Identification of different EEC types and nerve fiber types in human gastric mucosa

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1 Works for me

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ABSTRACT

Enteroendocrine cells are important regulators of gastrointestinal, digestive and metabolic function. Here we describe protocols for investigating nerve fiber populations that may innervate these cells, using immunohistochemistry and high resolution microscopy techniques in the human gastric mucosa and muscle.

MATERIALS

NAME	CATALOG #	VENDOR
Rabbit anti-GRP (Gastrin releasing peptide Bombesin) antibody	Bombesin-GRP	
Sheep anti-NOS (Nitric oxide synthase) neuronal antibody	V205	
Sheep anti-NPY (Neuropeptide Y) antibody	E2210	
Mouse anti-TH (tyrosine hydroxylase) antibody	22941	
Rabbit anti-VIP (Vasoactive intestinal peptide) antibody	7913	
Rabbit anti-Substance P antibody	SK1 SP	
Rabbit anti-Gastrin antibody	8007	
Goat anti-CGRP antibody	1780	
Rabbit anti-Ghrelin antibody	RY1601	
Goat anti-VAChT (vesicular acetylcholine transporter) antibody	1624	Phoenix Pharmaceuticals

1 Stomach tissue is collected from patients who were undergoing gastric sleeve surgery for obesity at the Renown Regional Medical Center, Reno, Nevada.

2 The tissue is placed in phosphate buffered saline (PBS: 0.15 M NaCl in 0.01 M sodium phosphate buffer, pH 7.2) that is kept cold on ice as soon as it is removed by the surgeon. The tissue in PBS on ice is then transferred to the laboratory and is dissected in ice-cold PBS within 1 hour of retrieval. Dissected pieces of tissue are placed in cold fixative (2 % formaldehyde plus 0.2 % picric acid in 0.1 M sodium phosphate buffer, pH 7.0) and kept overnight at 4°C. Tissues are then washed 3 times (10 min) with dimethyl sulfoxide (DMSO) and then 3 times (10 min) with PBS. Tissue samples are then stored in PBS-azide (PBS containing 0.1% sodium azide) and stored at 4°C until used. The tissue samples are trimmed to suitable size for sectioning (block face 1 cm wide, approximately) and placed overnight in PBS-sucrose-azide (PBS with 30% sucrose and 0.1% azide) then overnight in 50% PBS-sucrose-azide and 50% OCT mixture (Tissue Tek, Elkhart, IN, USA), before being trimmed, embedded in 100% OCT and frozen in isopentane cooled with liquid nitrogen.

3 Sections of 12 µm thickness are cut, allowed to dry at room temperature for 1 h on microscope slides (SuperFrostPlus®; Grale Scientific, Vic, Australia) and incubated with 10% normal horse serum plus 1% Triton X-100 in PBS for 30 min. Mixtures of primary antibodies for double staining were then placed on the sections that were left at 4°C overnight. The tissue was washed three times in PBS and incubated with appropriate secondary antibodies labelled with Alexa Fluor dyes for 1.5 h at room temperature. Sections were then washed 3 times with PBS before mounting with non-fluorescent mounting medium (Dako, Carpinteria, CA, USA). For all secondary antisera used, sections that were incubated without primary antibodies were used to investigate background staining and autofluorescence. There was no indication of non-specific binding of the secondary antibodies.

4 Using the LSM800 confocal microscope (Zeiss, Sydney, Australia), tile scans are taken with a 20x objective and used to determine relative fiber density by applying a grid-style analysis. Tile scans are exported to be analysed off-line using ImageJ software (imagej.nih.gov/ij/). Thresholding is applied to each image generating a representative 'mask' of the specific immunoreactivity. An offset grid of circles with 13.5µm diameter, with their centres spaced approx. 38µm apart, is placed over the thresholded image. If immunoreactivity is detected within, or intercepted with, a circle sampling area, that sampling area is determined as positive. Relative abundance of immunoreactivity is calculated as a percentage of positive sampling areas in relation to the total number of sampling areas in the muscle regions of the corpus. This analysis was repeated in tissues of four patients.

5 Fibers are visually determined to be co-bundled or co-localised in double stained sections using an AxioImager microscope (Zeiss) with a 40x objective lens. In the circular muscle, a region is selected in the one channel and the total number for visible fibers determined. Then using the second channel, the fiber is determined as either co-bundled, co-localised or single labelled. 25 fields of view are first analysed with 488 then 555 channels, then a further 25 fields of view are first analysed with 555 then 488 channels. The percentage of co-bundled and co-localised fibres is calculated. This analysis is repeated in tissues of four patients.