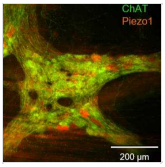

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Piezo proteins: incidence and abundance in the enteric nervous system with immunohistochemical techniques

Forked from Piezo proteins: incidence and abundance in the enteric nervous system with immunohistochemical techniques

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ABSTRACT

This protocol is to quantify the presence of both Piezo1 and 2 in enteric neurons throughout the gastrointestinal tract using immunohistochemistry on human samples that were obtained from patients undergoing surgery. The samples were taken from macroscopically unaffected areas. Using immunohistochemical techniques to determine the expression of Piezo channels in the ENS we investigated the co-expression of Piezo1 and 2 channels with a wide range of markers for enteric neuronal populations.

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FORK NOTE

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MATERIALS TEXT

a.Human samples of large and small intestines
b.Krebs solution containing (in mM): 117 NaCl, 11 Glucose, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$; Sigma-Aldrich (Schnelldorf, Germany)
c.4% paraformaldehyde;Sigma- Aldrich
d.0.002% picric acid; Sigma- Aldrich
e.0.1 mol/l phosphate buffer; Sigma- Aldrich
f.Phosphate-buffered saline (PBS): H_2O, Sodium Phosphate Monobasic, Sodium Phosphate Dibasic, NaCl; Sigma-Aldrich
g.0.1% NaN_3, Sigma- Aldrich
h.4% horse serum (HS) Sigma- Aldrich
i.poly-l-lysine- coated slides; Menzel (Braunschweig, Germany)
j.Glycerol; Sigma-Aldrich
l.epifluorescence microscope (Olympus, Germany)
m.Scion image software (Scion Corp., Frederick, MD, USA) on the computer
k. Antibodies:

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Antibody	Source	Diluition
Rabbit anti-PIEZO1 (NBP1-78446)	Novus Biological, Cambridge, UK	1:20000/2000
Rabbit anti-PIEZO2 (NBP1-78624)	Novus Biological, Cambridge, UK	1:2000
Human: Mouse Anti-HU Biotin (A-21272) Guinea pig/Mouse: Human anti-Hu	Thermo Fisher Scientific	1:50 1:10000
Goat anti-choline acetyltransferase (ChAT; AB144P)	Chemicon, Limburg, Germany	1:100
Guinea pig: Sheep anti-nitric oxide synthase (NOS; AB1529)	Chemicon, Limburg, Germany	1:1000
Guinea pig: Rabbit anti-vasoactive intestinal peptide (VIP; RIN-7161)	Peninsula laboratories international, Inc., USA	1:5000
Mouse/Human: Mouse Anti-vasoactive intestinal peptide (VIP; MaVIP)	East Acres Biological, Southbridge, MA, USA	1:6000/1:2000
Mouse/Human: Rabbit Anti- nitric oxide synthase (210 501R025)	Alexis, Enzo Life Sciences GmbH, Lörrach Germany	1:5000/1:2000
Goat anti-VR1(P19) (SC-12503)	Santa Cruz, Heidelberg, Germany	1:500/1:1000
Sheep anti-TH (AB1542)	Chemicon, Limburg, Germany	1:5000
Mouse anti-calbindin (300)	SWant, Marly, Switzerland	1:200
Rabbit anti-CGRP (CA-08-220)	Genosys, Cambridgeshire, UK	1:6000

Table 1. List of primary antibodies used.

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Antibody	Source	Diluition
Donkey Anti-Rabbit Cy2 (711-225-152)	Dianova, Hamburg, Germany	1:200
Donkey Anti-Rabbit Cy3 (711-165-152)	Dianova	1:500
Donkey Anti-Goat Cy5 (705-175-147)	Dianova	1:500
Donkey Anti-Goat Cy3 (705-165-147)	Dianova	1:500
Donkey Anti-Human AMCA (709-155-149)	Dianova	1:50
Streptavidin Cy3 (016-160-084)	Dianova	1:500
Donkey Anti-Mouse Cy3 (715-165-151)	Dianova	1:500
Donkey Anti-Sheep Cy2 (713-225-147)	Dianova	1:200

Table 2. List of secondary antibodies used.

1 Human samples of large and small intestines were taken from macroscopically unaffected areas and placed in an ice-cold-oxygenated sterile Krebs solution and immediately transferred to the laboratory. Tissues were then dissected in the ice-cold oxygenated sterile Krebs solution to obtain whole-mount submucosal and myenteric plexus preparations.

2 Tissue specimens were fixed overnight at room temperature in a solution containing 4% paraformaldehyde and 0.002% picric acid in 0.1 mol/l phosphate buffer and then washed (3 × 10 min) in phosphate buffer.

3 The preparations were then incubated in phosphate-buffered saline (PBS)/ NaN3 (0.1%)/horse serum (HS, 4%) for 1 h at room temperature followed by 48 h and 12 h incubation with the primary and secondary antibody, respectively. For the Piezo1 antibody, double and triple staining for the co-localization study was performed.

4 Specimens were washed in PBS, mounted on poly-l-lysine- coated slides and cover slipped with a solution of PBS (pH 7.0)/NaN3 (0.1) containing 65% glycerol.

5 The preparations were examined with an epifluorescence microscope (Olympus) equipped with appropriate filter blocks. Pictures were acquired with a video camera connected to a computer and controlled by Scion image software (Scion Corp., Frederick, MD, USA). Frame integration and contrast enhancement were employed for image processing.

6 Neurons stained with the antibodies were manually counted and picture of the stained ganglia were acquired.

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