Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol18Issue1abst008P.pdf

A chemogenetic approach to study the short chain fatty acid receptor FFA2

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Introduction: FFA2 and FFA3 are two G protein-coupled receptor subtypes activated by short chain fatty acids (SCFAs), endogenous ligands generated as a by-product of the breakdown of starches by the gut microbiota. Understanding the precise physiological roles of these receptors and their potential therapeutic utility has proven to be challenging. This reflects: 1) the co-expression of FFA2 and FFA3 in some tissues, 2) conflicting and contradictory results generated in transgenic mouse *knock-out* models and 3) that available FFA2 antagonists act only at the human and not rodent receptor orthologues. To address these challenges we have generated a transgenic *knock-in* mouse line expressing a DREADD (*Designer Receptor Exclusively Activated by a Designer Drug*) version of human FFA2 receptor (hFFA2-DREADD) such that it no longer responds to SCFAs but instead to a range of non-endogenously generated ligands, including sorbic acid (SA) [1]. The pharmacology of the modified receptor was assessed and colonic crypt cultures compared between the transgenic line and wild type mice (C57BL/6) for ligand-induced release of glucagon-like peptide-1 (GLP-1).

Methods: GLP-1 release assays were performed as previously described [2] using colonic crypts derived from C57BL/6 or hFFA2-DREADD expressing mice. Results were analysed as % of GLP-1 release, taking the response induced by 10μM 3-isobutyl-1-methylxanthine as 100%. Antagonists were incubated 15min prior to agonist treatment. Results represent the mean±SEM and derive from at least three independent experiments. * P<0.05; *** P<0.001 for significance versus vehicle data; $^{\$\$\$}$ P<0.001 for significance versus SA data, one-way ANOVA followed by Bonferroni post hoc test.

Results: 1mM SA was able to induce GLP-1 release in colonic crypts derived from hFFA2-DREADD mice (table) but not C57BL/6. This effect was similar to that obtained by treatment with a SCFA, propionate (10mM), in C57BL/6 mice (49.4±9.8**), suggesting that the mutated DREADD receptor retains the pharmacological properties of the wild type receptor in this tissue. In addition, the SA-mediated effect was inhibited by two selective human FFA2 antagonists, CATPB and GLPG0974 (table), confirming that this effect of SA is mediated exclusively by FFA2 receptor activation.

hFFA2-DREADD	Vehicle	SA	SA + CATPB	SA + GLPG0974
GLP-1 release (%)	18.3±1.5	50.7±4.3***	29.0±3.5*$^{\$\$\$}$	23.1±2.5$^{\$\$\$}$

Conclusions: These animals provide means to further assess roles of FFA2 and a model to predict efficacy of human specific antagonists of FFA2 prior to studies in humans.

References:

1. Hudson BD et al. (2012) *FASEB J.* **26**:4951-4965.
2. Bolognini D et al. (2016) *J.Biol.Chem.* **291:**18915-18931.