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Immunohistochemistry and high resolution microscopy of pig gastric enteroendocrine cells

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

Enteroendocrine cells are important regulators of gastrointestinal, digestive and metabolic function. Here we describe protocols for identifying, mapping and characterising these cells in the pig gastric mucosa using immunohistochemistry and high resolution microscopy techniques

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MATERIALS TEXT

MATERIALS

Goat anti-5HT antibody RRID:AB_572262 Immunostar Catalog #20079 (1:5000)
Rabbit anti-5-HT antibody RRID:AB_572263 Immunostar Catalog #20080 (1:2000)
Mouse anti-Gastrin/CCK antibody RRID: AB_2650429 Contributed by users Catalog #28.2 (1:2700)
Chicken anti-Ghrelin antibody RRID:AB_2041392 Abcam Catalog #ab15861 (1:800)
Rabbit anti-Gastrin antibody RRID:AB_2762851Contributed by users Catalog #8007 (1:3000)
Rabbit anti-Ghrelin antibody RRID:AB_2767291Contributed by users Catalog #RY1601 (1:5000)
Rabbit anti-HDC antibody RRID:AB2773044 Contributed by users Catalog #16045 (1:2000)
Rabbit anti-PYY antibody RRID:AB_1855194 Sigma-aldrich Catalog #HPA010973 (1:100)
Mouse anti-Somatostatin antibody RRID:AB_2783535 Contributed by users Catalog #S8 (1:1000)
Mouse anti-H+/K+ ATPase RRID:AB_565613 Millipore Catalogue #119100 (1:200)

1 Tissue sources and preparation

All procedures were conducted according to the National Health and Medical Research Council of Australia guidelines and were approved by the University of Melbourne Animal Experimentation Ethics Committee. Large White / Landrace crossbred pigs (30-35 kg females) were from the University of Melbourne School of Agriculture and Food. Pigs were sedated with a xylazil and ketamine mix and euthanised by cardiac injection of pentobarbital sodium (150 mg/kg).Tissues for haematoxylin and eosin staining and immunohistochemistry were removed, opened along the mesenteric border, and pinned flat, mucosa up, without being stretched. Segments were washed with phosphate buffered saline (PBS; 0.15 mol.L-1 NaCl in 0.01 mol.L-1 sodium phosphate buffer, pH 7.2) and fixed at 4°C overnight with Zamboni's fixative (2% w/v formaldehyde and 0.2% w/v picric acid in 0.1 mol.L-1 sodium phosphate buffer, pH 7.2). Tissues were washed three times with dimethyl sulfoxide and three times with PBS, before being stored in PBS-sucrose-azide (0.1% w/v sodium azide and 30% w/v sucrose in PBS) at 4°C.

2 Haematoxylin and eosin staining

Tissue was placed into histology cassettes and dehydrated through graded ethanol to histolene and embedded in paraffin. Sections (5 µm) were cut and stained with haematoxylin and eosin (H&E). Slides were coverslipped with Prolong diamond (Thermo Fisher) mounting medium. Slides were examined and photographed using an Axioplan microscope (Zeiss, Sydney, Australia).

3 Immunohistochemistry

Samples for immunohistochemistry were placed in PBS-sucrose-azide and OCT compound (Tissue Tek, Elkhart, IN, USA) in a 1:1 ratio overnight before being embedded in 100% OCT and snap frozen in isopentane cooled with liquid nitrogen.Cryosections (12 µm) were cut and air dried for 1 h on SuperFrostPlus® microscope slides (Menzel-Glaser; Thermo Fisher, Scoresby, Vic, Australia). They were then covered with normal horse serum (10% v/v with triton-X in PBS) for 30 min at room temperature and incubated with mixtures of primary antibodies (Table 1) overnight at 4°C. The preparations were then washed three times with PBS before a 1 h incubation with mixtures of secondary antibodies (Table 1) at room temperature. Sections were washed three times with dH2O and, in some cases, incubated with Hoechst 33258 nuclear staining solution (10 µg/mL Bisbenzimide-Blue in dH2O; Sigma) for 5 min. Slides were washed three times with distilled water before being mounted under coverslips with Dako fluorescence mounting medium (Agilent, Tullamarine, Vic, Australia). Slides were examined and imaged using an Axio Imager microscope (Zeiss), or an LSM800 or LSM880 confocal microscope (Zeiss).

4 Immunofluorescence image quantification

Sections for cell counts were imaged as tile scans with a nominal optical thickness of 7.7 µm using a 10x objective on the LSM800 confocal microscope (Zeiss). A 1.5 mm wide region from each imaged section, which contained the full thickness of the mucosa, was selected for analysis in Fiji (http://imagej.nih.gov/ij/). Cells from each channel were manually circled by one investigator and verified by a second investigator, and were counted as positive if their mean pixel intensity was clearly above a threshold determined from the background fluorescence. The total mucosal area was also measured in order to determine the cell density (positive cells per mm2 of mucosa). The number of positive cells in the luminal, middle, and submucosal portions of the mucosa were also determined. Data are presented as mean ± SEM, for n = 3 animals.