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The A_3 adenosine receptor: An enigmatic player in cell biology

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Abstract

Adenosine is a primordial signaling molecule present in every cell of the human body that mediates its physiological functions by interacting with 4 subtypes of G-protein-coupled receptors, termed A_1, A_{2A}, A_{2B} and A_3. The A_3 subtype is perhaps the most enigmatic among adenosine receptors since, although several studies have been performed in the years to elucidate its physiological function, it still presents in several cases a double nature in different pathophysiological conditions. The 2 personalities of A_3 often come into direct conflict, e.g., in ischemia, inflammation and cancer, rendering this receptor as a single entity behaving in 2 different ways. This review focuses on the most relevant aspects of A_3 adenosine subtype activation and summarizes the pharmacological evidence as the basis of the dichotomy of this receptor in different therapeutic fields. Although much is still to be learned about the function of the A_3 receptor and in spite of its duality, at the present time it can be speculated that A_3 receptor selective ligands might show utility in the treatment of ischemic conditions, glaucoma, asthma, arthritis, cancer and other disorders in which inflammation is a feature. The biggest and most intriguing challenge for the future is therefore to understand whether and where selective A_3 agonists or antagonists are the best choice.

Keywords: Adenosine A_3 receptor; Gene and tissue localization; Ischemic conditions; Inflammation; Cancer

Abbreviations: $\Delta\psi$, mithocondrial membrane potential; A_3AR, adenosine A_3 receptor; A_3KO, A_3 knock-out; ADA, adenosine deaminase; $ADA^{-/-}$, adenosine deaminase deficient; AP-1, activator protein-1; B_{max}, receptor density; Ca^+, calcium; CADO, 2-chloroadenosine; CCL2, chemokine (C-C motif) ligand 2; CHO-hA_3, Chinese Hamster Ovary cells transfected with human A_3AR; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)-*N*-methyl-5′-carbamoyladenosine; CREB, cAMP response element-binding protein; DAG, 1,2-diacylglycerol; ERK1/2, extracellular signal-regulated kinase 1/2; G-CSF, granulocyte-colony stimulating factor; GFAP, glial fibrillary acidic protein; GPCR, G-protein-coupled receptor; GSK-3β, glycogen synthase kinase; HIF-1α, hypoxia-inducible factor-α, IB-MECA, N^6-(3-iodobenzyl)-adenosine-5′-*N*-methylcarboxamide; IFN-γ, interferon γ; IL, interleukin; iNOS, inducible nitric oxide synthase; IP_3, inositol triphosphate; K_{ATP}, ATP-sensitive potassium; LAK, lymphokine-activated killer; LPS, lipopolysaccharide; MAP-2, microtubule-associated protein 2; MAPK, mitogen-activated protein kinase; MCP3, monocyte chemotactic protein-3; MEK, mitogen-activated protein kinase kinase; MIP-1α, macrophage inflammatory protein; mito, mitochondrial; mPTP, mitochondrial permeability transition pore; NECA, 5-*N*-ethylcarboxamide adenosine; NF-κB, nuclear factor-κB; NK, natural killer; p38, stress-activated protein kinase with molecular weight 38 kDa; PBMC, peripheral blood mononuclear cells; PI3K, phosphoinositide 3-kinase; PKB/Akt, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PTX, pertussis toxin; RBL, rat basophilic leukemia; TARC, thymus- and activation-regulated chemokine; TNF-α, tumor necrosis factor α; TRAIL, TNF-related apoptosis-inducing ligand.

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1. Introduction

1.1. A_3 receptor gene cloning, localization and structure

The A_3 adenosine receptor (A_3AR) is the only adenosine subtype which was cloned before its pharmacological identification. It was originally isolated as an orphan receptor from rat testis, having 40% sequence homology with canine A_1 and A_{2A} subtypes (Meyerhof et al., 1991) and was identical with the A_3AR later cloned from rat striatum (Zhou et al., 1992). Homologs of the rat striatal A_3AR have been cloned from sheep and human, revealing large interspecies differences in A_3AR structure. For example, the rat A_3AR presents only 74% sequence homology with sheep and human A_3AR, while there is 85% homology between sheep and human A_3AR. This is reflected in the very different pharmacological profiles of the species homologs, especially in terms of antagonist binding that has made characterization of this adenosine subtype difficult. Recently equine A_3AR has been cloned and pharmacologically characterized. Sequencing of the cDNA indicated that it has a high degree of sequence similarity with that of other mammalian A_3AR transcripts, including human and sheep (Brandon et al., 2006).

The A_3AR has been mapped on human chromosome 1p21-p13 (Atkinson et al., 1997) and consists of 318 aminoacid residues. Murrison et al. (1996) determined that the A_3AR gene contains 2 exons separated by a single intron of about 2.2 kb. The upstream sequence does not contain a TATA-like motif, but it has a CCAAT sequence and consensus binding sites for SP1, NF-IL6, GATA1 and GATA3 transcription factors. Involvement of the latter in transcriptional control of this gene would be consistent with a role of the receptor in immune function. The A_3AR is a G-protein-coupled receptor (GPCR) characterized by its C-terminal portion facing the intracellular compartment and 7 transmembrane spanning domains. In contrast to other adenosine receptors, the C-terminal region presents multiple serine and threonine residues, which may serve as potential sites of phosphorylation that are important for rapid receptor desensitization upon agonist application (Palmer & Stiles, 2000). Phosphorylation leads to a decrease of the number of receptors in the high-affinity state and a decrease of agonist potency to inhibit adenylyl cyclase activity. At the same time, the receptor is reversibly internalized in an agonist-dependent fashion (Trincavelli et al., 2002a).

1.2. Tissue localization of A_3 receptor mRNA and protein

The A_3AR has widely distributed its mRNA being expressed in testis, lung, kidneys, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon and eye of rat, sheep and humans (Zhou et al., 1992; Salvatore et al., 1993; Linden, 1994; Rivkees, 1994; Dixon et al., 1996). However, marked differences exist in expression levels within and among species. In particular rat testis and mast cells express high concentrations of A_3 mRNA, while low levels have been detected in most other rat tissues (Linden et al., 1993; Salvatore et al., 1993). Lung and liver have been found as the organs expressing high levels of A_3 mRNA in human, while low levels have been found in aorta and brain (Salvatore et al., 1993). Lung, spleen, pars tuberalis and pineal gland expressed the highest levels of A_3 mRNA in sheep.

The presence of A_3AR protein has been evaluated through radioligand binding, immunoassay or functional assay in a variety of primary cells, tissues (Table 1) and cell lines (Table 2). In the mouse brain a widespread, relatively low level of A_3AR binding sites, with a density (B_{max}) of 15 fmol/mg of protein in cerebellum, was found (Jacobson et al., 1993). Similar data were obtained in the rat and in gerbil and rabbit brain (Ji et al., 1994). Due to this very low expression, other authors reported that from in situ hybridization experiments, it was not possible to detect either the A_3 receptor gene or binding site in the central nervous system (CNS; Rivkees et al., 2000) and others described the expression of the A_3AR in thalamus and hypothalamus (Yaar et al., 2002). However, electrophysiological and biochemical evidence suggested the presence of A_3AR in the rat hippocampus (Dunwiddie et al., 1997; Macek et al., 1998; Lopes et al., 2003a) and cortex (Brand et al., 2001), and functional studies also indicated its presence in the brain (Jacobson et al., 1993; Von Lubitz et al., 1994; Haskó et al., 2005). In cardiomyocytes, there was no direct evidence of the presence of A_3AR (Peart & Headrick, 2007) but a plethora of studies reported that it was responsible for cardioprotection in a variety of species and models, including isolated cardiomyocytes and isolated myocardial muscle preparations (Tracey et al., 1997; Shneyvays et al., 1998; Thourani et al., 1999a; Shneyvays et al., 2001; Cross et al., 2002; Harrison et al., 2002; Germack & Dickenson, 2004; Headrick & Peart, 2005; Xu et al., 2006). In the rat mast cell line RBL-2H3, binding experiments detected a density of about 1 pmol/mg of protein (Olah et al., 1994; Ramkumar et al., 2003) and several authors reported a role for A_3AR in rat mast cell degranulation (Carruthers & Fozard, 1993; Fozard & Carruthers, 1993; Ramkumar et al., 1993; Hannon et al., 1995; El-Hashim et al., 1996; Fozard et al., 1996). In enteric neurons and epithelial cells, the A_3AR was evidenced by immunohistochemical studies (Christofi et al., 2001), and subsequently, it was quantified in colonic mucosa by radioligand binding experiments (Gessi et al., 2004a). In lung parenchyma and in human