Case Study

Custom Tools for GIP Receptor Drug Discovery

Introduction

Glucose-dependent insulinotropic polypeptide (GIP) receptor, a member of the G protein-coupled receptor (GPCR) family, serves as the primary mediator of GIP's physiological effects. Through its activation, GIP receptor signalling influences various cellular pathways, including those involved in insulin release, adipogenesis, and gastrointestinal function. Found primarily in pancreatic β-cells and adipocytes, GIP receptors mediate insulin release and impact lipid metabolism, implicating them in the development of obesity and related metabolic disorders.

Targeting GIP receptors presents a promising therapeutic strategy for managing obesity and type 2 diabetes, with pharmaceutical interventions offering potential avenues for improving metabolic health and addressing obesity-related complications. Understanding the intricate mechanisms underlying GIP receptor signalling provides valuable insights into the pathophysiology of metabolic diseases and facilitates the development of novel therapeutic interventions.

β-Arrestin Pathway

As a regulatory protein involved in signal transduction and cellular responses mediated by GPCR's, β-Arrestin plays a crucial role in receptor internalization, desensitization, and signalling regulation. In screening assays, β-Arrestin recruitment can serve as a surrogate marker for GPCR activation, offering a more comprehensive understanding of receptor pharmacology and potential therapeutic efficacy. Specific ligands can selectively recruit either β-Arrestin or promote G-protein activation, leading to biased signalling, which can have implications for drug development and provide opportunities for therapeutic strategies. In the context of GIP receptors, biased agonists that preferentially recruit β-Arrestin over G-protein activation may offer therapeutic advantages, such as improved insulin secretion or glucose homeostasis, while minimizing undesirable side effects associated with traditional G-protein activation. The interplay between β-Arrestin and G-protein signalling pathways underscores the importance of understanding their dynamics for the development of more targeted and effective therapeutics.

Custom Cell-Based Tools for Complex Receptors

- **Real-Time Monitoring of Receptor Interactions:** Generation of robust, stable cell lines to measure receptor interactions, providing real-time insights into GPCR signalling and modulation.
- **Biased Signalling Studies:** Cell-based tools designed to aid the investigation of biased signalling, enabling identification of selective modulators of either β-Arrestin or G-protein pathways.
- **Receptor Trafficking Tools:** Advanced assays enabling precise monitoring of ligand induced receptor internalization and trafficking.

Real-Time Monitoring of Receptor-β-Arrestin Interaction and Signalling

To enable discovery of modulators of receptor:β-Arrestin interaction and downstream signalling, SB Drug Discovery scientists have used NanoLuc® Binary Technology (NanoBiT, Promega) to generate robust stable cell lines to measure real-time interaction between receptors and β-Arrestin in live cells. NanoBiT is a structural complementation reporter system composed of a Large BiT (LgBiT) subunit and a small complementary peptide (SmBiT).

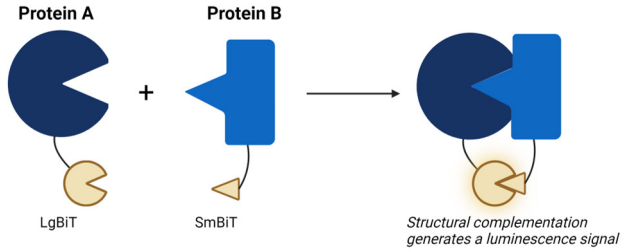

Figure 1. **Overview of NanoBiT® protein:protein interaction system.**

The LgBiT and SmBiT subunits are expressed as fusions to target proteins of interest and expressed in cells. When the two targeted proteins interact, the LgBiT and SmBiT subunits come together to form an active enzyme and generate a bright luminescent signal in the presence of substrate.

Assessment of SmBiT/LgBiT fusions

To identify optimal SmBiT/LgBiT positioning, a range of expression vectors were generated (Figure 2) and assessed using transiently transfected cells. The combination of GIPR-LgBiT and SmBiTβArr exhibited the best response to GIP and was used for all subsequent experiments (Figure 3).

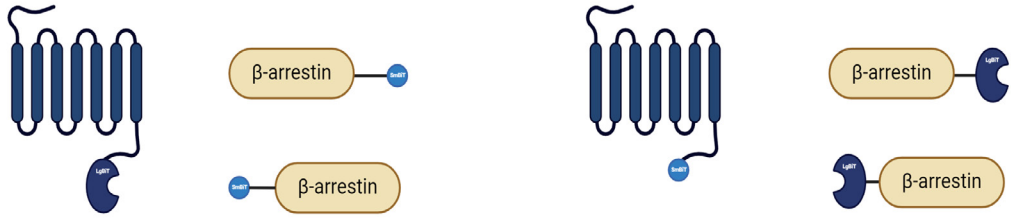

Figure 2. **Constructs generated to assess SmBiT/LgBiT tag position.**

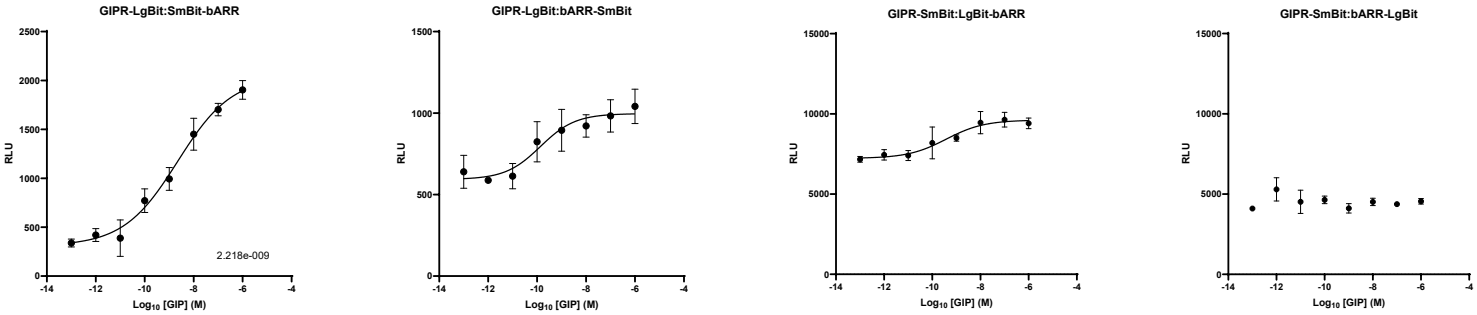

Figure 3. **Assessment of GIP receptor/β-Arrestin construct pairs using a transient NanoBiT luciferase assay.**

Stable Cell Line Generation

HEK293 cells were transfected with the optimal GIP receptor and β-arrestin expression vectors and maintained in media containing antibiotic selection until clonal isolated were apparent. Single cell clones were expanded from 96-well to 24-well plates and clones screened for functional activity in the NanoBiT luciferase assay.
The initial clone screen provided rapid assessment of GIP receptor/β-Arrestin activity in response to the ligand, GIP. A number of clones displayed a greater increase in luminescence compared to vehicle treated cells, suggesting GIP receptor activation and β-Arrestin recruitment.

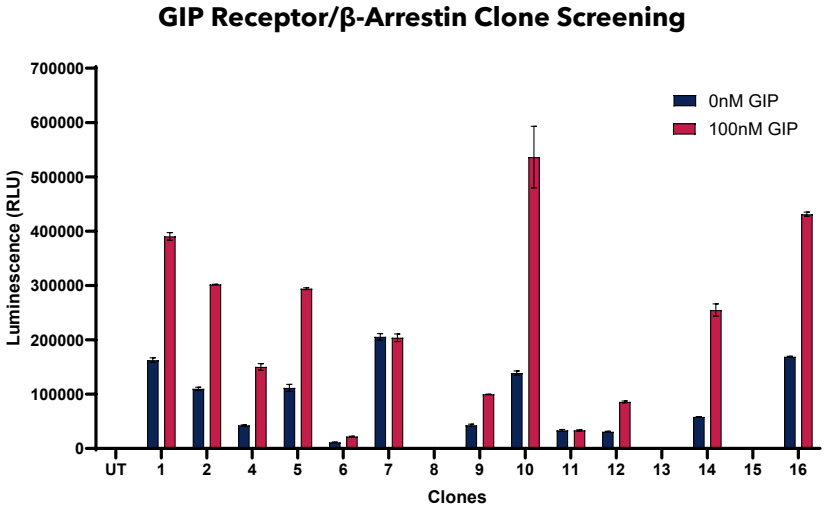

Figure 4. **Single cell clones were expanded and screened for GIP receptor/β-Arrestin interaction using NanoGlo® live detection reagent.**

The optimal clone was further characterized to assess the effect of GIP on β-Arrestin recruitment and measure changes in intracellular cAMP levels following GIP stimulation (Figure 5). Results showed an increased GIP potency in the cAMP assay (approx. 0.1 nM) compared to b-arrestin recruitment assay (1.3 nM). These findings are consistent with published literature.

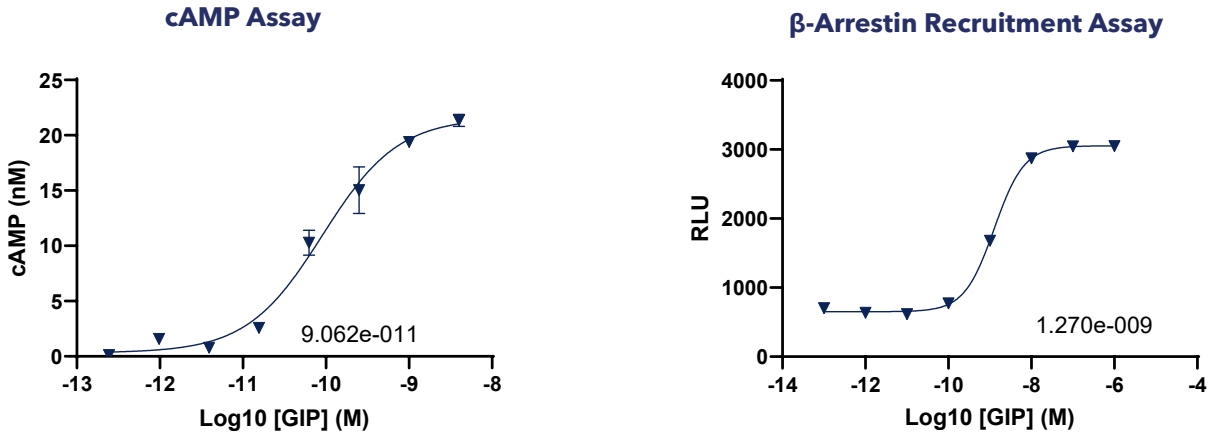

Figure 5. **Concentration response curves showing concentration-dependent increase in cAMP levels, measured using a cAMP HTRF assay (left) and GIP concentration-dependent increase in b-arr recruitment (right).**

GIP Receptor Internalization

To measure GIP receptor internalization, cells were transfected with HiBiT tagged GIP receptor and clonal isolated assessed for cell surface expression and response GIP using the Nano-Glo® HiBiT Extracellular Detection System. From the clone screen a number of isolated demonstrated an increase in luminescence over untransfected control (Figure 6), confirming cell surface expression of GIP receptor.

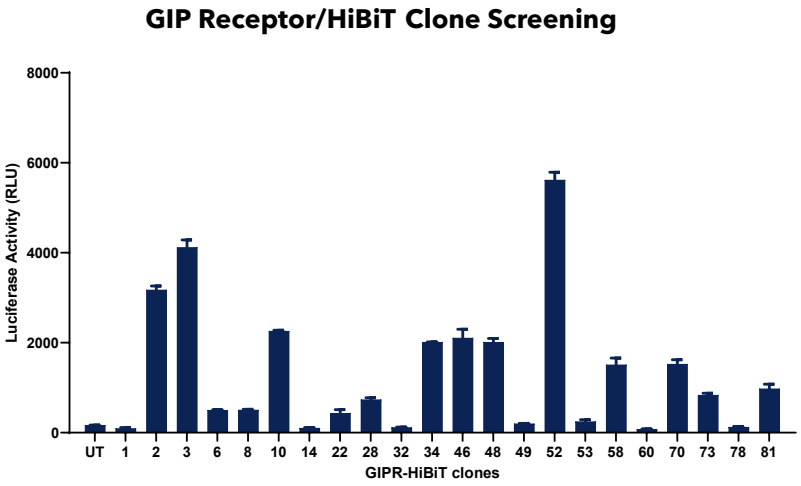

Figure 6. **HiBiT-GIP receptor clones were screened for cell surface expression using the Nano-Glo® HiBiT Extracellular Detection System to provide an initial assessment of GIP receptor expression. A number of clones showed detectable expression above background (untransfected) levels.**

The optimal HiBiT-GIP receptor clone was selected to assess cAMP response to GIP and receptor internalization. GIP displayed concentration-dependent activity in the cAMP assay with comparable EC_{50} value to that generated using the GIP receptor/β-Arrestin cell line. The HiBiT GIP receptor cell line also showed a concentration-dependent decrease in cell surface expression upon addition of GIP (Figure 7) with an EC_{50} value of approximately 3 nM.

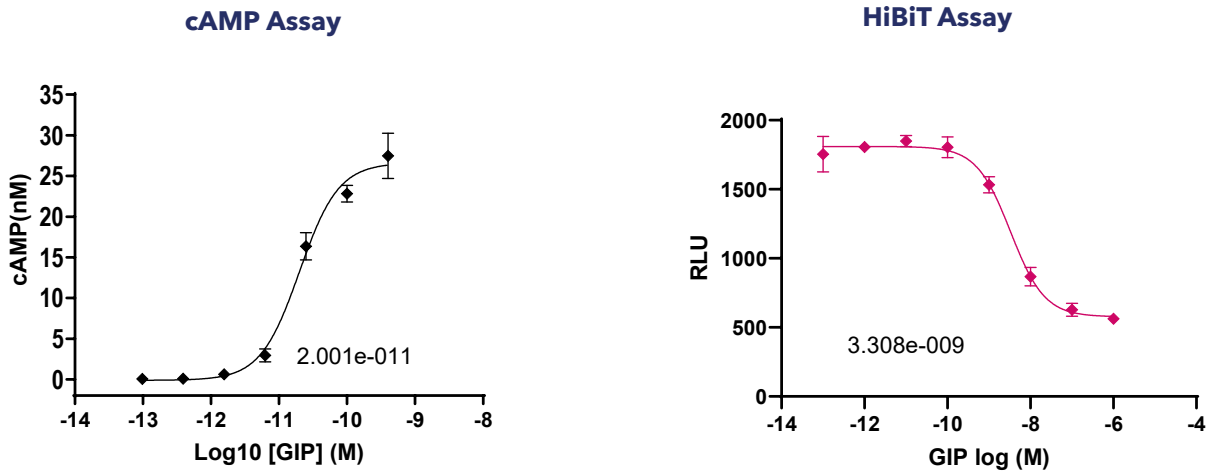

Figure 7. **HiBiT-GIP receptor cell line displayed a concentration-dependent reponse to GIP in both the cAMP assay (left) and HiBiT receptor internalization assay (right).**

Summary

SB Drug Discovery, specialized in custom cell-based tools for complex receptors offers promising therapeutic strategies for managing obesity and type 2 diabetes. By comprehensively understanding GIP receptor signaling, SB Drug Discovery provides novel avenues for pharmaceutical interventions aimed at improving metabolic health. Utilizing custom cell lines expressing GIP receptors and β-Arrestin, alongside cutting-edge screening assays that utilize the β-Arrestin pathway, deepens the understanding of GPCR activation. Biased agonists showcasing the potential for therapeutic advancements that enhance insulin secretion and glucose homeostasis while minimizing side effects.

Employing NanoLuc® Binary Technology (NanoBiT, Promega), robust, stable cell lines monitor receptor-β-Arrestin interactions in real-time, allowing precise identification of optimal receptor-β-Arretin interactions and measurement of downstream signaling. Additionally, advanced methodologies measure GIP receptor internalization and intracellular signaling changes. The HiBiT tag system allows cell surface expression and internalization dynamics to be accurately assessed, enhancing understanding of GIP receptor behavior under various conditions.

Advance your research with SB Drug Discovery's cell line generation services. We offer unparalleled expertise in delivering premium, custom cell line generation solutions that provide a competitive advantage.

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