Video Article

Isometric Contractility Measurement of the Mouse Mesenteric Artery Using Wire Myography

Jie Sun[1], Guang Ming Yang[2], Tao Tao[1], Li Sha Wei[1], Yang Pan[2], Min Sheng Zhu[1]

[1]Model Animal Research Center and MOE Key Laboratory of Model Animal and Disease Study, Nanjing University

[2]School of Pharmacy, Nanjing University of Chinese Medicine

Correspondence to: Yang Pan at y.pan2006@163.com, Min Sheng Zhu at zhums@nju.edu.cn

URL: https://www.jove.com/video/58064
DOI: doi:10.3791/58064

Keywords: Medicine, Issue 138, Mouse mesenteric artery, myograph, vascular smooth muscle contraction, small resistance vessel, drugs, Chinese herb

Date Published: 8/20/2018

Citation: Sun, J., Yang, G.M., Tao, T., Wei, L.S., Pan, Y., Zhu, M.S. Isometric Contractility Measurement of the Mouse Mesenteric Artery Using Wire Myography. *J. Vis. Exp.* (138), e58064, doi:10.3791/58064 (2018).

Abstract

The wire myograph technique is used to assess the contractility of vascular smooth muscles in response to depolarization, GPCR agonists/inhibitors and drugs. It is widely used in many studies on the physiological functions of vascular smooth muscle, the pathogenesis of vascular diseases such as hypertension, and the development of smooth muscle relaxant drugs. The mouse is a widely used model animal with a large pool of disease models and genetically modified strains. We introduced this method to measure the isometric contraction of mouse mesenteric artery in detail. A 1.4-mm segment of mouse mesenteric resistance artery was isolated and mounted on a myograph chamber by passing two steel wires through its lumen. After equilibration and normalization steps, the vessel segment was potentiated by a high-K^+ solution twice prior to the contraction assay. As an example of the application of this method in drug development, we measured the relaxant effect of a novel natural substance, neoliensinine, isolated from a Chinese herb, embryos of the lotus seed (*Nelumbo nucifera* Gaertn.) on mouse mesenteric arteries. The vessel segments mounted on the myograph chamber were stimulated with a high-K^+ solution. When the force tension reached a stable sustained phase, cumulative doses of neoliensinine were added to the chamber. We found that neoliensinine had a dose-dependent relaxant effect on smooth muscle contraction, thus suggesting that it bears potential activity against hypertension. In addition, as the vessel segment can survive at least 4 hours after mounting and maintain contractility induced by the high-K^+ solution for many times, we suggest that the wire myograph system may be used for the time-consuming process of drug screening.

Video Link

The video component of this article can be found at https://www.jove.com/video/58064/

Introduction

The small vessel myograph system used here was for measuring the isometric contraction of small resistance vessels with internal diameters ranging from 100 to 400 µm. Isolated small vessels (about 2 mm long) were inserted by two 40-µm diameter wires and were then mounted on the micrometer-side and transducer-side jaws sequentially. This myograph technique was first suggested in 1972[1] and then developed primarily by Mulvany and his colleagues[2,3,4,5,6]. It is now a mature technique with stable equipment, easy performance and a standard normalization procedure[7,8,9]. We utilized this method with some modifications for measurements in the mouse mesenteric artery.

Vascular smooth muscle lines the walls of almost all blood vessels. Their fundamental function is to generate forces through contraction in response to various stimuli. The normal contractility of vascular smooth muscle is essential for blood pressure regulation and nutrition supplement[10]. Abnormal regulation of blood pressure results in a variety of diseases, including hypertension, heart failure and ischaemia. Several studies have suggested that abnormal blood pressure is always associated with dysfunctional vascular smooth muscle contractility[7,11,12,13]. The myograph method allows investigation of isometric contractility of mouse vessels induced by various stimuli including vasoconstrictors, inhibitors and drugs. Successful measurements of contraction will help us understand the mechanisms of blood pressure maintenance and the pathogenesis of vascular smooth muscle-associated diseases and to explore novel therapeutic approaches.

Many Chinese herbs have been widely used for clinical treatment of vascular diseases; however, their effective ingredients usually remain unknown. Thus, isolation and identification of the effective components is very important for the development of novel drugs. Multi-wire myograph technology offers a simple approach for screening active components in herbs. We have reported several studies using the small vessel myograph system to investigate mouse mesenteric artery contraction and identified natural compounds with anti-hypertension activity[12,13,14]. Here, we describe the detailed protocol for the myograph method and assess the relaxant effect of neoliensinine isolated from embryos of lotus seed (*Nelumbo nucifera* Gaertn.)[14].

Protocol

Animal manipulations were approved by the Institutional Animal Care and Use Committee (IACUC) of the Model Animal Research Center of Nanjing University.

1. Solution Preparation

1. Prepare HEPES-Tyrode solution (H-T) using 137.0 mM NaCl, 2.7 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2 \cdot 6H_2O$, 5.6 mM D-glucose, and 10 mM HEPES, pH 7.3-7.4.
2. Prepare HEPES-Tyrode solution without calcium (Ca^{2+}-free H-T) using 140.6 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 5.6 mM D-glucose, and 10 mM HEPES, pH 7.3-7.4.
3. Prepare HEPES-Tyrode solution using 124 mM KCl (High K^+) using 15.7 mM NaCl, 124.0 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2 \cdot 6H_2O$, 5.6 mM D-glucose, and 10 mM HEPES, pH 7.3-7.4.

2. Experiment Preparation

1. Preheat H-T and High-K^+ solutions using a 37 °C water bath.
2. Turn on the myograph system, data acquisition hardware and computer.
3. Carefully fill all myograph chambers with 5 mL of H-T solution each.
4. Fill two Petri dishes with 20 mL of 4 °C H-T and Ca^{2+}-free H-T solutions, respectively, and store on ice.
5. Fill a 10-cm coated Petri dish with 20 mL of H-T solution, and maintain it at room temperature.

3. Mouse Mesenteric Artery Dissection

1. Euthanize an 8-12-week-old C57BL/6J female or male mouse by cervical dislocation. Pin the mouse down with its abdomen facing up.
2. Moisten the abdomen with 70% ethanol. Then, cut the skin with scissors along the ventral midline from the groin, and make incisions from the start of the first incision downwards to the legs on both sides. Pull the skin back on both sides; make similar incisions to open the peritoneum.
3. Using scissors, cut the oesophagus, the colon and other connective tissues to completely isolate the gastrointestinal tract with feeding vasculature from the body.
4. With forceps, move the isolated segment into the dish containing the cold H-T prepared in step 2.4, and gently rinse the tissue in H-T solution several times to wash off the blood.
5. Transfer the isolated segment into the coated Petri dish prepared in step 2.5, and perform mesenteric artery dissection at room temperature.
6. Smooth out the stomach, jejunum, ileum and caecum in a clockwise direction, and pin the stomach and caecum on the left and right-hand side, respectively.
7. Stretch the mesenteric vasculature bed and fix the intestine with pins to expose the dissected mesenteric arteries.
   Note: Under these conditions, the arteries are on top of the veins.
8. Turn on the transmission light source of a stereoscopic microscope, and dissect arteries under the microscope. Make sure that the entire tissue is immersed in the solution.
9. Clamp the adipose tissues around the arteries with forceps, and isolate the arteries by cutting off all the connective tissues with dissection scissors. Avoid injuring the arteries.

4. Arterial Mounting

1. Transfer and immerse the mesenteric artery tree into the cold Ca^{2+}-free H-T solution (prepared in step 2.4) by clamping the excess arteries with forceps.
2. Cut off a 1.4-mm portion of the artery proximal to intestinal wall of a mesenteric arcade, and use two forceps to open both sides of this artery segment carefully.
3. Prepare two segments of stainless steel wire 2.5 cm in length, and place them into the same dish.
4. Gently clamp one end of the artery using forceps, and carefully insert two wires into the lumen of the artery one by one with help of another forceps. Ensure that the wires are kept straight and do not touch the endothelium.
5. Using two forceps, clamp the two steel wires outside of the threaded vessel simultaneously, and carefully transfer the vessel from the Petri dish to a myograph chamber previously filled with H-T solution (step 2.3).
6. Screw the jaws apart to make space for mounting. Clamp both sides of one of the two inserted wires using two forceps, and place the vessel in the jaw gap (**Figure 1A**).
7. Wrap both sides of the clamped wire around screws of the jaw connected to the micrometer (**Figure 1B**).
8. Fix the left-hand screw by twisting clockwise. Straighten the wire using right-hand forceps, then fix the right-hand screw by twisting clockwise (**Figure 1C**). Make sure that the vessel is always inside the jaw gap, but do not touch the jaw to avoid damage.
9. Close the two jaws using the micrometer (**Figure 1D**). Make sure the two jaws are close enough but that they do not touch each other and that the unfixed wire is on the top of the fixed wire.
10. Using the right-hand forceps, carefully fold the unscrewed wire at the corner of the jaw connected to force transducer, and wrap it clockwise around the right-side screw (**Figure 1E**). Then, fix the screw. Repeat this step on the left side of the wire and fix the left-side screw (**Figure 1F**).
11. Move the jaws slightly apart by carefully rotating the micrometer (**Figure 1G**). Avoid stretching the vessel. Use forceps to move the wire at the micrometer side to the horizontal plane of the wire at the transducer side. Carefully rotate the micrometer so that the gap between the two jaws can just accommodate the two wires.

12. Repeat Steps 4.2 – 4.11 to mount arteries onto the other chambers. Connect all the chambers to the equipment, cover the chambers, attach the 100% oxygen supply and a temperature probe, and start heating to 37 °C. Open the charting software and press the **Start** button on the **Chart View** window to start recording.
13. Equilibrate for about 20 min.

5. Normalization

Note: In order to standardize the experimental conditions and to obtain reliable physiological responsiveness of vessels, a normalization procedure is necessary[15]. According to the relationship between the active force and internal circumference of the vessel, the wire myograph system has a standard normalization program to assess the internal circumference (IC) of the mounted vessel[5,8,9]. Briefly, to calculate IC (μm), read the micrometer and input the value as the X value and the transducer output force, *i.e.,* resting wall tension (mN/mm), as the Y value. The program will return a fitted curve of (X, Y) and calculate the IC corresponding to a transmural pressure of 100 mmHg (IC_{100}). The vessel is set to the normalized internal circumference (IC_1) when the active responsiveness is maximal.

1. Set forces to zero for all channels on the device, and equilibrate for another 1-2 min.
2. Select **Normalization settings** from the "**DMT menu**, and set up the parameters as follows:
   Eyepiece calibration (mm/div): 0.36; Target pressure (kPa): 13.3; IC_1/IC_{100}: 0.9; Online averaging time (seconds): 2; Delay time (seconds): 60.
   Click the **OK** button to close the **DMT Normalization Settings** window.
3. Select the channel of interest from the **DMT menu** to open a DMT normalization window for the corresponding channel. Enter the constant values into the window as follows: Tissue end-points a1: 0.1; Tissue end-points a2: 4; Wire diameter (μm): 40. The window displays the calculated vessel length as 1.40 mm.
4. Read the micrometer of the appropriate tissue chamber. Enter the value into the **Micrometer reading** box, and click **Add Point** button. This value is the initial value of X (X0). After a 60 s delay time, the window displays the force and the effective pressure (ERTP) corresponding to this micrometer value. Simultaneously, the **Micrometer reading** box becomes active.
5. Stretch the vessel being normalized by turning the micrometer in a counter-clockwise direction. Enter the micrometer value into the **Micrometer reading** box, and click **Add Point** button. Wait for a delay time of 60 s again.
6. Repeat step 5.5, continue to stretch the vessel, and add micrometer values until the window displays the value of "Micrometer X_1", which is the calculated micrometer setting required to stretch the vessel to its IC_1.
7. Set the micrometer to X_1 value.
   Note: The normalized tension is usually 1-2 mN.

6. Artery Contraction Recording

Note: All the solutions, including H-T and High-K^+ solution used in this section, were prepared in step 2.1.

1. After normalization, equilibrate the vessel in the chamber for 15-20 min.
   Note: These is no need to change the solution in this step.
2. Challenge the vessel with High-K^+ solution twice.
   1. To challenge the vessel, replace H-T solution with 5 mL of High-K^+ solution to induce contraction for 10 min, followed by washing with 5 mL of H-T solution 3-4 times.
      Note: Typical contraction has a maximal force over 3 mN and a constant sustained force around 2.5 mN[12]. If the first challenge generates a maximal force below 2.5 mN or the sustained force decreases with time or the second challenge generates a much lower force than the first-time dose, the vessel is discarded and will not be used for further investigation.
3. Challenge the vessel with 5 mL of High-K^+ solution to induce contraction. After 5 min, add 0.5 μL of the neoliensinine stock solution (10 mM in DMSO)[14] into the chamber to relax the vessel at a final concentration of 1 μM neoliensinine.
4. When the force is stable (this usually takes several minutes), add another 0.5 μL of neoliensinine stock solution into the chamber to increase the concentration to 2 μM. Add 1 μL of the stock solution each time to increase the concentration to 4, 6, 8 and 10 μM to generate the dose-response curve.
   Note: The stock and working concentrations vary among drugs.

Representative Results

We measured the isometric contractility of mouse mesenteric artery using a multi-wire myograph system and assessed the relaxant effect of neoliensinine purified from embryos of lotus seed (*Nelumbo nucifera* Gaertn.)[14]. The mouse mesenteric resistance artery was isolated, cleaned of connective tissues and cut into 1.4-mm segments. The artery segment was inserted by two steel wires in Ca^{2+}-free H-T solution in a Petri dish, and then the segment was mounted on two jaws of a myograph chamber (**Figure 2A**). After mounting the segment, the two wires were adjusted to be parallel, close but not touching each other (**Figure 2B**). Prior to the force measurement, the vessel segment was normalized and potentiated twice by High-K^+ solution so as to stabilize the vessel. During the normalization procedure, the vessel was stretched several times until reaching the value of $IC_{100,}$ and each stretch cycle included a robust contraction, rapid relaxation and a force maintenance in 60 s (**Figure 3**). The contraction of the vascular smooth muscle induced by High-K^+ solution usually showed two phases, a robust phase and a sustained phase (**Figure 3**). The vessel segment can be used for further experiments only if the High-K^+-evoked contraction appears normal and reproducible. A typical measurement with neoliensinine is represented in **Figure 4**. When the force tension induced by High-K^+ reached a sustained phase, we added cumulative doses of neoliensinine (1, 2, 4, 6, 8 and 10 μM) through the holes in the chamber cover. As the doses increased, the force reduced in a dose-dependent manner. The result indicated that neoliensinine is a vascular smooth muscle relaxant substance that potentially acts as a candidate anti-hypertension drug[14].

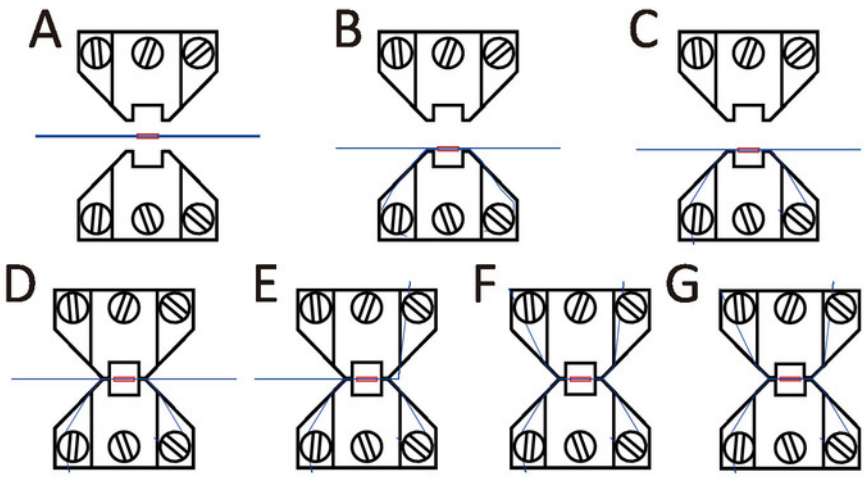

Figure 1: A schematic of arterial mounting procedure. The blue lines represent the wires, and the red rectangle represents the artery. Please click here to view a larger version of this figure.

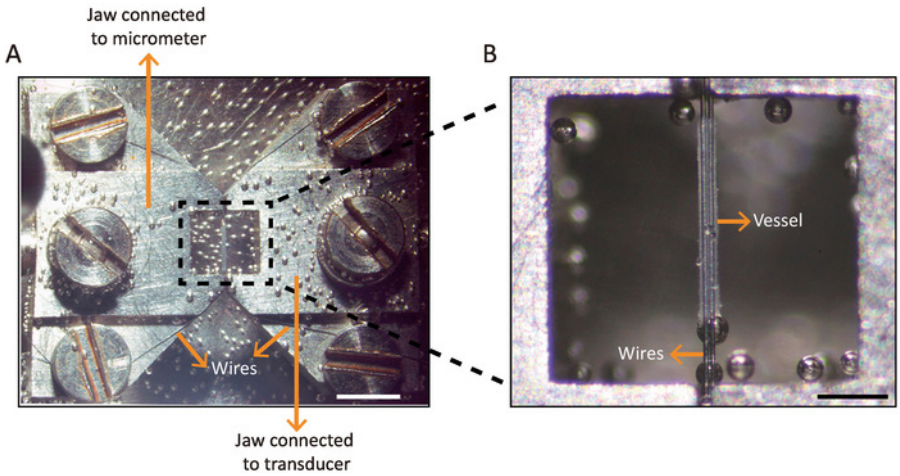

Figure 2: A mouse mesenteric artery segment mounted on the myograph chamber. (A) A mouse mesenteric artery segment mounted on two jaws using two steel wires. The white bar = 2 mm. **(B)** A microscopic image of the mounted mouse mesenteric artery segment in Panel (A). Black bar = 0.5 mm. Please click here to view a larger version of this figure.

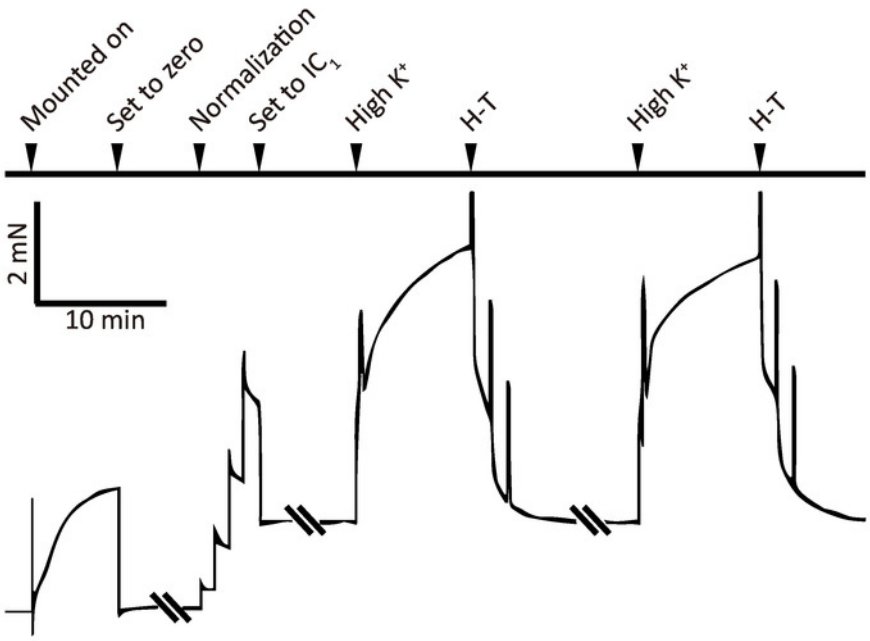

Figure 3: Representative original tracings showing the normalization procedure and potentiation by High-K^+ solution. After the second High-K^+ stimulation, the regular experiment can be performed. Please click here to view a larger version of this figure.

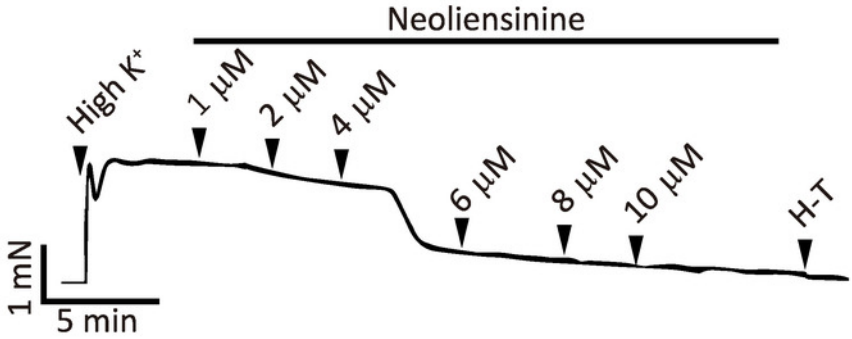

Figure 4: Representative tracing of mouse mesenteric artery that is contracted by High-K+ solution and then relaxed by adding accumulative doses of neoliensinine. As the doses increased, the force reduced in a dose-dependent manner Please click here to view a larger version of this figure.

Discussion

Hypertension is a widespread public health challenge due to its severe complications, including cardiovascular and kidney diseases[16]. Understanding the pathogenesis of hypertension and exploring more anti-hypertensive drugs has become an urgent task in this field. Blood pressure is generated and maintained by peripheral resistance of the circulation. According to Poiseuille's Law, the relatively small arteries generate a large proportion of circulatory resistance and serve as the dominant producer of blood pressure[3,10]. Thus, measurement of small-resistance arteries rather than large arteries is more suitable for studies of blood pressure. The wire myograph technology is one of the best modalities to study the physiological functions of small-resistance arteries and pathogenesis of vascular diseases.

The small vessel wire myograph system has been well documented in other reports and was used to measure contraction of rat mesenteric arteries[8] and mouse arteries such as the aorta[9]. Taking advantage of genetic manipulation, a variety of disease models and drug screening models, the mouse has become a widely used model animal in many fields. Therefore, here, we provided a modified protocol of this method for measurement of mouse mesenteric artery contraction. In this report, we successfully measured the contractility of mouse mesenteric arteries with modifications of the fundamental buffers and mounting steps. Many studies on *ex vivo* vasocontractility measurement used solutions containing $NaHCO_3$, such as Krebs solutions, to mimic physiological salt solution. However, such buffers need CO_2 to adjust the pH value throughout the measurement, resulting in the production of $CaCO_3$. We selected H-T solution as the buffer system and found it worked well. Since temperature has little effect on the pK_a value of HEPES, the pH value of the solution is conveniently adjusted at room temperature and is unchanged at 37 °C [17]. In addition, we use Ca^{2+}-free H-T solution when guiding the wires through the vessel lumen so as to prevent vessel constriction by Ca^{2+}. Another modification in this protocol is the mounting procedure. Some reports[8,9] and the device manual[5] recommend

guiding the second wire after fixing the first wire on the jaw. We find it works better when two wires are guided through the vessel lumen before mounting the vessel because this method may reduce possible damage of the transducer due to the limited chamber space.

Despite the high reproducibility of this method, we should pay more attention to some key steps. The most important is to avoid damage to vessels caused by forceps and scissors. During vessel dissection, the operator should use the forceps gently when stretching the adipose tissue and use the scissors carefully when cutting the connective tissues. In addition, clamping the vessel for fixation should be done gently, and damage to the endothelium should be avoided when guiding the wires because the endothelium-damaged vessel will give rise to abnormal responses, *e.g.,* the damaged vessel shows apparent force tension after stimulation with acetylcholine, while the normal vessel shows a relaxant effect. The explanation for this phenomenon is that the damaged endothelium cannot produce nitric oxide properly. Note that in the experiment involving endothelium-related contraction, the endothelium status should be tested prior to force measurement. In addition, we should also carefully mount the vessel on the jaws because the transducer is easily damaged if applied with a hard force. Finally, we usually do not use a constant increment value on the micrometer when performing normalization. The value of the increment is 30 or 20 µm initially and 10 µm after the effective pressure reaches 11-12 kPa. This method may reduce normalization time and may prevent overstretching, thereby attenuating vessel damage.

Although our investigation focused on mouse mesenteric arteries, this method can also be used for aorta, bronchi, and other small vessels including renal, brain and pulmonary arteries. Since this system includes four channels, it is convenient for measurement of four parallel samples simultaneously. In addition, an entire mesenteric vascular bed can provide at least four artery segments, it is thus very easy to design different experimental groups. According to our experience, each artery segment survives at least 4 hours and maintains good responses to the High-K^+ solution over at least 6 repetitions. This property is extremely useful for measurements of the effects of several additions of various candidate drugs. However, there are also limitations to the wire myograph system. The *ex vivo* wire myograph experiment is only able to measure isometric vasocontractility, but it should usually be combined with other measurements for complex analysis of the vessel.

In summary, we described a method for measurement of isomeric contractility in the mouse mesenteric artery using a multi-wire myograph system. This method can be used to assess the functions of vascular smooth muscle and to screen relaxants of smooth muscle.

Disclosures

The authors have nothing to disclose.

Acknowledgements

We thank Dr. Wei Qi He (Soochow University, Suzhou, China) and Dr. Yan Ning Qiao (Shaanxi Normal University, Xi'an, China) for the technical assistance. This work was supported by the National Natural Science Foundation of China (Grant 31272311, 81373295 and 81473420) and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (Grant No. ysxk-2016).

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