CHAPTER

Label-free impedance-based whole cell assay to study GPCR pharmacology

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CHAPTER OUTLINE

Methods in Cell Biology, Volume 149, ISSN 0091-679X, https://doi.org/10.1016/bs.mcb.2018.08.003

Abstract

The superfamily of G protein-coupled receptors (GPCRs) represents the largest group of cell surface receptors in the human body. It is estimated that around 40% of the drugs currently on the market target GPCRs. As only a very small number of GPCRs is targeted by these marketed drugs, the potential of GPCRs as novel drug targets remains enormous. As opposed to conventional in vitro assays, label-free cellular assays using a biosensor provide new opportunities for studying GPCRs. Integrated receptor-mediated responses are measured in real-time rather than a single downstream signaling pathway, without the need for the use of any label (e.g., fluorescent or radioactive). Here, we describe a protocol to study GPCR pharmacology using the label-free whole cell impedance-based biosensor system xCELLigence. This assay allows quantification of compound-induced GPCR-mediated responses in real-time. Finally, we have also discussed the analysis and interpretation of the results obtained with this assay using the $mGlu_2$ receptor as a model system.

1 INTRODUCTION

G protein-coupled receptors (GPCRs) are gatekeepers of signal transduction and represent the largest protein family in the human genome with over 800 members (Bjarnadóttir et al., 2006). Structurally, these receptors share a common architecture of seven transmembrane helices which are connected by three intracellular and three extracellular loops. Based on structural diversity GPCRs are classified into five subgroups: class A (rhodopsin), class B (secretin), class C (glutamate), adhesion, and frizzled/taste2 (Fredriksson, Lagerström, Lundin, & Schiöth, 2003; Rosenbaum, Rasmussen, & Kobilka, 2009).

GPCRs are located in the cell membrane where they recognize a variety of extracellular stimuli including photons, ions, small molecules, peptides, and proteins. Receptor activation by the endogenous agonist results in a conformational change in receptor structure that induces downstream signaling events via different intracellular proteins including heterotrimeric G proteins, arrestins, and kinases (Rajagopal & Shenoy, 2018). Between individual GPCRs these signal transduction pathways are very diverse. Different downstream signaling pathways can be activated and a variety of these pathways can be activated simultaneously. The pathways that are being activated can even depend on the receptor ligand, so-called biased signaling (Smith, Lefkowitz, & Rajagopal, 2018).

Traditionally, functional in vitro endpoint-based assays have been the golden standard for drug screening at GPCRs. These assays assess the ability of a compound to activate or inactivate a specific pathway and measure the functional effects at the end of the experiment. The last decade has shown a great increase of the number of label-free cellular assays that can monitor responses in real-time (Fang, 2015; Scott & Peters, 2010). Typically, these assays make use of a biosensor that measures cellular responses, including compound-induced responses which result from morphological changes of cells (Yu et al., 2006). In this way some of the downsides of traditional endpoint assays are avoided: no reporter genes or labels

(e.g., fluorescent, radioactive) are required and continuous measurements can be performed (Halai & Cooper, 2012). Label-free assays allow the use of many different cell types including recombinant cell lines, cell lines endogenously expressing the target of interest and even patient-derived cells (Hillger, Lieuw, Heitman, & IJzerman, 2017). The label-free assays that have been used so far to study GPCR responses are mostly based on measurement of changes in cell morphology. This has been achieved by optical measurements of cell morphology, e.g., EPIC (Corning Inc.) or by measuring cellular impedance, e.g., xCELLigence (ACEA BioSciences) (Lundstrom, 2017; Scott & Peters, 2010).

The xCELLigence real-time cell analyzer (RTCA) system is based on the measurement of the electrical impedance generated by cells attaching to gold sensor electrodes that are embedded on the bottom of microelectronic *E*-plates. A very weak electrical current is applied to the sensor electrodes where the AC excitation level is in the lower mV range, resulting in a current in the μA range. Relative changes in impedance (Z) are described by the dimensionless parameter cell index (CI), and can be monitored in real-time. The CI level at a given point in time is defined by the equation:

$$\mathrm{CI} = (Z_i - Z_0)\Omega/15\,\Omega$$

where Z_i is the impedance at the given point in time and Z_0 is the baseline impedance in the absence of cells, which is measure prior the experiment and defined as $\mathrm{CI} = 0$.

The total impedance measured consists of four components: (1) resistance of the electrolyte solution (most often culture medium) for which is corrected by a baseline measurement; (2) impedance at the electrode/solution interface; (3) impedance at the electrode–cell interface, mostly depending on cell adherence, which changes the local ionic environment at the electrode interface which alters impedance (Fang, Frutos, & Verklereen, 2008); and (4) the impedance of the cell, which is comprised of two components resistance (depending on cellular ionic strength) and reactance (depending on the cell membranes acting as imperfect capacitators). Overall impedance is thus a combination of many factors including viability, confluency, adhesion, and morphology of the cells and the ionic environment of the surrounding solutions.

Such cellular parameters are also affected upon activation or inactivation of GPCR signaling, resulting in changes in cellular impedance that can be monitored in real-time using the xCELLigence system (Fig. 1). Typically, GPCR-mediated activation results in increased cell adhesion and an overall increase in CI, while inactivation results in a lower CI indicating decreased adhesion (Scott & Peters, 2010). The xCELLigence system has shown potential in studying GPCR pharmacology of many different GPCRs using many different cell lines (e.g., Hillger et al., 2015; Klein Herenbrink et al., 2016; Nederpelt, Vergroesen, IJzerman, & Heitman, 2016), including the use of xCELLigence to study receptor activation kinetics (Nederpelt et al., 2017) and in vitro translational pharmacology, i.e., from target-binding kinetics to the duration of the functional responses of GPCR ligands (Doornbos et al., 2017).

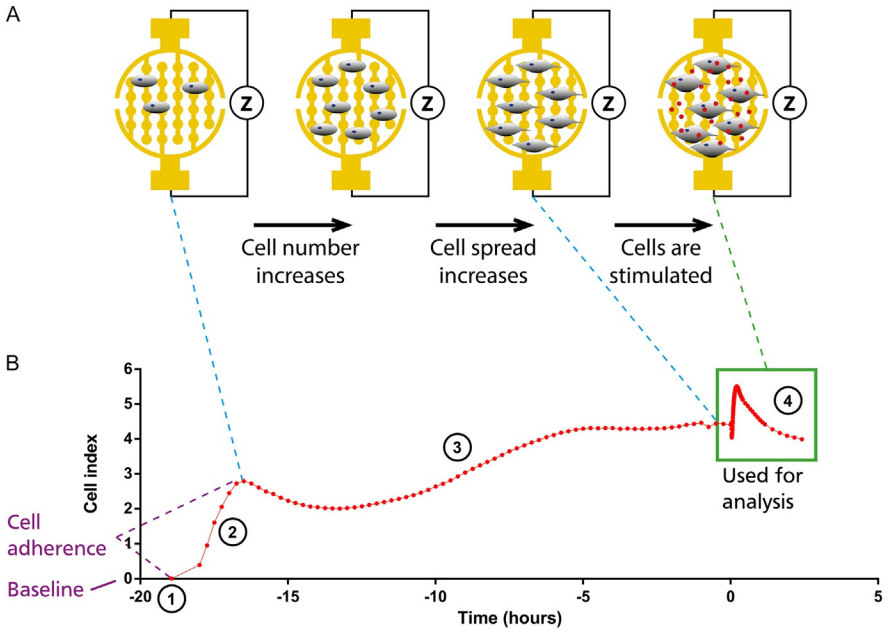

FIG. 1

Schematic representation of the impedance-based biosensor xCELLigence. (A) The biosensors measure impedance using gold electrodes mounted at the bottom of a microwell plate. Cells adhering to the bottom of the well and thus to the electrodes restrict the flow of current between the two electrodes and thus increase the impedance. The cell number, morphology, and viability affect the impedance generated. GPCR responses also induce alterations in cellular impedance, thereby enabling quantification of compound-induced receptor responses. (B) A typical xCELLigence graph is shown, in which the baseline measurement (1) is used to correct for background impedance generated by the culture medium–electrode interface. After cell seeding, impedance initially increases upon cell adherence (2), followed by a growth curve (3) in which the impedance is affected by cell proliferation and morphology changes. The impedance responses generated by compound-induced GPCR responses (4) are generally used for data analysis.

Here, we describe a protocol for evaluation and quantification of GPCR-mediated signaling using the $G\alpha_{i/o}$-coupled metabotropic glutamate receptor 2 ($mGlu_2$; a class C GPCR) as a model system. The cell line used is a CHO-K1 cell line overexpressing the $mGlu_2$ receptor. We describe the general experimental set-up followed by workflows designed to evaluate the preferred number of cells, receptor-mediated signaling, pathway-dependent signaling, and the evaluation and quantification of the potency of agonist- and inverse agonist-induced receptor-mediated responses.

2 MATERIALS

2.1 CHEMICALS

Chemicals are from general commercial sources and are of analytical grade and standard laboratory equipment is used, unless stated otherwise.

2.2 LABEL-FREE SYSTEM

2.2.1 System

xCELLigence real-time cell analyzer (RTCA) system (ACEA Biosciences, San Diego, CA, USA). Dual purpose (DP; 16 wells) and single plate (SP; 96 wells) systems are used. The systems are placed in a humidified incubator at 37 °C and 5% CO_2 and are connected to a laptop next to the incubator. RTCA 2.0 software is used for controlling the measurements and may be used for data analysis.

2.2.2 *E*-plates

PET *E*-plates 16 and 96 for the xCELLigence DP and SP system, respectively, were obtained from Bioké (Leiden, the Netherlands).

2.3 CELL CULTURE

2.3.1 Cells

Chinese hamster ovary cells (CHO-K1, CCL 61) were from ATCC (Rockville, MD, USA). CHO-K1 cells stably expressing the human $mGlu_2$ (CHO-K1_$hmGlu_2$) were from Janssen Research and Development (Beerse, Belgium).

2.3.2 Culture medium

Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) supplemented with 10% (v/v) fetal calf serum (FCS; Biowest, Nuaillé, France), 200 IU mL^{-1} penicillin, 200 µg mL^{-1} streptomycin, 30.5 µg mL^{-1} L-proline, and 400 µg mL^{-1} G418 (all from Duchefa Biochemie Haarlem, The Netherlands). Parental CHO-K1 cells were cultured in the same medium without G418.

2.3.3 Assay medium

For serum starvation serum-free culture medium was used which was supplemented with glutamate–pyruvate transaminase (GPT; 3 U/mL; Sigma) and additional sodium pyruvate (3 mM; Sigma) to reduce endogenous glutamate levels.

2.3.4 Phosphate-buffered saline (PBS) 1 × without calcium or magnesium. (Sigma D8537; 137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.47 mM KH_2PO_4).

2.3.5 Trypsin solution. 0.005% Trypsin in PBS containing 0.14 mM EDTA.

2.3.6 T75 tissue culture flasks (Sarstedt).

2.4 LIGANDS UNDER INVESTIGATION

Ligands are prepared in DMSO stock solutions prior to testing. If DMSO is not a suitable solvent, e.g., for reasons of solubility or stability, ligands can be dissolved in water or directly into assay medium.

3 METHODS

The following procedure describes how to perform a typical experiment to assess compound-induced receptor-mediated responses.

3.1 CELL CULTURE

Cells are cultured in a humidified incubator at 37 °C and 5% CO_2 and are subcultured bi-weekly at a ratio of 1:10 by trypsinization. For trypsinization add 1 mL trypsin solution to the plate after washing with PBS and incubate for maximally 5 min at 37 °C and 5% CO_2. Cells can be used for experiments from passage #3 up to #20. When preparing for experiments, cells are seeded such that a confluency of 80–90% is reached. One T75 tissue culture flask is generally sufficient for an experiment using a 96 wells *E*-plate.

3.2 xCELLigence ASSAY PROTOCOL

Overall, the steps in an experiment are:

- Experiment design and software set-up
- Baseline measurement
- Harvest, count, and seed cells
- Measure growth curve
- Serum starvation (optional)
- Stimulation
- Data analysis

3.2.1 Experiment design and software set-up

3.2.1.1 Design the plate lay-out such that stimulations are performed at least in duplo and that the time of addition (stimulation) is the same for the conditions that are to be compared.

3.2.1.2 Enter the plate design in the RTCA 2.0 software, which allows for data analysis of ligand potency and efficacy at the end of the experiment.

3.2.1.3 Enter measurement schedule in the software.

The software can record impedance at intervals at the convenience of the user, where also the number of measurements (sweeps) for each step can be chosen. Table 1 shows an example of an agonist stimulation experiment. Step 1 is used to measure the baseline impedance of the wells in the presence of only culture

Table 1 Example of a Measurement Schedule for Stimulation Experiments

Step	Auto	Sweeps	Interval	Unit	Remarks
1		1	1	Minutes	Baseline measurement
2		100	15	Minutes	Growth curve
3		82	15	Seconds	Stimulation
4	x	20	1	Minutes	
5	x	9	5	Minutes	
6	x	100	15	Minutes	
7		100	15	Minutes	Back-up step

medium. Step 2 follows the growth curve of the cells overnight. The compound-induced responses are followed every 15 s (step 3), which is later changed to every minute (step 4), every 5 min (step 5), and every 15 min (step 6). By selecting "auto" these steps are started automatically. The last step 7 is a back-up step that can be used in case of a problem.

3.2.2 Baseline measurement

3.2.2.1 Add 45 µL medium of 37 °C per well to the *E*-plate. Place the plate in the incubator for 30 min so that both the plate and the medium are warmed homogeneously.

3.2.2.2 Mount the plate in the machine and measure baseline (step 1). Remove the plate from the machine and allow to rest at RT for approximately 30 min.

3.2.3 Harvest, count, and seed cells

3.2.3.1 Detach cells from the culture plate by trypsinization, add 9 mL culture medium of 37 °C, and transfer to a 15 mL centrifuge tube (total volume ~10 mL).

3.2.3.2 Centrifuge the cell suspension for 5 min at 1000 rpm (0.2 rcf). Aspirate the supernatant and resuspend the cell pellet in culture medium of 37 °C (e.g., 6 mL).

3.2.3.3 Count the cells by mixing 10 µL cell suspension with 10 µL trypan blue. Add 10 µL to the cell counter slide (Biorad) to count viable cells in a cell counter (Biorad).

3.2.3.4 Dilute the cell suspension to the desired cell number. Add 50 µL of the cell suspension to the xCELLigence *E*-plate using an automated multichannel pipet. Leave the plate for 30 min at RT. Cells are now allowed to attach to the bottom of the wells.

3.2.4 Measure growth curve (generally overnight, 18–20 h)

3.2.4.1 Mount the plate in the xCELLigence machine and start step 2. Cells are now allowed to proliferate overnight, while a growth curve is measured continuously.

3.2.5 Serum starvation

Presence of the endogenous ligand can affect compound-induced receptor responses of both agonists and inverse agonists, as shown for glutamate

at the $mGlu_2$ receptor, for example (Doornbos et al., 2018). Therefore, a serum starvation step is used to deplete endogenous ligand levels from the medium. In this step, the culture medium is replaced by serum-free assay medium that does not contain the endogenous agonist. In the case of the $mGlu_2$ receptor, serum-free this assay medium is further supplemented with the glutamate depleting enzyme GPT and additional pyruvate, which is behaving as cofactor to also decrease endogenous glutamate levels produced by cells.

3.2.5.1 Pause the experiment 3 h prior to the stimulation.

3.2.5.2 Remove the plate from the xCELLigence machine and place it in the flow cabinet under sterile conditions.

3.2.5.3 Gently aspirate culture medium from all wells.

3.2.5.4 Wash once with 95 µL assay medium of 37 °C (be gentle).

3.2.5.5 Gently add 95 µL assay medium of 37 °C.

3.2.5.6 Mount the plate in the xCELLigence machine and resume the experiment.

3.2.6 Stimulation

3.2.6.1 Make compound dilutions in assay medium of 37 °C and subsequently transfer ~100 µL to a round bottomed 96-multiwell plate for compound addition. Place the 96-multiwell plate in the incubator for at least 30 min. Since DMSO affects the CI responses, make sure DMSO concentrations are below 1% and are equal in all wells.

3.2.6.2 Abort the proliferation step (step 2) and start the stimulation step (step 3) just before compound addition.

3.2.6.3 Add compounds in 5 µL of the compound dilution after 18 h of growth using preferably an automatic multichannel pipette or 96-well pipetting system in order to reduce the duration of addition, as much as possible.

3.3 DATA ANALYSIS

All raw data are obtained from the RTCA software 2.0 (ACEA Biosciences). Typically, compound-induced responses are normalized to delta cell index (ΔCI) or normalized cell index (nCI) after subtracting baseline (vehicle control) to correct for ligand-independent effects. Maximum or minimum peak responses are considered as the highest or lowest ΔCI or nCI level within 60 min after stimulation. Then, further data analysis is performed using Graphpad Prism (v7.00, GraphPad software, San Diego, CA, USA), from which concentration–response curves and bar graphs are generated. To obtain pEC50 and pIC50 values non-linear regression curve fitting is used, using a sigmoidal dose–response curve with variable slope. Data are shown as the mean ± SEM of at least three individual experiments performed in duplicate. Statistical analyses are performed as indicated in the figure legend. If *P*-values are below 0.05, observed differences are considered statistically significant.

3.4 OPTIONAL ADDITIONS TO THE PROTOCOL

- To evaluate the effect of a neutral antagonist on agonist-induced responses, a pre-treatment step can be added, e.g., 1 h or 30 min prior to the agonist stimulation. To this end, an additional step should be added to the schedule in the ACEA software.
- The potency and efficacy of allosteric modulators can be quantified using the xCELLigence. Initial experiments for evaluation of potency generally start with dose–response curves of the endogenous agonist in the presence of multiple increasing concentrations of a PAM or NAM. To this end, a co-addition of agonist and allosteric modulator is performed at the time of stimulation. A typical follow-up experiment consists of a dose–response curve of the allosteric modulator in the presence of an EC_{20} or EC_{80} concentration of the agonist in the case of PAMs or NAMs, respectively. The xCELLigence technique has been shown to be able to record such allosteric modulator behavior for GPCRs, including the muscarinic acetylcholine M_4 receptor and the $mGlu_2$ receptor (Chen et al., 2015; Doornbos et al., 2017), and may therefore be used to quantify responses of both PAMs and NAMs across GPCRs.

3.5 NOTES

- The bottom of the wells and the bottom of the plates should not be touched to prevent any damages that may affect the electrodes.
- As was shown here, serum starvation can be used in cases where GPCRs may react to factors from the serum.
- Air bubbles on the surface of the electrode will greatly affect the impedance measurements and should thus be avoided by gentle additions of fluids to the wells and by preventing pipetting air into the medium.

  The xCELLigence system works best with adherent cell lines. If suspension cell lines are to be used the wells can be coated, e.g., with fibronectin. Hillger et al. (2015) have performed an assay optimization study for the use of suspension cell lines on xCELLigence using various coatings.

4 RESULTS

4.1 CELL NUMBER TITRATION

To determine the optimal cell number for the experiments a cell number titration was performed using 20,000–80,000 cells per well. Cells were allowed to grow overnight for 18 h. As shown in Fig. 2, the cell index obtained was cell number dependent. After the experiment pictures were taken (Fig. 2B–E), showing that at 20,000 cells per well no confluency was obtained (Fig. 2B). At both 60,000 and 80,000 cells per well, the wells were overgrown and cells started to grow

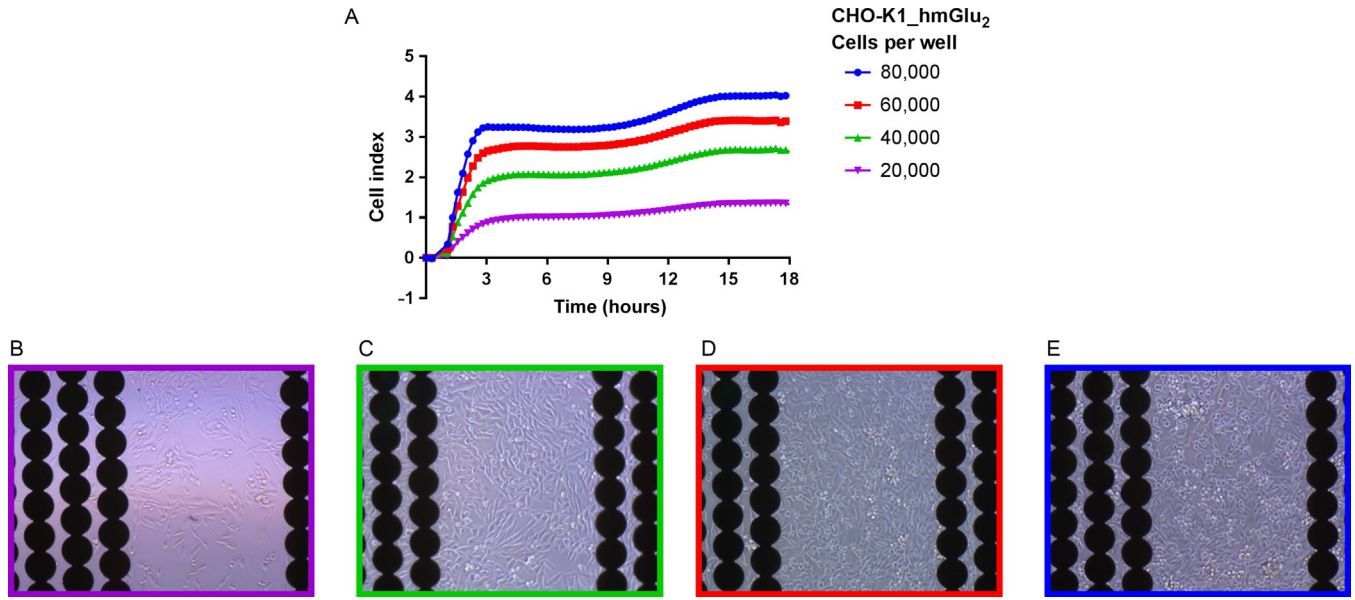

FIG. 2

Cell number titration. (A) Growth curve of CHO-K1_hmGlu$_2$ cells, seeded at different cell densities. Pictures were taken after 18 h, showing no confluency for (B) 20,000 cells per well. A confluent monolayer for (C) 40,000 cells per well and overgrown wells for (D) 60,000 and (E) 80,000 cells per well. xCELLigence traces (A) are from a representative experiment performed in quadruplicate. Pictures are from a representative experiment using VIEW *E*-plates, which contain an area without electrodes in the middle of the well-enabling microscopic evaluation.

on top of each other (Fig. 2D and E). The preferred cell number was 40,000 cells per well, since at this cell number a confluent monolayer was obtained just before compound addition (Fig. 2C).

4.2 RECEPTOR-SELECTIVE SIGNALING

To evaluate the receptor-mediated response of an agonist and an inverse agonist, compound-induced responses on CHO-K1_hmGlu$_2$ cells were compared to responses on parental CHO-K1 cells (Fig. 3). A high 1 μM concentration of the mGlu$_{2/3}$-selective synthetic agonist LY354740 was used for stimulation resulting

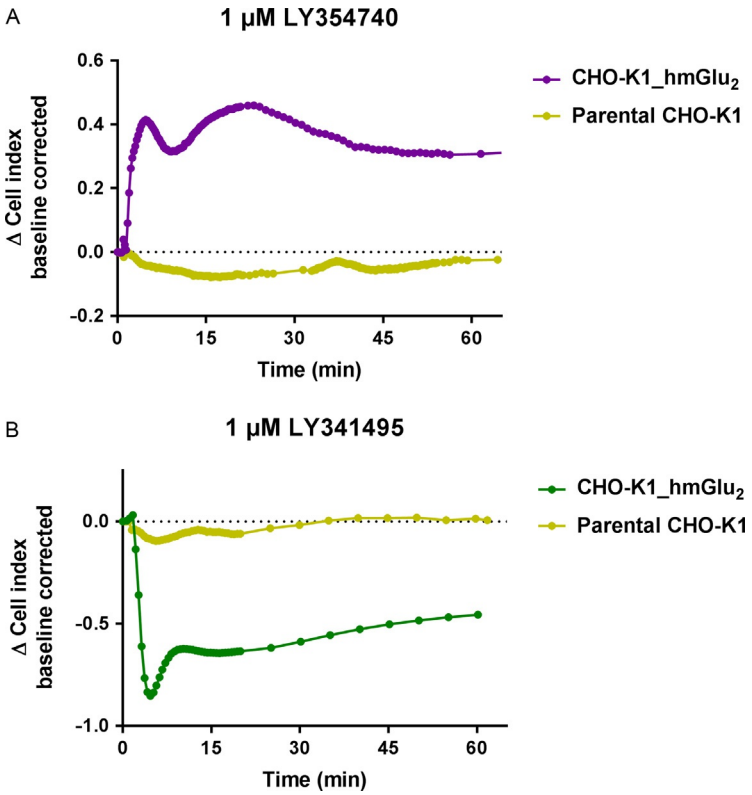

FIG. 3

Effect of mGlu$_2$ receptor ligands on parental cell line. (A) Agonist LY354740 and (B) inverse agonist LY341495-induced responses on CHO-K1_hmGlu$_2$ and parental CHO-K1 cells seeded at 40,000 cells per well. xCELLigence traces are from representative experiments performed in duplicate.

Data are adapted from Doornbos, M. L. J., Van der Linden, I., Vereyken, L., Tresadern, G., IJzerman, A. P., Lavreysen, H., & Heitman, L. H. (2018). Constitutive activity of the metabotropic glutamate receptor 2 explored with a whole-cell label-free biosensor. Biochemical Pharmacology, 152, *201–210. http://doi.org/10.1016/j.bcp.2018.03.026.*

in a positive maximum response around 5 min of 0.47 ± 0.04 ΔCI, then gradually decreasing with a second "shoulder" peak around 20 min (Fig. 3A). In contrast to this positive response of LY354740, 1 μM of the inverse agonist LY341495 induced a negative response on cell index after approximately 5 min (Fig. 3B), with a minimum peak level of −0.98 ± 0.06 ΔCI. The traces of both LY354740 and LY341495 eventually returned to baseline. These traces showed that for this receptor ligands with an opposite pharmacological characteristic result in opposite effects on cell morphology and thus impedance. Importantly, both agonist LY354740 and inverse agonist LY341495 did not induce a response on the parental CHO-K1 cells, which indicated that the responses of both compounds on the CHO-K-1_hmGlu$_2$ cells were mGlu$_2$ receptor mediated (Fig. 3A and B).

4.3 EVALUATION OF G PROTEIN COUPLING

To evaluate whether the effects induced by the agonist LY354740 and inverse agonist LY341495 were mediated via the G$\alpha_{i/o}$ protein, the selective G$\alpha_{i/o}$ inhibitor PTX was used (Mangmool & Kurose, 2011). Cells were seeded in culture medium containing 300 ng/mL PTX. This concentration was also used in the serum starvation step to keep it constant for the complete duration of the experiment. Responses of both LY354740 and LY341495 were lost in the presence of PTX, confirming that these compound-induced responses represented signaling via the G$\alpha_{i/o}$ protein (Fig. 4).

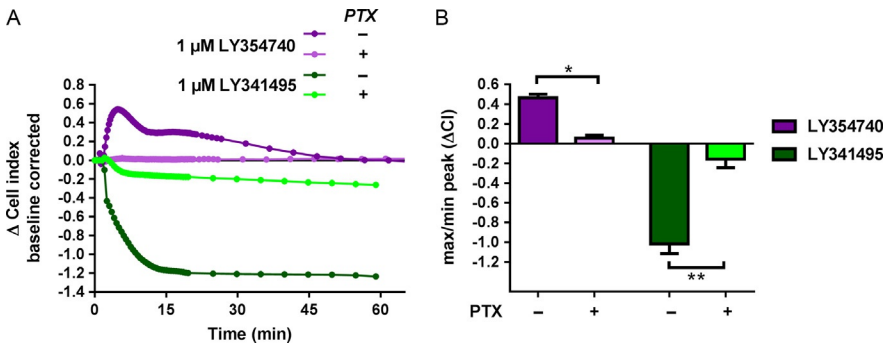

FIG. 4

Effect of PTX pre-treatment on mGlu$_2$ receptor ligand responses. (A) Responses of 1 μM agonist LY354740 and 1 μM inverse agonist LY341495 in the absence or presence of PTX (300 ng/mL). (B) Comparison of the maximum and minimum responses induced by 1 μM LY354740 and LY341495 in the absence of presence of PTX (300 ng/mL). xCELLigence traces are from representative experiments performed in duplicate. Bar graphs represent mean ± SEM of at least three individual experiments performed in duplicate. Statistical analysis was performed using a two-tailed unpaired Student's *t*-test. $*P < 0.01$, $**P < 0.001$.

Data are adapted from Doornbos, M. L. J., Van der Linden, I., Vereyken, L., Tresadern, G., IJzerman, A. P., Lavreysen, H., & Heitman, L. H. (2018). Constitutive activity of the metabotropic glutamate receptor 2 explored with a whole-cell label-free biosensor. Biochemical Pharmacology, 152, *201–210. http://doi.org/10.1016/j.bcp.2018.03.026.*

4.4 CONCENTRATION-DEPENDENT RESPONSES OF AGONIST LY354740 AND INVERSE AGONIST LY341495

Stimulation with increasing concentrations of agonist LY354740 and inverse agonist LY341495 resulted in concentration-dependent positive and negative ΔCI values, respectively (Fig. 5A). For LY354740 a concentration–response curve was obtained from the maximum ΔCI values resulting in a pEC_{50} value of 6.70 ± 0.24 (Fig. 5B). This potency was lower than observed in other assays such as Ca^{2+} mobilization (pEC50 7.4; Monn et al., 2015) and similar to the potency obtained

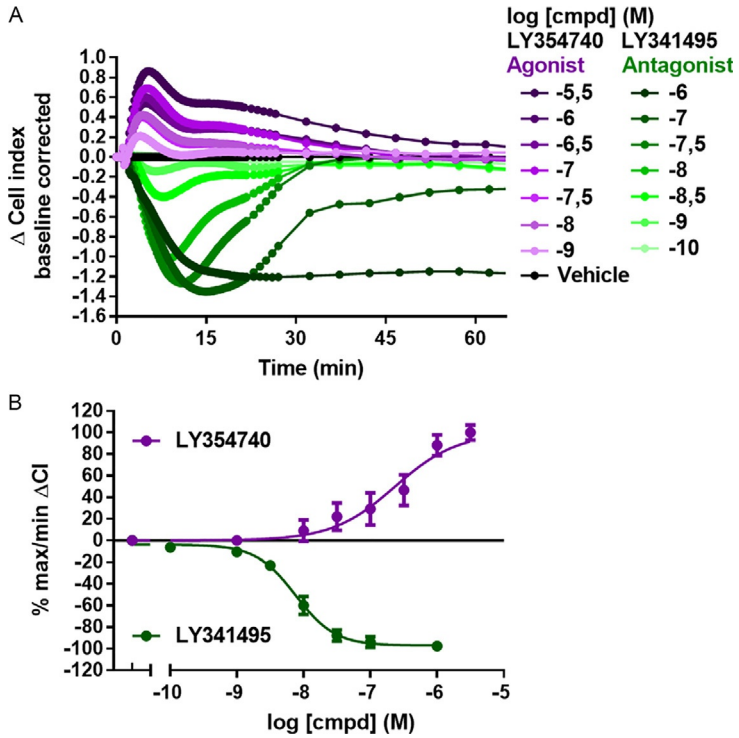

FIG. 5

Dose–response curves of $mGlu_2$ receptor ligands. (A) Cells were stimulated by increasing concentration of agonist LY354740 or inverse agonist LY341495. Both ligands induced concentration-dependent responses. (B) Concentration–response curves were obtained from the maximum (LY354740) or minimum (LY341495) ΔCI values and normalized from vehicle-induced ΔCI (0%) to maximum (100%) or minimum (−100%) ΔCI. xCELLigence traces are from representative experiments performed in duplicate. Concentration–response curves (*panel B*) represent mean ± SEM of at least three individual experiments performed in duplicate.

Data are adapted from Doornbos, M. L. J., Van der Linden, I., Vereyken, L., Tresadern, G., IJzerman, A. P., Lavreysen, H., & Heitman, L. H. (2018). Constitutive activity of the metabotropic glutamate receptor 2 explored with a whole-cell label-free biosensor. Biochemical Pharmacology, 152, *201–210. http://doi.org/10.1016/j.bcp.2018.03.026.*

in electrophysiology experiments on hippocampal slices (pEC50 6.9; Kilbride, Huang, Rowan, & Anwyl, 1998). For inverse agonist LY341495 concentration-dependent negative peaks were found. Minimum ΔCI were used to determine the potency, resulting in a pIC_{50} of 8.15 ± 0.09 (Fig. 5B). This value is in agreement with potencies reported before using cAMP, Ca^{2+}-mobilization assays and [^{35}S]GTPγS assays (Campo et al., 2011; Kingston et al., 1998).

5 CONCLUSIONS

The impedance-based xCELLigence assay is an easy-to-use assay that enables evaluation of GPCR pharmacology using living cells without the need for any labels. The assay can be used for a wide variety of cell types including those with low receptor expression such as endogenous cell lines or even patient-derived cells. A wide variety of compound-induced GPCR responses can be measured and quantified in real-time including the classical parameters potency and efficacy, but also receptor activation kinetics.

The $mGlu_2$ receptor was used here as a model system, showing that GPCRs can be well studied using this technique. Specifically, we showed that specific agonist- and inverse-agonist-induced receptor-mediated responses on the $mGlu_2$ receptor. Recent developments from the vendor (ACEA), such as increasing the number of *E*-plates per reader (MT system) and increasing the number of wells from 96 to 384 (HT system), will further increase the throughput of the system. Together this makes it an even more useful approach for studying GPCR pharmacology, but also for evaluation of larger series of GPCR ligands.

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