Biochemical Pharmacology 87 (2014) 321–331

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Different efficacy of adenosine and NECA derivatives at the human A_3 adenosine receptor: Insight into the receptor activation switch

Diego Dal Ben [a], Michela Buccioni [a], Catia Lambertucci [a], Sonja Kachler [b], Nico Falgner [b], Gabriella Marucci [a], Ajiroghene Thomas [a], Gloria Cristalli [a], Rosaria Volpini [a], Karl-Norbert Klotz [b,*]

[a] School of Pharmacy, Medicinal Chemistry Unit, University of Camerino, Via S. Agostino 1, I-62032 Camerino, Italy
[b] Universität Würzburg, Institut für Pharmakologie und Toxikologie, Versbacher Str. 9, D-97078, Würzburg, Germany

ARTICLE INFO

Article history:
Received 17 July 2013
Accepted 15 October 2013
Available online 23 October 2013

Keywords:
Adenosine receptor
NECA
A_3
Agonist
Efficacy
Molecular modeling

ABSTRACT

A_3 Adenosine receptors are promising drug targets for a number of diseases and intense efforts are dedicated to develop selective agonists and antagonists of these receptors. A series of adenosine derivatives with 2-(ar)-alkynyl chains, with high affinity and different degrees of selectivity for human A_3 adenosine receptors was tested for the ability to inhibit forskolin-stimulated adenylyl cyclase. All these derivatives are partial agonists at A_3 adenosine receptors; their efficacy is not significantly modified by the introduction of small alkyl substituents in the N^6-position. In contrast, the adenosine-5′-*N*-ethyluronamide (NECA) analogs of 2-(ar)-alkynyladenosine derivatives are full A_3 agonists. Molecular modeling analyses were performed considering both the conformational behavior of the ligands and the impact of 2- and 5′-substituents on ligand–target interaction. The results suggest an explanation for the different agonistic behavior of adenosine and NECA derivatives, respectively. A sub-pocket of the binding site was analyzed as a crucial interaction domain for receptor activation.

1. Introduction

Adenosine (Ado) is an ubiquitous metabolite that regulates the function of virtually every cell type via one or several of four subtypes of G protein-coupled receptors (GPCRs) known as A_1, A_{2A}, A_{2B}, and A_3 [1]. In human, the A_3 adenosine receptor (A_3AR) is highly expressed in particular in immune cells, lung, and liver and at lower densities in heart, aorta, and brain and it is involved in a variety of key physiological processes such as release of inflammatory mediators and inhibition of tumor necrosis factor-α production [2–6]. These data make A_3AR an attractive therapeutic target and the design and synthesis of potent and selective agonists for this adenosine receptor (AR) subtype could be helpful to provide tools for further characterization and evaluation of the physio-pathological role of the protein and for the development of new drugs with anti-inflammatory, anticancer, and cardioprotective potential [7–14].

Over the decades a large number of agonists with distinct patterns of selectivity for the different AR subtypes have been developed [1,3,15–19], with the Ado scaffold generally believed as mandatory for their development. However, a series of novel compounds structurally unrelated to Ado was discovered that potently stimulates AR subtypes [20,21]. Considering Ado derivatives, previous studies reported substitutions and modifications to the Ado core resulting in partial [22,23] or complete loss of agonist efficacy [24,25], while among others the 2-, N^6-, and 5′-positions were found to be key sites for tolerable modifications to the Ado scaffold without altering the agonistic properties.

In particular, substitution of the 2-position may dramatically affect the pharmacological characteristics of Ado and selective agonists for all subtypes except A_{2B}AR have been developed employing a structurally diverse array of substituents in this position. As an example, 2-chloro-N^6-cyclopentyladenosine (CCPA) was introduced as an A_1AR selective agonist [26,27] while CGS

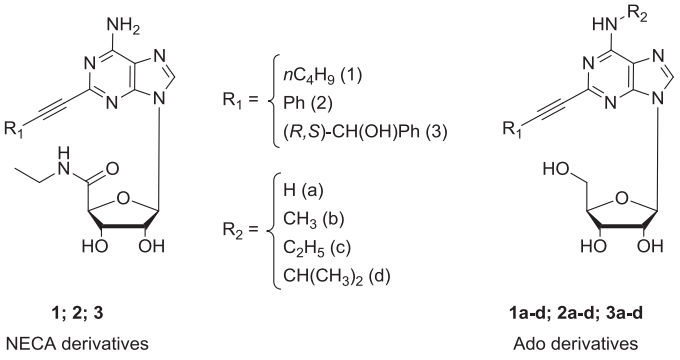
Chemical compounds studied in this article: Adenosine (PubChem CID: 60961); NECA (PubChem CID: 448222); HENECA (PubChem CID: 164437); PHPNECA (PubChem CID: 44339675).

Abbreviations: NECA, adenosine-5′-*N*-ethyluronamide; Ado, adenosine; GPCR, G protein-coupled receptor; AR, adenosine receptor; CCPA, 2-chloro-N^6-cyclopentyladenosine; HEMADO, 2-hexyn-1-yl-N^6-methyladenosine; CPA, N^6-cyclopentyladenosine; HENECA, 2-hexyn-1-yl-adenosine-5′-*N*-ethyluronamide; HEADO, 2-hexyn-1-yl-adenosine; PENECA, 2-phenylethynyladenosine-5′-*N*-ethyluronamide; PEADO, 2-phenylethynyladenosine; PEMADO, N^6-methyl-2-phenylethynyladenosine; PHPNECA, 2-(3-hydroxy-3-phenyl)propyn-1-yl-adenosine-5′-*N*-ethyluronamide; PHPADO, 2-(3-hydroxy-3-phenyl)propyn-1-yl-adenosine; PHPMADO, 2-(3-hydroxy-3-phenyl)propyn-1-yl-N^6-methyladenosine; MECA, adenosine-5′-*N*-methyluronamide; TM, transmembrane; EL, extracellular; ns, nanosecond; ps, picosecond.

* Corresponding author.
E-mail address: klotz@toxi.uni-wuerzburg.de (K.-N. Klotz).

0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.10.011

Table 1
Affinity (K_i nM) values of A_3AR agonists (values are from ref [40]).



cpd		R_1	R_2	A_3AR K_i (nM)
1	2-Hexyn-1-yl-adenosine-5′-*N*-ethyluronamide (HENECA)	nC_4H_9	H	2.4
1a	2-Hexyn-1-yl-adenosine (HEADO)	nC_4H_9	H	4.7
1b	2-Hexyn-1-yl-N^6-methyladenosine (HEMADO)	nC_4H_9	CH_3	1.1
1c	2-Hexyn-1-yl-N^6-ethyladenosine	nC_4H_9	C_2H_5	2.3
1d	2-Hexyn-1-yl-N^6-isopropyladenosine	nC_4H_9	$CH(CH_3)_2$	9.7
2	2-Phenylethynyladenosine-5′-*N*-ethyluronamide (PENECA)	Ph	H	6.2
2a	2-Phenylethynyladenosine (PEADO)	Ph	H	16
2b	N^6-Methyl-2-phenylethynyladenosine (PEMADO)	Ph	CH_3	3.4
2c	N^6-Ethyl-2-phenylethynyladenosine	Ph	C_2H_5	4.9
2d	N^6-Isopropyl-2-phenylethynyladenosine	Ph	$CH(CH_3)_2$	17
3	2-(3-Hydroxy-3-phenyl)propyn-1-yl-adenosine-5′-*N*-ethyluronamide (PHPNECA)	(*R*,*S*)-CH(OH)Ph	H	0.42
3a	2-(3-Hydroxy-3-phenyl)propyn-1-yl-adenosine (PHPADO)	(*R*,*S*)-CH(OH)Ph	H	3.3
3b	2-(3-Hydroxy-3-phenyl)propyn-1-yl-N^6-methyladenosine (PHPMADO)	(*R*,*S*)-CH(OH)Ph	CH_3	0.76
3c	2-(3-Hydroxy-3-phenyl)propyn-1-yl-N^6-ethyladenosine	(*R*,*S*)-CH(OH)Ph	C_2H_5	0.97
3d	2-(3-Hydroxy-3-phenyl)propyn-1-yl-N^6-isopropyladenosine	(*R*,*S*)-CH(OH)Ph	$CH(CH_3)_2$	2.3

21680 with a larger 2-(*p*-(2-carboxyethyl)phenylethylamino)-moiety is a potent A_{2A}AR agonist [28,29]. Further highly potent A_{2A}AR agonists were also identified in a series of 2-(*N*′-alkylidenehydrazino)Ados [30,31].

It turned out that activation of the A_3AR is more affected by structural changes of Ado than activation of other AR subtypes. In particular modifications in the 2- and N^6-position as well as the ribose will influence the efficacy of Ado derivatives at this receptor [32–37]. It was found out that the introduction of an alkynyl group in the 2-position is a successful strategy to develop Ado derivatives with high affinity for A_1 and A_{2A} AR. However, it proved to be a particularly successful approach for the development of high affinity agonists with remarkable selectivity for A_3AR [37–43]. [^{3}H]2-hexyn-1-yl-N^6-methyladenosine ([^{3}H]HEMADO) constitutes such an example and was recently introduced as an A_3AR selective radioligand [44].

Normally, Ado derivatives that were characterized as agonists at one receptor subtype would stimulate other subtypes as well if affinity allows for binding. A surprising observation was made that the above cited A_1AR agonist CCPA behaves as antagonist at the human A_3AR subtype in contrast to N^6-cyclopentyladenosine (CPA) that lacks the 2-chloro substitution [45]. A further previous report [35] described 2-substituted Ado derivatives with varying efficacy at the different ARs. Considering Ado derivatives presenting alkynyl substituents, it was reported that the introduction of 8-substituents in 2-alkynylAdos leads to the development of compounds presenting as partial agonists at A_{2A}AR [46], while the transfer of the same alkynyl substituents from the 2- to 8-position of Ado changes the pharmacological profile of the compounds from A_3AR agonist to antagonist [25,47].

These data prompted us to take a careful look at the functional behavior of selected 2-substituted Ado derivatives which we reported in previous studies as potent agonists of A_3AR [37,40]. These compounds (Table 1) represented a series of Ado derivatives bearing in the 2-position (ar)-alkynyl chains and in the N^6-position small alkyl groups. All derivatives are endowed with low nanomolar affinity and different degrees of selectivity for the human A_3AR [40,43].

Further modification of these compounds by the replacement of the hydroxyl group in the 5′-position of the sugar moiety with an *N*-ethylcarboxamido function (obtaining the NECA derivatives) showed to enhance A_3AR affinity and selectivity. In the present study, these molecules were tested for their ability to inhibit forskolin-stimulated adenylyl cyclase activity and hence to analyze their efficacy profiles at the human A_3AR. Molecular modeling analyses were then carried out to get a rationalization of the activities identified for the presented series of compounds. The crystal structure of the A_{2A}AR in complex with ZM241385 (PDB ID: 3EML [48]) was employed as template to develop a homology model for the A_3AR that was used for docking studies of the analyzed ligands.

2. Materials and methods

2.1. Biology

All AR agonists (Table 1) were synthesized as described earlier [40,49–52]. All other compounds including guanine nucleotides were from Sigma-RBI, Taufkirchen, Germany. [α-^{32}P]ATP was from PerkinElmer, Rodgau, Germany. Media and fetal calf serum for cell culture were from PanSystems, Aidenbach, Germany, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine and G-418 were purchased from Gibco-Life Technologies, Eggenstein, Germany. All other materials were from sources as described earlier [28,37].