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Expression of α-transducin, a chemoreceptive molecule, in endocrine and non-endocrine cells of the pig gastrointestinal tract

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Introduction

Several neural and humoral mechanisms are known to control gut functions before, during and after food ingestion and digestion. Different specialized epithelial cells, i.e. cells that form gustatory buttons distributed throughout the oral cavity, tongue, pharynx, and epiglottis are involved in taste perception. Gustatory cells are able to discriminate different gustatory stimuli: bitter, sweet, salty and sour. Monosodium glutamate and disodium guanylate represent another type of stimulus referred to as *umami*. Each of these stimuli can be transduced with different modalities, in such a way that gustatory substances can act on peculiar ionic channels (i.e. salty and sour) or via G-protein coupled taste receptors (TR), i.e. TR1 and TR2 (for sweet and bitter, respectively). The activation of these receptors elicits the depolarization of gustatory cells through secondary messenger pathways. The presence of cells expressing TRs, able to discriminate different types of tastes, has been described along the gastrointestinal tract (Rozengurt and Sternini 2007) and in some segments of the respiratory system (Osculati et al. 2007) of several animal species, such as mice and rats as well as in humans. The perception of sweet and bitter is based on the activation of two TR linked G-proteins, such as α-transducin and α-gustducin. The aim of the present study was to examine: 1) the presence and distribution of α-transducin in the pig gastrointestinal (GI) tract; 2) the characterization of cells expressing α-transducin; 3) the relationship between α-transducin cells and nerve fibers supplying the GI tract mucosa.

Materials and methods

Segments of the entire gastrointestinal tract (from the esophagus to the rectum) were harvested from 45 day-old piglets. Tissue specimens were fixed in Zamboni's solution and embedded in paraffin. Sections (5–10μm thick) were processed for indirect immunofluorescence using primary antibodies directed against α-transducin (1:800), α-gustducin (1:500), chromogranin A (CgA, 1:50), somatostatin (SOM 1:1000), gastrin/cholecystokinin (CCK, 1:1000), villin (1:50) and protein gene product 9.5 (PGP 9.5, 1:500). Following primary antibodies, sections were incubated with secondary antibodies: goat anti-rabbit FITC and goat anti-mouse Alexa 594. Immunostained slides were examined with a Zeiss fluorescence microscope. Images were cropped with a Polaroid DMC digital photocamera and minimal alterations (minor adjustment to brightness and contrast) were performed using Corel Photo Paint and Corel Draw.

Results

α-transducin immunoreactive (IR) cells were detected throughout the whole length of the gastrointestinal tract, with the exception of the esophagus. In the pylorus, in an area of 10 mm2, the α-transducin positive cell density was 296.25 ± 58.6. In the small bowel (50 villi each animal), cell density was 36.5 ± 16.4, 36.8 ± 10.9 and 27 ± 3.6 in the duodenum, jejunum and ileum, respectively. In the large bowel (50 crypts each animal), cell density was 16 ± 2.4, 15 ± 5.4 and 28 ± 6.8 in the cecum, colon and rectum, respectively. CgA-IR cells showed a pear or "bottle-like" shape with cytoplasmic processes extending up to the endoluminal surface of the mucosa. In the pylorus, all the α-transducin-IR cells also expressed CgA-IR, whereas this co-localization was 87.4 ± 16.3%, 90.1 ± 4.3% and 76.7 ±

5.3% in the duodenum, jejunum and ileum, respectively. α-transducin/CgA-IR co-expressing cells were 97.9 ± 4.2%, 94.9 ± 5.9% and 100% in the cecum, colon and rectum, respectively. None of the α-transducin- and α-gustducin-IR cells co-expressed either gastrin or SOM in the pylorus, whereas approximately 10% of α-transducin-IR cells co-localized with SOM or CCK in the jejunum. Few cells of the pylorus co-expressed α-transducin/α-gustducin-IR. Villin-IR cells ("brush cells") were identified all along the gut, with the highest density in the ileum. Finally, varicose nerve fibers positive for the pan-neuronal marker PGP 9.5 were identified running through the *lamina propria* of the mucosa in close proximity to α-transducin-IR cells.

Discussion

The results of the present study provide a strong morphological basis showing the distribution of α-transducin, a key molecule involved in TR signalling, along the pig gastrointestinal tract. The decreasing density of α-transducin-IR cells from the pylorus to the small bowel up to the colon, most likely reflects the role exerted by these cells in the lumen chemosensitivity related to the transit of bolus and its chemical changes (i.e. nutrients) during the digestion phase throughout the gut. Our findings appear to be very peculiar to the digestive system, as it has not been reported in the respiratory tract of other mammals (e.g. rat) (Osculati et al. 2007). Other important information that emerged from this study is the identification of α-transducin expression in enteroendocrine cells (as identified by the CgA marker) as well as in brush (villin positive) cells of the pig GI tract. These results strengthen the concept that the GI mucosa is characterized by a rather complex chemoreceptive power through which it contributes to digestive function. Chemoreceptive mechanisms may occur as a result of either intracellular chemical messenger activation or via the release of paracrine and/or endocrine bio-active messengers (e.g. CCK or SOM). These messengers may elicit neural reflexes underlying digestive processes or "protective" responses, such as vomiting or food aversion (Rozengurt and Sternini 2007).

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Abbreviations

CCK	gastrin/cholecystokinin
CgA	chromogranin-A
GI	gastrointestinal
IR	immunoreactive
SOM	somatostatin
TR	taste receptor

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