Video Article

Assessing Myogenic Response and Vasoactivity In Resistance Mesenteric Arteries Using Pressure Myography

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Abstract

Small resistance arteries constrict and dilate respectively in response to increased or decreased intraluminal pressure; this phenomenon known as myogenic response is a key regulator of local blood flow. In isobaric conditions small resistance arteries develop sustained constriction known as myogenic tone (MT), which is a major determinant of systemic vascular resistance (SVR). Hence, *ex vivo* pressurized preparations of small resistance arteries are major tools to study microvascular function in near-physiological states. To achieve this, a freshly isolated intact segment of a small resistance artery (diameter ~260 µm) is mounted onto two small glass cannulas and pressurized. These arterial preparations retain most *in vivo* characteristics and permit assessment of vascular tone in real-time. Here we provide a detailed protocol for assessing vasoactivity in pressurized small resistance mesenteric arteries from rats; these arteries develop sustained vasoconstriction - approximately 25% of maximal diameter - when pressurized at 70 mmHg. These arterial preparations may be used to study the effect of investigational compounds on relationship between intra-arterial pressure and vasoactivity and determine changes in microvascular function in animal models of various diseases.

Video Link

The video component of this article can be found at http://www.jove.com/video/50997/

Introduction

Small resistance arteries are major determinants of SVR and play an important role in pathophysiology of many diseases[1,2]. Conditions such as diabetes[3], pregnancy[4], ischemia-reperfusion[5],obesity and hypertension[6,7] are frequently associated with altered microvascular function. Vascular myography can not only provide important insights into changes in microvascular function in various diseases but also help identify therapeutic targets and evaluate the efficacy of vasoactive compounds. Vascular function has been studied using isolated small arteries under isometric or isobaric vessel conditions[8]. Detailed description of isometric myography is provided elsewhere[9]. However there are differences in data obtained from isometric versus isobaric preparations[10-12]. Since pressurized arterial preparations allow the study of microvascular function in near-physiological conditions, the obtained findings may correlate better with *in vivo* behavior of the vascular bed[8,13].

In 1902 Bayliss first described the effect of transmural pressure on vascular diameter[14]. He observed in small resistance arteries from various vascular beds of rabbits, cats and dogs that a decrease in pressure was followed by vasodilation, and an increase in pressure was followed by vasoconstriction. This phenomenon is known as myogenic response. Bayliss and subsequent investigators observed that in isobaric conditions small resistance arteries develop sustained constriction known as MT[15,16]. Both myogenic response and MT can be assessed by using pressure myography (PM) technique. PM is used primarily to determine vasoactivity of small arteries, veins and other vessels. In addition to assessing the effect of vasoactive compounds on vascular diameter, PM - as the name indicates - is used to assess intravascular pressure-mediated changes on vascular diameter. Over the last few decades advances in computer software, which enhanced video microscopy and glass pipette pulling, have made PM easier to perform. However, dissection of viable intact segments of small blood vessels remains tedious and sometimes challenging. Here we outline a detailed protocol to study myogenic response in small mesenteric resistance arteries isolated from rats.

Protocol

The examples shown here are from experiments approved by IACUC at Georgia Regents University - Protocol No: # 2011-0408

1. Preparation of Reagents

1. Prepare dissection solution stock: For 500 ml of stock dissection solution (5x), dissolve 21.18 g NaCl, 0.875 g KCl, 0.739 g $MgSO_4$, 1.049 g MOPS and 0.019 g EDTA in 450 ml of Milli-Q water. Adjust pH to 7.3-7.4 using 1 N NaOH. Make up the volume to 500 ml with Milli-Q water. Stock solution can be stored up to 7-10 days. See **Table 1** for a list of chemicals and their vendors. See **Table 2** for concentration in mM.
2. Prepare working dissection solution: Prepare fresh working dissection solution every day. For 100 ml working solution, dissolve 0.091 g glucose, 0.016 g NaH_2PO_4 and 0.022 g sodium pyruvate in 79.8 ml of Milli-Q water. Add 0.2 ml 1M $CaCl_2$ and 20 ml dissection solution stock to bring volume to 100 ml.
3. Prepare physiological salt solution (PSS): To prepare 1,000 ml PSS, dissolve 0.365 g KCl, 6.545 g NaCl, 0.296 g $MgSO_4$, 0.163 g KH_2PO_4, 2.072 g glucose, 2.184 g $NaHCO_3$ and 2.383 g HEPES in 950 ml of Milli-Q water. Adjust pH to 7.3-7.4 using 1 N NaOH. Make up the volume to 1,000 ml with Milli-Q water. Remove 2 ml of solution and replace it with 2 ml of 1 M $CaCl_2$. (Fresh PSS needs to be prepared daily)
4. Prepare calcium (Ca^{2+}) free PSS: For 100 ml PSS without Ca^{2+}, dissolve 0.036 g KCl, 0.654 g NaCl, 0.029 g $MgSO_4$, 0.016 g KH_2PO_4, 0.207 g glucose, 0.218 g $NaHCO_3$, 0.238 g HEPES, 0.015 g EGTA and 0.0026 g Sodium nitroprusside (SNP) in 95 ml of Milli-Q water. Adjust pH to 7.3-7.4 using 1 N NaOH. Make up the volume to 100 ml with Milli-Q water.

2. Preparation of Glass Cannulas

1. Pull glass pipettes to generate the 100-150 µm tipped cannulas using a pipette puller as per manufacturer's guidelines.
2. Bevel the glass cannula tips using a microelectrode beveller, fire polish them and bend the glass cannula tips by ~45° using a heater probe.
3. Load the cannulas into micropipette holder and attach the micropipette holder on to the perfusion chamber.

3. Preparation of Perfusion Chamber

1. Rinse perfusion chamber with Milli-Q water followed by dissection solution for 5 min each. Load the chamber with 2 ml of dissection solution.
2. Suction dissection solution through cannula using 10 ml syringe and fill carefully the entire cannula and the attached tubing without any bubbles. Apply suction gently to prevent generation of bubbles.
3. Prepare two sutures with a half-knot each using blunt forceps. Since ophthalmic monofilament nylon sutures (10-0, 0.2 metric) are used to prepare the knots that are only 1-2 mm in diameter, dissection microscope may be needed.
4. Visualizing under dissection microscope, use dissection forceps to load both cannulas with partially closed suture knots slightly away from the tip. Later these knots will be slid carefully onto the cannulated arterial ends and closed completely.

4. Collection of Mesenteric Artery Arcade from Sprague-Dawley Rats

1. Seek approval of the local Institutional Animal Care and Use Committee (IACUC) before conducting these experiments. House animals in the animal facility with controlled temperature and lighting and allow free access to water and a commercial rodent chow.
2. Anesthetize rats by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Confirm deep anesthesia by toe-pinch and if needed administer additional anesthetics.
3. After confirming the surgical anesthesia, euthanize the animal by decapitation. Follow AAALAC guidelines for utilizing appropriate methods for animal euthanasia.
4. Use a dissection scissor and a forceps to perform a mid-line laparotomy from pelvis to sternum. This is done in two steps: first, incise the skin and second, incise the underlying muscle layer. Care must be taken not to injure the intra-abdominal organs.
5. Cut the proximal end of intestine close to the pylorus and the distal end close to the ileo-cecal junction. Tie both ends separately to prevent leak of chyme and feces thus avoiding contamination of extracellular bathing solution. Incise the mesentery at its base near the feeding vasculature *i.e.*, superior mesenteric artery and transfer the entire small intestinal mesenteric bed to a 50 ml beaker containing ice-cold dissection solution.
6. Allow harvested tissue to stay in ice cold dissection solution for 5 min and rinse with fresh dissection solution to get rid of blood.

5. Isolation and Cannulation of 4th Order Mesenteric Artery

1. Pin down the proximal end of the intestine on the right hand side in a sylgard-coated dish. Extend the remaining intestine in a counter clockwise path, pinning the segment down to spread the mesentery and exposing the blood vessels (**Figure 1**). Note: We isolate arterial segments at room temperature. Otherwise we place the mesenteric arcade containing dish on ice. Some labs, including those at our institution, use chiller units to dissect arteries at 4 °C.
2. Under a stereo zoom microscope dissect out 3rd and 4th order small mesenteric arteries (~260 µm) parallel to the small intestine using small scissors. First dissect away all the covering fat. Then dissect out the vein and isolate the artery with V-shaped branch point. Be careful not to puncture the selected segment. Start dissecting the fat near a 2nd order branch and find the way to 3rd or 4th order vessels.
    1. Note: Arteries and veins can be distinguished based on their wall thickness - arterial wall is thicker than vein's. Moreover, when adjoining connective tissue is pulled gently perpendicular to the vessels, veins collapse readily while arteries don't. Since arteries with lumen diameter <400 µm are major sites of systemic vascular resistance, for this protocol we used 4th order rat mesenteric arteries (lumen diameter <300 µm).
3. Isolate a 4-5 mm section of artery parallel to the small intestine. Visualize all the 5th order branches embedding into small intestine and cut them slightly away from the origin of branches and preserve a portion. These preserved portions of branches serve as holding sites (with dissection forceps) for transferring arterial segments to a perfusion chamber and, subsequently guide their cannulation.
4. Then cut the arterial segments by making 2 incisions distal to the 5th order branches on each side of the artery and transfer it to the perfusion chamber (see **Figure 1C** and legend).

5. Cannulate one end of the vessels on one of glass micropipette (diameter: 100-150 µm) using dissection forceps by holding the tips of the arterial segment with dissection forceps. Slide the previously loaded partially closed suture onto the cannulated end and secure it. Note: Proximal end of the artery may be cannulated onto the glass cannula that is connected to servo-controlled pressure-regulating device to mimic *in situ* environment.
6. Attach a dissection solution loaded 10 ml syringe to the stopcock connected to this cannula such that dissection solution in the tubing connecting the cannula and stopcock merges with that in the syringe. Gently raise the syringe. The gravitational force on the solution will remove the intra vascular blood from the open end of the vessel. After removing the intra-arterial blood, close the stopcock.
Note: Alternatively, attach the stopcock to the pressure controller, turn it on and gently increase the pressure to 5-10 mm Hg to achieve the same result.
7. Tie the distal end of the vessels onto a second glass cannula by carefully bringing the other cannula as close as possible to the untied end of the arterial segment. Slide the previously loaded partially closed suture onto the cannulated end and secure it. Care must be taken not tug or pull on the arterial segments. Make sure that stopcocks attached to both cannulas are closed.
8. Transfer perfusion chamber on to the stage of inverted microscope equipped with live video recording.
9. Connect the stopcock of cannula tied to the proximal end of arterial segment to a servo-controlled pressure-regulating device and make sure that stopcock attached to the other cannula remains closed to maintain stable intraluminal pressure.
10. Next, attach the vacuum tubing to the suction port and the perfusion tubing to the perfusion port of the chamber.
Note: Beveled needle port is used for suction and blunt needle port for perfusion.
11. Start perfusion of vessel with warm PSS through single inline solution heater (37 °C, equilibrated with gas mixture: 5% CO_2, 5% O_2 and 90% N to maintain neutral pH and adequate oxygenation[17]) at 2 ml/min using a peristaltic pump. Turn the vacuum on as well. Place a thermistor in the chamber to monitor temperature continuously.
12. As the temperature of PSS in the chamber nears ~37 °C (usually within 5 min), slowly increase intraluminal pressure from 20 to 100 mmHg and check vessels for leaks. This is done using the automatic pressure setting of pressure regulator. Discard vessels with leak and replace with another segment. The vessels with leaks will not be able to hold the pressure.
13. Assess the arterial segment for bends while maintaining the pressure at 100 mmHg. Using the screw-lever, move the cannula to straighten the arterial segment. Do not over stretch the arterial segments; the goal is to mimic *in vivo* arterial segment length.
14. Reduce the pressure to 70 mmHg (to mimic *in vivo* pressure in the mesenteric arcade[18]) and allow the arterial segment to stabilize and develop myogenic tone. Arteries may be pressurized variably (40-70 mmHg) according to experimental strategy and vascular bed. A previously published review provides an excellent review of variability in MT in arterial segments from different vascular beds[8].

6. Measurement of Arterial Diameter

1. View arteries at 10X objective on a microscope equipped with a monochrome video charge-coupled device camera. Measure luminal diameter using video frame grabber and real-time edge-detection system. A list of equipment used is provided in **Table 3**.
2. Monitor and record vessel diameter continuously.
3. Observe for development of MT. Note: We observed that in rat mesenteric resistance arteries, at 70 mmHg, development of MT is characterized by ~20% decrease in diameter. MT varies according to vascular bed and animal species.
4. Confirm vascular viability by assessing vasoconstrictor and vasodilator responses to 1 µM phenylephrine (Phe) and 1 µM acetylcholine (ACh).
5. At the end of each experiment, determine passive diameter (PD) by incubating arteries in Ca^{2+}-free PSS for 20 min.

7. Myogenic Response

1. Reduce the pressure to 20 mm Hg and allow the diameter to stabilize. Increase the intraluminal pressure in incremental steps (20, 40, 60, 80 and 100) and at each pressure step allow arteries to achieve a stable diameter (usually within 5 min).
2. Reduce the intraluminal pressure to 20 mmHg and incubate the arterial segment in Ca^{2+}-free PSS containing 0.39 mM EGTA and 0.1 mM SNP. Allow the arterial diameter to stabilize (usually 15 min).
3. Repeat the pressure-step response in Ca^{2+}-free PSS containing 0.39 mM EGTA and 0.1 mM SNP.

8. Interpretation of Results and Calculation Of Data

1. Calculate the MT as the percent difference in diameter observed for Ca^{2+}-containing versus Ca^{2+}-free PSS at each pressure according to following calculation:

$$\frac{\text{diameter at pressure 70 mmHg in } Ca^{2+}\text{-free PSS} - \text{diameter at pressure 70 mmHg in } Ca^{2+}\text{-containing PSS}}{\text{diameter at pressure 70 mmHg in } Ca^{2+}\text{-free PSS}} \times 100$$

2. For arteries undergoing vasomotion the diameter may be calculated by averaging the plateau phase for 1 min. Express the collected data as percent of maximal relaxation (%PD) according to the relationship: %PD = 100 x [ΔD/PD]; ΔD is the difference between the diameter before and after addition of any investigational compound (*e.g.* Phe); PD is passive diameter (also the maximal diameter).

Representative Results

Schematic representation of a typical pressure myograph set-up is shown in **Figure 1**. The two ends of the vessel are cannulated with a glass micropipette and secured with sutures on both sides. Via tubing and an open stopcock, one cannula is connected to a servo-controlled pressure-regulator; the other cannula is connected to a closed stopcock. The chamber is perfused with PSS and vascular diameter changes are observed by an inverted microscope connected to a CCD camera.

The arterial segment pressurized at 70 mmHg is incubated in freshly prepared warm PSS, which flows through the arterial chamber at 2-4 ml/min and suctioned out. Arterial diameter is monitored and recorded using videomicroscopy and edge detection software. After ~40 min, arterial segments constrict spontaneously by 20-40% of their starting diameter (**Figure 2A**). In our hands small rat resistance arteries constrict by 25-30% (average varies according to settings, operator, and arterial bed). Then, functional viability is assessed by vasodilator and vasoconstrictor responses to ACh (1 µM) and Phe (1 µM), respectively (**Figure 2A**). While other vasodilators may be used, ACh induces endothelium-dependent vasodilation and thus is useful in assessing both endothelial as well as vascular smooth muscle viability. Subsequently the arterial segment is re-incubated in PSS and once the diameter stabilizes, it is ready for experiment. At the end of each experiment, arterial segments are incubated in Ca^{2+} free PSS to measure PD (**Figure 2B**). The diameters recorded in **Figure 2A** and **2B** are tabulated in **Figure 2C**. Absolute MT is the difference between PD and stable diameter achieved upon spontaneous vasoconstriction at 70 mmHg. Hence, the MT observed from the tracing shown is 33% of PD. As seen here, response to ACh (1 µM) is generally similar to that observed for Ca^{2+} free PSS. Note that in experiments assessing vasodilation, prior addition of a vasoconstrictor may be needed.

To determine myogenic response, rat mesenteric arterial segments are subjected to increasing intraluminal pressure steps between 20 and 100 mmHg. An example is shown in **Figure 3A**. The arteries are allowed to achieve a stable diameter after each step (~5 min; dashed lines). Subsequently, the same arterial segment is subjected to pressure-response in Ca^{2+}-free PSS with 0.39 mM EGTA and 0.1 mM SNP (**Figure 3A**). The diameter achieved at the end of each pressure step may be shown as a line graph (**Figure 3B**). MT calculated as the percent difference in diameter for Ca^{2+}-containing vs. Ca^{2+}-free PSS at each pressure may be shown as line or bar graph (**Figure 3C**).

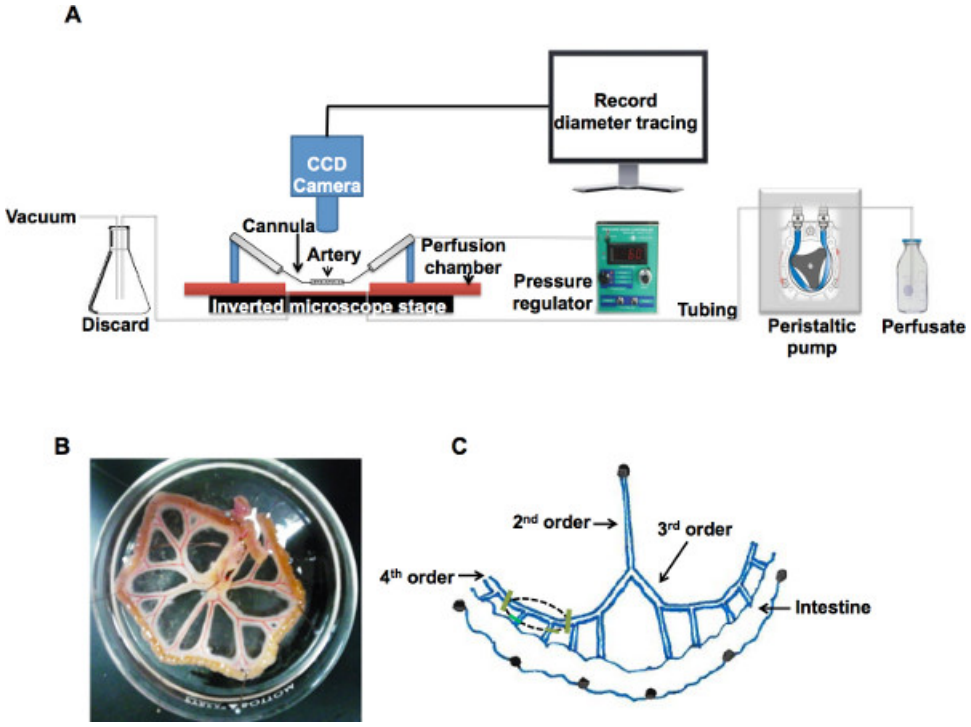
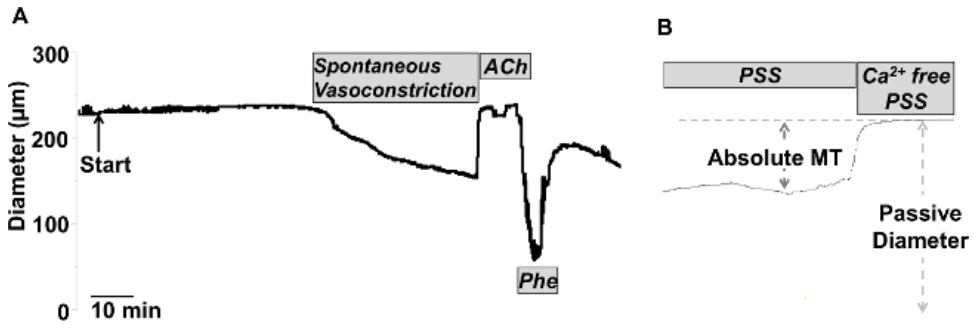


Figure 1: An illustration of pressure myograph set-up. (**A**) The key components are indicated. See **Table 3** for a list of all equipment. (**B**) Harvested mesenteric bed pinned on a sylgard-coated dish is shown. (**C**) A cartoon of mesenteric arterial arcade is shown. Black dots represent pin positions. The dashed section represents an arterial segment to be dissected. Small green bars indicate the incision sites on the artery. Click here to view larger figure.



C

Perfusate	Diameter (µm)
PSS (alone)	227
PSS (alone)—after 1hr	154
Ca^{2+} free PSS	230
Absolute Myogenic Tone	76

Figure 2: (**A**) As indicated by the tracing, diameter of small mesenteric arteries from rats, when pressurized at 70 mmHg, decreases spontaneously. Addition of ACh (1 µM) increased the diameter (to near-starting diameter). Addition of Phe (1 µM) to tissue bath decreases arterial diameter. (**B**) Incubation in Ca^{2+}-free PSS increases arterial diameter. (**C**)The diameter of a single pressurized arterial segment in various perfusates shown in A and B is tabulated.

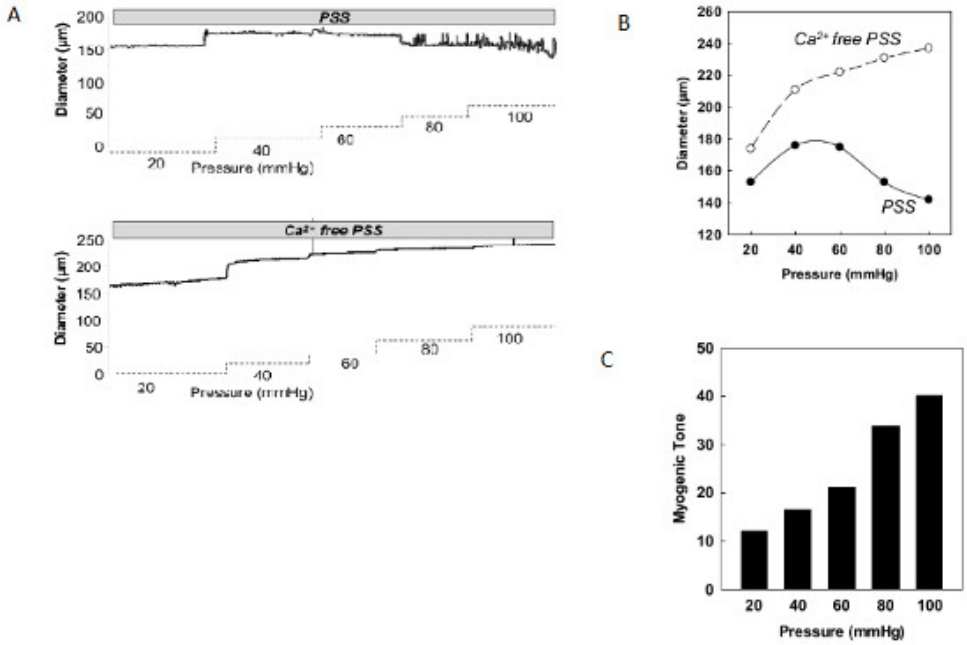


Figure 3: (**A**) Arterial diameter is recorded continuously while increasing intraluminal pressure incrementally in the presence of PSS and Ca^{2+}-free PSS. (**B**) Line curve of arterial diameter achieved at the each pressure step. (**C**) Bar graph of MT achieved at each pressure step. Click here to view larger figure.

Discussion

Critical steps, troubleshooting and modifications

In a typical isobaric vessel preparation, the artery is pressurized at 70 mmHg between two glass cannulas perfused with warm (37 °C) PSS. After 30-45 min, arteries develop MT, characterized by spontaneous decrease in diameter that stabilizes in 20-30 min. The resistance arteries from various vascular beds develop variable MT. For example rat resistance mesenteric arteries develop MT ~25% of PD, while cremastric arteries

may achieve MT ~40% of PD. Arteries that do not develop MT within 60 min should be discarded; this duration may vary according to vascular bed and species. Arteries with inadequate response to Phe and ACh should also be discarded.

pH and temperature of the PSS have a significant impact on the development of MT. pH of PSS, which sits for long periods without aeration, may increase. Additionally, at room temperature arteries are unlikely to develop MT. Hence the PSS should be aerated as soon as possible using the gas mixture indicated in the protocol section and temperature of the perfusion chamber should be monitored continuously and maintained at ~37 °C using a flow heater.

Since these experiments are 3-5 hr in duration, perfusion chambers and associated tubing are exposed to PSS for long periods; salt-precipitates can build up in both the chamber and tubing which may interfere with subsequent experiments. Hence it is critical to thoroughly wash the perfusion chamber and rinse the tubing with de-ionized water after each experiment. Similarly, care must be taken to thoroughly clean the sylgard-coated dish used for dissection with de-ionized water after each dissection.

Limitations

Despite its importance, PM has various limitations. First, the collective cost to procure PM equipment is high (~$22,000) and may be prohibitive for certain labs; a detailed list of equipment is shown in **Table 3**. Second, fresh vessels are needed for most experiments; hence a new animal is euthanized for each experiment, adding to the overall cost. Third, dissection of small mesenteric arteries is tedious and requires other instruments such as dissection microscope and microdissection tools, which are prone to damage. Fourth, there is a learning curve; gaining expertise in and establishing PM in a lab requires dedicated staff, time and effort.

Significance with respect to other methods and future applications

Isobaric and isometric experimental protocols are two major approaches used to determine vascular reactivity. In contrast to isobaric preparations, vasoactivity in isometric preparations is determined by measuring vascular smooth muscle tension using a wire myograph system. In addition to differences in equipment required for these two experimental protocols, agonist-induced contraction is different among these experimental approaches in regards to magnitude, time-course and direction of vascular wall tension[11,19]. Because of technical conveniences and limitations, both preparations serve important roles. For example, because it is easier to maintain microscopic focus on isometric preparations, they are often used for simultaneous measurement of vascular reactivity and changes in vascular smooth muscle Ca^{2+}. On the other hand, myogenic activity is best assessed in pressurized preparations that are considered to mimic *in vivo* physiological state closely. A detailed review of differences among these preparations is provided previously[19].

In conclusion, pressure myography is a reliable technique to study myogenic response in small resistance vessels at near-physiological conditions. Despite its limitations, PM has provided significant contributions to the understanding of changes in vascular function in normal and pathologic conditions[3-7,20-23]. Regulation of systemic vascular tone is highly complex and involves local and neuro-hormonal factors hence isolating the role of specific mechanisms regulating tone of vascular beds *in vivo* is difficult. In this regard, *ex vivo* pressurized arterial preparations serve as excellent surrogates. Those interested in the transduction mechanisms of MT and myogenic response are referred to previously published excellent reviews[15,19]. In the future we may see advances in equipment that integrate assessment of myogenic response and changes in downstream messengers such as Ca^{2+} though it is highly unlikely that we would see a reduction in equipment costs. However, as this technique is adopted by scientists with varied background, we will likely see its application to assess changes in microvascular function in diseases other than hypertension, diabetes and shock such as cirrhosis, dementia *etc.*

Disclosures

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