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Neuroimaging of human myenteric neurons

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Abstract

This protocol is to investigate the sensitivity of human colonic myenteric neurons to chemical and electrical stimulation using ultrafast neuroimaging technique with the voltage sensitive dye Di-8-ANEPPS. We compared tissue samples from different colonic sub regions. Tissue samples were taken during surgery (Medical school of Hannover, Germany), placed in ice-cold oxygenated Krebs solution for preparation and immediately transferred to our laboratory. Tissues were dissected in ice-cold oxygenated Krebs solution for preparation to obtain whole-mount myenteric plexus preparations. Samples were used for ultrafast neuroimaging technique combined with a voltage sensitive dye (VSD). In order to stain the ganglia, VSD Di-8-ANEPPS (20-40 µM) was applied directly into the ganglion at moderate pressure (≤ 0.5 bar). Ganglia were then stimulated by applying 100 µM nicotine or 1,000 serotonin on the surface of a ganglion. Electrical stimulation was performed with a self-designed platinum wire electrode with a tip diameter of 75 µm, placed onto interganglionic neuronal fiber tracts, applying 180 µA single-pulses for 0.5 ms. The preparations were examined with an inverted microscope equipped with an appropriate filter set. Pictures were acquired with a CMOS camera connected to a computer and controlled by Turbo SM 64 Software.

Materials

a. samples of human colon
b. Krebs solution for preparation containing in (mM): 117 NaCl, 11 Glucose, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$; Carl Roth GmbH 26 Co. KG (Karlsruhe, Germany)
c. Krebs solution for experiment containing in (mM): 117 NaCl, 11 Glucose, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 20 $NaHCO_3$, 2.5 $CaCl_2$; Carl Roth GmbH 26 Co. KG (Karlsruhe, Germany)
d. Di-8-ANEPPS and 4-Di-2-ASP; Thermo Fisher Scientific Inc. (Waltham, MA, USA)
e. 0.0135 % Pluronic® F-127; Thermo-Fisher Scientific Inc. (Waltham, MA, USA)
f. 0.135 % Dimethyl sulfoxide (DMSO); Acros Organics (Geel, Belgium)
g. 100 µM nicotine (GL9693); Glentham Life Sciences (Corsham, Great Britain)
h. Sylgard® 184; World Precision Instruments (Sarasota, FL, USA)
i. micropipettes: glass capillaries (GB100 F-10); Science Products GmbH (Hofheim, Germany) formed into micropipettes by a micropipette puller (P-1000); Sutter Instrument Company (Novato, CA, USA)
j. inverted microscope (Olympus Corporation, Hamburg Germany)
k. DaVinci 1K CMOS camera; SciMeasure Analytical Systems (Decatur, GA, USA)
l. Turbo SM 64 Software; Redshirt Imaging LLC (Decatur, GA, USA)

Troubleshooting

Steps

1 Samples of the human ascending colon, transverse colon, descending colon and sigmoidal colon/rectum were taken out during operation from patients. They were placed in ice-cold oxygenated Krebs solution for preparation and immediately transferred to the laboratory. Tissues were carefully dissected in the ice-cold oxygenated Krebs solution for preparation to obtain whole mount myenteric plexus preparations.

2 Tissue samples were pinned on a ring made of Sylgard®184 and transferred into a recording chamber where they were constantly perfused with 37°C oxygenated experimental Krebs solution.

3 Pre-staining to visualize the ganglia was performed by incubation with the fluorescent dye 4-Di-2-ASP (2.5 µM).

4 The voltage sensitive dye (VSD) Di-8-ANEPPS was dissolved in 1.5 ml experimental Krebs solution to which 0.135% DMSO and 0.0135% Pluronic® F-127 were added, so that it was used at a concentration of 20 µM.

5 In order to stain selected ganglia, a micropipette was filled with Di-8-ANEPPS and then positioned on the surface of the ganglion. The ganglion sheath was scratched with the tip of the micropipette and the VSD was applied into the ganglion at moderate pressure (≤ 0.5 bar).

6 The neuronal responsiveness were tested applying by a micropipette nicotine and then applying nicotine (100 µM) and serotonin (1,000 µM) on the surface of a ganglion at a pressure of 0.5 bar for 500 ms.

7 Electrical stimulation was performed with a self-designed platinum wire electrode with a tip diameter of 75 µm, placed onto interganglionic neuronal fiber tracts, applying 180 µA single-pulses for 0.5 ms.

8 The preparations were examined with an inverted microscope equipped with an appropriate filter set. Pictures were acquired with a CMOS camera connected to a computer and controlled by Turbo SM 64 Software.

9 Neuronal responsiveness were manually analysed.

Protocol references

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