Kinetic investigations into G protein activation and biased agonism at the dopamine D2 receptor

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Background-The dopamine D2 receptor (D2R) is a therapeutic target for the treatment of several neuropsychiatric disorders. The D2R can couple to all members of the heterotrimeric G α -i/o/z protein family but the physiological importance of coupling to distinct G protein subtypes remains unclear. It has been demonstrated that slow-dissociating agonists display time-dependent biased agonism between endpoints at different levels in the D2R signalling cascade(1). The aim of this study was to explore biased agonism at the level of coupling to different G proteins and how this might be influenced by agonist binding kinetics.

Methods-D2R expressing FLPIN HEK293 cells were stimulated by a panel of agonists with varied intrinsic efficacy and binding kinetics. G α -i/o/z activation was measured in live cells by BRET-based biosensors which detect dissociation of the G β -gamma subunit upon activation of the G protein(2). Biased agonism was quantified by analysing concentration response curves of agonists at different timepoints using an operational model of agonism(3).

Results-The D2R showed a coupling preference for G α A/B and G α z over G α i1/2/3 as indicated by the relative potency of high efficacy agonists. Time-dependent biased agonism was observed for some agonists relative to ropinirole primarily between G α i1/2/3 and G α z activation. Interestingly, all agonists, independent of their receptor binding kinetics, displayed a large increase in potency over time for G α z activation while the potency of agonists at all other G proteins either did not change, or changed in manner consistent with their dissociation rate from the D2R. We provide evidence that the large increase in potency over time at G α z is dependent on a slow rate of GTP hydrolysis.

Conclusion-We observed no correlation between association or dissociation rate of selected agonists and their efficacy to activate different G proteins. Subtle but statistically significant bias was detected between G α z and G α i1/2/3 activation for some agonists, and this pattern of bias changes over time. Finally, we have provided evidence that rates of G protein activation/deactivation in combination with ligand binding kinetics may underlie some observations of biased agonism.

1.Klein-Herenbrink, C. et al. (2016)*Nat. Commun.* 7,10842

2.Hollins, B. et al. (2009)*Cell. Signal.* 21,1015-1021

3.Kenakin, T. et al. (2012)*ACS Chem. Neurosci.* 3,193-203