Biased allosteric agonism and modulation of metabotropic glutamate receptor 5: implications for optimising preclinical neuroscience drug discovery

Emerging evidence for CNS disorders with glutamatergic dysfunction suggests the metabotropic glutamate receptor subtype 5 ($mGlu_5$) is a promising target. Current $mGlu_5$ allosteric modulators have largely been classified based solely on modulation of intracellular calcium (iCa^{2+}) responses to orthosteric agonists, resulting in a narrow classification of ligands, and potential unappreciated bias in receptor signalling. We assessed seven diverse $mGlu_5$ allosteric modulators across iCa^{2+}, inositol phosphate (IP_1) accumulation and phosphorylation of extracellular signal-regulated kinases (pERK1/2), to explore their potential for engendering biased agonism and/or modulation. Relative to the reference orthosteric agonist, *(S)*-3,5-dihydroxyphenylglycine (DHPG), all seven allosteric ligands exhibited biased agonism in HEK293A cells stably expressing $mGlu_5$, coupling more strongly to IP_1 accumulation and pERK1/2 over iCa^{2+} mobilisation. Disparities in iCa^{2+} and IP_1 suggests the presence of ion channel modulation, rather than solely Gq activation. These bias profiles were generally recapitulated at native receptors in cortical neuron cultures. Relative to DHPG, *(R)*-5-((3-fluorophenyl)ethynyl)-*N*-(3-hydroxy-3-methylbutan-2-yl)picolinamide (VU0424465) showed significantly greater potency at IP_1 accumulation (pEC_{50} = 8.70±0.13 vs 5.44±0.10, $p<0.05$ one-way ANOVA, Tukey's post-test, n=3-10), as well as pERK1/2 (pEC_{50} = 8.39±0.13 vs 6.31±0.25, $p<0.05$ one-way ANOVA, Tukey's post-test, n=3-10). Interestingly, *N*-(1,3-diphenyl-1*H*-pyrazolo-5-yl)-4-nitrobenzamide (VU29), and *N*-cyclobutyl-6-((3-fluorophenyl)ethynyl)picolin-amide (VU0360172), which showed no agonism for iCa^{2+} mobilisation in recombinant cells, showed robust agonism for both IP_1 and pERK1/2 (logR=7.41±0.11, 6.44±0.39 (IP_1) and 7.17±0.05, 7.02±0.18 (pERK1/2) respectively, n=3-10). Application of an operational model of agonism allowed quantification of biased agonism across $mGlu_5$ signalling pathways relative to DHPG (Fig. 1). Unappreciated biased agonism and modulation may contribute to unanticipated effects (both therapeutic and adverse) when translating from recombinant systems to preclinical models.

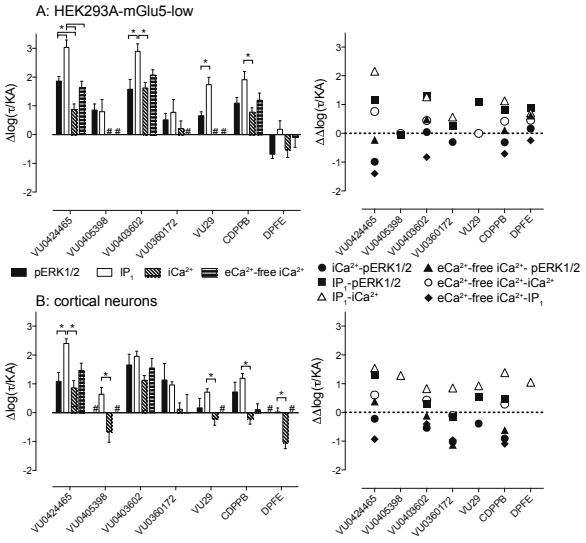

Fig 1. $mGlu_5$ allosteric ligands are biased agonists relative to DHPG in HEK293A-$mGlu_5$-low and cortical neurons.

The transduction coefficient ($\log(\tau/K_A)$) was derived as previously described (1) and normalized to that of DHPG ($\Delta\log(\tau/K_A)$). To calculate the degree of bias evident for different

ligands between different pathways, $\Delta\log(\tau/K_A)$ were subtracted from one another ($\Delta\Delta\log(\tau/K_A)$) to determine Log bias factors. Data for $\Delta\log(\tau/K_A)$ represent the mean ± SEM. Log bias estimates are mean only. * denotes significantly different comparisons, $p<0.05$, one-way ANOVA with Tukey's post-test. n=3-10 experiments performed in duplicate.

(1) Kenakin T et al (2012). *ACS Chem Neurosci* **3**(3): 193–203.