A Simple & Robust Cell-based Assay for Evaluating GLP-1R, GIPR, and GCGR Agonists

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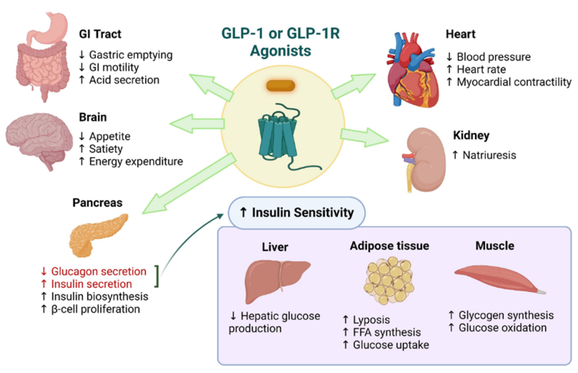
BACKGROUND

The rise in obesity over the last few decades has been associated with increases in related comorbidities such as cardiovascular diseases, type-2 diabetes, and certain types of cancer. Therefore, management of these comorbidities and control of body weight are highly desirable.

Better control of blood glucose levels in diabetic patients has been achieved with GLP-1R (glucagon-like peptide-1 receptor) agonists, which mimic GLP-1, a hormone that stimulates insulin release and inhibits glucagon secretion. GLP-1R agonists also promote a sense of satiety, helping patients with weight loss, and may reduce cardiovascular risks in diabetic patients. Recent studies have shown that dual or triple receptor agonists targeting GLP-1R, GCGR (glucagon receptor) and GIPR (glucose-dependent insulinotropic polypeptide receptor) may provide even better options to improve metabolism and weight management in obese and diabetic patients.

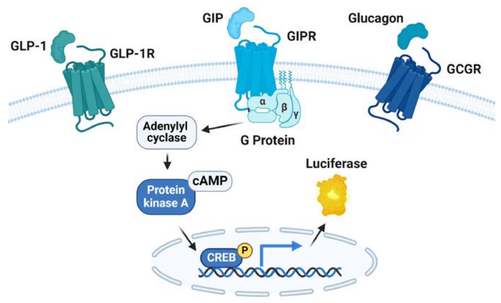


To support multi-receptor agonist development, we have generated and characterized three cell lines that respond to GCGR, GIPR, or GLP-1R activation by inducing luciferase activity. These cells were engineered to express GCGR, GIPR, or GLP-1R, and a firefly luciferase reporter under the control of CRE (cAMP response elements), which is induced by activation of transcription factor CREB. These cells produce a robust luciferase readout upon agonist stimulation.

CELL LINES AND METHODS

Cell Lines

Single clone, stable HEK293 (Human Embryonic Kidney 293) cell lines were engineered to conditionally express firefly luciferase, which is under the control of cAMP response elements (CRE). They also overexpress one of the human G-protein coupled receptors: GLP-1R (#78176), GIPR (#78589), or GCGR (#82187). Agonist activation of the receptor can be monitored by measuring luciferase activity.



Chemicals

Chemicals used in this study were purchased from MedChemExpress, R&D Systems, and GenScript. GLP-1 Fc-Fusion was produced at BPS Bioscience (#102006).

Methods

Experiments were performed in triplicate in white, clear-bottom cell culture plates. Cells were seeded in 90 µl/well of Opti-MEM™ medium (ThermoFisher Scientific) and allowed to attach overnight. The next day, serial dilutions of agonists were prepared in Opti-MEM™ and added to the cells (10 µl/well). Medium without agonist was added to the unstimulated control wells. The cells were then incubated at 37°C with 5% CO_2 for 5 hours. After incubation, 100 µl/well of ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to each well at 100 µl/well and rocked for 15 minutes at room temperature. Luminescence was measured using the BioTek Synergy 2 plate reader. The background luminescence was determined against a no-cell condition and was subtracted from all other values.

CELL LINE CHARACTERIZATION

The three clonal cell lines overexpress the receptor of interest (Figure 1) and display a strong increase in luciferase activity upon addition of the respective agonist, with GLP-1 inducting luciferase activity by about 300-fold, GIP by >40-fold, and glucagon by about 200-fold (Figure 2) whether measured in a 96-well format or in a 384-well format. Sensitivity was in the range of 0.05 nM for GLP-1, 0.4 nM for GIP, and 0.004 nM for glucagon. Finally, incubation times were optimized for each cell line to ensure signal stability when performing the bioassay (Figure 3).

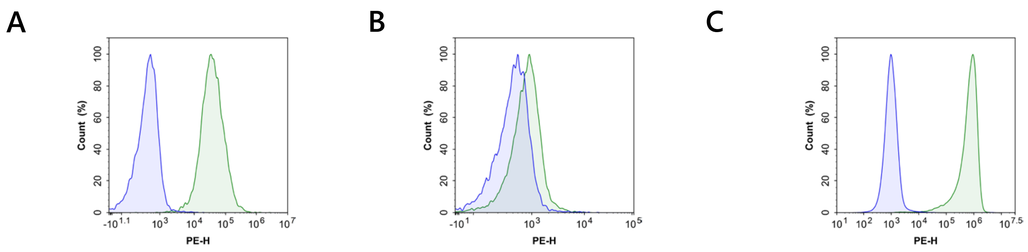


Figure 1. Cell surface expression of GLP-1R (A), GIPR (B), and GCGR (C). *Receptor expression was evaluated by flow cytometry using specific primary antibodies (R&D Systems, #FAB2814P in (A), #FAB8210P in (B), and MAB10296 in (C)), and seconday PE-conjugated anti-mouse IgG (BioLegend Poly4053). Overexpressing cells (green) were compared to parental luciferase reporter HEK293 cells (blue). Y-axis represents the % cell number. X-axis indicates the intensity of PE.*

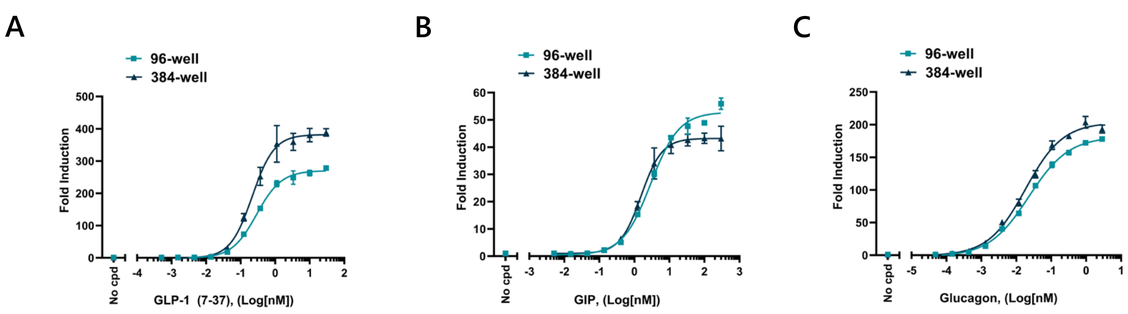


Figure 2. Agonist-induced luciferase activity in each cell line. *Cells were incubated with increasing concentrations of GLP-1* ***(A),*** *GIP* ***(B),*** *or glucagon* ***(C).*** *ONE-Step™ Luciferase reagent was added for 15 minutes at room temperature and luminescence was measured in a luminometer. Results are expressed as fold induction, with luminescence of unstimulated cells set to 1.*

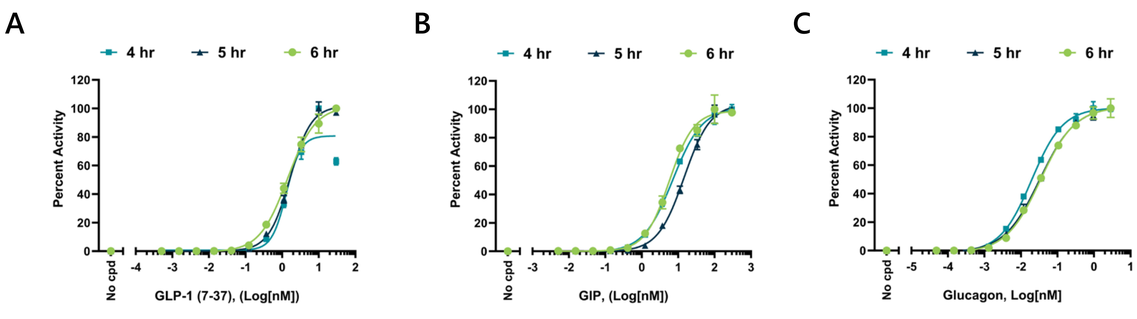


Figure 3. Signal stability in luciferase reporter cell lines. *Cells were incubated with increasing concentrations of GLP-1* ***(A),*** *GIP* ***(B),*** *or glucagon* ***(C)*** *for the indicated times. ONE-Step™ Luciferase reagent was added for 15 minutes at room temperature and luminescence was measured in a luminometer. Results are expressed as percent of maximum activity, with maximum set to 100%.*

AGONIST STABILITY IN SERUM

Drug developers may need to examine the stability of candidate agonists in serum, since these compounds remain in the bloodstream upon administration to patients. Numerous factors contribute to the degradation of agonists within this environment. These experiments indicate that our reporter cell lines are suitable systems to perform such assays.

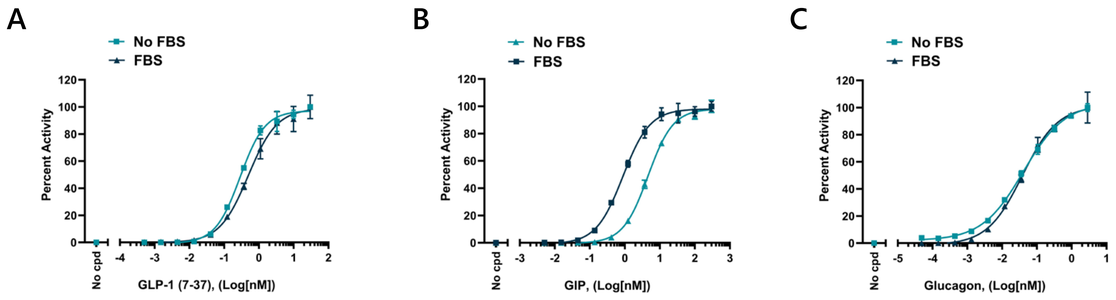


Figure 4. Agonist stability in serum. *Cells were incubated with increasing concentrations of GLP-1* ***(A),*** *GIP* ***(B),*** *or glucagon* ***(C)*** *for 5 hours. The agonist was diluted in medium containing 10% FBS (Fetal Bovine Serum) or in Opti-MEM™. ONE-Step™ Luciferase reagent was added for 15 minutes at room temperature and luminescence was measured in a luminometer. Results are expressed as percent of maximum activity, with maximum set to 100%.*

COMPARISON OF KNOWN AGONISTS

Reporter activation by 11 known GPCR analogs was assessed in the relevant cell lines. All analogs except for glucagon were assayed in GLP-1R/CRE cells. Tirzepatide, a dual-receptor agonist, and Retatrutide, a triple hormone receptor agonist, were assayed in all three cell lines.

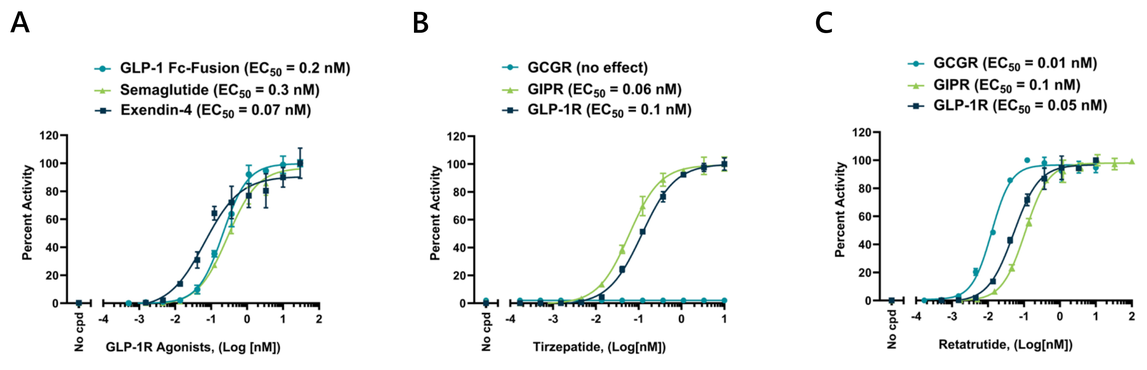


Figure 5. Comparison of analog potency in GLP-1R, GIPR, and GCGR/CRE Luciferase Reporter Cell Lines. *Cells were incubated with increasing concentrations of agonist for 5 hours before addition of ONE-Step™ Luciferase reagent and luminescence measurement.* ***(A)*** *GLP-1R agonists were assayed in the GLP-1R cell line;* ***(B)*** *Tirzepatide was assayed in all cell lines;* ***(C)*** *Retatrutide was assayed in all cell lines. Results are expressed as percent of maximum activity, with maximum set to 100%.*

Table 1: Analog EC_{50} in GLP-1R, GIPR, or GCGR/CRE Luciferase Reporter Cell Lines. *Luciferase activity resulting from incubation with 11 known GPCR analogs was measured in the relevant cell lines as described in Figure 5. All analogs were assayed in GLP-1R/CRE cells, except for glucagon. Retatrutide and tirzepatide were assayed in the three cell lines. Not applicable (n/a) indicates that the analog is not known to activate this receptor and was not assayed in the corresponding cell line. Where indicated, Standard Deviations (SD) of EC_{50} were calculated from 3 to 5 separate experiments, each run in triplicate (except GLP-1 Fc-fusion, for which n=2).*

For each condition, EC_{50} was determined by curve fitting using Prism software v 11.0.

Analog	GLP-1R, EC_{50}	GIPR, EC_{50}	GCGR, EC_{50}
GLP-1 (7-37)	0.92 ± 0.86 nM	n/a	n/a
GIP	No stimulation	3.6 ±1.5 nM	n/a
Glucagon	n/a	n/a	0.03 ± 0.01 nM
Retatrutide	0.14 ± 0.14 nM	0.4 ± 0.5 nM	0.02 ± 0.02 nM
Tirzepatide	0.09 ± 0.02 nM	0.1 ± 0.1 nM	No stimulation
Exendin-4	0.07 nM	n/a	n/a
Dulaglutide Moiety	0.2 nM	n/a	n/a
Lixisenatide	1 nM	n/a	n/a
Semaglutide	0.3 nM	n/a	n/a
GLP-1 Fc-Fusion	0.25 ± 0.07 nM	n/a	n/a
Danuglipron	0.3 nM	n/a	n/a

SUMMARY & CONCLUSION

Cell-based assays are pivotal in drug development, enabling the assessment of potential drug candidates' biological activity on living cells and providing insight into their mechanisms. They offer a more precise depiction of compound interactions with living cells compared to biochemical assays. Inducible reporter assays, particularly luciferase reporters linked to pathway-specific promoters, offer a straightforward, robust, and quantitative way to measure signaling activity in intact cells. These conditional reporter systems transcend various cell types and signaling pathways, enabling high-throughput screening of extensive compound libraries for inhibitory or activating functions.

Three single clone, stable HEK293 cell lines were generated, each overexpressing a hormone receptor (GLP-1R, GIPR, or GCGR) and a Luciferase Reporter under the control of cAMP response elements responding to hormone and analog stimulation by increasing luciferase expression. Luciferase activity can be measured using a simple luminometer and is directly proportional to receptor activation.

Our three cell lines produced a robust luciferase readout upon stimulation of the cell surface receptor and are ideal for screening candidate molecules and determining their EC_{50}.

- Experimentally determined EC_{50} were consistent with existing data
- **Strong induction signal**: 40 to 300-fold stimulation depending on the cell line
- **Stable signal**: luminescence maintained from 4 hours to 6 hours after analog addition
- **Amenable to high throughput**: similar results were obtained using 96-well and 384-well formats

References

(1) Coskun T, *et al.* 2022, *Cell Metabolism* 34: 1234-1247.
(2) Yang B, *et al.* 2022, *Molecular Metabolism* 66: 101638.
(3) Zhao X, *et al.* 2021, *Front. Endocrinol.* 12: 721135.