FFA2-Elicited Glucagon Like Peptide-1 Secretion: Roles of $G\alpha_i$ and $G\alpha_q$.

Introduction: The incretin hormone glucagon like peptide-1 (GLP-1) is secreted from the L-cells of the colon in response to short chain free fatty acids (SCFA) which are generated by the fermentation of non-digestible carbohydrates by the gut microbiota. This effect is mediated at least in part by signalling through the free fatty acid receptor 2 (FFA2) (1). FFA2 is reported to couple to both $G\alpha_i$ and $G\alpha_q$ (2). This study aims determine the signalling pathways which regulate GLP-1secretion in the L-cells of the colon.

Methods: Colons from adult C57BL6/NTac mice were digested with collagenase into cell structures resembling colonic crypts as previously described (3). After overnight incubation, the crypts were incubated for two hours in physiological saline, containing 1 mM acetate, 1 mM propionate, or 1 mM butyrate, in the presence or absence of 100 nM PTX (overnight) or 100 nM FR900359 (30 min). Active GLP-1 was assayed by ELISA in the culture supernatant and cell lysates. Amount released was expressed as a percentage of total GLP-1 content and was normalised to baseline secretion per mouse. Data are means ± SEM, statistical analyses are one-way or two-way ANOVA with *post hoc* Dunnett's or Sidak's, where appropriate.

Results: Propionate significantly increased GLP-1 secretion, while acetate and butyrate showed similar trends. Propionate-elicited secretion was unaffected by concomitant PTX incubation, but was abolished by the $G\alpha_q$ inhibitor FR900359 (see table 1). In colonic crypts from *Ffar2* -/- mice, no increases in GLP-1 secretion were observed with acetate, butyrate or propionate.

Table 1. Fold increases in GLP-1 secretion over baseline (n=3).

	***Ffar2* +/+**	***Ffar2* -/-**	**+ PTX**	**+ FR900359**
Acetate	1.47 ±0.09	1.17 ±0.19	1.97 ±0.35	-
Propionate	2.82 ±0.51	1.15 ±0.16	2.06 ±0.34	0.93 ±0.06
Butyrate	2.15 ±0.50	1.19 ±0.12	1.85 ±0.40	-

Conclusions: GLP-1 secretion in response to SCFAs appears to be downstream of FFAR2 coupling to $G\alpha_q$, whereas $G\alpha_i$ appears not to be involved. Potentially, FFA2 may be entirely responsible for GLP-1 secretion, rather than FFA3, which is believed to be expressed on the same cells. This study is the first time the new $G\alpha_q$ inhibitor FR900359 has been used to demonstrate the involvement of $G\alpha_q$ downstream of FFA2, or indeed any GPCR in the L-cell.

References

1. Tolhurst G. *et al.* (2012) *Diabetes* **61:** 364–371.
2, Brown *et al.* (2003) *JBC* **278:** 11312–11319.
3. Reimann F. *et al.* (2008) *Cell Metabolism* **8:** 532–9.