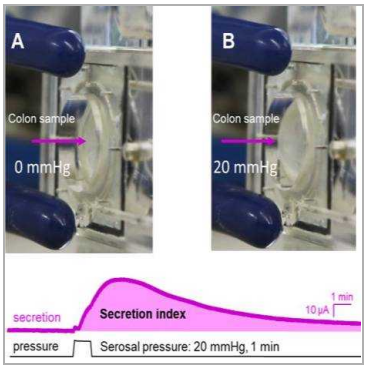

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Ussing chamber experiments for distension evoked secretion in mucosa/submucosa preparations from human colon

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ABSTRACT

This protocol is to investigate the mechanisms of mechanically induced secretion in human colon. For this purpose, we used the voltage clamp Ussing chamber method to gain insights into the distension induced secretion evoked by pressure application to the mucosal or serosal side in the human colon mucosal/submucosal samples. We could show that distension elicited a secretory response due to Cl- and HCO3- fluxes into the lumen. Prostaglandins played an important role in distension induced secretion, because the cyclooxygenase inhibitor piroxicam significantly reduced the distension induced secretion response. Subsequent application of tetrodotoxin revealed a minor, but significant nervous component. This was likely due to activation of mechnosensitive synapses because synaptic blockade by ω-conotoxin GVIA reduced the response to distension. Secretion was induced by tensile rather than compressive forces as preventing distension by a filter inhibited the secretion.

Keywords: Ussing chamber, distention, enteric nervous system, submucosal plexus, secretion

MATERIALS

a. Samples of human colon
b. Stereo Microscope SZ51, Olympus)
c. 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 & Co. KG (Karlsruhe, Germany)
d. 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 & Co. KG (Karlsruhe, Germany)
e. EasyMount Ussing Chamber System EM8-C, with VCC MC6 and MC2 Voltage/Current Clamp Amplifier, P2300 Easy Mount Ussing chambers, EM-CSYS-4 / EM-CSYS-2 Easy Mount holders, P2020 Easy Mount Electrodes, P2023-20 electrode tips (Physiologic Instruments, Reno, USA)
f. Homemade tissue holders (acrylic glass) with 0.5cm² or 1.08cm² tissue windows, equipped with 0.4mm insect pins for tissue mounting and stimulating electrodes (0.5mm platinum/iridium wire 90/10)
g. Stimulator Grass SD-9
h. PowerLab 8/35 8 channel analog to digital converter with LabChart 8 software
i. Temperature controlled circulating waterbath to maintain using chambers at 37°C
j. Ussing chamber pressure pump (see https://discover.pennsieve.io/datasets/68)
k. Pharmacological substances included (with the final concentration given in brackets): Tropisetron (1µM, ICS-205-930, Sandoz, Basel), Piroxicam (10µM, Sigma-Aldrich, Taufkirchen, Germany), Gadolinium (100µM, Gadolinium(III)-chlorid Hexahydrat, Sigma-Aldrich), Neostigmin (1µM, Sigma-Aldrich), Tetrodotoxin (1µM, Biotrend Chemikalien GmbH, Cologne, Germany), Atropine (1µM, Sigma-Aldrich), Prostaglandin E2 (Sigma-Aldrich), ω-conotoxin GVIA (500nM, Alomone Labs, Jerusalem, Israel)

1 Samples of human colon were placed in ice-cold oxygenated Krebs solution and washed by gentle agitation. Tissues were then dissected in fresh ice-cold oxygenated Krebs solution to obtain preparations containing the mucosa and all submucosal plexi.

2 Tissue samples were cut into pieces of 1cm² or 0.5cm² and mounted in the Ussing chamber holders. The holders were inserted into the Ussing chambers, and the chambers were filled with Krebs solution.

3 The tissue was equilibrated for 60min.

4 As a test for tissue viability, tissue resistance and the response to electrical field stimulation (EFS, 10Hz, pulse duration 1ms, 20V, 10sec) were recorded

5 For the mechanical stimulation both halves of the chambers were sealed with silicon elastomer plugs and the pressure pump was connected to the carbogen gas inlet of the mucosal or serosal chamber

6 Pharmacological substances were added on the serosal or mucosal side 30min before a mechanical stimulation.

7 For analysis, we calculated the integral of the response curve in the LabChart software