Development of an ex vivo receptor occupancy assay for the Class C GPCR $mGlu_5$

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Introduction: Receptor occupancy (RO) assays play an important role in drug development, as they confirm target engagement in the tissue of interest. We have recently developed HTL0014242, a negative allosteric modulator of $mGlu_5$, and demonstrated RO in rat hippocampus using autoradiography[1]. However, such methodology requires specialised equipment and thus we have established an ex vivo RO assay using a tissue homogenate filtration-based format. Here, we report assay development and validation using [^{3}H]M-MPEP and HTL0014242 in murine brain tissue.

Method: HTL0014242 was synthesized as established in[1]. Forebrain was dissected from six adult male CD-1 mice. Membranes were prepared from three mice as described previously[2] and protein linearity was performed to assess specific binding of [^{3}H]M-MPEP. Using forebrain membranes (30 µg/well), saturation and kinetic [^{3}H]M-MPEP binding experiments were performed. Competition binding assays were used to determine the $pK_i$of HTL0014242. All were carried out as described in[1]. In RO experiments tissue needs to be rapidly processed to avoid dosed compound dissociation, therefore tissue was prepared by crude homogenisation (Polytron Homogeniser; 7,000 rpm; 20 s) immediately prior to use in protein linearity assays. Using chosen conditions (1.4 mg homogenate/well), the preparation was employed to perform both steady-state saturation and kinetic association assays at 4°C to select conditions resulting in full $mGlu_5$ RO.

Results: Increasing protein concentration correlated with increased specific binding of [^{3}H]M-MPEP and 30 µg forebrain membranes/well resulted in high specific binding without radioligand depletion (Figure 1). Saturation and kinetic binding assays allowed K_d and association/dissociation rates to be calculated, and competition binding assays established that HTL0014242 displays high affinity for the murine $mGlu_5$ (Table 1). By using high [^{3}H]M-MPEP concentrations ($20xK_d$) but short incubation times, it was demonstrated that all $mGlu_5$ receptors in tissue homogenates were occupied by 10 minutes, as calculated by comparing specific [^{3}H]M-MPEP binding to the B_{max} determined in steady-state saturation binding studies (Figure 2).

Table 1. $mGlu_5$ ligand binding in murine forebrain. K_d of [^{3}H]M-MPEP was determined by saturation binding (SB) or kinetic binding (KB) assays. Data shown are mean ± SD and as aim was to establish methodology, an n of 2 was performed to limit animal use.

[^{3}H]M-MPEP				**HTL0014242**
K_d by SB (nM)	K_{on} (M^{-1} min^{-1})	K_{off} (min^{-1})	K_d by KB (nM)	pK_i
3.91 ± 0.16	25,777,542 ± 2,477,593	0.0471 ± 0.0262	1.79 ± 0.84	8.88 ± 0.24

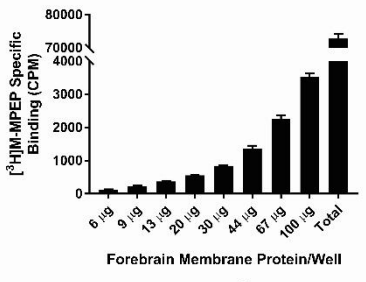

Figure 1. Binding of [^{3}H]M-MPEP to membranes purified from murine forebrain

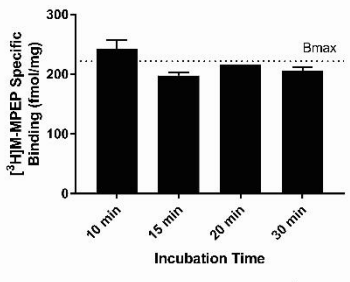

Figure 2. Association of $20xK_d$ [^{3}H]M-MPEP in crude homogenates of murine forebrain

Conclusion: Expression of $mGlu_5$ was confirmed in murine forebrain and binding of [^{3}H]M-MPEP and HTL0014242 was characterised. In tissue from dosed animals, performing a 10-minute association of $20xK_d$ [^{3}H]M-MPEP at 4°C would allow the radioligand to occupy all unliganded $mGlu_5$ but should prevent

dissociation of HTL0014242. This data will serve as the groundwork for the final HTL0014242 dosing study to confirm $mGlu_5$ occupancy in the murine brain.

References:

1. Christopher JA *et al.* (2015). *J Med Chem.* **58**: 6653-64.
2. Bradley SJ *et al.* (2011) *Mol Pharmacol.* **79**: 874-85.