Investigating pharmacological interactions between $mGlu_1$ and $mGlu_5$ using a constitutive heterodimer construct

Following the discovery that metabotropic glutamate receptor 5 ($mGlu_5$) exists as a covalently bonded homodimer(1), in house data lead us to believe that $mGlu_5$ may form a heterodimer with $mGlu_1$. Using $mGlu_1$ and $mGlu_5$ constructs with C terminals modified with $GABA_B$ tails, we hoped to understand whether these receptors would interact pharmacologically as a dimer.

The pharmacological properties of mGlu5 specific positive allosteric modulators, LSN-2814617 and CDPPB, were determined for $mGlu_1$, $mGlu_5$, $mGlu_5$ constitutive homodimer ($mGlu_{5.5}$), and $mGlu_{1/5}$ constitutive heterodimer ($mGlu_{1.5}$) cell lines using a Fluorometric Imaging Plate Reader (FLIPR). Briefly, stable cell lines expressing the above constructs were plated onto poly-D-lysine coated 96 well plates at 50,000 cells/well and incubated for 24 hours at 37 ºC with 95 % air and 5 % CO_2. The cells were then incubated in HBSS supplemented with 10 mM HEPES and 10 µM Fluo-4 AM and incubated for 1 hour at room temperature in the dark. A range of concentrations of group I selective agonist (S)-3,5-DHPG (150 nM to 30 µM) were assayed in combination with a range of concentrations of either LSN-2814617 (10 nM to 10 µM) or CDPPB (3 nM to 3 µM). Intracellular calcium levels were monitored before and after the addition of compounds, with data collection ranging from 1 image every second to 1 image every 3 seconds. Further assays were performed as above using an EC_{90} concentration of (S)-3,5-DHPG (10 µM) to assess a range of concentrations of the highly selective mGlu5 negative allosteric modulator MPEP (3 pM to 10 µM). Data was analysed using the operational ternary complex model with Graphpad Prism v6.0.

Our data showed that the $mGlu_5$ and $mGlu_{5.5}$ constructs had insignificant differences in their pharmacological properties with respect to LSN-2814617 ($\alpha\beta$ = 3.95 ± 0.32 for $mGlu_5$, 3.99 ± 0.39 for $mGlu_{5.5}$; $p > 0.05$ two-tailed Student's t-test), however, $mGlu_{1.5}$ was significantly different to the wild-type ($\alpha\beta$ = 1.68 ± 0.37; $p = 0.009$ two-tailed Student's t-test versus $mGlu_5$). The properties of CDPPB were not significantly different between $mGlu_5$ and $mGlu_{5.5}$ ($\alpha\beta$ = 8.84 ± 0.38 for $mGlu_5$, 13.9 ± 1.13 for $mGlu_{5.5}$; $p > 0.05$ two-tailed Student's t-test), but again $mGlu_{1.5}$ was significantly different to the wild-type ($\alpha\beta$ = 3.45 ± 1.26; $p = 0.005$ two-tailed Student's t-test versus $mGlu_5$). Similarly, there was no significant difference in the ability of MPEP to inhibit at (S)-3,5-DHPG response at the $mGlu_5$ and $mGlu_{5.5}$ constructs (EC_{50} = 8.89 ± 0.05 for $mGlu_5$, 8.59 ± 0.02 for $mGlu_{5.5}$; $p > 0.05$ two-tailed Student's t-test). There was a significant difference in the ability of MPEP to inhibit an (S)-3,5-DHPG response at the $mGlu_{1.5}$ compared to the wild-type and $mGlu_{5.5}$ (EC_{50} = 7.86 ± 0.09; $p = <0.005$). MPEP only inhibited 50% of the (S)-3,5-DHPG response in the $mGlu_{1.5}$ assays, but acted as a full inhibitor at the wild-type and $mGlu_{5.5}$ homodimer.

Our experiments have shown that there are significant differences between the pharmacological profile of allosteric modulators acting at the $mGlu_5$ homodimer compared to a $mGlu_{1.5}$ heterodimer. The $mGlu_1$ part of the heterodimer appears to reduce the activity of allosteric modulators at the $mGlu_5$ receptor. This is unlikely to be due to the modification of the C-terminal tail, as this effect was not present in the $mGlu_{5.5}$ homodimer construct. Further experiments, including assessing the binding properties of these constructs could elucidate whether these effects are by binding-site inhibition or functional inhibition, and whether the dimer exists *in vivo*.

(1) Romano et al (1998). *J Biol Chem* **271(45):** 28612–28616.