Application Note

Cell Analysis

From Classical to Online Monitoring of G-Protein-Coupled Receptor Stimulation in Living Cells

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Introduction

G-protein-coupled receptors (GPCRs) represent the largest family of transmembrane receptors. They are responsible for conveying extracellular signals to the inside of the cell via interactions with intracellular heterotrimeric G proteins. This interaction affects enzymes, ion channels, and other intracellular messengers. More than 800 GPCRs exist, mediating many molecular physiological functions, (for example, by serving as receptors for hormones, neurotransmitters, cytokines, lipids, small molecules, and various sensory signals such as light and odors). Due to their widespread occurrence and involvement in critical physiological functions, more than 50% of the current therapeutic agents on the market are targeted against GPCRs.

The biological functions and ligands are unknown for approximately 150 of these receptors, which are called orphan GPCRs. These have become a major focus of recent pharmacological research and development. The characterization of these GPCRs can provide new insights into cell biology and disease mechanisms, and allow the development of compounds specifically directed to one target.

All GPCRs possess seven transmembrane α-helices, linked by three intracellular and three extracellular loops, an extracellular N-terminal tail, and an intracellular C-terminal tail. The GPCR receives an extracellular stimulus (for example, small molecules, proteins, purines, lipids, ions, light, odorants, or pheromones) that induces a conformational change in the receptor, either facilitating or inhibiting the release of a G protein from the receptor. The released G protein in turn interacts with a diverse group of effectors that control intracellular messengers (Figure 1).

G proteins have three types of subunit: α, β, and γ. Most GPCRs couple to heterotrimeric G proteins, which comprise a $G_{\beta/\gamma}$ complex and a G_α subunit. The G_α subunits can be further subdivided into $G_{\alpha s}$ (G_s), $G_{\alpha i/o}$ (G_i), $G_{\alpha q/11}$ (G_q), and $G_{\alpha 12/13}$ ($G_{12/13}$) isoforms. Each of these isoforms has a distinct biological function (Figure 1).

G proteins normally exist as inactive, GDP-bound $G_{\alpha\beta\gamma}$ heterotrimers. During receptor activation, the GDP-bound G protein interacts with the intracellular face and C-terminus of the receptor, which induces a GDP-to-GTP exchange on the G_α subunit and a concurrent dissociation of G_α from the $G_{\beta\gamma}$ complex. The active GTP-G_α subunit and $G_{\beta\gamma}$ complex bind to their respective downstream effectors, which may be kinases, phosphatases, small GTPases, integral membrane proteins, or a multitude of other targets.

To turn off a ligand-mediated response or to adapt to a persistent stimulus, receptor-mediated signaling is eventually silenced by receptor desensitization. This negative feedback mechanism usually involves intracellular receptor phosphorylation. This phosphorylation triggers a transient receptor inactivation, either by preventing G protein reassociation or by receptor internalization.

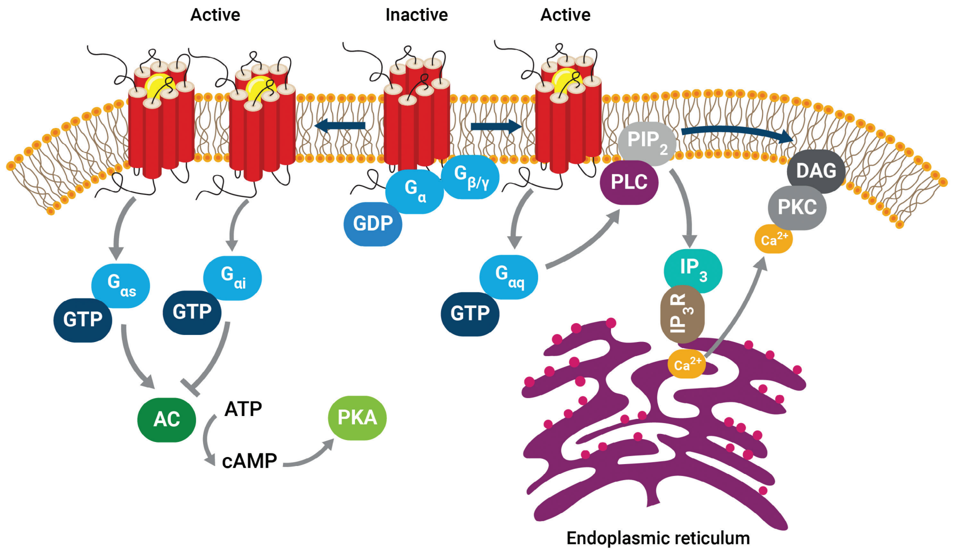

Figure 1. Schematic representation of GPCR signaling. Most GPCRs transmit extracellular signals via heterotrimeric G proteins, consisting of a G_α subunit and a $G_{\beta/\gamma}$ complex. Inactive G proteins are attached to the intracellular domain of a GPCR, and GDP is bound to the G_α subunits of these inactive proteins. Receptor agonists activate the intrinsic guanine nucleotide exchange factor (GEF) domain of the receptor, which mediates GDP/GTP exchange on the G_α subunit. The GTP-bound G protein is then released from the receptor and dissociates into its G_α and $G_{\beta/\gamma}$ subunits. The G_α subunit can be subdivided into four isoforms, $G_{\alpha s}$, $G_{\alpha i}$, $G_{\alpha q}$, and $G_{\alpha 12/13}$, each triggering an individual downstream signaling cascade. (1) Activated $G_{\alpha s}$ stimulates cyclic AMP (cAMP) production by activating adenylate cyclase (AC). Cyclic AMP serves as a second messenger and regulates other proteins, such as protein kinase A (PKA). (2) $G_{\alpha i}$ activation blocks cAMP production by inhibiting AC. (3) The release of $G_{\alpha q}$ activates phospholipase C (PLC). PLC catalyzes the cleavage of phospatidylinositol bisphosphate (PIP_2) to inositol triphosphate (IP_3) and diacylglycerol (DAG) at the plasma membrane. The release of IP_3 mediates the release of Ca^{2+} from the endoplasmatic reticulum by activating IP_3: receptor (IP_R). In conjunction, Ca^{2+} and DAG activate protein kinase C, which alters the function of numerous targets.

Compound discovery processes often use cell-based assays to monitor the functional activation of GPCRs. High-throughput functional assays, such as Ca^{2+} measurements and reporter gene assays, have frequently been used to screen chemical or peptide libraries for agonists and antagonists of various GPCRs. The traditional means of studying and screening for GPCR function in cell-based assays involve (i) labeling of cells with radioactive precursors or fluorescent reagents, (ii) measuring single molecular events such as Ca^{2+} release, inositol triphosphate generation, or changes in cAMP levels, and (iii) genetically engineering cell lines to overexpress reporter genes, such as β-galactosidase or luciferase. However, cell labeling and genetically engineered cell lines make such conventional assay systems more likely to generate artifacts.

The xCELLigence system is a microelectronic biosensor system for cell-based assays. It provides real-time, noninvasive, label-free cellular analysis for various research applications in compound development. Examples of applications are: toxicology, cancer research, cell death, proliferation, and viability, and virology. The xCELLigence system consists of the RTCA control unit with RTCA software, the RTCA analyzer, and either an RTCA single plate (SP), or RTCA multiple plates (MP) station. The RTCA SP or MP station fits inside a standard cell culture incubator, ensuring that the temperature, humidity, and CO_2 environment are controlled throughout each experiment.

The RTCA SP station accommodates one plate (called an E-Plate 96) at a time. However, the RTCA MP station can accommodate up to six E-Plates simultaneously. This enables the MP station to monitor up to six different experiments at the same time. Each E-Plate 96 has biosensor arrays integrated into the bottom of its wells. The contact between these electrodes and the cells alters the local ionic environment at the electrode/solution interface, leading to a change in measured impedance. The nature of the cell interaction with the electrodes (for example, increased cell adhesion or spreading) will also cause further changes in impedance. Electrode impedance, measured as changes in an arbitrary Cell Index (CI) value, can be used to monitor many cell parameters, (for example, adhesion, proliferation, viability, or death). It can even detect morphological changes. This impedance-based technology has been validated for proliferation and cytotoxicity assays,[1] cell adhesion and spreading,[2] cell culture quality control,[3] receptor tyrosine kinase activation,[4] mast cell activation,[5] and natural killer cell cytotoxicity.[6]

The benefits of the xCELLigence system for assays of GPCR function include:

- Real-time monitoring of endogenous receptors without the need for cell labeling or overexpression of reporters.
- Ability to use primary cells or disease-relevant cells.
- Dissection of discrete downstream signaling pathways during receptor stimulation.
- Ability to perform multiple stimulations in the same well, to assess receptor desensitization/resensitization or receptor cross-talk.
- Ability to assess partial agonism and inverse agonism.
- Ability to combine short-term receptor responses with long-term cellular responses, such as cell proliferation, cell cycle arrest, and cytotoxicity.

To determine if the stimulation of endogenously expressed GPCRs can be monitored by the xCELLigence system, cells were stimulated with various receptor agonists, triggering either G_s-, G_i-, or G_q-mediated downstream signals. The induction of G protein-specific signaling cascades, including stimulation of adenylate cyclase (G_s), inhibition of adenylate cyclase (G_i), and activation of PLC (G_q), may cause characteristic morphological changes of the cell. These changes can be monitored as changes in CI.

Receptor desensitization studies were also included to test the versatility of the impedance-based monitoring of GPCR stimulation. Pyrilamine, a specific histamine H_1 receptor antagonist, was used as a model of antagonist-mediated receptor silencing. To monitor endogenous receptor desensitization, cells were repeatedly stimulated with the same ligand, and the signal intensity of the individual stimulations was compared.

These experiments give an overview of how the xCELLigence system can be used for the dynamic and noninvasive monitoring of GPCR activation/inactivation in living cells.

Materials and methods

Cell culture

All cells were cultured in the absence of antibiotics in standard humidified incubators at 37 °C and 5% CO_2. CHO-K1 cells, obtained from the European Collection of Animal Cell Cultures (ECACC, Porton Down, Salisbury, UK), were maintained in F12-Ham's cell culture medium, including 10% FCS and 2 mM L-glutamine. HeLa cells (ECACC) were cultured in minimum essential medium (MEM) supplemented with 10% FCS, nonessential amino acids (NEAA), and 2 mM L-glutamine. HEK293 cells (ATCC, Wesel, Germany) were maintained in MEM supplemented with 10% FCS, NEAA, 2 mM L-glutamine, and 1 mM Na-pyruvate. Serum-starved cells were washed with PBS and cultured in medium containing 0.5% FCS for 3 hours before GPCR stimulation.

Compounds

Adenosine-5'-triphosphate (ATP) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). Histamine, human calcitonin, serotonin, and pyrilamine were obtained from Sigma Aldrich GmbH (Taufkirchen, Germany).

Impedance-based GPCR assay

The cellular response to GPCR stimulation was monitored on an RTCA MP instrument, comprising an RTCA analyzer, RTCA control unit with RTCA software, and RTCA MP station.The station is designed for the simultaneous analysis of six E-Plate 96 plates. The RTCA MP station remained in a humidified cell culture incubator (Binder CB210) throughout the experiments. Background impedance was measured with 100 µL cell culture medium per well. For standard experiments, 2.5×10^4 CHO-K1, 1×10^4 HeLa, and 3×10^4 HEK293 cells were plated per well to produce a semiconfluent cell culture 24 hours after seeding. The final volume of cell culture medium was adjusted to 200 µL per well. To allow equal distribution of cells, E-Plates 96 containing cells were preincubated for 30 minutes at room temperature. Then, plates were transferred to the RTCA MP station inside the incubator and cultured overnight.

Impedance was routinely recorded at 15-minute intervals to monitor cell culture conditions. GPCR stimulation was performed inside the incubator by replacing the E-Plate 96 lid with an RTCA Frame 96. Using the RTCA Frame 96, users can directly pipet into the wells while the E-Plate 96 is locked in the RTCA station. This allows continuous monitoring during compound administration. After administration of GPCR agonists, impedance was monitored at intervals of 15 to 30 seconds for at least 1 hour. For antagonist studies, the H1-histamine receptor antagonist pyrilamine was added to the cell culture medium 15 minutes before histamine administration. Unless otherwise stated, solvent-stimulated cells were used to establish a reference baseline. Results were baseline-adjusted and expressed as CI normalized to the time point of compound administration.

Results and discussion

Dynamic and quantitative monitoring of the activation of endogenous GPCRs in living cells

Calcitonin, synthesized in the thyroid gland, is a hormone known to participate in calcium metabolism. The calcitonin receptor has been shown to be a member of the seven-transmembrane, G protein-coupled receptor family. CHO-K1 cells endogenously express the calcitonin 1a receptor (C1a), which, upon calcitonin stimulation, releases G protein type G_s, activating adenylate cyclase and triggering cyclic AMP production, as well as activating protein kinase A.[7]

As shown in Figure 2A, administration of calcitonin (5 pM to 5 µM) to serum-deprived CHO-K1 cells induced a dose-dependent cellular response, measured as a transient increase in impedance/CI for at least 3 hours. This finding demonstrates that G_s-mediated signaling triggers a morphological change in CHO-K1 cells that can be monitored by the xCELLigence system. To determine the half-maximal effective concentration (EC_{50}) of calcitonin, the RTCA software was used to plot the normalized CI_{max} against the log concentration of calcitonin, generating a sigmoidal dose-response curve; the EC_{50} calculated from the curve was 5.1 nM.

Non serum-starved CHO-K1 cells, when stimulated with calcitonin, showed significantly lower signal intensity, less dynamic range, and less signal retention than serum-starved cells (Figure 2B). These findings demonstrate that the removal of growth factors by serum starvation increases the excitability of cells, leading to a more prominent signal.

As the state of the cell culture was

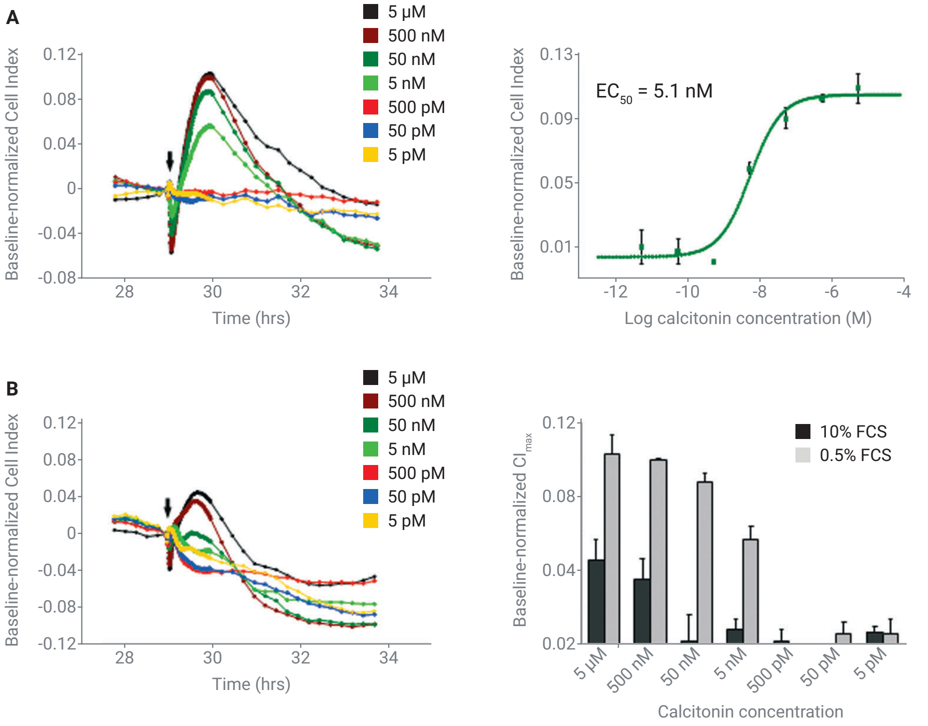

Figure 2. G_s-protein-coupled receptor stimulation by calcitonin in CHO-K1 cells. (A) Serum-starved cells (3 hours in 0.5% FCS) were stimulated with calcitonin (5 pM to 5 µM) and the cellular response was detected by the Agilent xCELLigence system (left panel). The calcitonin EC_{50} was calculated from the respective dose-response curve with RTCA software (right panel). (B) Calcitonin (5 pM to 5 µM) stimulation of non serum-starved CHO-K1 cells (left panel). Comparison of CI signal intensity in serum-starved versus nonstarved cells after calcitonin stimulation (right panel). Black arrows indicate the time of calcitonin administration.

suspected to have an effect on the impedance signal after receptor stimulation, CHO-K1 cells were seeded at different concentrations to yield cell cultures in different phases of proliferation, as characterized by the degree of confluence. Cell proliferation was monitored continuously to determine the optimal time-point for GPCR stimulation with 5 µM calcitonin. After 24 hours, cell culture medium was replaced with medium containing 0.5% FCS to improve cellular response. Figure 3 shows the CI plots from four cultures containing different numbers of cells. Each plot was normalized and baseline-adjusted to the corresponding unstimulated reference cell culture.

Calcitonin stimulation of serum-starved CHO-K1 cells triggered a change in impedance in all cell cultures. However, the signal pattern varied significantly. Semiconfluent cells showed an initial reduction in impedance followed by a rapid inversion to a positive amplitude. The observed signal shifted towards a more negative amplitude in the confluent cell culture, indicating that the signal shape depends on the cell number. The xCELLigence system allows the study of receptors and cells with high sensitivity under more physiological conditions and in real time.

To determine if stimulation of G_i-coupled GPCRs can also be monitored, CHO-K1 cells were stimulated with increasing concentrations of serotonin (1 pM to 100 µM). CHO-K1 cells endogenously express GPCR 5-HT1B,[8] a G_i-coupled receptor that inhibits cAMP production from ATP when stimulated with the neurotransmitter serotonin.
In serum-starved CHO-K1 cells, serotonin administration triggered a dose-dependent increase in CI within 30 minutes after compound addition (Figure 4). Using the RTCA software, an EC_{50} of 21 nM was calculated from the dose-response curve, demonstrating that G_i-mediated signaling can be quantified by this system.

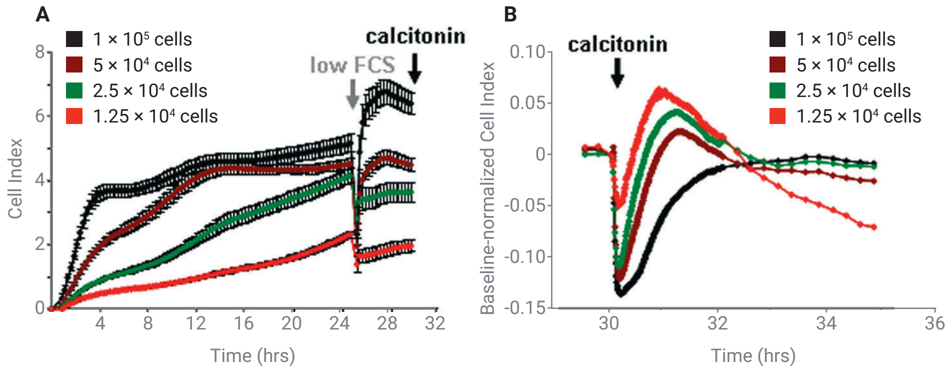

Figure 3. Calcitonin stimulation of CHO-K1 cells in different phases of proliferation. Titration of the number of serum-starved (gray arrow) cells reveals a cell number-dependent response after 5 µM calcitonin stimulation (black arrow).

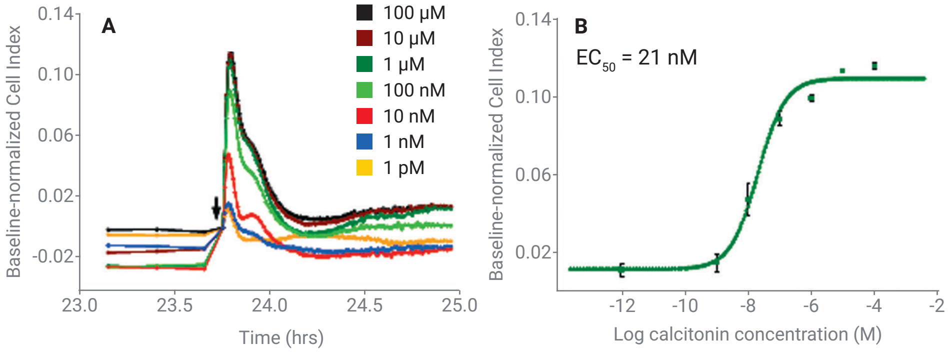

Figure 4. Stimulation of G_i-protein-coupled receptor by serotonin in CHO-K1 cells. Serum-starved cells (3 hours in 0.5% FCS) were stimulated with serotonin (100 µM to 1 pM) and the cellular response was detected by the Agilent xCELLigence system (left panel). An EC_{50} of 21 nM was calculated from a typical dose-response curve (right panel). The black arrow indicates the time of serotonin administration.

Next, the xCELLigence system was used to detect G_q-coupled receptor stimulation, which is linked to phospholipase C activation and hence Ca^{2+} release and activation of protein kinase C (Figure 5).

The nucleotide ATP stimulates purinergic receptors, such as P2Y, a family of mostly G_q-coupled receptors that can be found in almost all mammalian tissues. To investigate the cellular response to purinergic receptor stimulation, serum-deprived CHO-K1 cells were from ATP when stimulated with the neurotransmitter serotonin. In serum-starved CHO-K1 cells, serotonin administration triggered a dose-dependent increase in CI within 30 minutes after compound addition (Figure 4). Using the RTCA software, an EC_{50} of 21 nM was calculated from the dose-response curve, demonstrating that G_i-mediated signaling can be quantified by this system. As shown in Figure 5A, ATP caused a temporary dose-dependent increase in CI. The observed increase had two stages: a strong initial CI increase followed by a weaker secondary response. The EC_{50} was calculated from the first signal, which peaked approximately 5 minutes after ATP stimulation.

In another study, HeLa cells showed high responsiveness to histamine, the endogenous ligand of the G_q-coupled histamine H_1 receptor expressed in these cells.[9] Histamine (0.3 to 60 µM) triggered a dose-dependent increase in CI for at least 6 hours after compound administration (Figure 5B). In these cells, an EC_{50} of 1.6 µM was calculated for histamine, demonstrating that G_q-coupled receptor activation can be quantified by the xCELLigence system.

In summary, these findings show that G_s-, G_i-, and G_q-mediated signaling can all be dynamically monitored and quantified by the xCELLigence system. The continuous monitoring allowed by the xCELLigence system also reveals cell receptor functions that could not be found in a targeted study.

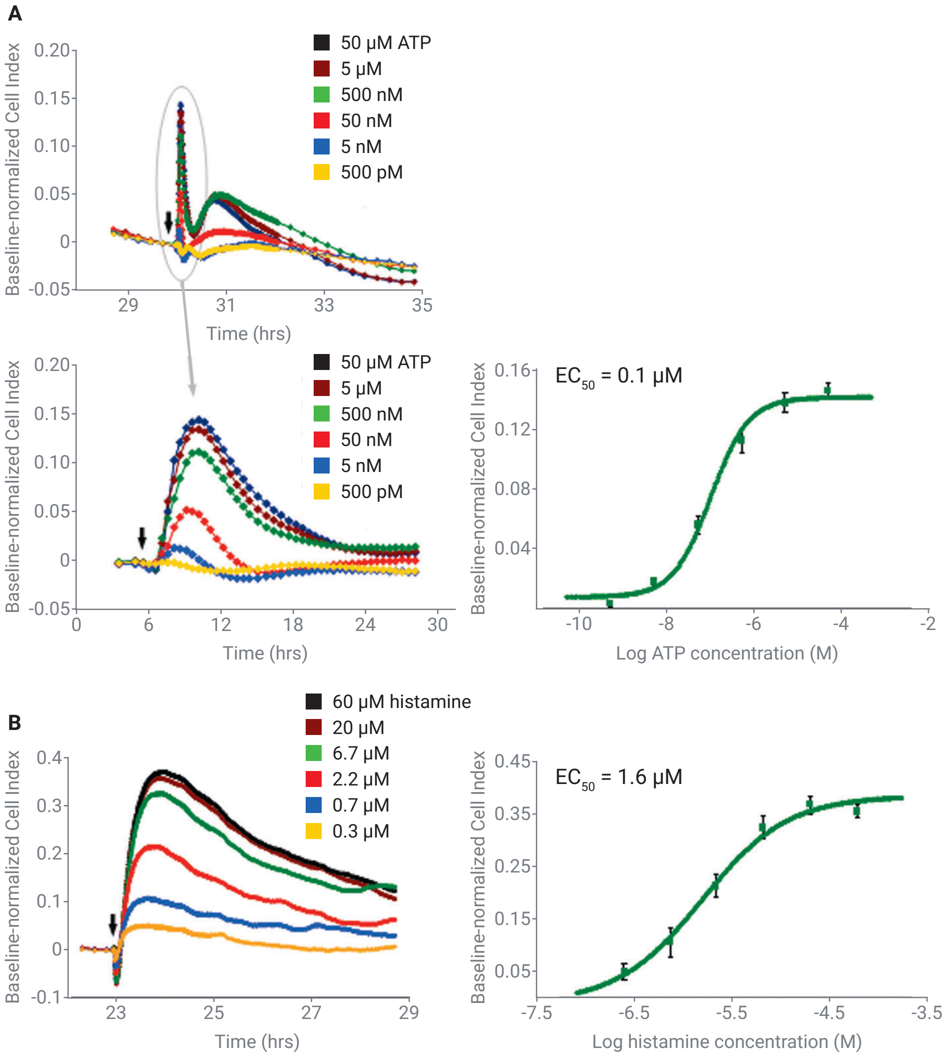

Figure 5. Stimulation of Gq-protein-coupled receptors in CHO-K1 and HeLa cells. (A) Serum-starved CHO-K1 cells (3 hours in 0.5% FCS) were stimulated with ATP (500 pM to 50 µM) and the cellular response was detected by the Agilent xCELLigence system (left panel). The CI response curves are shown up to 5 hours and 30 minutes after ATP administration. The CI was determined at 30-second intervals. The ATP EC_{50} was calculated with the RTCA software (right panel). (B) Analysis of endogenous GPCR stimulation by histamine (250 nM to 60 µM) in HeLa cells (left panel). The quantification of the histamine EC_{50} is shown in the right panel. Black arrows indicate compound administration.

Monitoring and quantification of antagonist-mediated receptor silencing

Antagonist-mediated receptor desensitization was performed to demonstrate the versatility of the xCELLigence-based GPCR assay. Pyrilamine, a specific histamine H_1 receptor antagonist, was used to desensitize HeLa cells to histamine administration. HeLa cells were pre-incubated with increasing concentrations of pyrilamine (12.8 pM to 1 µM) 15 minutes before they were stimulated with 5 µM histamine. CI signals were monitored after the addition of histamine. Figure 6 shows the resulting normalized and baseline-adjusted signals, which demonstrate a dose-dependent reduction of the cellular histamine response. Under these conditions, a complete signal inhibition was achieved with 1 µM pyrilamine. In contrast, pyrilamine concentrations below 1.6 nM did not show any major inhibitory effects. From this experiment involving HeLa cells stimulated with 5 µM histamine, an IC_{50} of 22 nM was calculated for the antagonist pyrilamine. In conclusion, antagonist IC_{50} values can be determined on the xCELLigence system to quantify the effectiveness of antagonist-mediated receptor silencing.

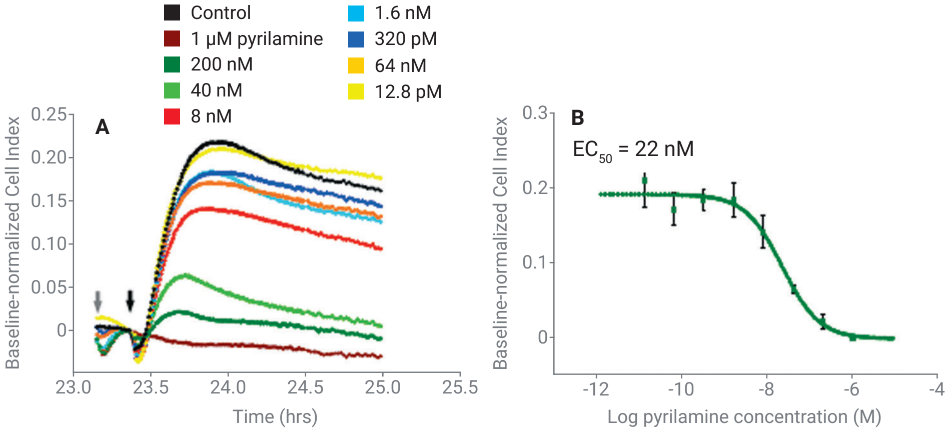

Figure 6. Inhibition of histamine stimulation by antagonist pyrilamine. Nonserum-starved HeLa cells were pre-incubated for 15 minutes with pyrilamine (12.8 pM to 1 µM), a specific H1 histamine receptor antagonist. Then, cells were stimulated with 5 µM histamine and the cellular response was monitored by the Agilent xCELLigence system. Pyrilamine prevented cellular stimulation in a dose-dependent manner (left panel). The IC_{50} calculation is shown in the right panel. Pyrilamine and histamine administration are indicated, respectively, by a gray and a black arrow.

Dynamic monitoring of endogenous receptor desensitization

GPCRs may also be silenced by intracellular phosphorylation to compensate for a prolonged exposure to an agonist, avoiding excessive receptor activation. This endogenous feedback mechanism mediates a transient receptor desensitization, preventing repeated stimulation of the cell within a certain time period. To test this, HEK293 cells were repeatedly stimulated with a nonsaturated concentration of carbachol, an acetylcholine receptor-specific agonist. HEK293 cells endogenously express the muscarinic acetylcholine receptor M_1, which is inactivated by intracellular phosphorylation and arrestin linking, preventing G_q binding.[10] As shown in Figure 7A, carbachol stimulation created a dose-dependent increase in CI for at least 1 hour. Yet, additional cellular response to carbachol (50 µM) was not detectable 4 and 8 hours after the first compound administration.

To investigate the velocity of receptor resensitization, carbachol-containing medium was washed off the cells 2 hours after the first stimulation. Two hours later, HEK293 cells were stimulated for a second and a third time with the same carbachol concentration. After carbachol removal, cells were sensitive to repeated stimulation (Figure 7B), albeit to a lesser extent, suggesting a partial recycling of the receptor. However, cells stimulated with low carbachol concentrations showed identical responses after the second and third stimulation, indicating that carbachol concentrations below 13 µM do not induce significant receptor desensitization in these cells.

These experiments clearly show that endogenous receptor desensitization, as well as receptor resensitization, can be continuously monitored and quantified by the xCELLigence system. Due to the high sensitivity of the xCELLigence, receptor studies can be conducted without receptor overexpression.

Cell-based assays are an essential tool for the investigation of GPCR activation and inactivation, particularly as a tool for screening GPCR agonists and antagonists. Conventional methods for studying and screening GPCR function in cell-based assays usually involve invasive techniques or genetically engineered cell lines. This application note demonstrates that the xCELLigence system allows the continuous monitoring of endogenous GPCRs in living cells. This experimental approach is label-free, sensitive, and noninvasive.

It has been shown that the activation of G_s, G_i, and G_q-mediated signaling triggers a characteristic cellular response that can be monitored by the xCELLigence system. The data obtained can generate a typical dose-response curve for quantifying the efficacy of an individual agonist (EC_{50}) or antagonist (IC_{50}). Notably, the data presented here were collected from cells with endogenous levels of GPCR expression, highlighting the sensitivity of this impedance-based GPCR assay. As this approach does not require engineered cell lines that overexpress GPCRs or certain reporter genes, it allows GPCR-mediated signaling to be investigated under more physiological conditions.

The main features that distinguish the xCELLigence system from other cell-based functional assays for GPCRs are:

- No pre- or postlabeling of cells is required, saving money and time.
- The assay is noninvasive and cellular destruction is not required. Multiple operations can therefore be performed on the same cells. For example, the same well can be subjected to multiple stimulations with the same agonist/antagonist.
- The detection methods are label-free, avoiding potential interference from the label.
- Since the system monitors cell attachment and morphology, which are integral components of cell viability, it can detect any compound that may be potentially cytotoxic or have other adverse effects.
- Cellular processes are monitored in real time. So, the assay provides a comprehensive record of the entire assay period and reveals cell receptor functions that would not be found in a targeted study.

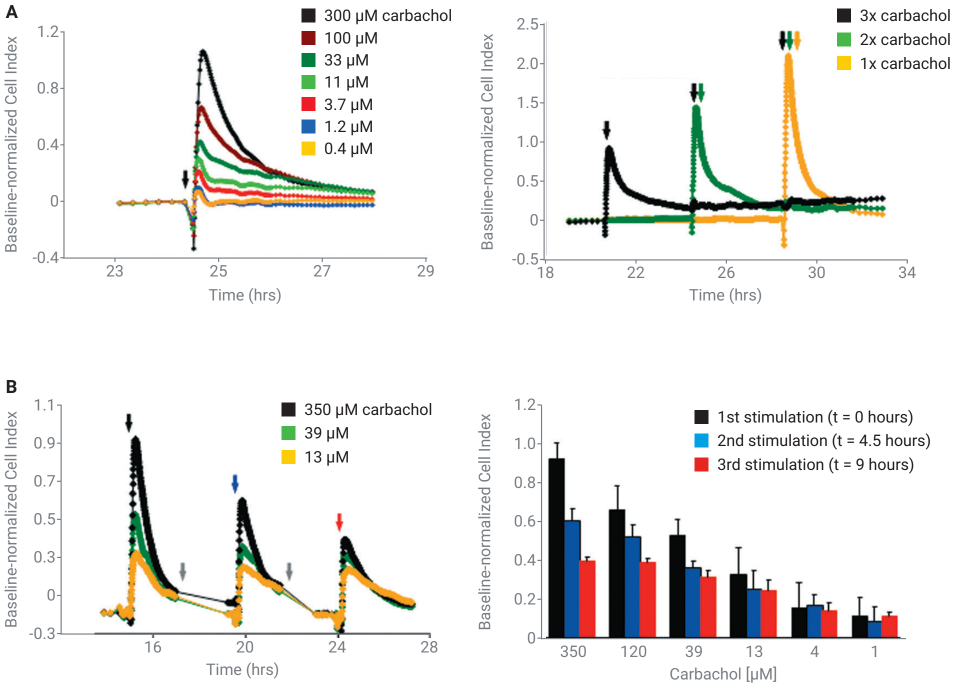

Figure 7. G-protein-coupled receptor desensitization after carbachol stimulation. (A) Administering carbachol (black arrow) to nonserum-starved HEK293 cells triggers an increase in CI in a dose-dependent manner (left panel). Cells were stimulated with 50 µM carbachol repeatedly (3x black arrow, 2x green arrow, 1x orange arrow) and the CI was continuously monitored. Additional carbachol administration after 4 and 8 hours did not trigger further cellular responses (right panel). (B) HEK293 cells were stimulated with carbachol repeatedly and cell culture medium was replaced (gray arrow) between stimulations. Signals were renormalized at the time of each individual carbachol administration (left panel). Signal intensity after repeated compound administration revealed dose-dependent desensitization/resensitization of carbachol receptors (right panel).

Since the CI is continuously recorded throughout the whole experiment, this assay provides comprehensive information about cellular events that occur before and after receptor activation. This allows the researcher to make informed decisions about the timing of the experiment, (for example, to optimize agonist/antagonist administration). These features of the xCELLigence let users examine receptor signaling in more detail, which could increase overall understanding of GPCR function.

References

1. Solly, K. *et al.* Application of Real-Time Cell Electronic Sensing (RT-CES) Technology to Cell-Based Assays. *Assay Drug. Dev. Technol.* **2004**, *2*, 363–372.
2. Atienza, J. M. *et al.* Dynamic Monitoring of Cell Adhesion and Spreading on Microelectronic Sensor arrays. *J. Biomol. Screen.* **2005**, *10*, 795–805.
3. Kirstein, S. L. *et al.* Live Cell Quality Control and Utility of Real-Time Cell Electronic Sensing for Assay Development. *Assay Drug Dev. Technol.* **2006**, *4*, 545–553.
4. Atienza, J. M. *et al.* Label-Free and Real-Time Cell-Based Kinase Assay for Screening Selective and Potent Receptor Tyrosine Kinase Inhibitors Using Microelectronic Sensor Array. *J. Biomol. Screen.* **2006**, *11*, 634–643.
5. Abassi, Y. A. *et al.* Label-Free, Real-Time Monitoring of IgE-Mediated Mast Cell Activation on Microelectronic Cell Sensor Arrays. *J. Immunol. Methods* **2004**, *292*, 195–205.
6. Zhu, J. *et al.* Dynamic and Label-Free Monitoring of Natural Killer Cell Cytotoxic Activity Using Electronic Cell Sensor Arrays. *J. Immunol. Methods* **2006**, *309*, 25–33.
7. George, S. E.; Bungay, P. J.; Naylor, L. H. Functional Coupling of Endogenous Serotonin (5-HT1B) and Calcitonin (C1a) Receptors in CHO Cells to a Cyclic AMP-Responsive Luciferase Reporter Gene. *J. Neurochem*. **1997**, *69*, 1278–1285.
8. Giles, H. *et al.* Characterization of a 5-HT1B Receptor on CHO Cells: Functional Responses in the Absence of Radioligand Binding. *Br. J. Pharmacol.* **1996**, *117*, 1119–1126.
9. Volpi, M.; Berlin, R. D. Intracellular Elevations of Free Calcium Induced by Activation Of Histamine H_1 Receptors in Interphase and Mitotic HeLa Cells: Hormone Signal Transduction Is Altered During Mitosis. *J. Cell Biol.* **1988**, *107*, 2533–2539.
10. Mundell, S. J.; Benovic, J. L. Selective Regulation of Endogenous G Protein-Coupled Receptors by Arrestins in HEK293 Cells. *J. Biol. Chem*. **2000**, *275*, 12900–12908.

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