Development of a novel fluorescent ligand binding assay for the long chain fatty acid receptor FFA1

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Estimation of affinity at the long chain free fatty acid (FFA) receptor FFA1 (GPR40), for example using radioligand approaches, is made problematic by moderate affinity, highly lipophilic ligands (1). Here we characterise a novel FFA1 fluorescent agonist (CellAura technologies, Nottingham), linked to a hydrophilic fluorophore (40Ag-Cy5). We show that 40Ag-Cy5 labels FFA1 receptors in HEK293 cells, and allows measurement of competing ligand affinities by imaging approaches.

Stably transfected 293TR cells expressed FLAG-tagged human FFA1 under control of a tetracycline (tet) inducible promoter. Fluo4 measurements of calcium mobilisation were as previously described (2). In binding experiments at 37^{o}C, cells on 96 well imaging plates were pretreated for 5 min with competing ligand in HEPES buffered saline (without BSA), followed by 40Ag-Cy5 for 30 min, a sufficient period to allow equilibrium binding. Following imaging on a confocal platereader (IX Ultra, MDC), granularity analysis identified 1 - 2 µm cell surface compartments, which quantified 40Ag-Cy5 binding. Concentration response and competition curves were fitted to pooled data using Graphpad Prism v5 to provide pEC_{50} or pIC_{50} values.

Calcium responses in 293TR FFA1 cells were observed to both 40Ag-Cy5, and its precursor without Cy5 modification (40Ag-congener), with respective pEC_{50} values of 5.8 ± 0.2 and 7.9 ± 0.3 (each n = 4). No effect of 40Ag-Cy5 (up to 10 µM) was observed in the absence of 1 µg / ml tet pretreatment to induce FF1 receptor expression. Similarly, 100 nM 40Ag-Cy5 labelled the plasma membrane of 293TR FFA1 cells only following tet incubation. 40Ag-Cy5 binding was displaced by a number of synthetic analogues (n = 3 – 4) including the antagonist GW1100 (pIC_{50} 6.0 ± 0.2, ref 3) the agonist GW9508 (pIC_{50} 6.8 ± 0.2, ref 3), 40Ag-congener (pIC_{50} 6.1 ± 0.2), and the thiazolidinedione troglitazone (pIC_{50} 6.1 ± 0.2). However the endogenous FFA oleic acid (up to 100 µM) did not fully displace 40Ag-Cy5 binding. By applying the Cheng-Prusoff correction for paired GW1100 competition curves using 100 nM or 1 µM 40Ag-Cy5 (GW1100 pIC50 5.61, n = 2), we obtained pKi estimates for 40-Ag Cy5 (6.2), and GW1100 (6.0 – equivalent to its functional pKb, ref 3).

Thus specific FFA1 receptor labelling by 40Ag-Cy5 enables imaging binding studies to determine competing ligand affinities. This should prove useful in investigations of novel ligands and the FFA1 receptor binding site, which have previously relied on indirect assays of functional activity (1).

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