MAPPING NUTRIENT-SENSING NEURONAL PATHWAYS IN THE INTACT INTESTINAL WALL USING CALCIUM IMAGING

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Luminal nutrients in the gastrointestinal tract are sensed by specialized enteroendocrine cells in the mucosal epithelium and this information is then conveyed to the enteric nervous system (ENS). It is well established that enteric nerve reflexes are essential for tuning intestinal activity to facilitate digestion and nutrient absorption. However, the complex architecture of the ENS has traditionally posed a significant challenge for studying its circuitry within the intact gut. Furthermore, whether specific enteric neural pathways are dedicated to detecting specific nutrients remains unclear. To address this, we used calcium imaging on intact small intestine preparations from transgenic Wnt1-cre;R26R-GCaMP3 mice, which express the fluorescent calcium indicator GCaMP3 in their ENS. Nutrient solutions of glucose (300 mM) as a model sugar, or acetate (100 mM) as a model short chain fatty acid, were perfused onto the mucosa whilst imaging the underlying enteric neurons. We also mimicked glucose transport or diffusion of acetate across the mucosa by pressure ejecting nutrients from a micropipette impaled through the epithelium to target the sensory nerve endings innervating the mucosa. *Post-hoc* immunohistochemical labeling was performed to classify the types of responding neurons based on their neurochemistry. Glucose and acetate perfused onto the mucosa both evoked Ca^{2+} transients in distinct patterns of enteric neurons. Although the percentage of total myenteric neurons that displayed Ca^{2+} responses to glucose and to acetate perfusion were comparable, the proportions of the different neurochemical subtypes of neurons that responded to glucose vs. acetate differed significantly. Glucose or acetate applied through the epithelium did not evoke Ca^{2+} transients in enteric neurons, suggesting that they are unlikely to directly stimulate mucosal nerve endings and are indeed first sensed at the mucosa. This optical approach and these findings allow us to map the enteric neural pathways activated by specific luminal nutrients. Taken together, our data shows that different components of the circuitry are activated by different nutrients.