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Protocol for High Resolution Manometry

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ABSTRACT

This protocol was submitted on behalf of the authors from the Tache Dinning lab, by the SPARC project. The goal was to create a protocol from a methods section.

Specimen retrieval and initial handling

1

Specimen retrieval and initial handling

Segments of descending colon (n = 30) or sigmoid colon (n = 4) were removed from 34 patients who underwent elective colorectal surgery (anterior resection) for non-obstructing distal large bowel cancer (n = 31), or recurrent sigmoid diverticulitis (n = 3), after obtaining prior informed consent. The length of colon resected was determined by the vascular territory of the inferior mesenteric artery, which spans from the splenic flexure to the upper rectum. The inferior mesenteric artery is routinely ligated in anterior resections, as such there is typically a length of macroscopically normal colon resected along with the diseased segment.

To reduce time between segment removal and motility recordings, an investigator was notified of surgery timing and immediately retrieved the specimen upon removal. The tumour with its mesentery and at least 5cm margin of normal colon attached, was removed from the rest of the colonic specimen and sent immediately histological review by surgical pathologist. The remaining segment of normal colonic tissue proximal to the diseased segment (median length = 25cm; range 15 – 36cm), was placed into warmed, pre-oxygenated Krebs solution:
in mM:

- 118 mM NaCl,
- 4.7 mM KCl ,
- 1.0 mM NaH_2PO_4,
- 25.0 mM $NaHCO_3$,
- 1.2 mM $MgCl_2$,
- 11 mM D-glucose,
- 2.5 mM $CaCl_2$

was taken to the laboratory.

Prior to placement in the organ bath, the specimen was cleared of residual blood and faecal content by washing with Krebs solution. All specimens were washed and placed in the organ bath within 20 minutes of removal from the patient. Following recordings, colonic specimens were required to be returned to the pathology department. Experimental recordings ranging 1 – 5.5 hours (median = 2.13 hours). All specimens had normal colour, and were not cyanotic. The study was approved by the Flinders Clinical Research Ethics Committee no. 50/07.

Experimental set up

2 *Experimental set up*

Using an experimental setup similar to that describe in previous studies, [1-3] the specimen was placed into a water-jacketed organ bath filled with 2 L of Krebs solution, maintained at 36-37^{o}C. Krebs solution was gassed continuously with carbogen (95% O_2/5% CO_2). A high-resolution manometry catheter taped with a wax film (Parafilm 'M' laboratory film; Bemis, Neenah, WI, USA) to a Perspex rod was inserted into the lumen of the specimen. The rod was fixed in the organ bath with the catheter placed against the mucosa. A suture thread with an attached alligator clip was

secured to the anal and oral ends of the organ bath. These clips attached to oral and anal end of the specimen to hold it in place (Figure 1). Between 3 to 4 isometric force transducers (depending on the specimen length) were attached to the superior aspect of the specimen, using alligator clips tied to silk threads, and spaced evenly along the length of the specimen (~5-7 cm intervals; details below; Figure 1). The clips were attached to the serosa in between the taenia coli. A resting tension of ~5g was applied to each transducer, which lifted the colon up causing maintained contact between the manometry catheter and the mucosa. While, both the high resolution manometry catheter and force transducers recorded mechanical colonic activity, data were only analysed from the manometry catheter. In 12 recordings, two suction electrodes were applied the colonic serosa for extracellular recording of colonic smooth muscle action potentials (Figure 1).

Specimens equilibrated in the organ bath for 20 minutes. A control period was then recorded in which no drugs were applied, for a minimum of 30 minutes. Following the control period, 1mM hexamethonium or 0.3mM lidocaine were added to the organ bath. To activate ascending neural pathways, electrical field stimulation (EFS; Grass SD9 Stimulator; Grass, Quincy, MA, USA; 10Hz, 0.5ms pulse width, 50V, 5 seconds train duration[TH1]) was applied across the anal end of th[PD2] [TH3] e preparation. To avoid confusion between spontaneous and EFS-evoked contractions, stimulations were applied after spontaneous contractions at a time interval less than half that of ongoing contractions.

Circular smooth muscle mechanical recordings

3 *Circular smooth muscle mechanical recordings – force transducers and high-resolution manometry (HRM)*

The isometric force transducers (Grass FT-03C; Grass, Quincy, MA, USA) were connected to two custom-made preamplifiers (Biomedical Engineering, Flinders University) and then to a PowerLab (model 16/35, AD Instruments, Bela Vista, NSW, Australia) and a Macintosh computer running Labchart version 6 (AD Instruments, NSW, Australia). Two high resolution manometry catheters were used to record intraluminal muscle contractions. The first, used for 12 recordings, was a fibre-optic catheter with 72 pressure sensors spaced at 1cm intervals. The fibre-optic catheter was attached to a spectral interrogator unit (FBG-scan 804; FOS&S, Geel, Belgium) and pressures were recorded in real time on a custom-written LabVIEW program (National Instruments, Austin, TX, USA). The second catheter, used for 22 recordings, was a commercially available, solid state high-resolution impedance manometry catheter (Sandhill Scientific, Unisensor USA Inc.) with 32 pressure sensors spaced at 1cm intervals. Pressure data was acquired at 10Hz using InSIGHT^{TM} (Sandhill Scientific, Unisensor USA Inc.). Impedance data was not used in this study. Pressure data acquired by either catheter was exported as text (*.txt) files for analysis in custom-made PlotHRM software developed by the authors (LW), written in Matlab (Mathworks, MA, USA) and Java (Sun Microsystems, CA, USA.

Drugs

4 *Drugs*

Potent neurotoxin tetrodotoxin (TTX) was considered, however this ion channel blocker was not used on account of the large dose required to induce an effect in a 2L organ bath, and the requirement that specimens be handled by pathologists following the experiment. Rather, lidocaine (0.3mM), another blocker of voltage dependent sodium channels was used. Hexamethonium (1mM) was also used. Both drugs were purchased from Sigma-Aldrich, Castle Hill, Australia.