Profiling of Metabotropic Glutamate Receptors using radioligand binding & functional assays

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Summary

In spite of the potential of metabotropic receptors for therapeutic applications, advances in drug discovery have been limited by availability of robust assays for primary screening, lead identification and optimization programs and compounds profiling. Here we present the pharmacological validation of functional and radioligand binding assays for all human metabotropic glutamate receptors expressed in recombinant clonal cell lines using inducible or stable expression systems. These assays were used to characterize orthosteric agonists and antagonists, allosteric agonists, neutral allosteric modulators, positive allosteric modulators (PAM) and negative allosteric modulators (NAM). Different read outs are available and allow to select the most relevant assay for a specific drug discovery or profiling programs. When possible, assays based on natural coupling for each receptor were developed and compared to generic assays based on calcium flux readouts. Indeed use of endogenous agonist and assay matching the natural coupling of a metabotropic glutamate receptor seems to be more predictive. (Methods and further information available upon request).

Results

Different assays were developed for each metabotropic receptor pharmacological characterization in 96 and 384 well formats. Most of the receptors were expressed in inducible cell lines. $mGlu_5$ cell line was used as a model to validate different assays and optimized conditions for allosteric modulators screening. Radioligand binding assay was developed with both orthosteric or allosteric radioligands (Fig. 1). K_i obtained with different competitors including NAM (MPEP and Fenobam) and PAM (CDPPB) were similar to those described previously (Malherbe *et al.*, 2003; Porter *et al.*, 2005, Chen *et al.*, 2007). Regarding $mGlu_5$ functional assays, an aequorin assay was optimized for NAM testing (Fig.2) with data comparable to the radioligand assay. Interestingly, this aequorin assay showed that most PAM compounds also behaved as ago-allosteric compounds, able to activate $mGlu_5$ in absence of orthosteric agonist. This was a limitation for this assay because preincubation of cells with these PAM triggered an aequorin depletion when reference agonist was injected at EC_{20} (Fig. 3). IPOne™ HTRF was also validated (Fig.4) and allowed to detect inverse agonist activities for NAM like MPEP or Fenobam (Fig. 5) that cannot be detected using a calcium flux assay. In parallel, different assays were validated for each mGlu receptor (Fig. 5-12) and resulted in robust assays suitable for both primary functional screening and hit to lead follow up. Using Aequorin assay, testing of NAM was very reproducible and allowed to obtain potencies similar to those described in the literature. Again most compounds used in PAM testing did show ago-allosteric activity (activity in absence of added orthosteric ligand) for several mGlu receptors. cAMP HTRF™ assay gave excellent results with $mGlu_2$ (Fig. 6) and $mGlu_7$ (Fig; 11) for characterization of PAM and NAM whereas SPA- GTPγS was used for $mGlu_4$ (Fig. 9), $mGlu_6$ (Fig. 10) and $mGlu_8$ (Fig. 12). Such assay targeting natural coupling of these metabotropic receptors did also show a different profile for some compounds (Fig. 6 and 7) and most of the time higher potency for the test compounds.

Fig. 1: $mGlu_5$ Radioligand binding assay

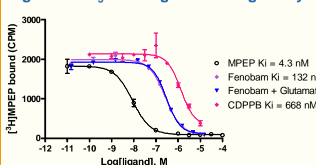


Fig. 2: $mGlu_5$ Aequorin assay for NAM

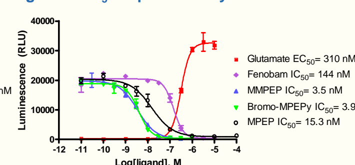


Fig. 3: $mGlu_5$ Aequorin assay for PAM

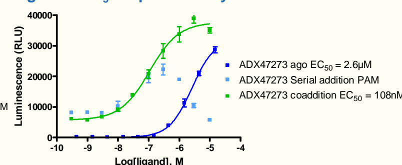


Fig. 4: $mGlu_5$: IPOne™ HTRF assay

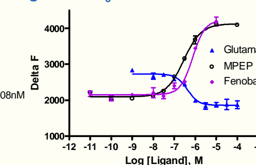


Fig. 5: $mGlu_1$: Aequorin functional assay

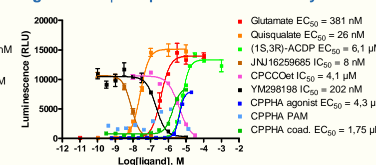


Fig. 6: $mGlu_2$: cAMP HTRF™ functional assay

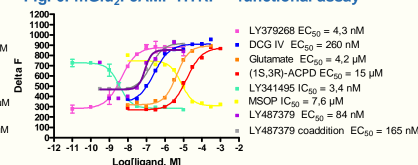


Fig. 7: $mGlu_2$: Aequorin functional assay

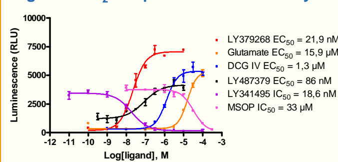


Fig. 8: $mGlu_3$: Aequorin functional assay

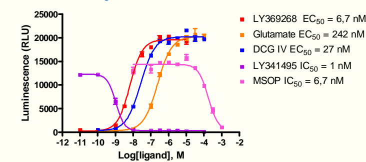


Fig. 9: $mGlu_4$: GTPγS functional assay

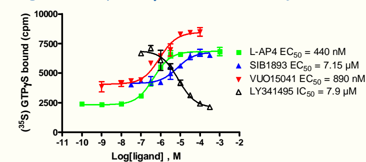


Fig. 10: $mGlu_6$: GTPγS functional assay

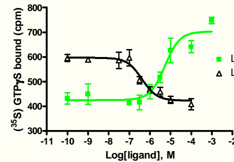


Fig. 11: $mGlu_7$: cAMP HTRF™ functional assay

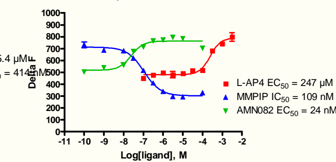
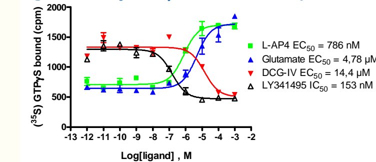


Fig. 12: $mGlu_8$: GTPγS functional assay



Conclusions

- The stable expression of different metabotropic glutamate receptors in inducible or stable clonal cell lines allowed development of functional and radioligand binding assays suitable for HTS as well as for molecular pharmacology.
- Aequorin assay is well adapted to functional HTS allowing to identify both PAM and NAM in the same screening. Further use of neutral allosteric modulator like 5-MPEP helped for binding site characterization.
- Allosteric agonism of PAM for $mGlu_5$ were observed in all conditions tested and were not competed by orthosteric antagonists.
- GTPγS, cAMP or IPOne HTRF™ functional assays are suitable for characterization of PAM and NAM as well as inverse agonist.
- Binding assays completed the panel of tests to discriminate the putative binding sites of allosteric modulators.
- $mGlu_1$, $mGlu_2$, $mGlu_3$, $mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$ are available for testing and profiling and are currently used for further radioligand and functional assays.
- $mGlu_6$ natural coupling assay is under current development.

References

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