

Impedance-based cellular analysis - GPCRs

Monitoring of endogenous GPCR signaling with the **Bionas Discovery™ adcon reader**

INTRODUCTION

G-protein coupled receptors (GPCRs) represent the largest and most ubiquitous family of seven-transmembrane receptors and thus have become the most popular target class for therapeutic intervention. The **Bionas Discovery™ adcon reader** for impedance-based cellular analysis provides a powerful tool for the investigation of GPCR functions in receptor target validation studies without the use of any label and in real-time. Furthermore, the system allows substance addition without the need to stop the measurement for plate handling.

In this study, impedance-based measurement using the **Bionas Discovery™ adcon reader** was used to distinguish the kinetic response profiles of functional endogenous GPCR receptor activities in U2OS cells (human osteosarcoma).

RESULTS

U2OS cells endogenously express histamine, somatostatin and β -adrenergic receptors, coupled to three different subtypes, namely G_q , G_i and G_s . The cells were treated with histamine, somatostatin

isoproterenol (all 10 μ M) resulting in an activation of the appropriate receptors.

In Figure 1, the kinetic impedance responses of the three main G-protein receptor subunits are shown.

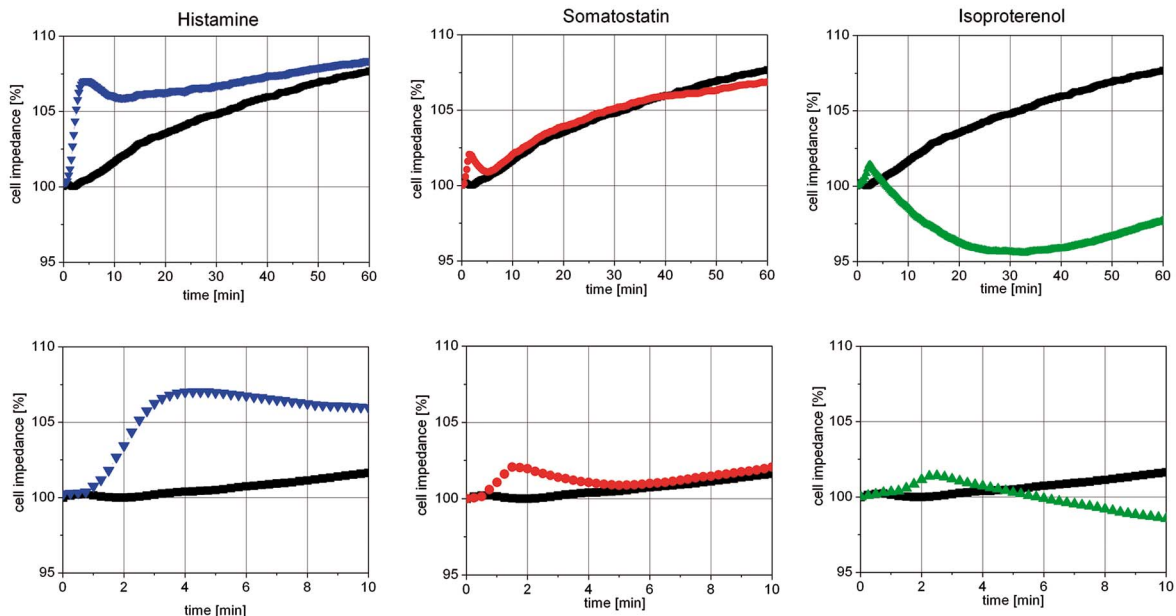
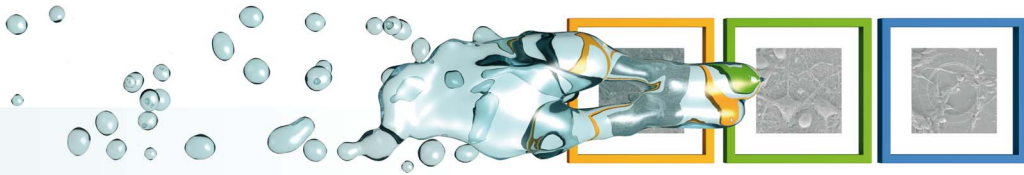


Figure 1: Impedance measurement of three receptor subtypes of U2OS cells

Upper row: Impedance was initially increased after the addition of histamine (G_q , blue), somatostatin (G_i , red) and isoproterenol (G_s , green) followed by a decrease which was different in size and shape for the three GPCR subunits.

Lower row: Enlargement of the initial 10 min after receptor activation showing the immediate change of impedance signal.





The effect of isoproterenol was inhibited by the β -adrenoceptor antagonist alprenolol, which decreased the isoproterenol-induced effect by around 50%, indicating a specific action at G_i .

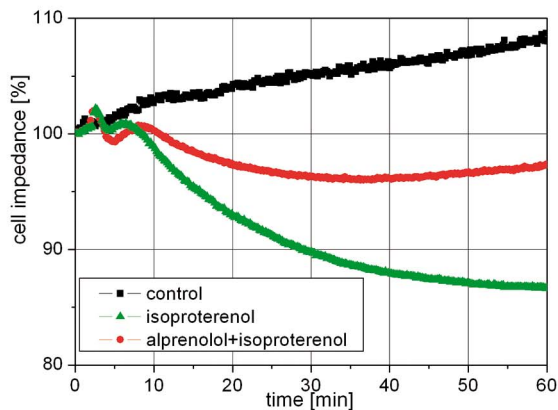


Figure 2: Inhibition of the β -adrenergic receptor activation by the antagonist alprenolol.

MATERIALS & METHODS

Analysis in the Bionas Discovery™ adcon reader. The **Bionas Discovery™ adcon reader** consists of the plate station, the analyzer and a PC as user interface. The plate station, positioned into the incubator, accommodates the adcon plate in 96-well format with interdigitated electrodes at the bottom of each well for impedance measurement providing information about cell viability, morphology, adhesion/confluence, proliferation and membrane integrity. Cellular adhesive alterations, e.g. upon stimulation of receptors, are recorded in real-time. The data are analyzed with the **Bionas Discovery™ adcon software**.

GPCR assay. Prior to the experiment, the plate station was placed inside an incubator and connected with the external analyzer. U2OS cells (human osteosarcoma) were seeded at a density of 1×10^4 cells/well in an adcon plate.

Cell growth was monitored with the **Bionas Discovery™ adcon reader** under standard cell cultivation conditions (5% CO_2 , 37°C) for 24h. 45 min prior to receptor activation by histamine, somatostatin and isoproterenol (all 10 μM), the plate station was removed from the incubator and the cultivation medium was changed to Hank's buffer + 0.1% BSA + 20 mM HEPES + P/S at RT. Ligands of G-protein subunits were added in Hank's buffer during the ongoing measurement at RT and impedance was monitored for the following 60 min. Prior to the addition of isoproterenol, cells were preincubated with alprenolol for 15 min.

CONCLUSION

With the **Bionas Discovery™ adcon reader** endogenous GPCRs were monitored upon stimulation. The immediate change of impedance after activation of the receptors designates the sensitivity of the real-time readout. A major advantage of the system is the feature to activate receptors during the real-time data acquisition.

The **Bionas Discovery™ adcon reader** significantly reduces plate handling and enables the real-time monitoring of fast cellular events without any interruption which triggers unique response profiles within minutes of activation, shown here in U2OS cells for three GPCR subtypes.

AUTHOR

S. Ortinau, PhD, Bionas GmbH

CONTACT

Bionas GmbH, Friedrich-Barnewitz-Straße 3
18119 Rostock, +49 (0) 381 5196 442
www.bionas-discovery.com
sales@bionas-discovery.com