Aromatic interactions effect on binding and function of agonist at Serotonin 5-HT_{2C} receptor. Homology Modeling, Docking, Molecular Dynamics, and Experimental study.

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Activation of the human serotonin (5-hydroxytryptamine, 5-HT) 5-HT_{2C} G protein-coupled receptor (GPCR) is therapeutic for neuropsychiatric disorders and obesity, however, activation of 5-HT_{2A} and 5-HT_{2B} GPCRs is associated with adverse psychiatric and cardiac effects, respectively. Ligands specific for activation of 5-HT_{2C} receptors have been difficult to achieve because the 5-HT_2 GPCR family shares ~75% transmembrane (TM) sequence identity. For the human 5-HT_{2A} receptor, molecular modeling and mutagenesis results indicate conserved TM helix 6 aromatic residues W6.48, F6.51, and F6.52 are important for ligand binding and agonist vs. inverse agonist/antagonist functional activity (Kroeze et al., 2002), however, corresponding results for the 5-HT_{2C} receptor have not been reported. Accordingly, novel GPCR homology molecular modeling studies, molecular dynamics (MD) simulations, and mutagenesis experiments were undertaken here to characterize molecular determinants for 5-HT2C specific activation by novel (vs. known) ligands. Affinity and function (phospholipase C [PLC] signaling) of 5-HT in comparison to the novel 5-HT_{2C} agonist/5-$HT_{2A/2B}$ inverse agonist (–)-*trans*-(2*S*,4*R*)-4-phenyl-2-*N*,*N*-dimethylamino-1,2,3,4-tetrahydronaphthalene (PAT) (Booth et al., 2009), and several PAT analogs (Vincek & Booth, 2009), were assessed at the wild type (WT) and W6.48A, F6.51A, and F6.52A point-mutated recombinant human 5-HT_{2C} receptors in HEK cells. Homology model development for WT and point-mutated 5-HT_{2C} receptors, ligand docking, and MD simulations in a lipid POPC membrane (Cordova-Sintjago et al., 2012) were undertaken to delineate ligand–receptor molecular interactions at the 5-HT (orthosteric) binding pocket. At the WT 5-HT_{2C} receptor model, docking results indicate interactions in the binding pocket are different for PAT analogs compared to 5-HT. There were possible π-π stacking interactions between the PATs C(4) phenyl moiety and the 5-HT_{2C} W6.48 indole moiety. The PATs aromatic tetrahydronaphthalene moiety docked close and nearly parallel to the 5-HT_{2C} F6.51 aromatic ring, suggesting π-π stacking interactions. No direct interactions were observed between the PAT analogs and the 5-HT_{2C} F6.52 residue. At the W6.48A and F6.51A 5-HT_{2C} receptor models, PATs had no relevant interactions (ditto for the F6.52A model). For 5-HT, two distinct poses docked at the WT 5-HT_{2C} model and neither pose indicated π-π stacking interactions between 5-HT and W6.48, however, in both poses, the 5-HT indole moiety interacted with F6.51 and F6.52. Experimental studies using the point-mutated 5-HT_{2C} receptors indicated W6.48 is essential for binding to PAT analogs with a C(4) phenyl moiety but not with a C(4) cyclohexyl moiety. Lesser affinity differences were noted at the F6.51A 5-HT_{2C} receptor and no differences for the F6.52A 5-HT_{2C} receptor, in comparison to WT receptors. The W6.48 residue and to a lesser extent the F6.51 and F6.52 residues were required for PAT-mediated activation of 5-HT_{2C} PLC signaling. Interestingly, at the F6.51A, F6.52A, and W6.48A 5-HT_{2C} receptors, the C(4) cyclohexyl analog was an agonist whereas it was an inverse agonist at the WT 5-HT_{2C} receptor. Ligand docking and MD simulations showed that the C(4) cyclohexyl moiety undergoes conformational changes that apparently differentially stabilize agonist vs. inverse agonist 5-HT2C conformations, involving the F6.51, F6.52, and W6.48 residues. Results suggest ligand binding and functional interaction with W6.48, F6.51, and F6.52 residues may be different for 5-HT_{2C} receptors in comparison to 5HT_{2A} receptors, which informs 5HT_{2C}-specific ligand design for drug development purposes. Moreover, computational results help predict and explain experimental outcomes for drug discovery purposes. Support: NIH RO1 DA023928, DA030989, MH081193. Keywords: GPCR, homology modeling, docking, molecular dynamics, serotonin. Booth et al., Eur. J. Pharmacol. 615:1, 2009; Booth & Vincek, Tetrahedron Lett. 50:5107, 2009; Cordova-Sinjago et al., Int. J Quant. Chem. 112:140, 2012; Kroeze et al., Curr Top Med Chem. 2:507, 2002.