermore, activation of A_{2A} ARs in hippocampal astrocytes under physiological conditions inhibits glutamate uptake via glutamate (GLT)-1 transporters and stimulates astrocytic glutamate release (Li *et al.* 2001; Nishizaki *et al.* 2002; Matos *et al.* 2012), explaining the potentiation of glutamatergic transmission.

Besides this regulation of proliferation and survival of astrocytes, adenosine also has effects on their secretory functions. In fact, A_1 AR activation induced nerve growth factor (NGF) and S100b release from astrocytes (Ciccarelli *et al.* 1999). A_{2B}

ARs have shown to increase the levels of interleukin (IL)-6 (Schwaninger *et al.* 1997), while the activation of A_3 ARs induced chemokine (C-C motif) ligand 2 (CCL2) secretion (Wittendorp *et al.* 2004; Rosito *et al.* 2012) and A_{2A} AR stimulation was reported to inhibit inducible nitric oxide synthase (iNOS) expression and nitric oxide (NO) production in astrocyte cultures (Brodie *et al.* 1998). Moreover, these neuroprotective mechanisms are particularly relevant in neuropathological conditions where brain injury results in a massive release of ATP and adenosine and leads to reactive astrogliosis with enhanced production of inflammatory cytokines, increased expression of major histocompatibility complex (MHC)-II and augmented production of free radicals (Hasko *et al.* 2005). Indeed, cytokines and growth factors can also interact with astrocyte ARs, influencing their function and expression (Abbracchio and Ceruti 2007). For instance, exposure of astrocytes to IL-6 upregulates A_1 AR expression (Biber *et al.* 2001) while tumor necrosis factor alpha (TNF-α) exposure presents a dualistic and opposite effect on A_{2B} AR functioning after short- and long-term cytokine cell exposure (Trincavelli *et al.* 2004; Trincavelli *et al.* 2008). These dualistic time-dependent effects induced by TNF-α on A_{2B} AR-mediated action on astrogliosis are differently modified by inflammatory mediators in a time-dependent manner, which might advocate the adenosinergic system as a promising target to selectively modulate cerebral damage progression in the acute and chronic phases.

Microglial involvement in neurodegenerative diseases is well-established, being microglial activation and neuroinflammation shared features of these neuropathologies (Polazzi and Monti 2010 for review). Studies regarding AR subtypes expressed on microglial cells do not completely correlate, probably because the expression profile is influenced by the level of microglial activation (Dare *et al.* 2007). A_1 ARs have shown antiproliferative and anti-inflammatory properties in microglia (Tsutsui *et al.* 2004; Haselkorn *et al.* 2010) whereas activation of A_{2A} AR stimulates proliferation (Kust *et al.* 1999), upregulates both cyclooxygenase (COX)-2 (Fiebich *et al.* 1996) and NGF (Heese *et al.* 1997) and mediates process retraction (Orr *et al.* 2009). Very recently, it was observed that adenosine augments IL-10 production by activated microglia acting on A_{2B} ARs (Koscsó *et al.* 2012). Finally, there is scarce evidence for functional A_3 ARs in microglial cells, namely suggesting a role in suppression of TNF-α production (Lee *et al.* 2006). The effects of adenosine will thus depend on the densities of receptor subtypes (which might change upon different stages of activation), their different affinities and signaling pathways triggered, as well as on the cellular microenvironment (summarized in Table 1).

There is also an important role of the adenosinergic system in the control of the impact of infiltrating blood cells in neuroinflammation. Of note, there is evidence on the anti-inflammatory potential of the A_{2A} AR activation on macrophages (Hasko *et al.* 2000), monocytes (Hasko *et al.* 2007), dendritic cells (Panther *et al.* 2003) and T cells (Csoka *et al.* 2008; Lappas *et al.* 2005), which may further explain the neuroprotective effects afforded by A_{2A} ARs.

These observations are in agreement with the theory proposed by Sitkowsky and Ohta (2005), that the immune system is regulated by two danger signals: an initial insult would first initiate a proinflammatory response and to avoid excessive collateral damage the tissue would issue a second danger signal which would downregulate inflammation. They suggested that adenosine, namely through A_{2A} ARs, would be a key "OFF" signal, coordinating different stages of inflammation and redirecting immune responses (Sitkovsky *et al.* 2004). However, studies in A_{2A} AR knockout mice, demonstrate that in the brain A_{2A} AR antagonists have an anti-inflammatory effect (Yu *et al.* 2004; Yu *et al.* 2008; Li *et al.* 2009), contrasting with A_{2A} AR effects in peripheral organs. Recent findings try to explain these controversies, demonstrating a critical role of extrasynaptic glutamate derived from brain insults in the modulation of A_{2A} AR functional effects in the regulation of neuroinflammation (Dai *et al.* 2010).

In sum, the role of the A_{2A} AR in neurodegeneration seems to depend upon the timing of the disease and the peculiar mechanisms at the basis of its etiopathology.

Table 1: Summary of the positive and detrimental actions of the different adenosine receptor subtypes regarding neuronal function and neuroinflammation.

	G- Protein Coupling	Positive Actions	Negative Actions
A1	▪ G_i	▪ ↓ Excitotoxicity ▪ ↑ Production of neuroprotective mediators	
A_{2A}	▪ G_s; G_{olf}	▪ Facilitates neurotransmission ▪ Facilitates neurotrophic factor action ▪ ↓ Inflammation	▪ ↑ Excitotoxicity ▪ ↓ A1 function ▪ ↑ Astroglial proliferation
A_{2B}	▪ G_s; G_q	▪ ↑ Production of neuroprotective cytokines ▪ ↑A1 expression	▪ ↑ Astroglial proliferation
A3	▪ G_i	▪ ↑ Astrocyte apoptosis ▪ ↑ Production of neuroprotective mediators	

Adenosine in different brain disorders

a) Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia in the elderly, and is characterized by progressive memory loss and cognitive deterioration (Blennow *et al.* 2006).

Defining neuropathological features are: a selective synaptic failure and neuronal loss in several brain regions, especially the cerebral cortex and the hippocampus; the deposition of extracellular amyloid plaques containing the Aβ peptide and the formation of intraneuronal neurofibrillary tangles (NFT) (Querfurth and La Ferla 2010).

It is believed, particularly from genetic studies, that an imbalance between production, clearance and aggregation of Aβ peptides is crucial to AD onset (Tanzi and Bertram 2005). In addition, the severity of the cognitive impairment correlates with the levels of oligomers rather than with the total Aβ burden (Lue LF *et al.* 1999), proving to be synaptotoxic (Klyubin *et al.* 2008). Moreover, aggregation of Aβ oligomers into fibrils gives rise to diffuse amyloid plaques which are associated with neuronal death, neuritic dystrophy, dendritic spine loss and abnormal axons (Knowles *et al.* 1999), leading to synaptic loss and disruption (Stern *et al.* 2004). This is an early change and structurally correlates with the cognitive impairment (Arendt 2005 for review).

Furthermore, there is an apparent contribution of neuroinflammatory processes in AD pathophysiology (Eikelenboom *et al.* 2006; Heneka and O'Banion 2007) and activated microglia and astrocytes were found to be associated with amyloid plaques (Wisniewski and Wiegel 1991; Stalder *et al.* 1999). There is also conflicting evidence concerning whether Aβ activates a glial inflammatory response or if glial dysfunction precedes amyloidogenesis (Polazzi and Monti 2010).

Adenosine, acting mainly through A_1 and A_{2A} ARs, can modulate cognition and memory (Sebastião and Ribeiro 2009a). There is increasing evidence suggesting redistribution and changes in the density of adenosine receptors in AD (Gomes *et al.* 2011). Autoradiography and binding studies in humans with AD have consistently shown a loss of A_1 ARs in the hippocampus, especially in the dentate gyrus (Jansen *et al.* 1990; Kalaria *et al.* 1990; Ulas *et al.* 1993; Deckert *et al.* 1998). There are also studies demonstrating decreases in A_1 ARs in the striatum (Ikeda *et al.* 1993), the temporal cortex and thalamus of AD patients (Fukumitsu *et al.* 2008). In contrast, the levels and functional activity of both A_1 and A_{2A} ARs seem to be increased in the frontal

cortex of these patients (Albasanz *et al.* 2008), which is in agreement with observations from a transgenic mouse model of AD (Arendash *et al.* 2006).

Notwithstanding, an immunolocalization study revealed increased immunoreactivity of A_1 ARs in neurons with NFT and in dystrophic neurites of senile plaques in the hippocampus and frontal cortex (Angulo *et al.* 2003). Moreover, the same study exposed the positive influences of the A_1 AR on amyloid precursor protein (APP) processing and in tau phosphorylation, slowing down the progression of the disease (Angulo *et al.* 2003).

There is more limited data about the distribution of A_{2A} ARs in AD, but interestingly it has been found an increased expression of A_{2A} ARs in microglial cells in the hippocampus and cerebral cortex (Angulo *et al.* 2003). As previously stated, chronic stressful conditions elicit upregulation of A_{2A} ARs (Rebola *et al.* 2005), encouraging the modulation of these receptors as a neuroprotective strategy. *In vitro* and *in vivo* studies using A_{2A} AR antagonists almost completely prevented Aβ-induced synaptic loss and neurotoxicity (Dall'Igna *et al.* 2003; Dall'Igna *et al.* 2007; Canas *et al.* 2009), raising the possibility of reversing neuronal deficits. The precise mechanism of this neuroprotection is still to be unveiled, but the brain anti-inflammatory properties (Geiger *et al.* 2006), the protection against free radicals (Leite *et al.* 2011) and the control of glutamate excitotoxicity (Cunha 2005) derived from A_{2A} AR blockade may all have an influence. Nevertheless, it is somewhat surprising the cognitive enhancement derived from A_{2A} AR blockade, since A_{2A} AR stimulation *in vitro* is known to facilitate acetylcholine secretion and enhances glutamatergic transmission in the hippocampus (*e.g.* Jin and Fredholm 1997). Indeed, currently the most used drugs in AD induce cholinergic enhancement.

A considerable amount of information has arisen, regarding the benefits of adenosine receptor manipulation in AD, and this derives greatly from the impact of caffeine in both animal AD experimental models and epidemiological studies. Caffeine is a natural methylxanthine and a non-selective A_1 and A_{2A} AR antagonist (Fredholm *et al.* 1999), and neuroprotective benefits from long-term intake have been reported.

In AD transgenic mice, long-term administration of caffeine improved cognitive performance, and reduced the levels of soluble Aβ fragments and of Aβ peptides (Arendash *et al.* 2006). More recently, a similar study has demonstrated sustained reductions in plasma Aβ and decreases in both soluble and deposited Aβ in hippocampus and cortex (Cao *et al.* 2009). The same group has also shown that caffeine has the ability to reverse the pre-installed memory deficits and the pre-existing considerable Aβ burden in these models (Arendash *et al.* 2009). These effects are

mimicked by selective A_{2A} AR antagonists, strengthening the idea that A_{2A} ARs are responsible for these beneficial effects.

There is also a limited number of epidemiological studies conducted to determine the association between long-term (years to decades) caffeine consumption and the incidence of AD. Recent systematic reviews and meta-analyses from cohort and case-control studies have found a trend towards the protection against cognitive decline in AD, with highest benefit among the eldest age group (Barranco Quintana *et al.* 2007; Santos *et al.* 2010). However, the heterogeneous methodologies and results preclude definite statements in this topic.

Besides these insights on the role of adenosine in AD pathophysiology there is also strong evidence concerning the possible role of caffeine and ARs in other conditions of memory impairment. Of note, caffeine has also influence on brain acetylcholine, leading to concentration increases in the prefrontal cortex of rats (Acquas *et al.* 2002), an effect likely mediated by the A_1 AR (Maemoto *et al.* 2004). Moreover, caffeine prevents in both rodents and humans the acute memory loss induced by scopolamine (Riedel *et al.* 1995; Botton *et al.* 2010). This highlights the specific correction of the cholinergic pathway involved in caffeine memory-enhancing effects. However, selective A_{2A} AR blockade did not reproduce these results suggesting that these receptors do not affect general processes of memory impairment but instead play a crucial role restricted to neurodegenerative conditions involving an insidious synaptic deterioration leading to memory dysfunction (Cunha *et al.* 2008).

There is clearly a need of more basic research on the role of the adenosinergic system in AD pathophysiology and associated cognitive impairment in order to understand its prophylactic and/or therapeutic potential.

b) Parkinson's Disease

Parkinson's Disease (PD) is a common neurodegenerative disorder characterized by a progressive loss of the dopaminergic neurons from the substantia nigra pars compacta (SNc) in the striatum, which leads to the typical motor symptoms of akinesia, bradykinesia, rigidity, resting tremor and postural abnormalities (Morelli *et al.* 2009). The etiology of the disease is still unknown but factors such as protein misfolding and aggregation and oxidative stress associated to mitochondrial dysfunction and excitotoxicity are important features in PD pathogenesis (Dauer and Przedborski 2003).

Currently, it is accepted that movement is regulated through integration of corticothalamic information from the striatum and output from the internal globus pallidus (Gpi) and substantia nigra pars reticulata (SNr) under tonic dopaminergic conditions (Albin *et al.* 1989). The GABAergic MSNs make up the major striatal output pathways – the monosynaptic D_1R-expressing direct pathway and the striatopallidal D_2R-expressing indirect pathway. Thus, dopamine acting through the different receptors in the direct and indirect pathways leads to movement facilitation and inhibition, respectively (Kulisevsky and Poyurovsky 2012). The important roles of dopamine in this control are lost in PD. Therefore, the introduction of 3,4-dihydroxy-L-phenylalanine (L-DOPA) and of dopamine agonists was a successful strategy to restore the deficits in the motor loop. However this only provides a symptomatic relief, with no influence on disease progression and has troubling long-term consequences such as motor fluctuations and L-DOPA-induced dyskinesias (Stacy and Galbreath 2008; Jankovic 2005).

ARs, especially the A_{2A} subtype, have a prominent role in several aspects of PD pathophysiology. A_{2A} ARs show the ability to control D_2R function through both the formation of heteromers and intracellular signaling pathways (Schiffmann *et al.* 2007) and therefore can alter each other's pharmacological properties such as affinity and desensitization in an antagonistic manner (Xie *et al.* 2007). The strong expression of A_{2A} ARs in the striatum, namely the post-synaptic co-localization with D_2R in GABAergic striatopallidal MSNs (Hillion *et al.* 2002) pinpoints a rationale for the use of A_{2A} AR antagonists as an alternative or adjunctive to the dopamine-based therapies. In addition, A_{2A} ARs are also expressed in GP and their blockade reduces extracellular GABA concentration, potentiating motor activity induced by L-DOPA (Rosin *et al.* 2003; Simola *et al.* 2006).

There is a large number of epidemiological and experimental studies in rodent and primate non-human models of PD, which confirm the motor activity enhancement effects mediated by acute A_{2A} AR blockade (Morelli *et al.* 2010; Wei *et al.* 2011) and can ameliorate symptoms even as monotherapy (Pinna *et al.* 2007). Moreover, synergistic interactions between L-DOPA and A_{2A} AR antagonists have also been described suggesting that they may be co-administered to potentiate the motor stimulant effects (Rose *et al.* 2006; Hodgson *et al.* 2010). Similar studies on the effects of chronic A_{2A} AR antagonism in animal models have shown analogous findings. With the co-administration of A_{2A} AR antagonists after the onset of motor fluctuations or L–DOPA-induced dyskinesia, it was allowed a reduction in the doses of dopaminomimetic compounds with potentiation of motor improvements without worsening dyskinesia

(Armentero *et al.* 2011 for review). More recently, evidence from knockout studies pointed towards a possible contribution of A_{2A} ARs in different brain regions and cellular elements other than the post-synaptic striatopallidal neurons in the attenuation or reversal of L-DOPA induced motor complications, a subject that requires further investigation (Wei *et al.* 2011).

Taken together, this data from non-clinical studies propelled clinical trials testing the use of selective A_{2A} AR antagonists in PD patients. Indeed, five clinical trials are currently underway (phases I to III) to analyze the therapeutic potential of adenosine A_{2A} AR (Armentero *et al.* 2011 for review). The most compelling evidence from these studies comes from the highly selective A_{2A} AR antagonist istradefylline. Bara-Jimenez and coworkers (2003) reported that at low doses of L-DOPA, istradefylline potentiated the antiparkinsonian response with less dyskinesia compared with that induced by optimal dose L-DOPA alone. In patients with dyskinesia, istradefylline has recently shown consistent and sustained increases in OFF time (Factor *et al.* 2010) but most studies in the field reported increased (although not troublesome) dyskinesias. However, changes in the design of the clinical trials such as dose selection, eventual concomitant caffeine intake and disease stage of the target population may provide different outcomes in the future.

In regard to the neurodegenerative process, A_{2A} AR blockade also reduces dopaminergic cell loss, although the mechanism for this neuroprotective role has not been uncovered yet (Chen *et al.* 2007). The previously stated ability of the A_{2A} AR to control glutamate release may be implicated, as well as the aforementioned modulation of glia-mediated neuroinflammation in PD. Moreover, antagonism of A_{2A} AR also influences the blood-brain barrier permeability and the traffic of peripheral immune cells (Varani *et al.* 2001). Neuroprotection may also be related with the inhibition of monoamine oxidase B (MAO-B) reported with A_{2A} AR antagonists (Petzer *et al.* 2009), decreasing oxidative stress and hazardous byproducts (Sagi *et al.* 2007). Additionally, there is increasing evidence that neurodegeneration begins with a synaptic dysfunction, which later evolves into an overt damage of neurons (Dauer and Przedborski 2003). Accordingly, the recent work of Gomes and colleagues has shed some light on the role of the A_{2A} AR in the control of corticostriatal circuits through glial cell line-derived neurotrophic factor (GDNF) modulation of glutamatergic and dopaminergic nerve terminals (Gomes *et al.* 2006; Gomes *et al.* 2009).

Hence, A_{2A} AR antagonism has a plethora of beneficial effects, not only relieving motor deficits in established PD but also showing a broader disease-modifying potential, arresting neurodegeneration and slowing progression. Nonetheless, concise

evidence on the specific mechanisms involved and their relevance is still scarce. In spite of the obstacles there is an exciting prospect for A_{2A} AR antagonists as alternative or adjunctive therapies in PD, as further basic and clinical investigation goes on.

c) Huntington's Disease

Huntington's Disease (HD) is an autossomal dominant neurodegenerative disease characterized by progressively worsening chorea, psychiatric disturbances and cognitive impairment. The pathological hallmark of HD is the degeneration of striatal GABAergic enkephalinergic MSN's (Mitchell *et al.* 1999). The molecular origin of this degeneration has been ascribed to a genetic mutation that leads to the production of an abnormal form of the protein Huntingtin (The Huntington's Disease Collaborative Research Group 1993). The mechanisms of pathogenicity remain unknown but it has been suggested both a loss of function of the normal Huntingtin, as well as a gain of function related to the toxic properties of the mutated protein (Cattaneo *et al.* 2005). Indeed, several lines of evidence support the idea that the mutated Huntingtin induces corticostriatal glutamatergic dysfunction. This includes increased glutamate release and decreased astrocytic glutamate clearance, together with increases in activation of NMDA receptors and triggering of mitochondrial dysfunction (Popolli *et al.* 2007). Moreover, recent evidence demonstrated increased immune activation of macrophages and microglia in HD, pointing towards a role for immune dysfunction in this brain pathology (Björkqvist *et al.* 2008).

The high density of A_{2A} ARs in the striatum, either pre-synaptically in the corticostriatal afferents modulating glutamate release as well as in the post-synaptical MSNs, supports pathophysiological relevance of this receptor in HD (Rosin *et al.* 1999; Hettinger *et al.* 2001). The observation of decreased expression of A_{2A} ARs in the striatum of early HD patients (Glass *et al.* 2000) and of transgenic mice (Blum *et al.* 2003) further reinforced their contribution to pathogenesis.

Several HD animal model studies (both pharmacological and genetic knockout) in different phases of the disease revealed changes in A_{2A} AR expression, density and/or signaling. Although expression of the A_{2A} ARs in the striatum is markedly decreased during HD progression (Chiang *et al.* 2005), striatal cells expressing the mutant Huntingtin have shown aberrant increases in A_{2A} AR signaling (Varani *et al.* 2010; Chou *et al.* 2005; Tarditi *et al.* 2006). Despite such controversies, A_{2A} ARs remain a potential target in HD.

As previously stated, adenosine acting on A_{2A} ARs at pre-synaptic sites, promotes glutamate release (Popoli *et al.* 2002) and is thus detrimental to neurons, while at post-synaptic, extra-synaptic sites and glial cells there is a speculative neuroprotective potential. Studies from HD animal models, regarding activation and blockade of A_{2A} ARs have yielded conflicting findings in relation to neurodegeneration since both A_{2A} AR agonists and antagonists were capable of producing neuroprotection (Popoli *et al.* 2007 for review). A recent study in A_{2A} AR knockout mice showed worsened motor performance and survival, supporting the idea that early and chronic blockade of A_{2A} ARs may not be beneficial (Mievis *et al.* 2011). This might reflect the pre- versus post-synaptic effects of A_{2A} ARs, and is in line with the unexpected ability of A_{2A} AR antagonists to potentiate NMDA-mediated toxicity (Popoli *et al.* 2007). The same controversy exists in regard to mitochondrial and metabolic dysfunction with findings of either protection or increase of striatal MSN lesion (Alfinito *et al.* 2003; Fink *et al.* 2004).

Moreover, it has been shown that A_{2A} AR agonists can enhance ubiquitin proteossome system (UPS) activity (Chiang *et al.* 2009), which may be of value since Huntingtin aggregate formation and UPS dysfunction are major features of HD (Wang *et al.* 2008).

In addition, normal Huntingtin has beneficial antiapoptotic actions and contributes to the production and delivery of BDNF, namely to striatal targets, and this supply is decreased in HD (Zuccato and Cattaneo 2007). BDNF is particularly important for the survival and plasticity phenomena of corticostriatal synapses (Cattaneo *et al.* 2005) and A_{2A} AR agonists are able to transactivate the TrkB BDNF receptor and regulate BDNF levels (Sebastião and Ribeiro 2009b). Indeed, A_{2A} ARs have shown a crucial role in this regulation (Tebano *et al.* 2010), which provides another neuroprotective mechanism afforded by adenosine. However, BDNF can also contribute to cell death, depending on the receptor activated (Gomes *et al.* 2011), which further complicates this controversy.

Finally, A_{2A} AR antagonism is generally accepted as a neuroprotective strategy, through the control of the neuroinflammatory component of neurodegenerative diseases, including HD. Furthermore, A_{2A} ARs expressed in bone marrow-derived inflammatory cells have proved to be important contributors to striatal damage in transgenic models of HD (Huang *et al.* 2006).

It appears that the disease stage and time-frame, the drug administration protocol, and the clinical manifestations might play critical roles in evaluating the future therapeutic potential of A_{2A} AR ligands (Popoli *et al.* 2008). HD is thus a special case

where the complexity of the disease combines with the complexity of A_{2A} AR pharmacology, possibly yielding biphasic neurotoxic-neuroprotective effects and making it difficult to establish if this is a target of interest.

d) Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the CNS, classically defined as an auto-reactive T-cell-driven damage directed towards oligodendrocytes and the myelin sheath surrounding central axons, with features of mononuclear cell infiltration and inflammatory damage (Prat and Antel 2005). However, more recent work has indicated prominent and widespread neuronal damage early in the disease (Vogt *et al.* 2009). This led to the hypothesis of MS as an inflammatory demyelinating and neurodegenerative disease but it remains elusive if there is a sequence of events or distinct pathological processes culminating in disease (Herz *et al.* 2010). Research focus has thus shifted towards the involvement of cellular and molecular mechanisms in this neuroimmune crosstalk. In addition, astrocytes and microglia have shown a dual role in MS, both promoting damage and inflammation as well as restoring the damaged tissues (Williams *et al.* 2007; Muzio *et al.* 2007).

Besides the roles in neuro-glial and glia-glial communication, not much is known regarding purinergic signaling in MS. Adenosine levels are decreased in the blood of MS patients, together with an increase of TNF-α (Mayne *et al.* 1999). In addition, blood immune cells and glial cels from MS patients show decreased expression of the A_1 AR, suggesting a dysfunction of this receptor in the pathogenesis of the disease (Johnston *et al.* 2001). Consistently, studies using experimental autoimmune encephalomyelitis (EAE), the most widely used animal model of MS, found that A_1 AR stimulation protects against neuroinflammation, demyelination and oligodendrocyte cytotoxicity (Tsutsui *et al.* 2004). This is further supported by the recent finding that suppression of EAE-induced neuroinflammation and neurobehavioral deficits by glucocorticoid treatment is accompanied by a concurrent increase in A_1 AR expression in monocytoid cells (Tsutsui *et al.* 2008). Moreover, adenosine directly promotes oligodendrocyte progenitor cell differentiation and myelination (Stevens *et al.* 2002) and stimulates their migration via A_1 ARs (Othman *et al.* 2003).

More recently, chronic intake of caffeine has shown the surprising ability to upregulate A_1 ARs, transforming growth factor-beta (TGF-β) and to suppress interferon-gamma (IFN-γ) in EAE guinea pigs (Chen *et al.* 2010). This data supports the view of A_1 AR-mediated shift from Th1 to Th2 cell function in the attenuation of pathology

(Chen *et al.* 2010) and provides a neurobiological basis for epidemiological investigation into the possible relationship between caffeine consumption and development of multiple sclerosis in humans.

Furthermore, pharmacological blockade of A_{2A} AR protected mice from EAE induction, highlighting the critical role of adenosine in the modulation of EAE, although the exact mechanisms remain to be clarified (Mills *et al.* 2008).

In the murine EAE model of MS, adenosine can also exacerbate immunopathological mechanisms. Infiltrating lymphocytes into the CNS requires the expression of CD73 either on T cells or in the CNS for disease induction (Mills *et al.* 2008). This means that the anti-inflammatory effects of adenosine can be offset by the effect on the access of pathogenic T-cells to the CNS.

Moreover, it was demonstrated that CD39 is expressed in a subset of forkhead box P3 (FOXP3)-expressing regulatory T cells, the numbers of which are strikingly reduced in patients with relapsing-remitting MS (Borsellino *et al.* 2007). These cells are suggested to play a role in the suppression of autoimmune damage, and their reduction in MS might impair the control of IL-17-mediated inflammation (Fletcher *et al.* 2009). In addition, lymphocytes from MS patients have shown an increased CD39 activity, which may provide a protective mechanism by fostering AR-mediated anti-inflammatory actions (Spanevello *et al.* 2010).

Besides CD73 and CD39, also ADA is involved in lymphocyte functioning in MS. ADA is known to control growth, proliferation, differentiation and migration of lymphocytes (Pérez-Aguilar *et al.* 2010). Since lymphocytes from MS patients exhibit reduced ADA activity (Vivekanandhan *et al.* 2005), this may further contribute to their infiltration in the CNS.

There is mounting evidence on the link between adenosine and the etiology of MS. The multifaceted role of this molecule and the myriad of integrative mechanisms in the control of immune responses open new avenues for treatments to be pursued in the future, as additional understanding is provided.

Discussion

The future prospects for adenosine-system-based therapies in chronic neurodegenerative diseases seem promising. However, in spite of the vast contributions of ARs to neuroprotection mechanisms and to the regulation of inflammation in the last few years, there are still several open questions which deserve special attention.

Adenosine elicits a multitude of effects in different cells, and factors such as expression, density, localization, transducing systems, and functions of ARs (particularly the A_{2A}) are subject to modifications upon brain activity or pathology. The same promiscuity occurs with the institution of pharmacological modulation of ARs or adenosine metabolism pathways and with caffeine intake, especially in a long-term basis. Moreover, the sources and pathways of generation of extracellular adenosine are still not fully uncovered in order to understand the dynamics of these receptors.

In regard to the experimental models of neurodegeneration, it is difficult to draw conclusions on the precise role of ARs in chronic conditions, since evidence derives in great part from acute injury models and some do not take into account the age of the animal. This is particularly relevant since conditions like AD and PD are strikingly prevalent in the elderly. Furthermore it is uncertain the translational potential of such studies into the human brain.

It seems well established that A_1 AR activation affords neuroprotection while blockade of A_{2A} ARs attenuates the burden of most brain disorders. As previously proposed, this would mean that a combination of AK inhibitors and antagonists of A_{2A} ARs would achieve the most neuroprotective potential (Cunha 2005). However this impact can only be predicted if more light is shed on when and how extracellular adenosine levels change in the brain (including synapse, neuro-glial domain, glia-glial domain or gliovascular domain), the blood-brain barrier and in infiltrating myeloid cells. In addition, these fluctuations may vary with disease stage and between diseases, further complicating the scenario.

The recognition of the dual role of neuroinflammation on neurodegeneration and neuroprotection and the importance of the adenosinergic system in the control of the balance between tissue injury and repair also turned attention towards ARs as modulators of glial and immune functions. The apparent primordial physiological protective function of adenosine following acute insults may be overshadowed by its reduced ability to protect against more chronic injury. Adenosine signals are endogenously protective, but imperfect and inconsistent. For instance, blocking the A_{2A}

AR in the brain to suppress inflammation may exacerbate peripheral proinflammatory effects. Moreover, interactions between activation of astrocytes and microglia are likely of great importance in this modulation since each of the glial cells expresses ARs and has the opposite effects of enhancement of immune responses and limiting of CNS inflammation. It is thus fundamental the understanding of gliotransmission and of the mechanisms encompassing other glial interplays in the progression and outcome of neurological diseases (Verkhratsky et al 2012 for review).

In regard to the specific neurodegenerative disorders, there are other particular caveats worth mentioning. In AD it is still difficult to disentangle the impacts of A_{2A} AR blockade on cognitive impairment from those related to disease pathophysiology itself. A putative target to be tested is related to cholinergic function. There is a deficiency in cholinergic projections in AD and presynaptic α-7 nicotinic acetylcholine receptors are essential for cognitive processing. Since the levels of such receptors are increased in early AD (Ikonomovic *et al.* 2009) and the activation of A_{2A} ARs exerted control of α-7 nicotinic currents in models of hippocampus (Fernandes *et al.* 2008), it can be proposed another mechanism of regulation of plasticity phenomena through A_{2A} AR inhibition.

The last years of research revolutionized the classic view of PD. Indeed, neurodegeneration extends into locations other than the striatum and also occurs in different nerve terminals. In addition, the basal ganglia pathways of motor control are far more intricate than previously thought, with plenty of interactions and feedback loops. Moreover, the complete characterization and the development of ligands targeting A_{2A} AR-containing heteromers must be considered in the future. These show unique pharmacological properties and are more selective in localization than each of the constituent receptors, while allowing us to better comprehend the disease pathophysiology (Ciruela *et al.* 2011 for review). Of note, the observation of heteromeric complexes and synergistic effects of A_{2A} and mGlu5 receptors in the striatum (Ferré *et al.* 2002; Kachroo *et al.* 2005) underline the potentialities of the simultaneous blockade of these receptors in PD treatment.

The discrepancies in the effects of A_{2A} ARs in HD have been the major issue in the development of new therapies. A_{2A} AR agonists seem to be mostly beneficial in late stages of degeneration while the protective effects of A_{2A} AR blockade depend on the strength of post-synaptic inhibition. Huntingtin alters the function and the expression of A_{2A} ARs, which further challenges this hypothesis. Here too, the formation of A_{2A} AR-containing heteromers provides a more specific and useful target. Very recently, a study with T1-11, a dual-action drug with weak A_{2A} AR agonist potential that inhibits

ENT-1, hindering adenosine uptake, substantially delayed HD progression (Huang *et al.* 2011). This dual function provides a new therapeutic concept that may be applicable to other neurotransmitter systems and facilitate the development of new drugs for neurodegenerative diseases.

The investigation of the specific role of the adenosinergic system in MS pathophysiology is still in its infancy. Most studies have rather focused on ATP receptors. However, MS can be seen as a model of protective autoimmunity, in which the brain natural repair mechanisms are impaired (Martino 2004). A_1 ARs seem to be important in the restoration of such mechanisms and induction of increases in extracellular adenosine seems a reliable strategy, namely through the enhancement of ectonucleotidases function.

Neurotrophic factors have pleiotropic effects in the brain beyond the neuronal trophic support and there is a lack of studies on the BDNF-mediated neuroprotection in pathological conditions. The same applies to the excitotoxicity mechanisms involved in neurodegenerative disorders (Dong *et al.* 2009).

The open questions may be answered with the exploration of the potentialities of the more recently revealed A_{2B} and A_3 ARs. Likewise, the development of more agonists and antagonists of ARs, allosteric enhancers or AR-containing heteromers, with different affinities and properties tries to bring some insight, namely targeting an adequate balance of pre- versus post-synaptic action and minimizing side effects.

In addition, the action of ectonucleotidases in the governation of purinergic signaling in ATP versus adenosine receptors deserves special attention ever since the existence of oligomeric complexes between them was demonstrated (Schicker *et al.* 2009). This adds to the network of overlapping biological reactions and the crosstalk of signaling pathways achieved in membrane microdomains (Lasley 2011 for review). It is hence noteworthy to mention the bolstering of ectonucleotidase function as a therapeutic alternative.

The intracellular metabolic pathways of adenosine are also subject of research as targets of adenosine augmentation therapies: inosine promotes axonal growth in the CNS and acts on ARs, yielding significant but not totally clear preclinical evidence in the treatment of neuroinflammatory disorders; xanthine oxidase inhibitors reduce free radical production and ultimately lead to increases in adenosine; urate has gained recognition because of its antioxidant effects and neuroprotective potential in MS models. Moreover, recent epidemiological and clinical studies show an inverse relationship between urate exposure and PD, serving as an independent prognostic

biomarker (Morelli *et al.* 2010). However such an approach is of little value in influencing disease pathophysiology, but rather providing symptomatic relief.

It was also observed that pathologically increased expression of AK is linked to astrogliosis in many CNS pathologies, leading to reductions in adenosine tone and leaving neurons more susceptible to damage (Boison 2008). Thus, it is logical the proposition of AK inhibitors in the treatment of such disorders. Instead, the significant side effects of the systemic use of these drugs pushed forward a new era of focal approaches of augmentation of nucleoside function, as already tried for epilepsy. This includes intelligent and ideally suited management strategies such as polimer-based adenosine delivery, stem cell-based adenosine delivery and gene therapy.

Conclusions

In this regard, there is an entire spectrum of (patho)physiological mechanisms and facets that are subject to adenosinergic regulation. Therapies based on this approach will depend on the timing of treatment, with respect to the therapeutic window, the stage and progression of damage and the duration and monitoring for both beneficial and adverse events. This precise time-space coincidence will only be achieved through more awareness on how adenosine signals are perceived, discriminated, sustained and terminated.

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Annex A

"Potencial das terapias baseadas no sistema da adenosina no tratamento de doenças Neurodegenerativas" – Resumo

Introdução

A adenosina é reconhecida como uma molécula de sinalização extracelular capaz de coordenar a homeostasia em todas as células, com efeitos na proteção e reparação tecidular. No entanto, no sistema nervoso central, esta molécula também exerce ações neuromodulatórias específicas no controlo da transmissão e plasticidade sinápticas e na coordenação de circuitos neuronais. Esta atividade dupla do sistema adenosinérgico levou à consideração da adenosina como um alvo terapêutico no tratamento de doenças neurológicas. Parte deste papel da adenosina parece estar relacionado com a regulação de processos neuroinflamatórios e modulação das respostas imunológicas, particularmente em circunstancias patológicas, conferindo um potencial neuroprotetor e uma oportunidade de controlo de doenças neurodegenerativas e neuroimunes.

Os efeitos combinados da adenosina na viabilidade neuronal e na inflamação suscitaram o interesse de vários grupos nesta área de investigação, e existem evidências emergentes que encorajam terapias baseadas neste sistema.

Objetivos

Este artigo de revisão bibliográfica tem o objetivo de sumarizar as evidências atuais no que diz respeito aos mecanismos mediados pela adenosina na fisiopatologia de distúrbios neuroimunes (focando doenças neurodegenerativas e autoimunes), assim como salientar as oportunidades terapêuticas e o potencial futuro do sistema adenosinérgico.

A Adenosina no Sistema Nervoso Central

São conhecidos quatro subtipos de recetores de adenosina – A_1, A_{2A}, A_{2B} e A_3. Todos eles são recetores metabotrópicos acoplados à proteína G, mas cada um tem

um perfil farmacológico, uma distribuição tecidular e vias de sinalização característicos. Ao nível cerebral, os recetores A_1 e A_{2A} demonstram um impacto particular na neuromodulação. Os recetores A_1 são mais abundantes no córtex cerebral, cerebelo, hipocampo e corno posterior da espinal medula, enquanto que os recetores A_{2A} predominam nos neurónios médios espinhosos do estriado. No entanto, ambos os recetores têm uma distribuição alargada no sistema nervoso central, embora com níveis de expressão mais baixos.

Estudos eletrofisiológicos e bioquímicos sobre o papel da adenosina nos circuitos neuronais concluíram que esta molécula é capaz de inibir a excitabilidade dos neurónios e a transmissão sináptica. Isto é conseguido através de inibição pré-sináptica tónica da libertação de neurotransmissores causada pela ativação dos recetores A_1, em específico nas sinapses excitatórias. Além disso, também existem contribuíções pós- e extra-sinápticas da ativação A_1, que controlam a resposta a estímulos excitatórios. Por outro lado, a ativação pré-sináptica de recetores A_{2A} facilita a libertação de vários neurotransmissores. Com efeito, a observação de co-localização e interação funcional entre estes dois subtipos de recetor no hipocampo levou à proposição de que um heterómero composto pelos recetores A_1 e A_{2A} funcionaria como um interruptor sináptico dependente da concentração de adenosina, produzindo nomeadamente efeitos opostos na libertação de glutamato. Desta forma, os recetores A_1 parecem ter importância neuroprotetora perante concentrações baixas de adenosina, evitando ativação sináptica excessiva, enquanto que os recetores A_{2A} são estimulados com concentrações mais elevadas, particularmente em condições de stress e antagonizando a ativação A_1, promovendo excitotoxicidade e sustentando fenómenos de plasticidade neuronal.

Para além dos efeitos nos mecanismos neuro-secretores, os recetores de adenosina têm ainda a capacidade de interagir funcionalmente com outros recetores, transportadores, e sistemas de sinalização intracelular, ajustando e adaptando a ação de vários neurotransmissores, neuromoduladores e fatores neurotróficos.

Os aumentos de adenosina extracelular resultam da libertação por um transportador equilibrativo, de dano celular ou da hidrólise de nucleotidos de adenina extracelulares por nucleotidases. As ectonucleotidases são estimuladas mediante condições nóxicas, provavelmente em resposta a um aumento de ATP extracelular, gerando níveis elevados de adenosina que constituem uma oportunidade terapêutica ao governar a sinalização purinérgica em circunstâncias patológicas. A adenosina é metabolizada a nível intracelular pela adenosina cinase e pela adenosina deaminase, vias que também são objeto de investigação.

A Adenosina na Neuroglia / Neuroinflamação

As células gliais, originalmente encaradas como um mero suporte estrutural dos neurónios, têm a capacidade de adaptação e de resposta a estímulos ambientais, nomeadamente detetando e influenciando a função neuronal e comunicando com outras células gliais e vasculares. O sistema adenosinérgico ganhou popularidade como mediador desta comunicação intercelular, com especial relevância em circunstâncias patológicas onde esta interação pode ser modulada e gerar neuroprotecção.

Os astrócitos apresentam uma interação bidirecional com os neurónios que é parcialmente mediada pela adenosina. Os recetores de adenosina dos astrócitos são responsáveis pela regulação da sua proliferação, sobrevida e apoptose. Para além disso, estes recetores têm importância no controlo de secreção de fatores neutróficos e citoquinas, os quais também podem influenciar a função e expressão dos próprios recetores de adenosina.

O envolvimento da microglia e a neuroinflamação são componentes importantes de doenças neurodegenerativas que também são objeto de controlo por parte da adenosina, assim como o impacto da infiltração de células imunes periféricas.

As propriedades pleiotrópicas do sistema da adenosina envolvem também um controlo da neuroinflamação crónica. Foi proposto que a adenosina, nomeadamente através dos recetores A_{2A} produz um sinal “OFF” que coordena os diferentes estadios de inflamação e redireciona as respostas imunes após uma resposta proinflamatória inicial. No entanto, no sistema nervoso central esta ação não é clara e os resultados são controversos.

A Adenosina em Patologias Neurológicas

a) Doença de Alzheimer

A doença de Alzheimer (AD) é a forma mais comum de demência no idoso e é caracterizada por uma perda de memória e deterioração cognitiva progressivas. Em termos neuropatológicos, a AD resulta da deposição de placas de amiloide Aβ e formação de emaranhados neurofibrilares (NFT). Estas alterações estão associadas a morte neuronial, neuritos distróficos e disrupção sináptica assim como a processos neuroinflamatórios e de disfunção glial. Existem estudos que indicam uma diminuição da densidade dos recetores A_1 em zonas importantes para o processamento cognitivo, embora se verifique um aumento e uma maior atividade deste recetor no córtex frontal,

especialmente em neurites distróficas. A adenosina exerce, desta forma, influências positivas no processamento da proteína precursora de amiloide e na fosforilação tau, desacelerando a progressão da doença. Por outro lado, o bloqueio dos recetores A_{2A} previne quase completamente a neurotoxicidade e a perda sináptica induzida por Aβ. O mecanismo exato para esta proteção é desconhecido mas envolve provávelmente propriedades anti-inflamatórias, proteção contra radicais livres e o controlo de excitotoxicidade.

Outra linha de evidência do papel da adenosina na AD deriva dos benefícios cognitivos do consumo a longo prazo de cafeína observado em modelos animais e em estudos epidemiológicos. A cafeína é um antagonista não seletivo dos recetores A_1 e A_{2A} e manifesta uma tendência protetora e de reversão do declínio cognitivo. No entanto não é claro se o bloqueio A_{2A} é o responsável por estes efeitos, ou se por outro lado atuará mais especificamente em processos mais insidiosos de deterioração sináptica.

b) Doença de Parkinson

A doença de Parkinson (PD) é caracterizada por uma perda progressiva dos neurónios dopaminérgicos da substância negra pars compacta (SNc) de etiologia desconhecida, resultando em sintomas de acinésia, bradicinésia, rigidez, tremor em repouso e anomalias posturais. Na sua etiopatogenia está envolvida uma perda do controlo dopaminérgico das vias de inibição e facilitação do movimento.

Os recetores A_{2A} estão envolvidos em vários aspetos da fisiopatologia da PD. A sua alta expressão no estriado e a formação de heterómeros com recetores de dopamina D_2 (D_2R) suscita a possibilidade da sua manipulação como terapia alternativa ou complementar às baseadas na dopamina. Com efeito, vários estudos epidemiológicos e experimentais em modelos animais confirmam um efeito de melhoria da atividade motora com o antagonismo dos recetores A_{2A}, tanto em monoterapia como em conjunto com L-DOPA, mostrando um efeito sinérgico e uma redução dos efeitos laterais dos compostos dopaminomiméticos. No entanto, estudos clínicos contrastam com estas evidências e aguardam resultados mais sólidos para a aprovação destes fármacos.

Para além do benefício sintomático, o bloqueio A_{2A} apresenta também um potencial modificador da doença, diminuindo a degeneração neuronial e impedindo a progressão, embora o mecanismo exato para esta proteção ainda estar por descobrir.

c) Doença de Huntington

A doença de Huntington (HD) é caracterizada por uma coreia de agravamento progressivo, distúrbios psiquiátricos e défice cognitivo. É uma doença autossómica dominante em que ocorre degeneração dos neurónios medios espinhosos (MSNs) GABAergicos encefalinérgicos do estriado, que está relacionada com a forma mutada da proteina Hungtingtina.

A observação de diferentes graus de expressão, densidade e sinalização dos recetores A_{2A} consoante a fase da doença, aponta um alvo potencial na HD. A HD parece ser um caso especial em que ambos o bloqueio e a ativação do recetor A_{2A} parecem apresentar propriedades neuroprotectoras. Tal poderá ser explicado através de um balanço entre a ação pré- versus pós-sináptica ou então pelos múltiplos mecanismos envolvidos na fisopatologia da HD em que o recetor A_{2A} intervém. A título de exemplo, agonistas A_{2A} têm a capacidade de transactivar o recetor trkB do BDNF (cuja produção e distribuíção é assegurada pela Hungtingtina normal) e atuam contra a formação de agregados de Hungtingtina, enquanto que o antagonismo A_{2A} tem efeitos no controlo do componente neuroinflamatório da HD, inclusive na modulação de células inflamatórias periféricas infiltrantes.

d) Esclerose Múltipla

A eslerose múltipla (MS) é uma doença inflamatória desmielinizante e neurodegenerativa, definida por um dano autorreativo mediado por células T nos oligodendrócitos e na bainha de mielina dos axónios centrais.

Para além do papel da adenosina no controlo da comunicação neuroglial e glia-glial, há muito pouca informação sobre a contribuição da sinalização purinérgica para a imunopatologia da MS. A adenosina, atuando nos recetores A_1, protege contra a neuroinflamação, desmielinização e citotoxicidade. Além disso, este recetor apresenta níveis de expressão reduzidos em MS, sugerindo que a sua disfunção pode estar envolvida na patogénese da doença. Existem ainda evidências do papel do recetor A_1 na transição de uma resposta imune Th1 para Th2, atenuando a patologia da MS.

Outro aspeto da fisiopatologia da MS controlado pela adenosina é a infiltração linfocitária no sistema nervoso central. Esta infiltração necessita da expressão de ectonucleotidases na superfície dos linfócitos. Por outro lado, se essa expressão estiver aumentada, pode também contribuir para o aumento da concentração de adenosina e para o controlo da inflamação. Existe, portanto uma controvérsia na

estratégia terapêutica a adotar, contrabalançando os efeitos anti-inflamatórios com a infiltração de células T patogénicas.

Discussão

As perspetivas do futuro de terapias baseadas na adenosina em doenças neurodegenerativas crónicas é animadora. No entanto, apesar das descobertas nos últimos anos acerca da vasta contribuição dos recetores de adenosina para mecanismos neuroprotetores e na regulação da inflamação, existem ainda várias questões em aberto que merecem especial atenção.

A adenosina desencadeia uma multiplicidade de efeitos em diferentes células, e fatores tais como a expressão, densidade, localização, sistema de transdução de sinal, e funções dos recetores da adenosina estão sujeitos a modificações consoante a atividade cerebral ou na presença de patologia. A mesma promiscuidade ocorre com a instituição de modulação farmacológica dos recetores ou das vias de metabolização da adenosina assim como após o consumo de cafeína, particularmente a longo-termo. Além disso, as fontes e as vias de geração de adenosina extracelular ainda não estão completamente caracterizadas de forma a compreender a dinâmica destes recetores.

Relativamente aos modelos experimentais de neurodegeneração, é difícil tecer conclusões sobre o papel específico dos recetores da adenosina em doenças crónicas, visto que grande parte das evidências é derivada de modelos de lesão aguda e por vezes não levam em conta a idade do animal. Isto tem particular relevância uma vez que doenças como AD e PD são altamente prevalentes no idoso.

Parece bem estabelecido que a ativação dos recetores A_1 confere neuroprotecção enquanto que o bloqueio dos recetores A_{2A} atenua a patologia na maioria das doenças cerebrais. Isto significaria que uma combinação de inibidores da adenosina cinase e antagonistas A_{2A} resultaria no maior potencial neuroprotector. Contudo, este impacto só poderá ser previsto assim que seja clarificado como e quando é que se alteram os níveis cerebrais (incluindo sinapse e domínios neuro-glial, glia-glial e gliovascular) de adenosina extracelular. Além disso, tais flutuações podem variar consoante a doença e o seu estadio, o que complica este cenário.

O reconhecimento do papel duplo da neuroinflamação na neurodegeneração e neuroproteção, aliado à função de controlo do balanço entre dano e reparação tecidular do sistema adenosinérgico, levou à investigação das potencialidades da adenosina na modulação das funções gliais e imunes. O papel primordial aparente da adenosina de proteção fisiológica em danos agudos contrasta com a capacidade reduzida de proteção em condições crónicas. Os sinais mediados pela adenosina são

endogenamente protetores , mas imperfeitos e inconsistentes. Por exemplo, o bloqueio dos recetores A_{2A} cerebrais para suprimir inflamação pode exacerbar efeitos proinflamatórios periféricos. Aliás, as interações com astrócitos e microglia têm provavelmente uma grande importância nesta modulação, visto que cada uma destas células gliais apresenta recetores de adenosina e têm efeitos opostos de promoção das respostas imunes e de inibição da inflamação do sistema nervoso central.

No que concerne às doenças neurodegenerativas em específico, existem também alguns aspetos que merecem discussão.

Na AD ainda é difícil distinguir os impactos do bloqueio A_{2A} no défice cognitivo daqueles que estão relacionados com a fisiopatologia da doença em si. Um alvo putativo a ser testado está relacionado com a função colinérgica. Foi observado que os recetores α-7 nicotínicos da acetilcolina estão aumentados no início da doença e que podem ser controlados pela ativação dos recetores A_{2A}. Visto que há um défice de projeções colinérgicas em AD, o bloqueio de recetores A_{2A} pode assim ter ainda mais um mecanismo de regulação de fenómenos de plasticidade neuronial.

Os últimos anos de investigação revolucionaram a visão clássica da PD. Na verdade, a neurodegeneração estende-se a outros locais e outros terminais e os circuitos de controlo motor dos gânglios da base são muito mais intrincados, dificultando a compreensão do papel da adenosina na sua fisiopatologia. Além disso, a caracterização e desenvolvimento de ligandos que tenham como alvo heterómeros que contenham o recetor A_{2A} devem ser considerados no futuro. Estes têm propriedades farmacológicas únicas e são mais seletivos em localização do que cada um dos recetores constituintes, permitindo uma melhor perceção dos mecanismos envolvidos na doença.

As discrepâncias dos efeitos dos recetores A_{2A} na HD têm sido o maior entrave no desenvolvimento de novas terapias. Agonistas A_{2A} parecem apresentar maior benefício em estadios tardios de degeneração enquanto que os efeitos protetores do bloqueio A_{2A} dependem do grau de inibição pós-sináptica. Também aqui a formação de heterómeros que contenham o recetor A_{2A} constitui um alvo mais específico e oportuno.

A investigação do papel específico do sistema adenosinérgico na fisiopatologia da MS está ainda no seu início. A MS pode ser vista como um modelo de autoimunidade protetora, em que os mecanismos naturais de reparação cerebral estão impedidos. Os recetores A_1 parecem ser importantes na restauração desses mecanismos e a indução de aumentos de adenosina extracelular parece uma estratégia plausível.

As múltiplas questões em aberto poderão obter resposta com a exploração das potencialidades dos recetores A_{2B} e A_3, assim como através do uso de novos agonistas e antagonistas, moduladores alostéricos e de heterómeros com diferentes afinidades e propriedades, criando nomeadamente um balanço adequado entre efeitos pré- e pós-sinápticos. A ação das ectonucleotidases também tem um papel importante na sinalização purinérgica de recetores de ATP versus adenosina, após se ter verificada a formação de complexos oligoméricos entre estes.

Também foi observado que a astrogliose reativa presente em muitas patologias cerebrais está associada a um aumento patológico da expressão de adenosina cinase. O uso lógico de inibidores desta enzima gerou, porém, efeitos laterais sistémicos significativos que incentivaram uma nova era de abordagens de aumento focal de adenosina. Aqui estão incluídas estratégias inteligentes como a distribuição por polímeros, a distribuição por células estaminais e a terapia génica.

Conclusões

Existe, portanto, um espectro inteiro de mecanismos fisio(pato)lógicos que estão sujeitos a regulação adenosinérgica. Terapias baseadas nesta abordagem irão depender do timing de tratamento, no que diz respeito à janela terapeutica, ao estadio e progressão dos danos e à duração e monitorização dos efeitos benéficos e adversos. Esta coincidência tempo-espaço será somente alcançada através de uma melhor compreensão sobre o modo como os sinais da adenosina são percebidos, discriminados, sustentados e terminados.