Nature Reviews Gastroenterology & Hepatology | Published online 5 July 2017; doi:10.1038/nrgastro.2017.91

NEUROGASTROENTEROLOGY

Tasting the gut — enterochromaffin cells are chemosensitive and modulate sensory neurons

> EC cells formed synapse-like structures with 5-HT_3R-labelled nerve fibres

A new study published in *Cell* provides a remarkably detailed characterization of gut epithelial enterochromaffin cells (ECs), showing that they are able to sense luminal metabolites, irritants and catecholamines, and subsequently alter the function of sensory nerves.

EC cells are specialized enteroendocrine cells, located in the intestinal epithelium. Despite their limited abundance (representing <1% of intestinal epithelial cells), these cells produce >90% of the body's serotonin. "EC cells have been implicated in visceral inflammation and pain, but little is understood about their cellular physiology or precisely how they communicate with the nervous system," explains author David Julius.

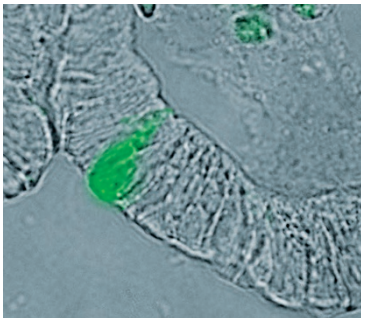
Spearheaded by first authors Nicholas Bellono and James Bayrer, the researchers sought to define the role of EC cells in governing nervous system responses to intestinal molecules. As a first step, they generated mouse-derived intestinal organoids with GFP-labelled EC cells, thereby enabling single-cell interrogation. "Thus, we had a renewable source from which to identify EC cells for detailed physiological, pharmacological and genetic profiling in a native-like environment, where the cellular architecture is maintained," says author Holly Ingraham.

Using whole-cell patch clamping, the investigators demonstrated that EC cells are electrically excitable, owing to their expression of K^+, Na^+ and Ca^{2+} voltage-gated ion channels. These data supported a sensory function, prompting the researchers to screen compounds for their ability to excite EC cells. Of 30 compounds screened, chosen for their known presence in the gut, consistent activation of EC cells was observed for allyl isothiocyanate (AITC; a pungent agent found in mustard and wasabi), the fatty acid fermentation products isovalerate, isobutyrate and butyrate, and the catecholamines dopamine, epinephrine and norepinephrine.

EC cells were markedly enriched for transcripts encoding sensory receptors or channels, notably *Trpa1* (encoding the AITC receptor TRPA1), *Olfr558* (encoding an olfactory receptor of which isovalerate is an agonist) and *Trpc4* (encoding TRPC4, a key component of the catecholamine signal transduction pathway). Further investigations demonstrated the ability of agonists for these proteins to induce EC cell responses, positioning EC cells as chemosensors capable of detecting numerous varied stimuli.

Next, the investigators examined the mechanisms of serotonin release resulting from EC cell stimulation. In an elegant series of experiments, they simultaneously measured the change in cytoplasmic Ca^{2+} levels in organoid-contained EC cells and whole-cell currents in adjacent 'biosensor' HEK293 cells (expressing the serotonin-gated ion channel 5-HT_3R) in response to stimulation by epinephrine, AITC or isovalerate. These stimuli elicited a Ca^{2+} response in EC cells, followed by substantial 5-HT_3R currents in biosensor cells, demonstrating that the serotonin released from EC cells in response to chemical stimuli has local effects. Conversely, blocking the respective chemosensory signalling cascades (for example, by inhibiting TRPC4) suppressed biosensor cell currents.

In mouse intestinal tissue sections, the researchers found that EC cells formed synapse-like structures with 5-HT_3R-labelled nerve fibres, as suggested by expression of presynaptic and postsynaptic markers. Moreover, the addition of norepinephrine to mouse colonic epithelial tissue led to strong responses in afferent mechanosensitive neurons that access the EC-cell-containing mucosa. This effect was blocked by TRPC4 inhibition or 5-HT_3R antagonism, and similar results were shown for isovalerate. Norepinephrine and isovalerate also increased the sensitivity of nerve fibres to colonic mechanical stimulation, indicating a role for the EC-cell–5-HT_3R signalling axis in modulating gut mechanosensitivity.



Intestinal organoid containing GFP-labelled enterochromaffin cell. Courtesy of H. Ingraham and D. Julius.

"We are now working toward investigating *in vivo* roles for EC-cell–neural signalling in gastrointestinal pain and motility," the authors reveal. They also hope to determine how synapses between EC cells and sensory neurons are maintained despite constant epithelial cell turnover.

Hugh Thomas

ORIGINAL ARTICLE Bellono, N. W. *et al.* Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell* **170**, 1–14 (2017)

FURTHER READING Spohn, S. N. *et al.* Non-conventional features of peripheral serotonin signalling — the gut and beyond. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 412–420 (2017)