Molecular determinants for agonist and inverse agonist ligand binding at the serotonin 5-HT_{2C} receptor: Homology modeling, ligand docking, molecular dynamics, and experimental studies.

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Serotonin 5-HT_{2C} G protein-coupled receptor (GPCR) agonist ligands demonstrate antiobesity effects and antipsychotic activity, but, inverse agonist/antagonist ligands cause weight-gain and other untoward metabolic side effects, observed with many currently available antipsychotic drugs. Importantly, 5-HT_{2C} agonist ligands must be specific because activation of 5-HT_{2A} and 5-HT_{2B} subtypes leads to psychotomimetic effects and cardiotoxicity, respectively—the only approved 5-HT_{2C} agonist drug (for obesity), lorcaserin, carries this liability due to its inability to fully distinguish between the phylogenetically closely-related 5-HT2 subtypes that have about 75% identical sequence homology in the transmembrane domains. In the absence of crystal structures for most GPCRs, including, 5-HT_{2C}, this work involved building a human 5-HT_{2C} homology model using the human β_2-adrenergic receptor (β2AR) crystal structure as template. The 5-HT_{2C} model was equilibrated in a POPC membrane model (Cordova-Sintjago et al., 2012) and used for docking and molecular dynamics (MD) studies to investigate the ligand–receptor binding interactions that lead to agonist vs. inverse agonist activity at the 5-HT_{2C} GPCR. Ligands used in the study included the multifunctional 5-HT_2 ligand, (–)-*trans*-(2*S*,4*R*)-4-phenyl-2-*N*,*N*-dimethylamino-1,2,3,4-tetrahydronaphth-alene (PAT), a 5-HT_{2C} agonist with 5-$HT_{2A\text{-}2B}$ antagonist/inverse agonist activity (Booth et al., 2009), and its corresponding 4-(3'-Br-phenyl) and 4-(3'-CF_3-phenyl) analogs, a 5-HT2C agonist and inverse agonist, respectively. Computational docking and MD results were tested for validity by experimental studies that measured ligand affinity and function regarding phospholipase C (PLC) signaling at wild-type (WT) vs. point-mutated human recombinant 5-HT_{2C} receptors expressed in clonal cells. In the course of the experiments, the crystal structure of the 5-HT_{2B} receptor was reported, which was found to have a very close structural correlation (RMS= 2.3 Å) with the 5-HT_{2C} model built by homology to the β2AR structure, corroborating the validity of molecular model.

Initial docking and MD outcomes suggested distinct interactions of 3'-Br-PAT and 3'-CF_3-PAT at the WT 5-HT_{2C} receptor model involving residues S3.36, S5.43, F5.47, W6.48, N6.55, N6.55, C7.45, C7.45, S7.46A, and M6.47. Accordingly, point-mutations were made to the human 5-HT_{2C} cDNA such that alanine was substituted for each of these amino acids (as well as lysine and serine in the case of N6.55 and C7.45, respectively), in turn, and affinity and function of each of the PAT analogs was assessed at the WT receptor and compared to results obtained with the point-mutated receptors.

Key results of the experimental studies validated the computational studies and indicated key residues governing ligand binding interactions that lead to agonist vs. inverse agonist function at the 5-HT_{2C} receptor. For example, at the N6.55A point-mutated 5-HT_{2C} receptor, 3'-Br-PAT was an inverse agonist and 3'-CF_3-PAT was an agonist ligand, a reversal of their function observed at WT receptors. These and other results of this study will be detailed toward the goal of drug discovery targeting the therapeutically important 5-HT_2 GPCRs. Support: *NIH RO1 DA023928, DA030989, MH081193*. Keywords: GPCR, homology modeling, docking, molecular dynamics, serotonin 5HT_{2C} GPCR. References: Booth et al. *Eur. J. Pharmacol*. 615:1, 2009; Cordova-Sinjago et al., *Int. J Quant. Chem*. 112:140, 2012