



MUSCLE BATH – CONTRACTILITY TESTING

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References:

For bladder:

Bjorling DE, et al. Response of the isolated guinea pig bladder to exogenous and endogenous leukotrienes. *J Urol* 1994;152:1281.

Busser BW, et al. Lipopolysaccharide upregulates bradykinin 1 receptors in the isolated mouse bladder. *J Urol* 1998;160:2267.

<http://www.jove.com/video/51807/bladder-smooth-muscle-strip-contractility-as-method-to-evaluate-lower>

For aorta:

<http://www.jove.com/video/52324/measurement-smooth-muscle-function-isolated-tissue-bath-applications>

PREPARATION

1. Prepare solutions required (normal and high K⁺ versions of Krebs's bicarbonate-buffered bath solution; recipes at end of document).
2. Turn on water baths and circulating pumps. Ensure proper temperature settings (37° C).
3. Turn on PC and PowerLab and Grass stimulator. ***Leave current amplifier (Med-Lab Stimusplitter II) OFF until ready for use to reduce risk of accidents***
4. Load settings file to start software with channel setup pre-loaded. *After initial channel setup, you should save a settings file to the desktop and use it to launch the application. The number of channels, Units (g), sampling rate (40 hz), input range for individual channels (5 mV), etc. are all saved. You can also pre-set the weight you use for the 2-point calibration to speed up that process.*
5. Two Point Calibration Procedure – to be performed each day before the start of experiments.
 - a. Select “Zero all inputs” in Commands menu to re-zero all input channels.
 - b. Begin recording data using the Start button on the bottom right.



- c. Gently hang a 5 g weight from each force transducer for a period of 5-10 seconds. Be careful to avoid unnecessary swinging or oscillation.
 - d. Stop recording after you have done this on each channel (you must stop recording to proceed to the next step).
 - e. Highlight an area on Channel 1 that includes before and after the weight was hung.
 - f. Click on the triangle next to the channel name on the right side and select "Units conversion".
 - g. Select the region of the trace corresponding to zero, and click the arrow to calibrate for 0 g, and repeat for the region of the trace corresponding to 5 g.
 - h. Repeat for each channel individually.
6. After calibration, fill each bath with 10 ml normal Krebs's solution and turn on oxygen (95% O₂/5% CO₂) flow. Ensure oxygen is bubbling in each bath, and adjust valves accordingly (this should not be necessary in most cases). Avoid excessive oxygen flow resulting in frothing of the fluid.
 7. Bring animals to laboratory and prepare instruments necessary (balance, forceps, and scissors). Place ~100 ml of normal Krebs's solution on ice.

TISSUE PREPARATION AND MOUNTING

8. When ready to begin experiment, euthanize animal and record weight prior to excising bladder. Remove and weigh bladder (gently squeeze if necessary to remove urine) as quickly as possible, then place into ice cold Krebs for further dissection (NOTE: Animals **must** be euthanized according to ACUC protocol specification).
9. Cut the bladder in half longitudinally, from base to dome. Cut in half again if 4 strips per mouse are required.
10. Begin recording in LabChart software immediately before mounting strips.
11. Strips are suspended by tying a silk ligature (3-0 or 4-0) around each end of the strip, approximately 1-2 mm from the edge to avoid the ligature slipping off when tension is applied.
12. Ligature is first secured to the anchoring rod, via stainless steel hook. The other end is secured to the force transducer. Raise the bath to submerge the muscle strip in the oxygenated 37° C Krebs's solution.
13. Remove excess slack from the tissue by adjusting the height of force transducer by turning micrometer to which it is mounted. Do not apply tension to the strip yet.
14. Allow 30 minutes of incubation after all strips are hung. Then apply a baseline tension to each strip (amount varies by species, 1.5 g of starting tension for mouse bladder strips, 2.0 g for rats). Optimal baseline tension can be determined by performing a series of preliminary studies testing the response of tissues to carbachol after using baseline tensions of 0.5 to 2.0 g. Slowly increase the tension over a period of 15-30 seconds.



15. Allow 15 minutes of incubation time, at which point, drain the baths and fill with fresh Kreb's solution. Incubate an additional 15 min.

MEASURING CONTRACTILITY AND RESPONSE TO AGONISTS

16. Drain the normal Kreb's solution and add "High K⁺" Kreb's solution containing an equimolar swap of KCl for NaCl. Allow the strips to reach peak contraction (approximately 30 seconds to plateau), and then rinse (drain and fill) TWICE with normal Kreb's.
17. After 15 minutes, perform a third rinse. Wait an additional 15 minutes before proceeding.
18. Begin electrical field stimulation protocol by turning on Med-Lab Stimusplitter II. THIS DEVICE PUTS OUT VERY HIGH CURRENTS and care must be taken to ensure no one is making adjustments to baths. Nobody should be standing near the baths, and everyone nearby should be notified not to approach the baths during current application. NOTE: performance of electrical field stimulation requires availability of platinum or stainless steel electrode in the tissue holder on either side of the suspended tissue. Current passes between the electrodes, stimulating the tissue.
19. The frequency response curve is typically performed using 12 V and 1 ms pulse parameters (selectable on Grass stimulator). A 5-10 second duration pulse is applied (turn off stimulator once tissue response has reached plateau) at increasing frequencies, with two minutes of rest between each current pulse.

Recommended frequency range: 2, 4, 8, 12, 20, 30 Hz.
20. Rinse baths, and allow a rest period of 30 minutes before proceeding with cumulative carbachol response curve. Use this time to prepare fresh drug and perform necessary dilutions.
21. Add lowest dose (starting at ~1-10 nM) of carbachol, wait ~45 seconds for response to develop before proceeding with next dose. Low doses may not produce any effect. Once an effect is visible, the tissue response should dictate the timing of additional doses (i.e., wait for response to plateau). Higher doses of carbachol and some other agonists may take longer to reach full effect.
22. Washout following response to highest dose (~30uM), includes TWO rinses, and an additional third rinse 15 minutes later, followed by a minimum of 15 minutes of additional rest before additional tissue stimulation.
23. Further testing (antagonists or other drugs of interest) is generally performed after these standard procedures are completed, and therefore later steps will vary according to the goals of the experiment. Data analysis is performed offline, and responses normalized to maximal contractile response to KCl or carbachol or tissue weight. For this reason, the "wet weight" of the individual strip should be recorded at the end of the experiment. Pharmacological characterizations of drug



or antagonist actions (IC_{50} , EC_{50} , E_{MAX} , etc) are done with the aid of software (ie, Graphpad Prism, KaleidaGraph, Origin, etc)

End protocol

MATERIALS

Standard PC (with USB port) for data visualization, storage, and offline analysis.

Muscle baths (also referred to as tissue or organ baths, available from Radnoti/ADInstruments, DMT, and others)

AD Instruments (ADI) PowerLab 8/35 (ADI#PL3508/P; analog-to-digital signal converter)

Bridge amplifier (ADI 8 channel bridge #FE228)

Grass FT03 force transducers (requires adapter cable for newer ADI Bridge Amps #MLAC11)

Syringe or bottle-top pump for accurate dispensing of 10ml bath volumes (for accurate drug dilutions)

Grass S48 Stimulator (any model designed to deliver square-wave pulses with requisite parameters, including S44, S88, S88X)

Med-Lab Stimusplitter II (current amplifier designed to deliver pulses to multiple baths simultaneously. This item is no longer made, but the alternative is to deliver the pulses to one or two baths at a time)

Platinum-plate stimulating electrodes on plastic inserts. (Custom-made, however commercial solutions are available through Harvard Apparatus or AD Instruments)



| Kreb's Solution | | | | 1X | 10X Stock Solution | |
|--|--------------------------------------|-----------|--------|----------------------|---------------------------|-----------|
| | chemical | conc (mM) | fw. | g/L | for 1L | for 2L |
| (1) | NaCl | 120 | 58.44 | 7.0128 | 70.128 | 140.256 |
| (2) | KCl | 4.7 | 74.55 | 0.350 | 3.504 | 7.008 |
| (3) | KH ₂ PO ₄ | 1.2 | 119.96 | 0.144 | 1.440 | 2.879 |
| (4) | MgCl ₂ *6H ₂ O | 1.2 | 203.3 | 0.244 | 2.440 | 4.879 |
| (5) | NaHCO ₃ | 25 | 84.01 | 2.100 | 21.003 | 42.005 |
| ADD THE FOLLOWING TO diH2O to make 1X solution from 10X stock (start with ~85% of final volume) | | | | | | |
| ADD Glucose and CaCl ₂ first, stir, and then 10X stock | | | | | | |
| | | | | final volume: | 1L | 2L |
| (6) | D-glucose | 10 | 180.2 | | 1.802 | 3.604 |
| (7) | CaCl ₂ *2H ₂ O | 2.5 | 147.02 | | 0.368 | 0.735 |
| | | | | | 10X Stock Solution | |
| HIGH K+ Krebs's Solution | | | | | for 1L | |
| (1) | NaCl | 4.7 | 58.44 | 0.27467 | 2.74668 | |
| (2) | KCl | 120 | 74.55 | 8.946 | 89.460 | |
| 3-5 same as above | | | | | | |
| must also add 6+7 same as above | | | | | | |