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Use of a transmembrane domain I minigene to disrupt dopamine D3 receptor quaternary structure

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Introduction: The human dopamine D_3 receptor (hD_3R) is a class A G protein-coupled receptor. We reported that hD_3R forms dimers and oligomers, described likely quaternary arrangement of this receptor (1) and its regulation by the binding of certain antagonist ligands (2). In this study, we explore whether the oligomerization of hD_3R has functional significance.

Method: We adopted a competitive inhibition strategy in which the receptor is co-expressed alongside a minigene encoding a single transmembrane domain (TM) from the same receptor (TMI in this study). Interaction of hD_3R with the TM minigene may limit or disrupt receptor-receptor interactions if the sequence of the minigene provides a receptor-receptor interaction interface.

Results: The hD_3R and the TMI minigene were modified at the N-terminus with SNAP-tag and HALO-tag, respectively and expressed transiently in Neuro2A cells. Labelling of cells with cell impermeant dyes SNAP-surface-549 and HALO-AlexaFluor-488 allowed the detection of both constructs at the cell surface. Subsequently, Homogenous Time Resolved-FRET (htrFRET) using Tag-lite technology was employed. Labelling of cells with a combination of HALO-Lumi4Tb and increasing concentrations of SNAP-Red followed by htrFRET analysis showed the presence of hD_3R-TMI minigene complexes at the cell surface. Neuro2A cells were then transiently transfected with varying amounts of VSV-SNAP-hD_3R, with or without a HA-HALO-TMI minigene construct and labelled with SNAP-Lumi4Tb and SNAP-Red. Fluorescence emission at 620 nm (reflecting cell surface expression of the receptor) and 665nm (reflecting protein-protein interaction) were then measured concurrently and correlated. This resulted in a linear relationship. However, the slope of the linear regression obtained in the presence of the TMI minigene was reduced substantially compared to that for hD_3R expressed alone (1.00 ± 0.06 and $0.67\pm0.03^{**}$, mean ± S.E, n=4,**, $p<0.001$; Student's *t* test), indicative of reduced proximity between hD_3R monomers and hence alteration of receptor oligomer structure. Spatial Intensity Distribution Analysis studies on cells able to express both hD_3R-tagged with monomeric eGFP and the TMI minigene also generated data consistent with reduced dimeric/oligomeric forms of hD_3R in the presence of the TMI minigene. Regions of interest containing predominantly hD_3R dimers/oligomers were $38.4\pm5.9\%$ and $11.1\pm3.2\%^{**}$ (mean ± S.E, n=4, **, $p<0.001$; Student's *t* test) in the absence and presence of TMI minigene, respectively.

Conclusions: These data are compatible with the minigene disrupting hD_3R oligomer organisation and provide further evidence that TMI-TMI interactions contribute to the generation of hD_3R dimer/oligomers.

References:

1) Marsango S. et al. (2015) *J.Biol.Chem*. **290**:15146-62;
2) Marsango S. et al. (2017) *Sci.Rep*. **7**:2134.