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The use of fluorescence correlation spectroscopy to investigate binding site stoichiometry for monomeric and dimeric fluorescent ligands at the neuropeptide Y Y1 receptor

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Introduction: Dimeric peptide antagonists of the Neuropeptide Y Y1 receptor (Y1R), such as GR231118 are characterised by non-surmountable properties compared to parent monomers, such as BVD15[1,4]. Recently we have synthesised mono-labelled Cy5 derivatives of both monomeric (Cy5_mono; I-K(Cy5)-P-R-Y-R-L-R-Y-$CONH_2$)[4] and dimeric peptides (Cy5_dimer; ((Cy5)-I-E-P-Dap-Y-R-L-R-Y-$CONH_2$)$_2$ single N-terminal tag). Here we use these ligands, in combination with fluorescence correlation spectroscopy (FCS), to explore how they interact with Y1R complexes in HEK293 cells, and whether binding stoichiometry differs between monomers and dimers.

Methods: FCS solution measurements were taken at 1-100nM peptide in HBSS/0.1%BSA at 21°C. Receptor binding measurements were taken at 21°C on the upper membrane of HEK293 cells expressing the SNAP-tagged Y1R, incubated with 1-30nM peptide, or 0.2µM SNAP AF647 as control, in HBSS/0.1%BSA (30mins; 37°C; 0%CO_2) as described[3]. Fluorescent fluctuations were analysed by autocorrelation analysis and photon counting histogram to obtain diffusion co-efficients (D) and particle brightness (MB). Data are given as mean±SEM, where MB is represented as a percentage of reference, Cy5-NHS-ester. Analysis was performed using one-way ANOVA followed by Tukey's test.

Results: 10nM peptide solution measurements gave D values of 124.5±7.5µm^2/s (n=24) and 111.1±5.6µm^2/s (n=11) for Cy5_mono and Cy5_dimer; non-aggregating peptide behaviour was confirmed by correspondence between FCS calculated and input concentrations and no change in MB. Cell based FCS measurements, in the presence of 10nM peptide, demonstrated similar D values for Y1R bound Cy5_mono (0.6±0.1µm^2/s; n=26 cells: 4 experiments) and Cy5_dimer (0.7±0.1µm^2/s; n=42:4), equivalent to SNAPsurface-AF647 labelled receptor controls (0.6±0.1µm^2/s; n=32:4). There were no significant changes in MB for bound monomer and dimer ligands as free ligand concentration was varied to change receptor occupancy. Y1R bound Cy5_mono MB was 45.0±6.7% at 1nM (n=29:4) and 39.1±5.1% at 30nM (n=20:4). However, at 10nM, the brightness of Y1R bound Cy5_dimer was significantly greater (59.9±6.3%; n=38:4) than for Cy5_mono (38.2±4.3%; n=24:4; $P<0.05$).

Conclusions: MB measurements support single-site occupancy of Y1R complexes by Cy5_mono, as expected for the predominantly monomeric Y1R reported in HEK293 cells[2]. However Cy5_dimer binding may promote clustering of Y1R complexes.

References:

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