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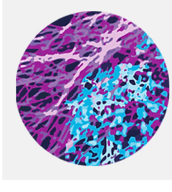

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How Can Membrane-Based Assays Revolutionise Drug Discovery for G Protein-Coupled Receptors?

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Overview

PURPOSE: Develop a safe, membrane-based screening tool for $G\alpha_i$-coupled GPCRs.

METHODS: A BRET-based Gi-CASE biosensor[1] assay was used to screen $G\alpha_i$-coupled CB_1R and CB_2Rs in whole-cell and membrane-based formats.

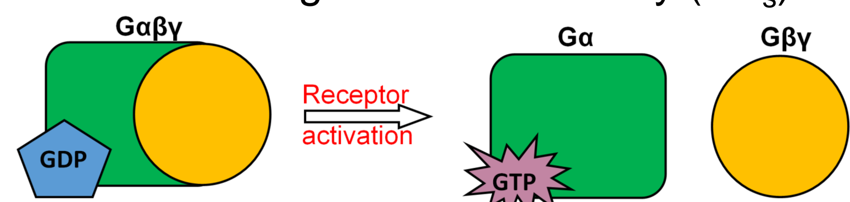
RESULTS: The potencies and efficacies of tested compounds agree with whole-cell and literature data. The assay can be applied to other $G\alpha_i$-coupled GPCRs.

Introduction

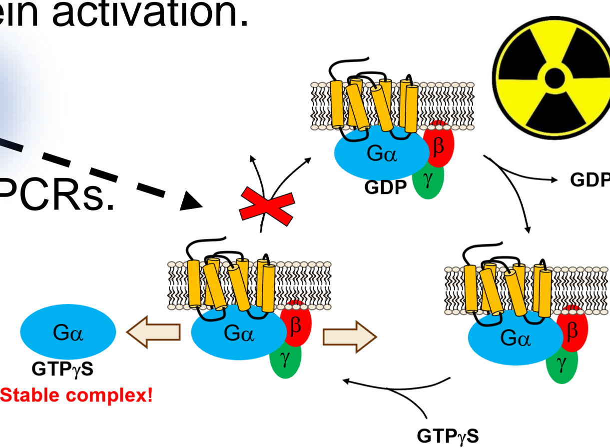
OBJECTIVE: Create a rapid, cheap, safe and effective membrane-based drug screening assay for $G\alpha_i$-coupled GPCRs using G protein activation biosensors[1].

BACKGROUND:

- ✓ Around 700 FDA-approved drugs target GPCRs[2].
- ✓ Adenylyl cyclase-linked GPCRs signal via stimulatory ($G\alpha_s$) or inhibitory ($G\alpha_i$) G proteins.



- ✓ Sensitive GPCR screening assays are essential for early-phase drug discovery and often involve whole-cell assays for detecting G protein activation.
- ✓ The [^{35}S]-GTPγS assay is a useful yet **radioactive** membrane-based assay for finding novel drugs at GPCRs.
- ✓ Thus, we describe a 384-well BRET-based $G\alpha_i$ protein for determining cannabinoid receptor activity.



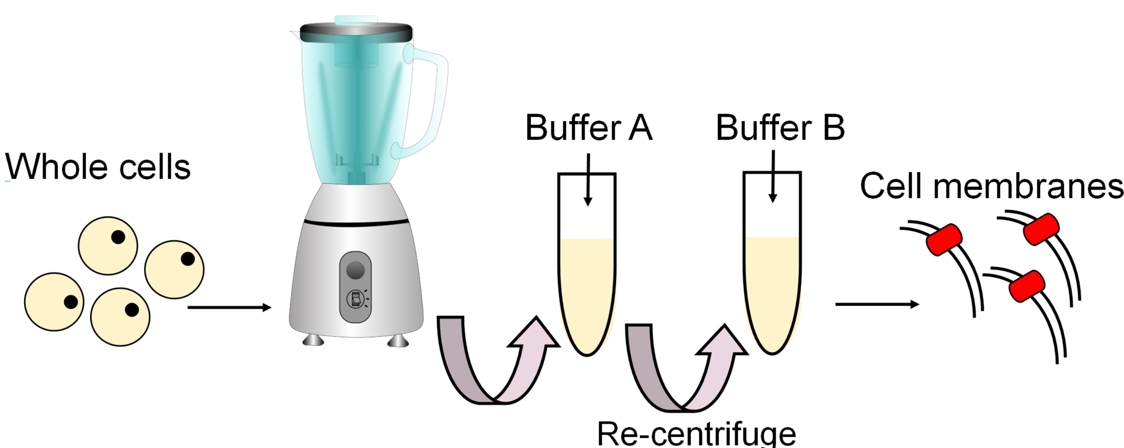
Methods

MEMBRANE PREPARATION:

- ✓ 20 mL Buffer A was added to cell pellet from 2xt175 flasks and homogenized. Mixture was centrifuged, supernatant discarded and the pellet was suspended in 0.9 mL Buffer B.

Buffer A: 10 mM HEPES, 10 mM EDTA pH 7.4

Buffer B: 10 mM HEPES, 0.1 mM EDTA pH 7.4

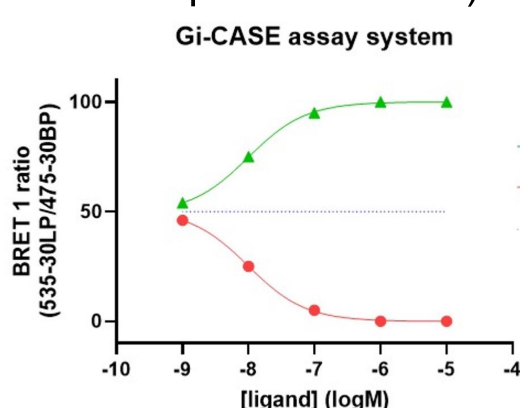


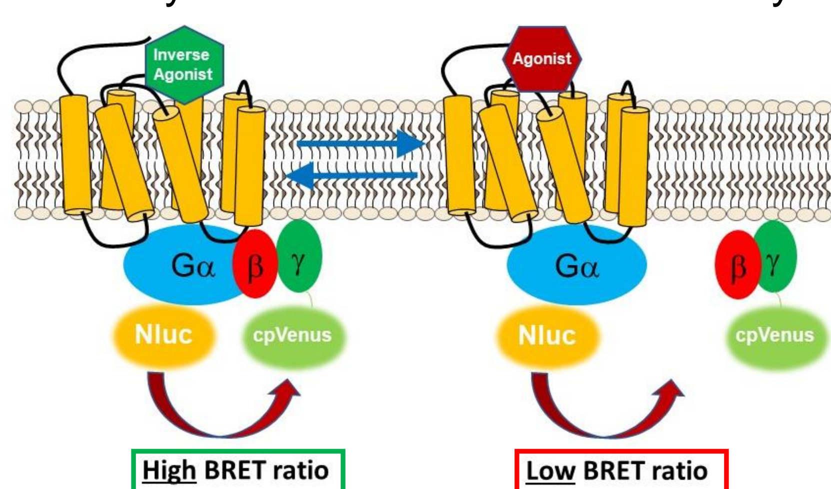
MEMBRANE-BASED ASSAY:

- ✓ CB_1R and CB_2R Gi-CASE membranes (5 µg/well) in assay buffer (HBSS, 0.02% pluronic F127, 0.5% BSA, 5 mM HEPES) and 50 µM furimazine were stimulated with HU-210, HU-308, rimonabant, and SR-144,528.

WHOLE-CELL ASSAY*:*

- ✓ CB_1R and CB_2R Gi-CASE cells in assay buffer (HBSS, 0.5% BSA, 5 mM HEPES) and 10 µM furimazine) were stimulated similarly to the membrane-based assay.





DATA ANALYSIS:

- ✓ BMG PHERAstar FSX plate reader was used. Raw data were converted to a ratio (535-30LP/475-30BP). Data analysis in GraphPad PRISM 9.2 and normalised to vehicle.

Results

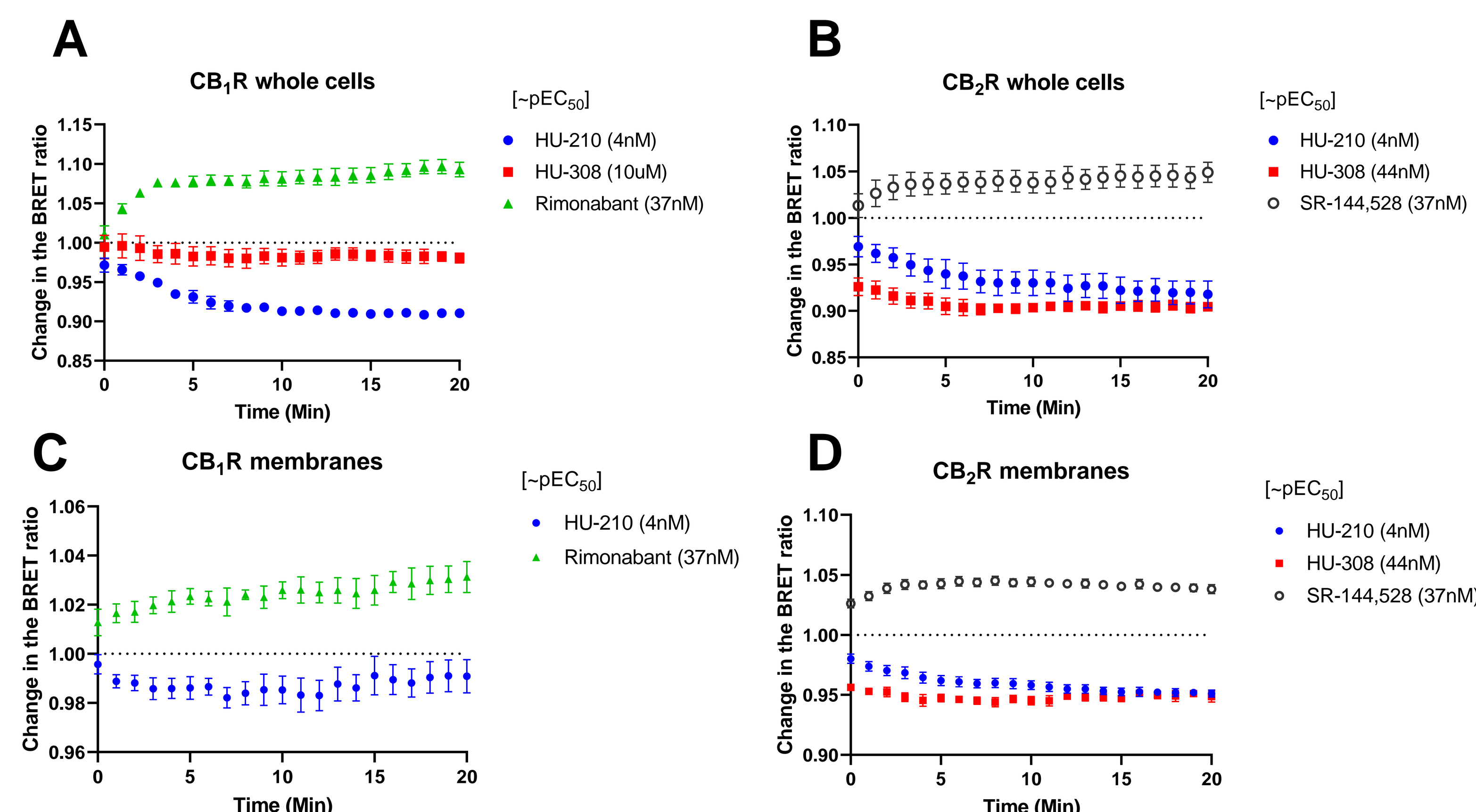


Figure 1: **Time courses following agonist and inverse agonist stimulation of (A) CB_1R and (B) CB_2R in whole cells and (C) CB_1R and (D) CB_2R membranes.** Gi-CASE biosensor incorporated[1]. Data are shown as mean ± SEM, with n≥3.

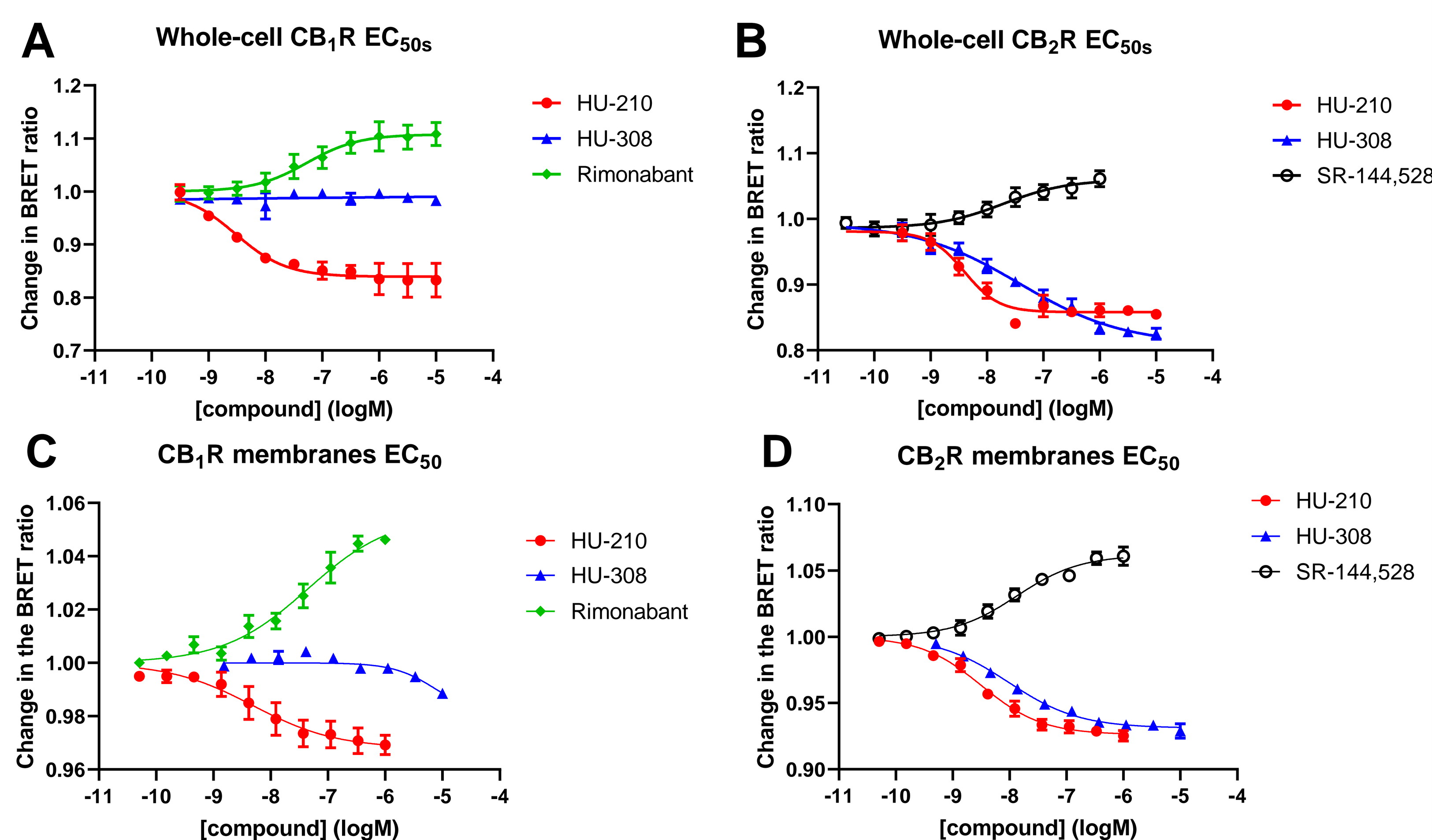


Figure 2: **Agonist and inverse agonist stimulation of (A) CB_1R and (B) CB_2R in whole cells and (C) CB_1R and (D) CB_2R membranes.** Gi-CASE biosensor incorporated[1]. Data are shown as mean ± SEM, with n≥3.

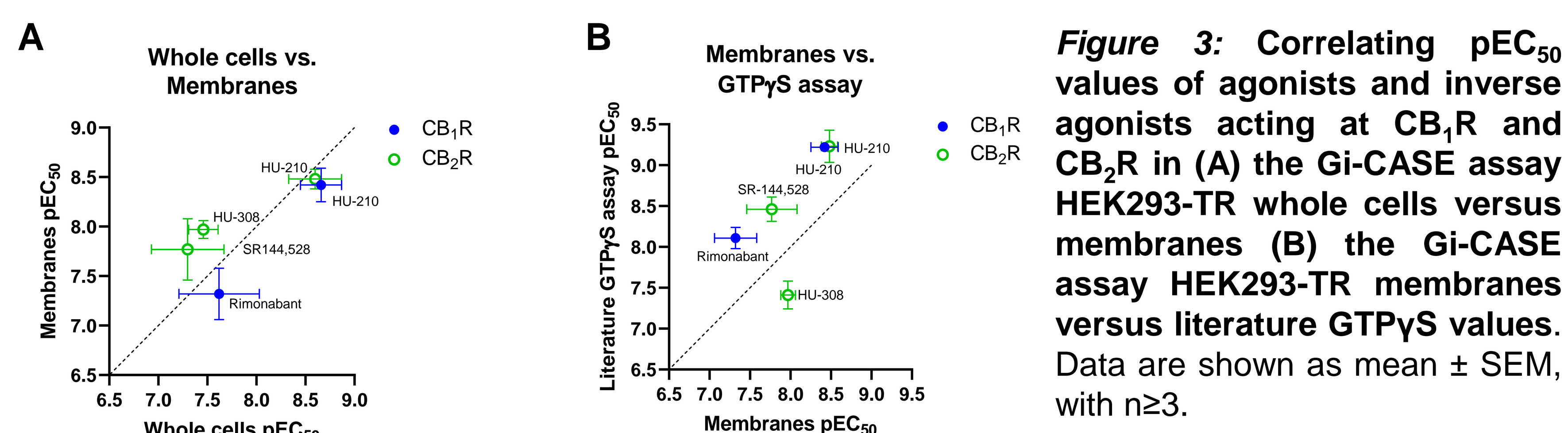


Figure 3: **Correlating pEC_{50} values of agonists and inverse agonists acting at CB_1R and CB_2R in (A) the Gi-CASE assay HEK293-TR whole cells versus membranes (B) the Gi-CASE assay HEK293-TR membranes versus literature GTPγS values.** Data are shown as mean ± SEM, with n≥3.

Conclusions

KEY FINDINGS:

- ✓ Agonist stimulation decreased, while inverse agonists increased the BRET signal in membranes with EC_{50} and efficacy evaluations in-line with previous reports[2-4].
- ✓ Comparable parameters and EC_{50} values using whole cells versus membranes at 37°C.

FUTURE DIRECTIONS:

- ✓ The 384-well membrane-based microscale assay as described is applicable for screening novel agonists and inverse agonists of other $G\alpha_i$-coupled GPCR and
- ✓ orphan GPCRs as part of wider drug discovery efforts.

Acknowledgements

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