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Mapping The Ligand Binding Pocket Of The Short-Chain Fatty Acid Receptor hFFA2

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The G protein-coupled receptor FFA2 has generated great interest for its potential use as a therapeutic target in inflammatory and metabolic disease treatment. FFA2 responds to short-chain fatty acids (SCFAs), which are metabolic by-products of gut microbiome activity (1). Understanding the binding pocket of FFA2 would greatly contribute to drug discovery efforts and aid development of novel compounds to probe the function of FFA2. To date, studies into ligand binding at FFA2 have been limited to functional assays employed in combination with mutagenesis to identify residues important for receptor activation (2). Described residues of interest include R180 and R255, two arginine residues thought to anchor the SCFA carboxylate, as well as the histidine residues H242 and H140. In order to extend these studies, we have employed a tritiated FFA2 antagonist to measure ligand binding to human FFA2.

Radioligand binding assays carried out in cell membranes were employed to determine total binding of radioligand AZ136821499 to the receptor. Non-specific binding was determined in the presence of 10 μM CATPB (4). Displacement assays were used to calculate unlabelled ligand affinities in terms of K_i. Data are shown as mean ± SE with n=3 and analysis was performed using an unpaired *t* test.

Saturation binding experiments suggest that AZ136821499 binds with high affinity to human FFA2 (K_d = 7.5 ± 0.4 nM). Interestingly, experiments at FFA2 mutants with key residues replaced by alanine reveal that radioligand affinity is affected only modestly at R255A (K_d = 13.0 ± 0.5) and H242A (K_d = 13.4 ± 0.9), while H140A increased radioligand affinity (K_d = 2.8 ± 0.1). In contrast, detectable specific binding was abolished at R180A and R180A-R255A mutants. Displacement assays at respective mutants showed that both C3 and synthetic agonist compound 1 (3) lose most of their ability to bind FFA2-R255A and hFFA2-H242A, while C3 binding to FFA2-H140A could only be detected at very high concentrations. Compound 1 was also able to bind FFA2-H140A, but with

Table 1. Calculated pK_i values of endogenous and synthetic FFA2 ligands for WT and mutant receptor (*p ≤ 0.05 vs. WT, **p ≤ 0.01 vs. WT)

Receptor	C3	Compound 1 (3)	CATPB (4)	GLPG0974 (5)
WT	2.96 ± 0.11	6.91 ± 0.12	7.87 ± 0.08	7.88 ± 0.08
R255A	No binding	No binding	7.19 ± 0.20*	7.59 ± 0.09
H242A	No binding	Weak binding	7.63 ± 0.07	8.04 ± 0.04
H140A	Weak binding	5.36 ± 0.14**	7.99 ± 0.09	8.56 ± 0.10*

substantial loss of affinity. In contrast, antagonists CATPB and GLPG0974 show only minor or no reduction in affinity at FFA2-R255A and an increased affinity at FFA2-H140A in case of GLPG0974.

In summary, interaction between AZ136821499 and FFA2 seems to depend mostly on R180, making this residue potentially a key in anchoring FFA2-specific ligands. R255 and H140 seem to play an important role in SCFA and synthetic agonist binding, but their mutation to alanine barely affects interaction with antagonists, suggesting differences in agonist versus antagonist binding determinants. These findings will now be employed to build a more comprehensive FFA2 homology model, allowing us to improve existing ligands and generate new compounds resulting in development of novel pharmacological tools and potential therapeutics. (We thank Astra-Zeneca for providing the tritiated hFFA2 antagonist.)

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(4) Hudson BD *et al*. (2012). *J Biol Chem* **287:** 41195–41209.

(5) Pizzonero M *et al*. (2014). *J Med Chem* **23:** 10044-10057