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Getting personal: Endogenous adenosine receptor signaling in lymphoblastoid cell lines

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ABSTRACT

Genetic differences between individuals that affect drug action form a challenge in drug therapy. Many drugs target G protein-coupled receptors (GPCRs), and a number of receptor variants have been noted to impact drug efficacy. This, however, has never been addressed in a systematic way, and, hence, we studied real-life genetic variation of receptor function in personalized cell lines. As a showcase we studied adenosine A_{2A} receptor (A_{2A}R) signaling in lymphoblastoid cell lines (LCLs) derived from a family of four from the Netherlands Twin Register (NTR), using a non-invasive label-free cellular assay. The potency of a partial agonist differed significantly for one individual. Genotype comparison revealed differences in two intron SNPs including rs2236624, which has been associated with caffeine-induced sleep disorders. While further validation is needed to confirm genotype-specific effects, this set-up clearly demonstrated that LCLs are a suitable model system to study genetic influences on A_{2A}R response in particular and GPCR responses in general.

1. Introduction

The majority of therapeutic drug targets to date are within the G protein-coupled receptor (GPCR) superfamily, a class of membrane-bound proteins [1,2]. As such, GPCRs have been widely and intensively studied for the development of new therapeutics. Among the most well-studied members of this group are the adenosine receptors, a family comprising of 4 different subtypes: A_1, A_{2A}, A_{2B} and A_3 [3]. The various subtypes have been implied in a broad range of diseases and (patho)-physiological conditions, such as a variety of respiratory and inflammatory conditions for the A_{2A} or cardiovascular disorders for the A_1 [4]. Likewise, a wide variety of compounds selectively activating, inhibiting or modulating these receptors are available to date [3,4]. Some of these have even been or are currently in clinical trials [3,4]. Adenosine itself has been long approved for treatment of supraventricular tachycardia [3] and one A_{2A}R antagonist, istradefylline, has made it to the market as adjuvant drug therapy for Parkinson's disease in Japan [5].

In the emerging era of personalized medicine, it is paramount for drug development to better understand the effects of a drug

Abbreviations: hA_1AR, human adenosine A_1 receptor; $hA_{2A}AR$, $A_{2A}R$, human adenosine A_{2A} receptor; $hA_{2B}AR$, human adenosine A_{2B} receptor; hA_3AR, human adenosine A_3 receptor; ADORA2A, adenosine A_{2A} receptor gene; AR, adenosine receptor; cAMP, cyclic adenosine 5′-monophosphate; CB2, cannabinoid receptor 2; CI, Cell Index; Δ CI, Δ cell index or delta cell index; DMSO, dimethylsulfoxide; FCS, fetal calf serum; EBV, Epstein–Barr Virus; EC_{50}, half maximal effective concentration; EC_{80}, 80% maximal effective concentration; GPCR, G protein-coupled receptor; IC_{50}, half maximal inhibitory concentration; K_i, equilibrium inhibition constant; LCL, lymphoblastoid cell line; NTR, Netherlands Twin Register; PBS, phosphate buffered saline; RTCA, real-time cell analyzer; SNP, single nucleotide polymorphism.

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not only in the overall population, but in the individual patient as well [6]. Genetic differences between individuals can affect drug action. Accordingly, several examples linking GPCR polymorphisms to diseases and drug response variation already exist [7–11], which include many commonly targeted GPCRs [11] such as purinergic [12,13], cannabinoid [9,10] and adenosine [14–16] receptors. Specifically for the adenosine A_{2A} receptor, Single Nucleotide Polymorphisms (SNPs) have been associated with for instance anxiety [17,18], caffeine intake [17], or vigilance and sleep [14]. Despite these examples of statistical association of genotype and condition, as well as extensive mutational characterization of the adenosine receptors, little is known about the direct functional effect of receptor polymorphisms or SNPs. Therefore, an ideal set-up would be to use patient-derived material as a model system to study the influence of polymorphisms on receptor response.

Lymphoblastoid cell lines (LCLs) are one of the most common choices for storing a person's genetic material [19,20] and can be used to study GPCR function as has been shown recently [21]. For example, Morag, Kirchheiner [22] studied the influence of a few GPCR antagonists on LCL growth. We recently published an even more direct way of measuring receptor function, including agonist and antagonist concentration-effect curves [21]. Using a newly developed, highly sensitive label-free cellular assay technology [21,23,24], we have shown that it is possible to measure an individual's GPCR response in LCLs using the cannabinoid receptor 2 as example [21]. In such label-free assays one can monitor drug effects on an intact cell in real-time, rather than being limited to a static, one-molecule-detection of ligand binding or second messenger accumulation, as is usually employed in GPCR and adenosine receptor research [3,23–25].

In the current study we have applied this label-free methodology to assess personal adenosine A_{2A} receptor function in LCLs. We characterized A_{2A}R signaling with various types of ligands including endogenous and synthetic agonists, partial agonist and antagonists, among which istradefylline. To allow conclusions about genotype in relation to receptor response, we compared responses between the individuals of a family of four from the Netherlands Twin Register [26]. This family consisted of two genetically unrelated individuals, the parents, as well as their children, which were monozygotic twins. Confirming the comparability of monozygotic twins' responses is one of the standard ways to control for genotype-unrelated effects, and thereby assess a system's suitability for genetic studies [26,27].

2. Material and methods

2.1. Chemicals and reagents

Fibronectin from bovine plasma, Roswell Park Memorial Institute (RPMI) 1640 cell culture medium (25 mM HEPES and $NaHCO_3$), NECA, adenosine and ATP were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). CGS21680, ZM241385 and CCPA were purchased from Abcam Biochemicals (Cambridge, United Kingdom), Cl-IB-MECA from Tocris Bioscience (Bristol, United Kingdom) and istradefylline from Axon Medchem (Groningen, The Netherlands). BAY60-6583 was synthesized in-house. LUF compounds were synthesized as described by van Tilburg, von Frijtag Drabbe Kunzel [28] for LUF5448 and LUF5631, van Tilburg, Gremmen [29] for LUF5549 and LUF5550 and Beukers, Chang [30] for LUF5834. All other chemicals and reagents were of analytical grade and obtained from commercial sources, unless stated otherwise.

2.2. Lymphoblastoid cell line generation

The lymphoblastoid cell lines (LCLs) were generated from participants of the Netherlands Twin Register (NTR, VU, Amsterdam, The Netherlands) [26]. The LCLs were generated by the Rutgers Institute (Department of Genetics, Piscataway, NJ, USA) using a standard transformation protocol [26], according to a previous publication [21]. Peripheral B-lymphocytes were transformed with Epstein–Barr Virus (EBV) by treatment with filtered medium from a Marmoset cell line in the presence of phytohemaglutinin (PHA) during the first week of culture [19,20,31]. Cultures were maintained for 8–12 weeks to expand the EBV transformed lymphocytes and subsequently cryopreserved.

2.3. Cell culture

LCLs from a family of four individuals, two parents (genetically unrelated; called Parent 1 and Parent 2) and their monozygotic twin (genetically equal; called Twin 1 and Twin 2), were used for the experiments presented in this manuscript. According to culture conditions described in a previous publication [21], cryopreserved cells were thawed and resuscitated. LCLs were grown as suspension cells in RPMI 1640 (25 mM HEPES and $NaHCO_3$) supplemented with 15% FCS, 50 mg/ml streptomycin, 50 IU/ml penicillin, at 37 °C and 5% CO_2 and were subcultured twice a week at a ratio of 1:5 on 10 cm ø plates. LCLs were disposed of after maximally 120 days in culture.

2.4. qPCR

RNA from LCLs was isolated using RNeasy Mini kit (QIAGEN, Venlo, The Netherlands). The RNA was treated with optional on column DNase digestion using DNase I (QIAGEN) and converted to cDNA using Superscript III (Invitrogen, Bleiswijk, The Netherlands). cDNA was run on custom designed 384 well qPCR plates from Lonza (Copenhagen, DK), in accordance with a previous publication [32]. These plates contained primers for 379 GPCRs as well as 3 RAMPs, together with primers for Rn18s and genomic DNA (Primers are listed in Engelstoft et al. [32]). Genomic DNA sample was used as calibrator and the relative copy number was calculated as stipulated previously [32].

2.5. Label-free whole-cell analysis (xCELLigence RTCA system)

2.5.1. Instrumentation principle

Cellular assays were performed using the xCELLigence RTCA system [23] in accordance with previously published protocols [21,33]. Briefly, the real-time cell analyzer (RTCA) measures the whole-cell responses using a detection system based on electrical impedance. Impedance is generated through cell attachment to gold electrodes embedded on the bottom of the microelectronic E-plates, which changes the local ionic environment at the electrode-solution interface. Relative changes in impedance (Z) are recorded in real-time and summarized in the so-called Cell Index (CI), a dimensionless parameter. The CI at any given time point is defined as $(Z_i - Z_0)\ \Omega/15\ \Omega$, where Z_i is the impedance at each individual time point. Z_0 represents the baseline impedance in the absence of cells, which is measured prior to the start of the experiment and defined as 0. As cells adhere to the electrodes, impedance and the corresponding CI increase proportionally. Changes in cell number and degree of adhesion, as well as cellular viability and morphology are directly reflected in the impedance profile [23,24]. Such cellular parameters are also affected upon activation of GPCR signaling, thereby allowing real-time monitoring of cellular signaling events [23].

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