Defining the quaternary organization of the human dopamine D_3 receptor and its regulation by receptor antagonist ligands

The dopamine D_3 receptor is a class A rhodopsin-like G protein-coupled receptor (GPCR) primarily coupled to inhibitory G-proteins. It has been reported that the human D_3 receptor (hD_3R) can form dimers and oligomers and we have previously defined the residues from within each of transmembrane domains (TMs) I, II, IV, V, VI and VII, as well as the intracellular helix VIII involved in the formation of the interfaces that allow hD_3R monomers to interact and form two distinct dimeric and a 'rhombic' tetramer species (1).The relative abundance of different hD_3R species at the cell surface of mammalian cells was here investigated to define the steady-state proportion of each form and to determine whether this can be regulated by cellular challenges. To do so we adopted a method that combines confocal microscopy imaging and spatial intensity distribution analysis (SpIDA) (2). This method is based on fitting fluorescence intensity histograms obtained from regions of interest (RoI, selected within single images) to obtain density maps of fluorescent molecules along with their quantal brightness (QB), which indicates their oligomeric state. We generated Flp-In™ T-REx™ 293 cells stably and constitutively expressing hD_3R modified at the C-terminus by the incorporation of monomeric EGFP (hD_3-mEGFP) and selected three different clones exhibiting different hD_3R expression levels (Table 1). SpIDA on these clones showed a QB (expressed as monomeric equivalent units, MEU (3)) indicating that hD_3R-mEGFP was present as a mixture of monomers and dimer/oligomers at the cell surface and that the size of the complexes and the percentage of dimer/oligomers increased with receptor density (Table 1).To test the hypothesis of a dependency between the proportion of dimer/oligomers and the receptor density we treated clone 8 cells with 20 mM sodium butyrate with the aim of enhancing hD_3-mEGFP receptor expression level. SpIDA revealed that the number of receptor per μm^{-2} increased 1.5 times after sodium butyrate treatment and concurrently so did the complexity of the quaternary structure of hD_3R and the percentage of dimer/oligomers at the cell surface (Table 1). Subsequently we tested whether dopamine receptor ligands could influence the quaternary structure of hD_3R. SpIDA showed that long-term treatment with the selective hD_3R antagonists etioclopride (10μM) or spiperone (10μM) did not significantly alter expression levels of hD_3R but although etioclopride treatment did not alter the quaternary structure of hD_3R spiperone treatment did, favouring the monomeric state (Table1).

Table 1 Summary of SpIDA results

hD3-mEGFP clone N	Treatment	N	Rec.μm⁻²	QB (MEU)		%Dimers Oligomers
2	Not treated	n=3x44 RoI	57.10±1.30	1.66±0.07	*	73.48%
6	Not treated	n=3x44 RoI	37.10±1.12	1.36±0.05	ns	49.98%
8	Not treated	n=3x44 RoI	45.71±1.26	1.28±0.03	**	53.78%
8	20 mM Sodium Butyrate	n=2x44 RoI	70.97±2.72	1.65±0.19	***	77.27%
8	10μM Spiperone	n=2x44 RoI	47.51±1.94	1.11±0.10	**	27.27%
8	10μM Etioclopride	n=1x44 RoI	45.57±1.97	1.35±xx	ns	55.81%

ns, not statistically significant; *,p>0.05; **,p>0.001 and ***p>0.0001 vs the indicated control or clone 8 not treated.

Our data provides further evidence of the co-existence of different hD_3R species at the cell surface of mammalian cells. The equilibrium among these distinct forms can vary with the receptor density and can be modulated by receptor ligand binding. Ongoing studies are aimed to selectively disrupt the quaternary structure of the hD_3R to determine the functional role of the different species in the signalling of this GPCR.

(1) Marsango S *et al*. (2015). *J Biol Chem* **290**: 14785–14796.
(2) Godin AG *et al*. (2011), *Proc. Natl. Acad. Sci. U S A* **108**: 7010–7015.
(3) Pediani JD *et al*. (2016) *J Biol Chem* (in press).