Profiling of G protein-coupled receptor (GPCR) stimulation by small compounds in live cells

Introduction

G protein-coupled receptors (GPCRs) are a large family of transmembrane proteins which are involved in many physiological and pathological processes. Extracellular stimuli result in cellular responses, which lead to ordered and dynamic redistribution of intracellular contents. Cellular responses are receptor-type specific. Accordingly, GPCRs are divided into four subclasses, $G_{i/o}$, $G_{q/11}$, G_s, and $G_{12/13}$.

Real-time label-free cell assays provide subtype-specific profiles without the requirement of labeling. However, available assays are based on a multiple-step analysis with additional antagonists or other responsemodifying agents, which is especially problematic in cases where the antagonist for a specific pathway is yet to be discovered.

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a real-time label-free detection method that allows the detection of changes on sensor surfaces, such as ligand binding, molecular rearrangements and cell adhesion. Thanks to its multi-parametric approach, several parameters can be followed in real-time (Figure 1). For example, peak angular position signal (PAP) detects the subtle mass redistributions taking place in cells during GPCR activation. In contrast, peak minimum intensity (PMI) is affected by the amount of light reflected back at the SPR coupling angle. This way, different MP-SPR responses alone or in combination with each other allow the study of different GPCR pathways in a single-step assay without the need for prior cell treatments, use of antagonists or pathway-modulating compounds.

Materials and methods

HeLa, CHO-K1 or A431 cells were immobilized on SPR102-AU sensors by pipetting detached cell suspensions over the sensors. For HeLa cells, sensors were first coated with human fibronectin. Sensors with cells were cultured at 37°C to full confluence. Cell confluence, morphology and monolayer integrity were confirmed under a light microscope.

The measurements were done using MP-SPR Navi™ 220A NAALI instrument with a flow rate of 20–30 µL/min at 37°C. The running buffer was equivalent to the basal media used for cell cultures. Samples were injected for 15 minutes. The reference sample, not including active compounds, was injected into the second channel. After the experiment, cells were treated with trypan blue, and cell viability was assessed under a microscope

Results and discussion

A431 cells were stimulated with the agonists - bradykinin and epinephrine. Bradykinin binds to the bradykinin B2 receptor, activating Gq pathways, while epinephrine binds to the β2-adrenoceptor, activating Gs pathways (Figure 2). Stimulation of A431 cells with different concentrations of epinephrine and bradykinin result in unique time-dependent PAP profiles (Figure 3). CHO-K1 and HeLa cells were stimulated with histamine; histamine binds to histamine H1 and H3 receptors, which activates Gq and Gi pathways, respectively. This resulted in similarly unique PAP profiles (data not shown).

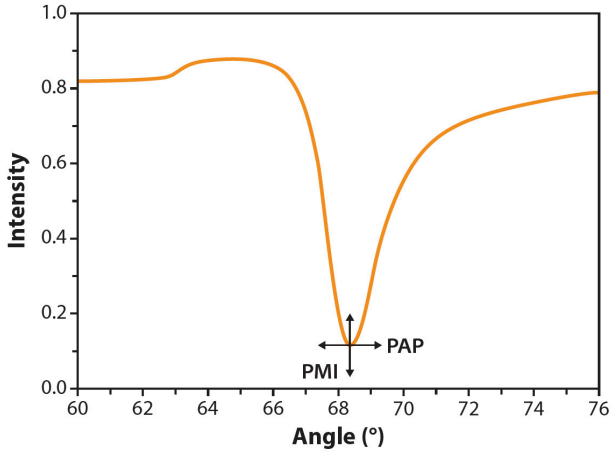

Figure 1. MP-SPR instruments measure full SPR curve continuously, and several parameters can be followed in real-time, including peak angular position signal (PAP) and peak minimum intensity (PMI).

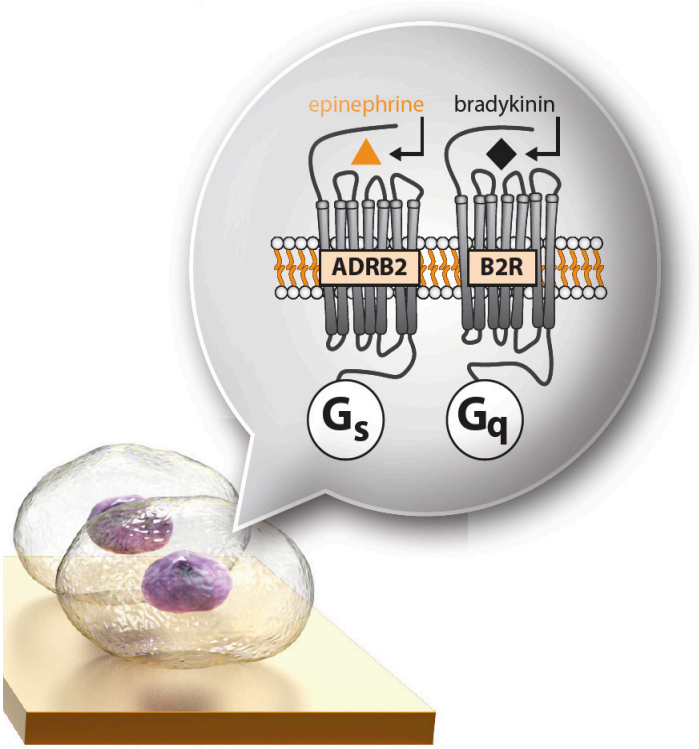

Figure 1. Stimulation of β2-adrenoceptor with epinephrine results in the activation of Gs pathways, while bradykinin B_2 receptor activates Gq pathways.

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Besides PAP, several other SPR signal responses display consistent and pathway-dependent changes. When plotting these signal responses against the PAP response, especially PMI, resulted in unambiguously distinguishable patterns for each analyzed GPCR signaling pathway (Figure 4).

To confirm that the observed cell response is specific to GPCRs, cells were either treated with histamine receptor antagonists and G protein inhibitors or were not transfected with histamine receptors. As a result, these cells did not response to treatment with 300nM histamine.

Comparison of MP-SPR PAP responses with responses measured using resonant waveguide grating (another optical method used for label-free cell measurements) revealed comparable concentration-response curves.

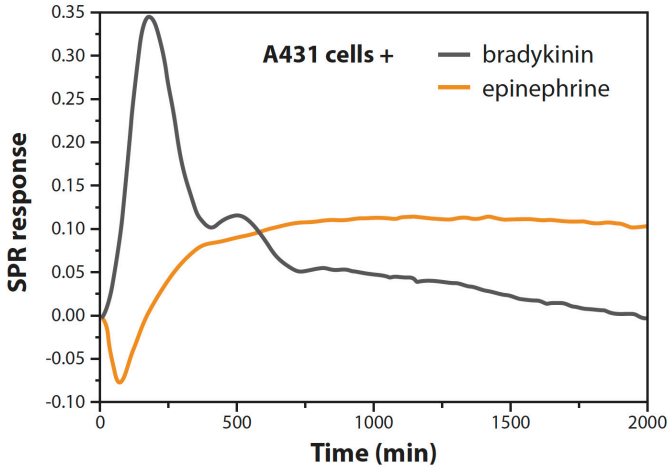

Figure 3. Stimulation of A431 cells with either bradykinin or epinephrine results in unique PAP profiles.

Conclusions

GPCRs are widely studied as targets for new drug compounds due to their central role in physiological processes. Combining dynamic angular (PAP) and intensity (PMI) shifts of the full SPR curve peaks, MP-SPR was used to separate activated GPCRs in live cells into subtype-specific signaling pathways without the use of antagonists or other response modulating agents.

Original article

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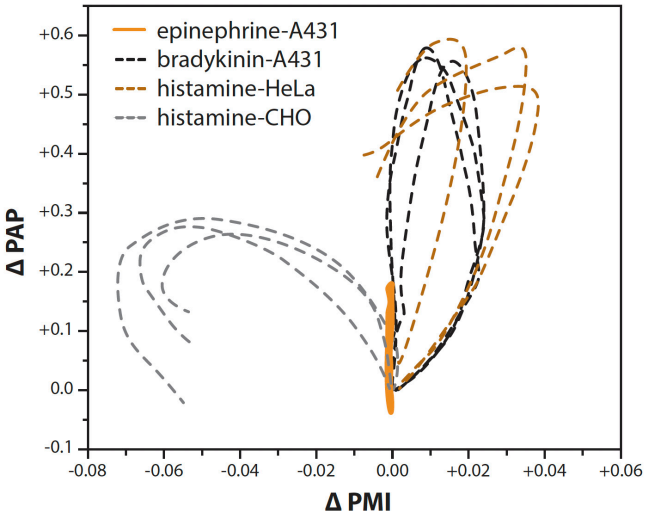

Figure 4. Two-parameter PMI-PAP response profiles, in which PAP (peak angular position) response is plotted against PMI (peak minimum intensity) response, displaying unambiguously distinguishable patterns for different agonists and cell lines.

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 400 KONTIO, 410A KAURIS

Accessories: flow cell for live cells

Sensor surface: SPR102-AU

Software: MP-SPR Navi™ Controller, DataViewer, TraceDrawer™ for MP-SPR Navi™

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