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Probing biased/partial agonism at the G protein-coupled A_{2B} adenosine receptor☆

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ABSTRACT

G protein-coupled A_{2B} adenosine receptor (AR) regulates numerous important physiological functions, but its activation by diverse A_{2B}AR agonists is poorly profiled. We probed potential partial and/or biased agonism in cell lines expressing variable levels of endogenous or recombinant A_{2B}AR. In cAMP accumulation assays, both 5′-substituted NECA and C2-substituted MRS3997 are full agonists. However, only 5′-substituted adenosine analogs are full agonists in calcium mobilization, ERK1/2 phosphorylation and β-arrestin translocation. A_{2B}AR overexpression in HEK293 cells markedly increased the agonist potency and maximum effect in cAMP accumulation, but less in calcium and ERK1/2. A_{2B}AR siRNA silencing was more effective in reducing the maximum cAMP effect of non-nucleoside agonist BAY60-6583 than NECA's. A quantitative 'operational model' characterized C2-substituted MRS3997 as either balanced (cAMP accumulation, ERK1/2) or strongly biased agonist (against calcium, β-arrestin). N^6-substitution biased against ERK1/2 (weakly) and calcium and β-arrestin (strongly) pathways. BAY60-6583 is ERK1/2-biased, suggesting a mechanism distinct from adenosine derivatives. BAY60-6583, as A_{2B}AR antagonist in MIN-6 mouse pancreatic β cells expressing low A_{2B}AR levels, induced insulin release. This is the first relatively systematic study of structure–efficacy relationships of this emerging drug target.

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1. Introduction

Structure–efficacy relationships of the A_1, A_{2A} and A_3 adenosine receptor (AR) agonists have been studied extensively in recent years [1–5]. This knowledge has led to the identification of many partial/biased agonists, including 5′-truncated nucleosides as antagonists and partial agonists for the A_3AR [3,6], and as full agonists for A_1 [5] and A_{2A} ARs [4], which have potential clinical applications [5,7,8]. The structure–efficacy relationships of agonists at the A_{2B}AR have not been previously probed.

The A_{2B}AR plays a critical role in many physiological and pathophysiological conditions, such as taste [9,10], inflammation [11–17], cancer [18–22], ischemic conditions [23,24], erectile function [25], diabetes [26–28], stem cell differentiation [29] and oxidative stress [30]. Despite its recently demonstrated importance, the nature of A_{2B}AR activation is still poorly understood. The role of the A_{2B}AR has been reported controversially, e.g. both agonists and antagonists have been demonstrated to have anti-inflammatory effects [31–33]. Those seemingly contradictory results could be, at least in part, because of promiscuous G protein

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Abbreviations: BAY60-6583 (BAY, LUF6210), 2-[[6-amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]acetamide; cyclic AMP (cAMP), 3′5′-cyclic adenosine monophosphate; CGS21680, 2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; CHO, Chinese hamster ovary; CPA, N^6-cyclopentyladenosine; CPCA, adenosine-5′-*N*-cyclopropyluronamide; CV1808, 2-phenylaminoadenosine; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular-signal-regulated kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPCR, G protein-coupled receptor; HEK, human embryonic kidney; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; IB-MECA, N^6-(3-iodobenzyl)-adenosine-5′-*N*-methyluronamide; KRBB, Krebs ringer bicarbonate buffer; LUF5833, 2-aminophenyl-6-(1*H*-imidazol-2-ylmethylsulfanyl)pyridine-35-dicarbonitrile; MRS3534, 2-(2-(indol-3-yl)ethyloxy)adenosine; MRS3997, 2-(2-(6-bromo-indol-3-yl)ethyloxy)adenosine; MRS5911, N^6-(4-iodophenyl)adenosine; NECA, adenosine-5′-*N*-ethyluronamide; *R*-PIA, *R*-N^6-(phenylisopropyl)adenosine; PSB603, 8-[4-[4-(4-chlorophenyl)piperazide-1-sulfonyl)phenyl]]-1-propyl-xanthine.

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coupling of the A_{2B}AR depending on the tissues and cells investigated or pathways measured; the lack of A_{2B}AR selective agonists until recently; and most importantly, a severe deficiency of understanding of the pharmacological properties of A_{2B}AR agonists used in some early studies. It is known that an agonist in one biological system might be a partial agonist or even an antagonist in other systems. Also, it is possible that different agonists attain variable maximum effects in specific signaling pathways, or a specific agonist has variable maximum effects in different signaling pathways. Biased agonists have been reported for several AR subtypes, but not the A_{2B}AR [34,35].In the present study we investigate various A_{2B}AR agonists of distinct structural classes (Fig. 1) in activating four signaling pathways under the direction of the A_{2B}AR using several cell lines, including T24 cells, a human bladder cancer cell line endogenously expressing the A_{2B}AR, and HEK293 cells expressing both endogenous and recombinant human A_{2B}ARs.

2. Materials and methods

2.1. Materials

2.1.1. Agonists

We selected four chemical classes of synthetic A_{2B}AR agonists: (A) 2-substituted adenosine analogs: MRS3997 [36], MRS3534 [37], CV1808 [38]. (B) N^6-substituted adenosine derivatives: MRS5911 [39], *R*-PIA [40]. (C) 5′-substituted adenosine analogs, NECA (adenosine-5′-*N*-ethyluronamide) and CPCA (5-N′-cyclopropyl-carboxamidoadenosine. (D) Non-nucleoside 3,5-dicyanopyridine agonists LUF5833 and BAY60-6583 (LUF6210, termed hereafter 'BAY') [41,42], which were synthesized at Leiden/Amsterdam Center for Drug Research (Leiden, The Netherlands). MRS3997, MRS3534 and MRS5911 [39] were synthesized at NIDDK, National Institutes of Health (Bethesda, MD, USA). Adenosine, NECA, CPA, CPCA, CV1808, *R*-PIA and probenecid were from Sigma (St. Louis, MO). Cl-IB-MECA was from Tocris (Minneapolis, MN).

2.1.2. Cell lines

Human cell lines (ATCC, Manassas, VA) were selected for endogenous expression of the A_{2B}AR at variable levels: T24 bladder cancer cells; 1321N1 astrocytoma cells; WM266 melanoma cells; PC-3 prostate cancer cells and HEK293 cells expressing both endogenous and recombinant human A_{2B}ARs. Other species were represented in: COS-7 monkey kidney cells endogenously expressing the A_{2B}AR (ATCC, Manassas, VA); MIN-6 mouse pancreatic β cells expressing the endogenous mouse A_{2B}AR (obtained from Jürgen Wess, NIDDK, NIH); and PathHunter CHO cells expressing the recombinant mouse A_{2B}AR and an engineered β-arrestin 2 (DiscoverX, Fremont, CA). The reason we used this mouse A_{2B}AR cell line was that a similar version of PathHunter CHO cells from DiscoverX expressing the human A_{2B}AR did not produce a robust response.

2.1.3. Additional materials and kits

Luminescence assay kit for β-arrestin2 translocation was from DiscoveRx (Fremont, CA). Calcium dye kit was from Molecular Devices (Sunnyvale, CA). AlphaScreen cyclic AMP kits and SureFire p-ERK1/2 (Thr202/Tyr204) assay kits were from PerkinElmer (Boston, MA). Superscript III First Strand Synthesis Supermix kit was from Invitrogen (Carlsbad, CA). The gene-specific FAM-labeled MGB Taqman probes were from Applied Biosystems (Life Technologies, Grand Island, NY). All other materials are from commercial sources and are of analytical grade.

2.2. cAMP accumulation assay

Cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 μmol/ml glutamine. Cells were plated in 96-well plates in 100 μl medium. After 24 h, the medium was removed and cells were washed three times with 100 μl DMEM, containing 50 mM HEPES, pH 7.4. Cells were then treated for 30 min with agonist and/or test compound in the presence of rolipram (10 μM) and adenosine deaminase (3 units/ml). The reaction was terminated by removal of the supernatant, and cells were lysed upon the addition of 100 μl of lysis buffer (0.3% Tween-20). For determination of cAMP production, an AlphaScreen cAMP kit was used according manufacturer's instructions.

2.3. Intracellular calcium mobilization assay

Cells were grown overnight in 100 μl of media in 96-well flat bottom plates at 37 °C at 5% CO_2 or until approx. 90% confluency.

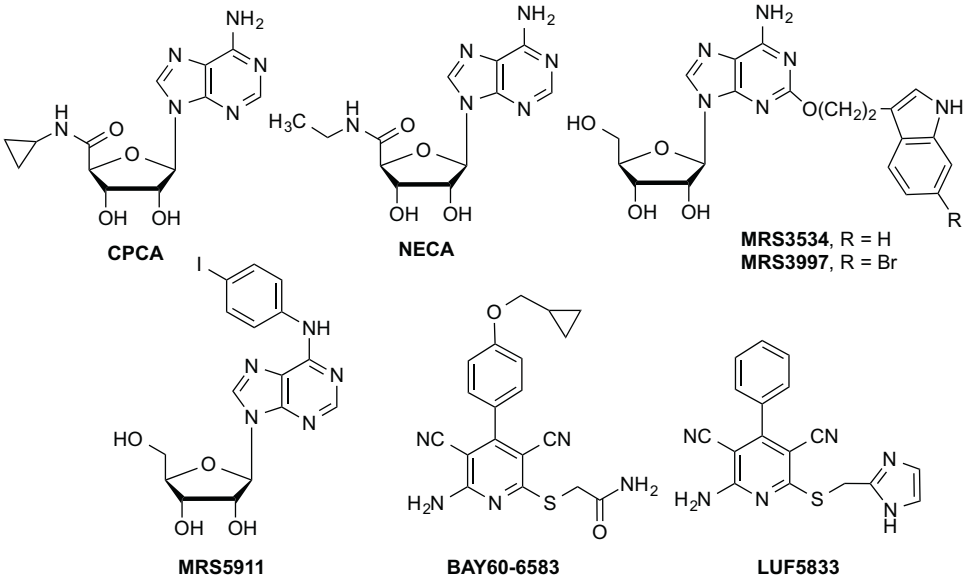

Fig. 1. Chemical structures of representative agonists used in the present study. Non-nucleoside agonists: BAY and LUF5833; 5′-substituted: NECA, CPCA, 2-substituted: MRS3997 and MRS3534. N^6-substituted: MRS5911.

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