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Original article

Non-invasive optical biosensor for assaying endogenous G protein-coupled receptors in adherent cells

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Abstract

Introduction: Screening drugs against G protein-coupled receptors (GPCRs) — the single largest family of drug targets in the human genome — is still a major effort in pharmaceutical and biotech industries. Conventional cell-based assays generally measure a single cellular event, such as the generation of a second messenger or the relocation of a specific protein target. However, manipulation or engineering of cells is often a prerequisite for these technologies to achieve desired sensitivities. The present study is focused on the use of non-invasive and manipulation-free optical biosensors for assaying endogenous GPCRs in adherent cells. **Methods:** Resonant waveguide grating (RWG) biosensor was applied to manifest ligand-induced dynamic mass redistribution (DMR) within the bottom portion of adherent cell layer. The DMR signatures mediated through the activation of several endogenous GPCRs in cells were characterized. Endogenous receptor panning was examined at cell system level by using a panel of agonists known to activate many GPCRs, and also at family receptor level by determining the efficacies of a set of family-specific agonists. **Results:** Three major types of optical signatures were identified; each was correlated with the activation of a class of GPCRs, depending on the G protein with which the receptor is coupled (i.e., G_q, G_s and G_i). The characteristics of DMR signals, mostly the amplitude and kinetics of a DMR event, were dependent on the doses of agonists and the expression levels of endogenous receptors. All three classes of endogenous receptors were found in human epidermoid carcinoma A431 cells. Interestingly, the dose-dependent switching from one type of DMR signal to another was observed for several GPCR agonists examined. A small panel of P2Y receptor agonists exhibited distinct efficacies in three cell lines examined. **Discussions:** The RWG biosensors were applicable to study the activation of endogenous GPCRs. Like second messengers or gene expression, the DMR signals obtained could be considered as novel and quantifiable physiological responses of living cells mediated through GPCRs and used for studying receptor biology.

Keywords: Resonant waveguide grating biosensor; G protein-coupled receptor; Dynamic mass redistribution; Receptor panning

1. Introduction

G protein-coupled receptors (GPCRs) are a super family of cell surface membrane proteins, and are involved in almost all physiological regulatory mechanisms in the human body as well as in the development and progression of many diseases (Rockman, Koch, & Lefkowitz, 2002; Schoneberg, Schulz, & Gudermann, 2002). Together with the ability for therapeutic intervention by small molecular medicines, GPCRs represent one of the largest classes of highly druggable targets in the human genome (Ma & Zemmel, 2002; Fredriksson & Schioth, 2005), and are a proven class of targets for drug discovery (Drews, 2000). Given that the current GPCR-based drugs only target ~ 50 well-characterized GPCRs (Fang, Lahiri, & Picard, 2003) and there are about 140 newly classified orphan receptors (Hopkins & Groom, 2002), GPCRs remain a popular class of targets in drug discovery today. However, a significant gap exists between the current pharmaceutical R and D spending and the productivity of drug discovery (i.e., the number of new drug entities entering the market). To reconcile the situation, drug discovery paradigm has started shifting from current target-directed to systems biology-centered approaches (Butcher, 2005; van der Greef & McBurney, 2005). The new paradigms proposed start with a biological or physiological response, and greatly emphasize the use of living systems for drug screens. This creates strong demand in cell-based assays

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to identify, optimize and test a potential drug candidate with significantly improved productivity.

Most of the current cell-based assays primarily rely on the measurement of a specific cellular response, including the generation of a second messenger (e.g., Ca^{2+}, cAMP), the dynamic relocation of a target tagged with a fluorescent molecule, and the expression of a reporter gene (Milligan, 2003). Generally, these technologies require manipulations or engineering of cells, such as loading of fluorescent molecules into cells or over-expression of a target with or without a fluorescent tag. These manipulations may alter the cellular physiology of the target receptor. Together with the possible interference of many compounds with the detection technology, current cell-based assays suffer imperative limitations.

Recently, we have developed a non-invasive and manipulation-free cell assay technologies, termed MRCAT (Mass Redistribution Cell Assay Technologies) (Fang, Ferrie, Fontaine, Mauro, & Balakrishnan, 2006). The MRCAT utilizes resonant waveguide grating (RWG) biosensor to perform online monitoring of the ligand-induced dynamic mass redistribution (DMR) within the bottom portion of adherent cells. The DMR signal obtained is an integrated cellular response, which is resulted from the ligand-induced dynamic, directed, and directional redistribution of cellular targets or molecular assemblies. The MRCAT not only permits studying cell activities including signaling and its network interactions (Fang, Ferrie, Fontaine, & Yuen, 2005; Fang, Li, & Peng, 2005), but also enables high throughput screening of compounds against endogenous receptors (Li, Ferrie, & Fang, 2006). Previously, we had described the DMR signature of G_q-coupled receptors (Fang et al., 2006). Here we applied the MRCAT to characterize the DMR signals mediated through the activation of endogenous G_s- and G_i-coupled receptors. The resultant DMR signals were used for receptor panning at both cell system- and family receptor-levels.

2. Materials and methods

2.1. Reagents

Adenosine amine cogener, anandamide, ATP, interleukin-8 (IL-8), interferon-γ-inducible protein-10, oleoyl-L-α-lysophosphatidic acid (LPA), NECA, thrombin, and UK14304 were purchased from Sigma Chemical Co. (St. Louis, MO). A-77636, BRL 54443, clonidine, epinephrine, HTMT dimaleate, oxotremorine M, oxymetazoline, propranolol, forskolin, NKH447 and SKF38392 were obtained from Tocris Chemical Co. (St. Louis, MO). Fluo-3 was obtained from Molecular Probes (Eugene, OR). Angiotensin II, apelin, bombesin, bradykinin, DAMGO, dynorphin A, endothelin-1, galanin, SLIGLR-amide, glucagon, α-melanocyte stimulating hormone (α-MSH), motilin, neurokinin A, neuromedin N, neuropeptide Y, neurotensin, nociceptin, substance P, and urotensin II were obtained from Bachem (King of Prussia, PA). Corning® Epic™ 96well biosensor microplates were obtained from Corning Inc (Corning, NY), and cleaned by exposure to high intensity UV light (UVO-cleaner, Jelight Company Inc., Laguna Hills, CA) for 6 min before use.

2.2. Cell culture

Human epidermoid carcinoma A431 cells, Chinese hamster ovary (CHO) cells, and engineered CHO cells overexpressing rat muscarinic receptor subtype 1 (CHO-M1) were obtained from American Type Cell Culture. For cell culturing, cells were grown in appropriate medium supplemented with 10% fetal bovine serum (FBS), 4.5 g/l glucose, 2 mM glutamine, and antibiotics. The medium was Dulbecco's modified Eagle's medium (DMEM) for A431 cells, and Kaighn's modification of Ham's F12 medium (F12K) for both CHO and CHO-M1 cells. Appropriate amount of cells at passage 2 to 8 suspended in 200 μl the corresponding medium containing 10% FBS were placed in each well of a 96well microplate. After cell seeding, the cells were cultured at 37 °C under air/5% CO_2 until ~ 95% confluency was reached.

2.3. Fluo-3 Ca^{2+} mobilization assay

A431 at passage 3 to 5 were grown in Costar™ 96well clear cell culture microplates until ~ 95% confluency, washed twice with 1× HBSS (1× regular Hank's balanced salt solution, 20 mM HEPES buffer, pH 7.0) in the presence of 2.5 mM probenicid, and labeled in the same buffer containing 4 μM Fluo-3 for 1 h at room temperature. The cells were then washed twice, and maintained in 100 μl 1× HBSS containing 2.5 mM probenicid. The assay was initiated by transferring 100 μl GPCR agonist solution to each well and calcium signal was recorded over 6 min with a 6 s interval using HTS7000 BioAssay Reader (PerkinElmer Life Science, Boston, MA).

2.4. Dynamic mass redistribution (DMR) optical biosensor assays

Corning® Epic™ angular interrogation system with transverse magnetic or p-polarized TM_0 mode was used for all studies. The detailed instrumental setup and assay protocols had been previously described (Fang, Ferrie, Fontaine, & Yuen, 2005). Briefly, all compound solutions were prepared using 1× HBSS containing minimal amount of dimethyl sulfoxide, while the cultured cells were washed and maintained with 100 μl the corresponding medium without any serum. The sensor microplate containing cells was placed into the optical system. The cells were then treated with 50 μl 1 × HBSS buffered solution in the absence and presence of a compound for about 1 h, and the cell responses were recorded throughout the assay. For A431 cells, a starvation step (i.e., continuous culturing in the DMEM without any serum for about 20 h) was introduced to induce the cells into a quiescent state before the assays. The unit of the responses indicated was a change in pixel of the central position of the resonant band of each sensor as imaged by a CCD camera (Fang, Li, & Peng, 2005).

2.5. Statistical analysis

Unless specifically mentioned, three replicates were carried out for each measurement or each compound. The standard