The nutrient sensing capacity of mouse enterochromaffin cells is dictated by gastrointestinal location

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Introduction. Enterochromaffin (EC) cells within the gastrointestinal (GI) tract provide ~90% of total body serotonin (5-HT). The release of 5-HT from these cells plays a diverse role in a number of important physiological functions. In particular, 5-HT release modulates gastrointestinal (GI) motility (Keating & Spencer, 2010) and recent evidence highlights a key role of gut-derived 5-HT in metabolic control (Sumara *et al*. 2012, Crane *et al*. 2015). Evidence also suggests an increase in 5-HT following nutrient ingestion (Zelkas *et al.* 2015), and short chain fatty acids (SCFA) predominantly synthesised by intestinal bacteria are associated with an increase in circulating 5-HT (Yano *et al*. 2015). Since exposure of EC cells to luminal nutrient content differs over the length of the gut, we aimed to isolate primary EC cells from the duodenum and colon of individual mice, and determine whether distinct regional differences exist in the capability of EC cells to sense nutrients, particularly with regards to sugars and free fatty acids (FFA).

Methods. EC cells were isolated from the mucosal layer of duodenum and colon tissue from euthanised C57/B6 mice according to our earlier method (Raghupathi *et al*. 2013). EC cell purity was determined by immunocytochemical staining for 5-HT and TPH1. Relative mRNA expression for nutrient sensing receptors and transporters for sugar (GLUT1, 2 & 5 and SGLT1 & 3), sweet tastant (T1R3) and FFA (FFAR1-4, GPR84) sensing, was determined by real-time PCR. EC cell activation by nutrients, shown as increased intracellular Ca^{2+}, was determined using flow cytometry and the Ca^{2+} indicators, Fura Red and Fluo-3. Nutrient-stimulated 5-HT secretion was measured by 5-HT ELISA. GI motility was measured using a distention-evoked peristalsis method in *ex vivo* tissue segments. Data are expressed as mean ± SEM.

Results. Our approach produces highly purified EC cell cultures in the duodenum (containing 5-HT: 85.9 ± 9.9%, TPH1: 93.8 ± 6.2%) and colon (5-HT: 97.7 ± 1.2%, TPH1: 88.8 ± 3. 7%). Duodenal and colonic EC cells differentially express a number of nutrient sensing transporters and receptors, particularly for sugars and free fatty acids. Colonic EC cells were more sensitive to acute glucose stimulation, with an increase in cytosolic Ca^{2+} and 5-HT secretion following exposure to 300 mM glucose, while duodenal EC cells responded to 500 mM glucose. Duodenal, but not colonic EC cells respond acutely to 300 mM and 500 mM fructose and 300 mM sucrose stimulation. Equimolar amounts of the non-metabolisable sugar, α-MG, did not increase 5-HT from EC cells. SCFA do not acutely stimulate EC cell 5-HT release. GI motility was inhibited in colon and duodenum segments in response to 500 mM luminal glucose.

Conclusion. EC cells can be isolated with high purity from different regions of the mouse GI tract, allowing for direct comparisons of this cell population along the length of the gut. EC cells express a number of nutrient sensing receptors and transporters. The nutrient sensing capabilities within the gut are location-dependent, as EC cells differentially sense and respond to glucose, fructose and sucrose at concentrations consistent with luminal nutrient ingestion. Thus, we highlight the complexity in understanding EC cell physiology, and identify differences in the regional sensing repertoire of EC cells to dietary sugars. By understanding the nutrient sensing mechanisms present in EC cells, we gain significant insight into how different nutrients, by augmenting 5-HT release, can affect physiological processes such as GI motility and metabolic control.

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