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Immunohistochemistry and high resolution microscopy of rat gastric enteroendocrine cells

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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 rat gastric mucosa using immunohistochemistry and high resolution microscopy techniques

MATERIALS

NAME	CATALOG #	VENDOR
Rabbit anti-5-HT antibody RRID:AB_572263	20080	Immunostar
Goat anti-5-HT antibody RRID:AB_572262	20079	Immunostar
Mouse anti-Gastrin/CCK antibody RRID: AB_2650429	28.2	
Chicken anti-Ghrelin antibody RRID:AB_2041392	ab15861	Abcam
Rabbit anti-Gastrin antibody RRID:AB_2762851	8007	
Mouse anti-TUJ1 antibody RRID:AB_10063408	801202	BioLegend
Goat anti-CGRP antibody RRID:AB_2783523	1780	
Rabbit anti-Ghrelin antibody RRID:AB_2767291	RY1601	
Rabbit anti-VIP antibody RRID:AB_2783533	7913	
Mouse anti-VIP antibody RRID:AB_2783532	V31(asc)	
Goat anti-VAChT antibody RRID:AB_2315530	H-V007	Phoenix Pharmaceuticals
Mouse anti-TH antibody RRID:AB_572268	22941	Immunostar
Rabbit anti-HDC antibody RRID:AB2773044	16045	
Rabbit anti-PYY antibody RRID:AB_1855194	HPA010973	Sigma-aldrich
Mouse anti-Somatostatin antibody RRID:AB_2783535	S8	

1 Experiments were conducted on Sprague-Dawley rats of 250-350 g. Procedures were approved by the University of Melbourne Animal Ethics Committee. Rats were supplied with food and water ad libitum prior to the experiments.

2 Rats were anaesthetised with a mixture of ketamine (55 mg/kg) and xylazine (9 mg/kg) prior to being perfused transcardially with heparinised phosphate buffered saline (PBS: 0.15M NaCl, 0.01M sodium phosphate buffer, pH 7.2) followed by fixative (2% formaldehyde, 0.2% picric acid in 0.1M sodium phosphate buffer, pH 7.0). The stomach was
removed, dissected, and post-fixed overnight at 4°C in the same fixative, before being cleared with 3 x 10 min washes in dimethyl sulfoxide, 3 x 10 min washes in PBS and then stored in PBS-sucrose-azide (30% sucrose, 0.1% sodium azide in PBS) at 4°C. Tissue was then equilibrated overnight at 4°C in a 1:1 solution of PBS-sucrose-azide and OCT compound
(Tissue Tek, Elkhart, IN, USA) before being embedded and frozen in OCT. Tissue from the antrum and corpus was analysed from 4 animals.

3 Cryosections (12 µm) were cut onto Superfrost Plus microscope slides (Menzel-Glaser; Thermo Fisher, Scoresby, VIC, Australia), air dried for 1 hour and then blocked in a solution of 10% normal horse serum with 1% Triton-X100 in PBS for 30 min at room temperature. Mixtures of up to 3 primary antibodies from different species were diluted and applied to slides overnight at 4°C. Slides were washed with 3 changes of PBS and then incubated with an appropriate mixture of Alexa fluor labelled secondary antibodies for 90 min at room temperature. After a further 3 changes of PBS, coverslips were applied with Dako fluorescence mounting medium (Agilent, Tullamarine, VIC, Australia). Samples for which the primary antibodies were omitted were analysed to investigate background staining
and autofluorescence and were used to set appropriate thresholds for quantification.

4 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, Sydney, Australia). A 1.5 mm wide region from each imaged section, which contained the full thickness of the mucosa, was selected and each separate channel, as well as a merged image, were converted to tif format for analysis in Fiji (http://imagej.nih.gov/ij/). Images were converted to greyscale and the
pixel scale was entered from microscope metadata. Cells from each channel were manually circled and were counted as positive if their mean grey value for pixel intensity was above a threshold determined from negative control images. The total mucosal area was also measured in order to determine the cell density (positive cells per mm2 of mucosa) and the number of positive cells in the top versus bottom half of the mucosa were also determined.