

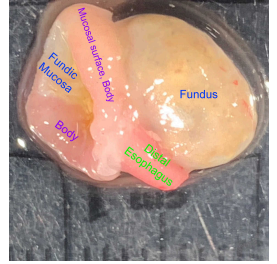


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Pressure measurement in intact mouse fundus

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We use this protocol and it's working

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Abstract

Each region of the stomach has distinct functional characteristics to ensure coordinated gastric storage and emptying. Inhibitory and excitatory enteric neurotransmission plays an important role in the adaptive relaxation of the fundus and the emptying of its contents. This protocol describes a procedure to examine the functional and mechanical properties of this specific region of the stomach by measuring intraluminal pressure in the isolated intact fundus. This method can be used to explore the motor function of the fundus under a variety of experimental conditions or disease states while largely preserving its three-dimensional shape.

Materials

Silk suture: 8-0, cat# 2023 , Mani Inc, Japan

Organ bath: cat# 158505, Radnoti LLC, Covina, CA

Pressure transducer: PX260, Edwards Lifesciences, Irvine, CA

Infusion pump: PHD Ultra syringe pump, cat# 70-3007 or 70-3005, Harvard Apparatus, Holliston, MA

Stimulator: RADSTIM Stimulator, cat# RS1000, Radnoti LLC, Covina, CA, USA

Data acquisition system: DataQ Instruments, Akron, OH

WinDaq software: Version 2.87, DataQ Instruments, Akron, OH

Prostaglandin F2 α : cat# 4214, Tocris, Minneapolis, MN

Atropine: cat# A0132, Sigma-Aldrich, St. Louis, MO

Guanethidine: cat# G-8520, Sigma-Aldrich, St. Louis, MO

L-NAME: cat# N5751, Sigma-Aldrich, St. Louis, MO

SNP: cat# 71780, Sigma-Aldrich, St. Louis, MO



Isolation of Gastric Fundus

1h 9m

- 1 Euthanize mice according to IACUC approved method (i.e., CO₂ asphyxiation). 5m
- 2 After abdominal incision, excise the stomach by transecting the lower esophagus and the pylorus. 1m
- 3 Quickly place stomach in Sylgard-coated dissecting dish filled with cold Krebs solution and continuously bubbled with carbogen (95% O₂ and 5% CO₂).
Kreb's Solution:
 - NaCl 120mM
 - KCl 5.9mM
 - NaHCO₃ 25mM
 - Na₂H₃PO₄ 1.2mM
 - MgCl₂ • 6H₂O 1.2mM
 - CaCl₂ 2.5mM
 - dextrose 11.5mM.
- 4 Isolate the fundic portion of the stomach from the remaining regions by cutting through the corpus below the gastroesophageal junction, leaving about 3mm of corporal tissue attached to the fundus. 5m
- 5 Gently remove the stomach contents. 1m
- 6 Immobilize the fundus on the bottom of the dissecting dish using insect pins inserted into the edges of the corpus. 3m
- 7 With the aid of a stereoscope, insert a 4F double lumen catheter into the esophageal stump and through the gastroesophageal junction, taking care not to evert the mucosa. Secure in place with suture. Flush both lumens with Kreb's solution to ensure that air bubbles have been removed. 15m
- 8 Close the distal edges of the fundus with a horizontal mattress suture using 8-0 silk (8-0 ophthalmic silk, cat# 2023, Mani Inc., Japan), keeping the mucosal surfaces in apposition and using the reflected edge of the corpus to support the stitches. The boundary between the fundus and body can be identified by the difference in mucosal color. 30m



- 9 Close the region below the base of the esophagus with a purse-string suture. 10m
- 10 Test for fluid leakage: Attach a 1mL syringe to the catheter and slowly infuse Kreb's solution into the fundic lumen. Verify the absence of bubbles from the suture line. 5m

Measurement of pressure in the fundus

- 11 Position the intact fundus between platinum electrodes in a 5ml organ bath (cat# 158505, Radnoti LLC, Covina, CA) that has been filled with Kreb's solution, maintained at 37°C and continuously aerated with carbogen. 5m
- 12 Attach one lumen of the catheter to a calibrated pressure transducer (PX260, Edwards Lifesciences, Irvine, CA) and the other lumen to an infusion pump (PHD Ultra syringe pump, cat# 70-3007 or 70-3005, Harvard Apparatus, USA). Monitor intraluminal pressure continuously using a Data-Q acquisition system driven by WINDAQ software (DATAQ Instruments, Inc. USA). Many commercially available acquisition systems are adequate for this purpose. 5m
- 13 Equilibrate tissue for 45-60 minutes. 1h

Generation of nerve-mediated relaxation responses in contracted fundus

- 14 After equilibration, slowly infuse Kreb's solution into fundus at 10 μ l/min while continuously measuring intraluminal pressure. 10m
- 15 With 100 μ l instilled into the lumen, pre-contract the fundus with prostaglandin F₂ α (10 μ M) under non-adrenergic, non-cholinergic conditions (i.e., in the presence of atropine 1 μ M, and guanethidine 10 μ M). 5m
- 16 Once the isovolumetric pressure in response to PGF₂ α reaches a plateau, generate nerve-mediated relaxation responses using electrical field stimulation (EFS, 30V, 0.5ms pulse duration, 10 seconds) over a range of frequencies (2-30Hz) delivered by an electronic stimulator (RADSTIM Stimulator, cat# RS1000, Radnoti, Covina, CA, USA). 10m
- 17 Post-junctional relaxation responses can be examined by exposing the fundus to a nitric oxide donor such as sodium nitroprusside (SNP 10 μ M). 5m
- 18 Withdraw the infused fluid from the lumen and wash the tissue by changing the Kreb's solution in the organ bath 2-3 times over 15 minutes. 15m



Evaluation of nitric oxide-mediated relaxation in the fundus

- 19 To evaluate the contribution of nitric oxide to the relaxation response, add a nitric oxide inhibitor (N ω -nitro-L-arginine methyl ester, 10 μ M or N ω -nitro-L-arginine, 10 μ M) to the organ bath for 15 minutes prior to evoking neurogenic relaxation responses as above (repeat steps 14-18).

40m

Pressure measurement in response to contractile stimuli

- 20 Slowly infuse Kreb's solution into fundus at 10 μ l/min while continuously measuring intraluminal pressure.
- 21 To evaluate receptor-independent contractile efficiency of the intact fundus, expose the preparation to high extracellular potassium by replacing the solution in the organ bath with a modified Kreb's solution in which the concentration of KCl is increased to 120mM, with a proportional reduction in the concentration of NaCl.

10m

5m

Measure weight of fundus

- 22 At the end of the experiment, remove the catheter, open the fundus along the suture line and weigh the tissue after blotting excess liquid on filter paper.

5m

5m