Development of Radioligand Membrane Binding Assays for Compound Screening

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Introduction
G-Protein coupled receptors (GPCRs) are involved in many diseases and act as the targets for many therapeutic small molecules.  To date radioligand binding assays remain the gold standard for determining GPCR binding affinities.  However, new methods are needed to improve the efficiency of high throughput screening.  These assays are available in many formats including cell-based and membrane-based methods.  The latter format is advantageous as it is more amenable to high-throughput screening.  Membrane-based assays offer a number of advantages over cell-based assays, including reduced assay times, lower costs and higher-throughput.  However, membrane-based assays have also been shown to be more sensitive to batch-to-batch variability, which can lead to increased assay failure rates.  In this study, we report the development of high-throughput membrane binding assays for mGluR2 and mGluR3 receptors, which can be used in parallel to functional assays for metabotropic GPCRs.  mGluR2 and mGluR3 receptors are involved in a variety of diseases including obesity, inflammatory diseases, pain, mood disorders, and more.  These receptors are of particular interest due to their role in the development of novel drugs for the treatment of these diseases.

Materials and Methods
Stable Cells: Metabolite Glutamate Receptors - mGluR2 HEK293T (Multispan Inc., Cat # MG293T) and mGluR3 HEK293T (Multispan Inc., Cat # MG393T).
Membrane preparation: Membranes (Multispan Inc., Cat # MHG1189, MCG1190) were prepared from cell lines expressing full-length mGluR2 and mGluR3 receptors.

Compounds: Compounds were solubilized in the following solvents: DMSO, EtOH

Radioligand Binding Assay: Cellular solubilized (50 µg/mL cytosol) were incubated with 35S radioligand.  Control agonist shows expected EC50 values for mGluR2 receptor.  B. Dose-dependent GTPγS assay for mGluR2 receptor expressed in HEK293T cells using SPA beads (Perkin Elmer, Cat # RPNQ0001).  Control agonist shows expected EC50 values for mGluR2 receptor.

Figure 3.

Conclusions
• Our mGluR2 and mGluR3 assay development results show comparable pharmacology to data reported in the literature for both functional and binding assays including GTPγS binding, cAMP, Ca++ and CaM binding assays.
• Compounds can be screened and ranked by both radioligand binding and radioligand functional assays for signaling activity in the cells or membranes in parallel to radioactive binding assays that measure physical interactions of receptors binding sites.
• These validated functional and binding assays are being used to identify compounds acting as positive allosteric modulators (PAMs), negative allosteric inhibitors (NAI), agonists, and antagonists of mGluR2 and mGluR3.