Video Article

Assessing Murine Resistance Artery Function Using Pressure Myography

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Abstract

Pressure myograph systems are exquisitely useful in the functional assessment of small arteries, pressurized to a suitable transmural pressure. The near physiological condition achieved in pressure myography permits in-depth characterization of intrinsic responses to pharmacological and physiological stimuli, which can be extrapolated to the *in vivo* behavior of the vascular bed. Pressure myograph has several advantages over conventional wire myographs. For example, smaller resistance vessels can be studied at tightly controlled and physiologically relevant intraluminal pressures. Here, we study the ability of 3rd order mesenteric arteries (3-4 mm long), preconstricted with phenylephrine, to vaso-relax in response to acetylcholine. Mesenteric arteries are mounted on two cannulas connected to a pressurized and sealed system that is maintained at constant pressure of 60 mmHg. The lumen and outer diameter of the vessel are continuously recorded using a video camera, allowing real time quantification of the vasoconstriction and vasorelaxation in response to phenylephrine and acetylcholine, respectively.

To demonstrate the applicability of pressure myography to study the etiology of cardiovascular disease, we assessed endothelium-dependent vascular function in a murine model of systemic hypertension. Mice deficient in the α_1 subunit of soluble guanylate cyclase ($sGC\alpha_1^{-/-}$) are hypertensive when on a 129S6 (S6) background ($sGC\alpha_1^{-/-S6}$) but not when on a C57BL/6 (B6) background ($sGC\alpha_1^{-/-B6}$). Using pressure myography, we demonstrate that $sGC\alpha_1$-deficiency results in impaired endothelium-dependent vasorelaxation. The vascular dysfunction is more pronounced in $sGC\alpha_1^{-/-S6}$ than in $sGC\alpha_1^{-/-B6}$ mice, likely contributing to the higher blood pressure in $sGC\alpha_1^{-/-S6}$ than in $sGC\alpha_1^{-/-B6}$ mice.

Pressure myography is a relatively simple, but sensitive and mechanistically useful technique that can be used to assess the effect of various stimuli on vascular contraction and relaxation, thereby augmenting our insight into the mechanisms underlying cardiovascular disease.

Video Link

The video component of this article can be found at https://www.jove.com/video/50328/

Introduction

Pressure myograph systems are used to measure the physiological function and properties of small arteries, veins and other vessels. An intact small segment of an artery or vein is mounted onto two small glass cannulas and pressurized to a suitable luminal pressure, allowing the vessel to maintain most of its *in vivo* characteristics (**Figures 1** and **2**). The near physiological condition in a pressure myograph reflects the *in vivo* behavior of the vascular bed, allowing the investigation of intrinsic properties (*e.g.* myogenic tone) of isolated vessels. Some of the advantages of pressure myography over wire myography, where muscle contraction is assessed by direct mechanical coupling to a force transducer [1], include (i) that micro resistance arteries, which define the overall resistance developed in vascular system, can be studied, whereas wire myograph is limited to larger conduit arteries, (ii) that the risk to damage the endothelium is reduced since no wires need to be passed through the vessel lumen, (iii) that the natural shape of the vessel is better maintained, and (iv) that vessel dimension can be studied at a wide range of pressures or shear stress [2].

Studying micro vessels can be more informative than studying larger conduit arteries to help understand the pathophysiology and molecular mechanisms that contribute to altered vascular tone in cardiovascular diseases such as hypertension. For example, impairment of endothelial function associated with feeding mice a high fat diet for 8 weeks could be demonstrated in 2nd-order mesenteric arteries [3] but not in aortic rings (**Figure 3**). Another advantage of pressure myography is that intrinsic myogenic constriction of the pressurized vessel is present, and that the role and function of the endothelium in this phenomenon can be studied. Here, we describe the use of a pressure myograph to study vascular reactivity of mouse mesenteric 3rd order resistance arteries in a setting of impaired nitric oxide (NO)-cGMP signaling.

Protocol

1. Preparation of Solution

1. 500X EDTA stock: weigh 500 mg EDTA-Na_2•$2H_2O$ and dissolve in 50 ml deionized water. Store at room temperature.
2. KCl depolarizing solution.
    1. Prepare K10X stock solution: 3.69 g NaCl, 18.64 g KCl, 0.36 g $MgSO_4$ anhydrous, 0.41 g KH_2PO_4, 0.46 g $CaCl_2$•$2H_2O$. Dissolve in 250 ml deionized water. Store at room temperature.
    2. For 100 ml final KCl depolarizing solution 1X: Dissolve 0.21 $NaHCO_3$, 0.18 g glucose in 90 ml deionized water. Add 10 ml of K10X stock solution. Add 95 µl of 500X EDTA solution. Mix well. Store at 4 °C. Use within one week.

3. Prepare fresh HEPES-PSS solution on the day of experiment (For 1 liter).
    1. 6.96 g NaCl, 0.35 g KCl, 0.288 g $MgSO_4$•$7H_2O$, 0.1605 g KH_2PO_4, 2.38 g HEPES. Dissolve in 100 ml deionized water. Mark as flask A.
    2. 0.312 g $CaCl_2$•$2H_2O$. Dissolve in 100 ml deionized water. Mark as flask B.
    3. 1.25 g $NaHCO_3$, 0.991 g glucose. Dissolve in 98 ml deionized water. Mark as flask C.

4. Add 2 ml of 500X EDTA in flask C. Take 700 ml deionized water in a big flask. Pour contents of flask A, B and C in big flask with continuous vortexing. Adjust the pH to 7.4 with NaOH.

2. Drugs Used

1. Phenylephrine (10^{-2} M): Dissolve 20.37 mg in 10 ml deionized water. Aliquot 1 ml and store at -80 °C. Serially dilute to obtain 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mol/L on the day of experiment.
2. Acetylcholine (10^{-2} M): Dissolve 18.17 mg in 10 ml deionized water. Aliquot 1 ml and store at -80 °C. Serially dilute to obtain 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mol/L on the day of experiment.

3. Mice

Male WT and $sGC\alpha_1^{-/-}$ mice on the S6 and B6 background were studied throughout this study. The $sGC\alpha_1^{-/-}$ were generated as described previously [4,5]. Housing and procedures involving experimental animals (mice) were approved by the Subcommittee on Research Animal Care of Massachusetts General Hospital, Boston, MA.

4. Measurement of Vascular Reactivity in Mesenteric Artery

1. Euthanize the mice with pentobarbital (200 mg/kg, i.p.). Open the abdominal cavity and dissect out the mesenteric tissue. Isolate and dissect a mesenteric artery of 3rd order free of connective and adipose tissue, and place the artery in ice-cold HEPES-PSS solution pre-equilibrated with 95% O_2 / 5% CO_2 for 15 min. Cut rings of 2-3 mm length with no branching from the mesenteric artery. Differentiating between arteries and veins at micro vessels level is problematic when there is no blood in the vessels. Therefore, we recommend identifying the vessel while it is still intact in the animal before dissecting it out.
2. Fill in the glass cannulas in the pressure myograph chamber (DMT Model 110P&11P version 1.31, **Figure 2**) with HEPES-PSS solution with the help of a 10 ml syringe through inlet and outlet valves. Filling of cannula should be done gently and carefully as excessive pressure can damage the fragile transducer connected to cannulas. After filling cannulas close both inlet and outlet valves tightly.
3. Mount one end of the vessel onto right cannula and carefully tie it with one fine strand of nylon suture. With the help of a syringe, flush and fill in the vessel with HEPES solution via inlet valve. Bring in the left cannula closer to the vessel and mount the other end of vessel onto it. Tie it with nylon suture. Fill the chamber with up to 10 ml with HEPES solution. Check for the leak by gently pushing HEPES solution via inlet valve with the help of a syringe.
4. Install the chamber on the myograph and under the video camera. Start the oxygen and heat (37 °C) in the chamber. Connect the inlet valve with tube P1 from the first HEPES solution reservoir, while the valve is closed. Let any bubble and solution pass through the tube until there are no bubbles in the tube. Open the valve.
5. Connect the left valve of the chamber with tube P2 coming from pressure regulator. Again check for any bubbles. There should not be any bubbles or leakage in the entire system.
6. Go to the pressure menu on the myo-interface panel or in the software and turn ON the pump while turning OFF the flow.
7. Open the program and click COLLECT. Vessel should be seen on the screen now (**Figure 3**). The vessel is visualized with a camera fitted on a microscope, allowing monitoring and analysis of the vessel lumen, vessel diameter, and wall thickness.
8. Gradually raise the interluminal pressure via the P1 valve by selecting P1 pressure in the myo-interface panel or in the program and enter the pressure value as follows; 5 mmHg → 10 → 20 → 40 → 60 mmHg. Vessel sometime tends to twist or convolute while pressure is increasing; adjust the tension or strain accordingly with the help of vertical or longitudinal micropositioner (**Figure 2**). Equilibrate the vessel at 60 mmHg and 37 °C at least for 45 min. Change the bath solution once with pre-warmed HEPES during equilibration.
9. Apply 10 ml of KCl depolarizing solution, prewarmed at 37 °C, to the bath to fully depolarize smooth muscle cells and achieve maximum constriction. After stable constriction, rinse the bath with HEPES solution 3 times every 10 min.
10. Check the viability of the vessel and integrity of the endothelium by stable and reproducible responses to the addition of phenylephrine (10^{-5} M) and acetylcholine (10^{-5} M). Rinse 3 times with HEPES solution every 10 min.
11. Add phenylephrine (10^{-5} M) to preconstrict the vessel, and when a stable constriction has been obtained, perform a cumulative concentration-vasodilation response curve (CRC) by sequential addition of increasing doses of acetylcholine (10^{-9}, 3 x 10^{-9}, 10^{-8}, 3 x 10^{-8}, 10^{-7}, 3 x 10^{-7}, 10^{-6}, 3 x 10^{-6}, and 10^{-5} M). After last dose, rinse 3 times with HEPES solution every 10 min before starting new CRC.

12. Determine the passive lumen diameter by applying Ca^{2+}-free PSS containing 2 mM EGTA. From the recorded tracings measure lumen diameter for each dose response (**Figure 4**), which will be used to calculate all parameters.
13. Express all acetylcholine relaxation responses as percentage change in lumen diameter after phenylephrine preconstriction compared with the difference between calcium-free diameter and diameter after phenylephrine constriction, using the following equation:
14. Percentage dilation = 100% x $[(D_x - D_i)/(D_{Ca\text{-}free} - D_i)]$, where D is the measured lumen diameter and x, i, and Ca-free denote arterial diameters at each dose of agonist (x), initial diameter following phenylephrine constriction (i), and in Ca^{2+}-free buffer (Ca-free).

5. Statistical Analysis

All continuous measurements are expressed as mean ±SEM. The vascular reactivity in mesenteric arteries is analyzed by repeated-measures two-way ANOVA. In all cases, $P < 0.05$ is considered statistically significant.

Representative Results

NO is centrally involved in maintenance of blood pressure homeostasis both in humans [6] and in animal models [7]. The ability of NO to control vascular smooth muscle relaxation is mediated by soluble guanylate cyclase (sGC), a heme-containing heterodimeric enzyme that generates cGMP [8]. Recently, a blood pressure-modifying genetic variant was identified in a locus that contains the $sGC\alpha_1$ and $sGC\beta_1$ genes, illustrating the relevance of sGC in regulating blood pressure in humans [9]. Interestingly, male mice deficient in the α_1 subunit of sGC ($sGC\alpha_1^{-/-}$) are hypertensive when on a 129S6 (S6) background ($sGC\alpha_1^{-/-S6}$) [5] but not when on a C57BL/6 (B6) background ($sGC\alpha_1^{-/-B6}$) [4]. We used pressure myography to study vascular relaxation in response to acetylcholine in mesenteric resistance arteries isolated from WT and $sGC\alpha_1^{-/-}$ mice on the S6 and B6 backgrounds and preconstricted with phenylephrine (**Figure 4**). We studied resistance arteries because they define ultimate resistance and compliance of the vascular system and thus directly regulate blood pressure. $sGC\alpha_1$-deficiency was associated with a decreased ability of a cetylcholine to induce vascular relaxation, regardless of the genetic background of the mice studied (**Figure 4** and **5**). However, endothelium-dependent relaxation was more markedly impaired in $sGC\alpha_1^{-/-}$ mice on the S6 background than in $sGC\alpha_1^{-/-}$ mice on the B6 background (**Figure 5**) [10]. Together, these findings suggest that the decreased sensitivity of the vasculature to endothelial-dependent relaxation may contribute to the strain-specific hypertension in $sGC\alpha_1^{-/-}$ mice.

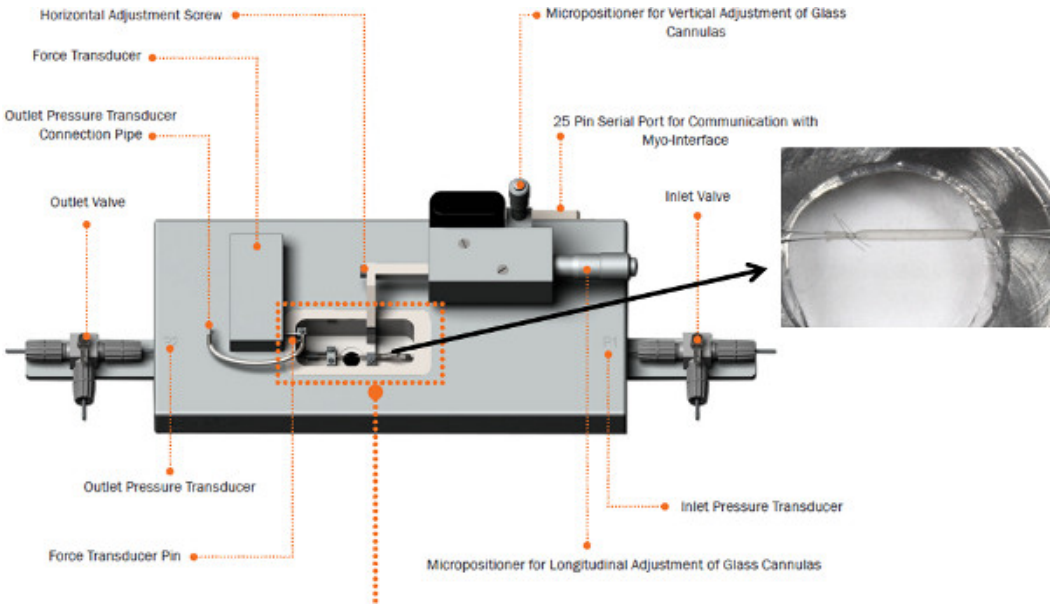

Figure 1. Schematic depicting the various parts of the pressure myograph chamber (DMT). Inset shows a mounted vessels on cannulas in the chamber. Click here to view larger figure.

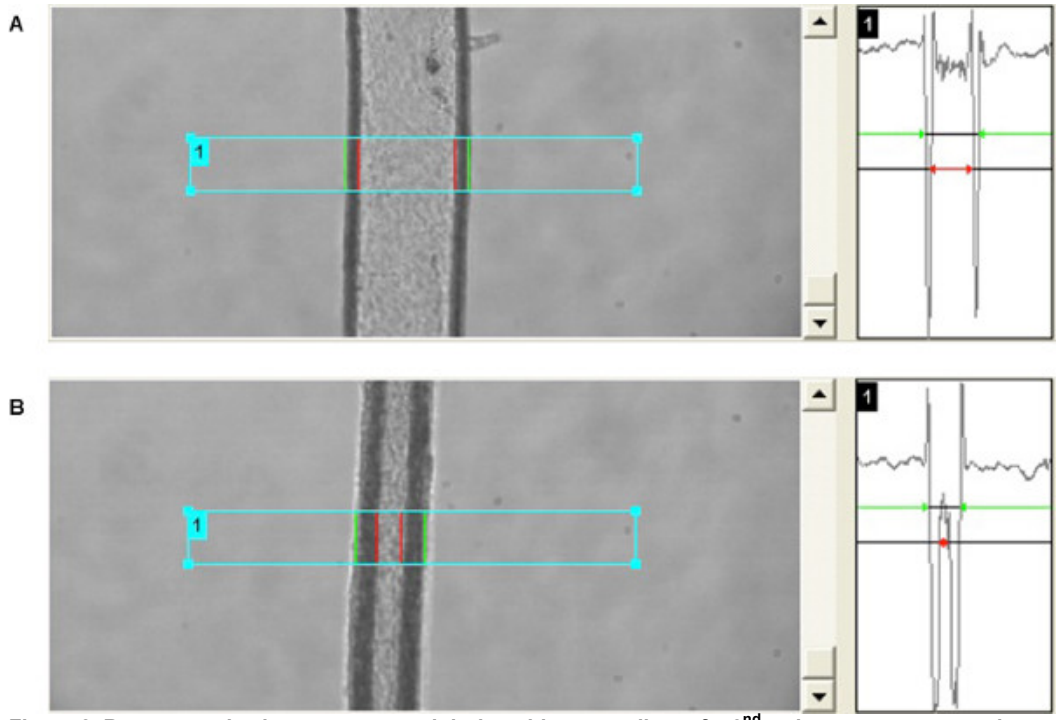

Figure 2. Representative images, captured during video recording, of a 2nd order mouse mesenteric artery under (A) basal and (B) phenylephrine-induced constriction. The outer diameter and vessel lumen are delineated by green and red lines, respectively. The traces in the right panels represent the signal intensity, measured in the area defined by the blue box in the images on the left, for outer diameter (green arrows) and lumen diameter (red arrows).

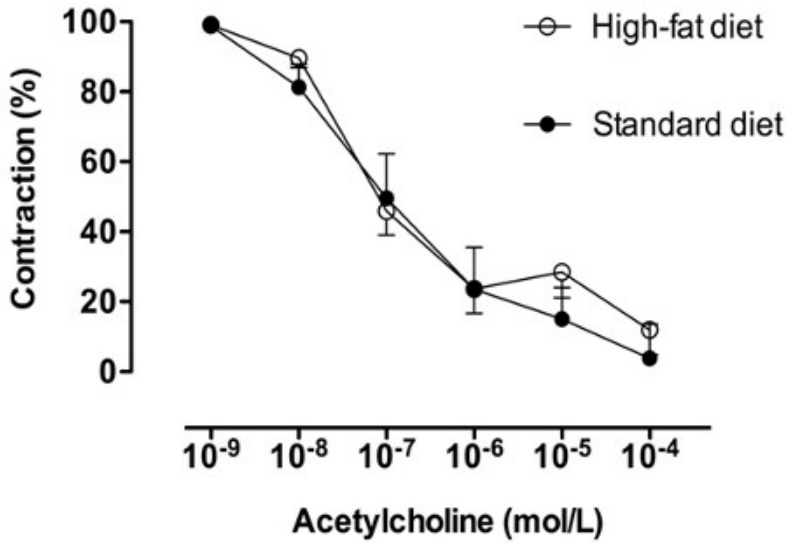

Figure 3. Endothelial dysfunction in mice fed with high-fat diet for 8 weeks was not apparent in aortic rings. Endothelial function was measured by the vasorelaxation response induced by acetylcholine (10^{-9} to 10^{-5} M) in phenylephrine (10^{-5} M) pre-contracted aortic rings. Standard diet, wild-type mice fed a standard diet (n=8); High-fat diet, wild-type mice fed a high-fat diet for 4-6 weeks (n=13).

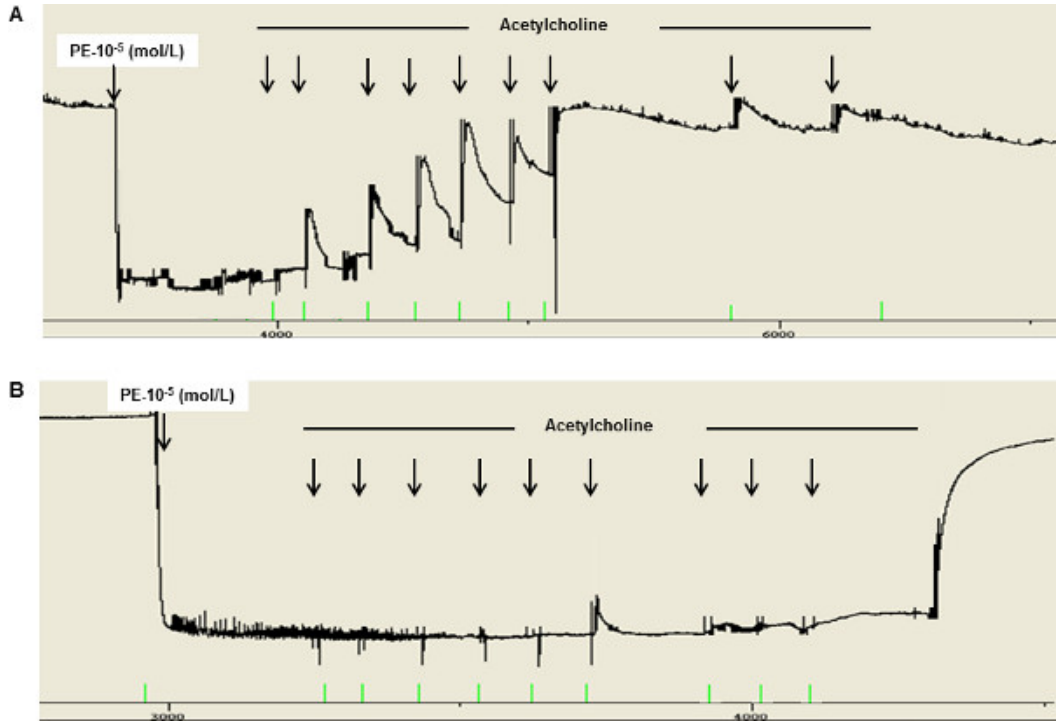

Figure 4. Representative original tracings of a dose response curve of acetylcholine on phenylephrine-proconstricted 2nd order mesenteric arteries isolated from a WTB6 (A) and a sGCα$_1^{-/-S6}$ mouse (B). Arrows represent cumulative addition of increasing doses of acetycholine (10^{-9} to 10^{-5} M). The X axis represents time (in seconds), the Y axis represents lumen diameter (in μM). Click here to view larger figure.

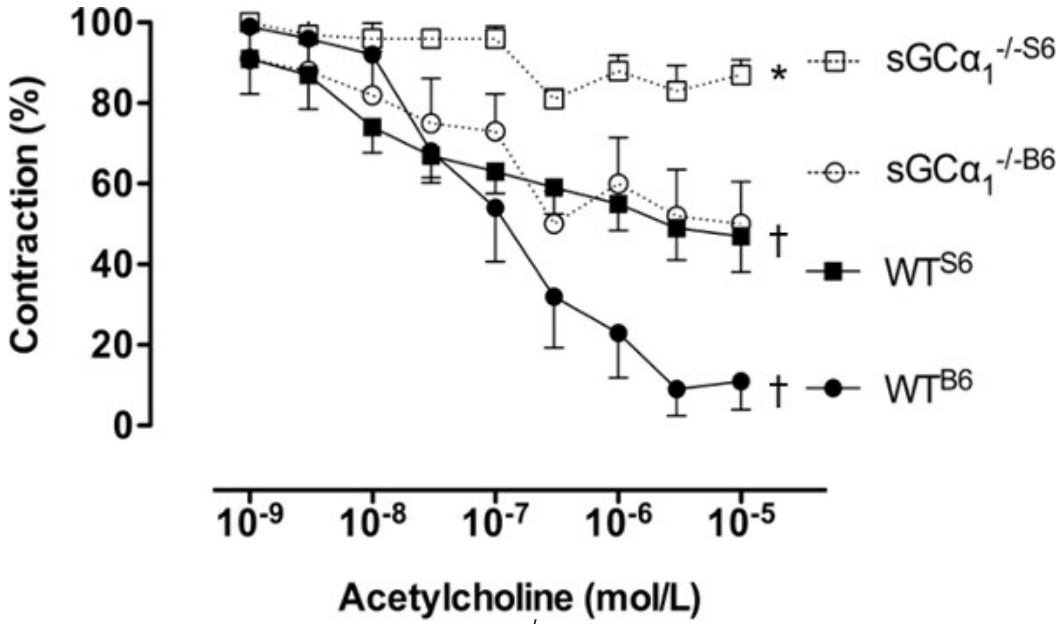

Figure 5. The strain-specific hypertension in sGCα$_1^{-/-}$ mice is associated with greater impairment of vascular reactivity in sGCα$_1^{-/-S6}$ mice than in sGCα$_1^{-/-B6}$ mice. The ability of acetylcholine (10^{-9} to 10^{-5}) to induce relaxation is quantitated in phenylephrine-precontracted (10^{-5} M) mesenteric arteries from male wild-type (WT, closed symbols, full line) and sGCα$_1^{-/-}$ (open symbnols, dashed line) mice on the B6 (circles) or S6 (squares) genetic background. Acetycholine-induced vascular relaxation is impaired to a greater extent in sGCα$_1^{-/-S6}$ than in sGCα$_1^{-/-B6}$ mice. N=3,5,3 and 6 for sGCα$_1^{-/-S6}$, sGCα$_1^{-/-B6}$, WTS6, respectively. †*P < 0.05 vs sGCα$_1^{-/-B6}$, P < 0.05 vs. sGCα$_1^{-/-}$ of the same genetic background. Adapted form Buys, *et al.* (2012).

Discussion

Mice are the experimental model of choice for many investigators, in part because of the possibility to introduce genetic modifications, thereby generating mouse models for human pathophysiology. The vasoactive status of small resistance but not of larger conduit vessels largely defines the regulation of blood flow throughout the vascular system [11]. The size of resistance arteries in small animals, such as mice, prevents the use

of a wire myograph to study microvascular function. Pressure myographs not only overcome this limitation but also allow for the measurement of vascular function under near physiological conditions.

We previously reported that male mice deficient in the $sGC\alpha_1$ are hypertensive when on a 129S6 (S6) background ($sGC\alpha_1^{-/-S6}$) [5] but not when on a C57BL/6 (B6) background ($sGC\alpha_1^{-/-B6}$) [4]. We hypothesized that this strain-related disparity in blood pressure is due to the differences in vascular reactivity between $sGC\alpha_1^{-/-}$ mice on the B6 and S6 backgrounds. Using a pressure myograph, we studied endothelium-dependent vasorelaxation responses in 3rd order mesenteric arteries. Acetylcholine is a muscarinic receptor agonist that binds to its receptor in the endothelium to activate endothelial nitric oxide synthase (eNOS) via increasing intracellular Ca^{2+} concentration. NO can subsequently activate sGC in the underlying smooth muscle cells layer to induce relaxation.

Using pressure myography to compare the ability of endothelial derived NO to vasorelax mesenteric arteries isolated from WT and $sGC\alpha_1^{-/-}$ mice on the S6 and B6 background, we identified differences in vascular reactivity between $sGC\alpha_1^{-/-}$ mice on the B6 and S6 backgrounds [10]. $sGC\alpha_1$-deficiency was associated with impaired endothelium-dependent vasorelaxation in both S6 and B6 mice. However, this impairment was greater in $sGC\alpha_1^{-/-S6}$ than in $sGC\alpha_1^{-/-B6}$ mice. The decreased ability of acetylcholine to induce vasorelaxation in S6 mice likely contributes to the increase in blood pressure observed in $sGC\alpha_1^{-/-S6}$ mice but not in $sGC\alpha_1^{-/-B6}$ mice. Although elevated blood pressure is often attributed to renal abnormalities [12], these results strengthen the emerging hypothesis that primary abnormalities of vascular smooth muscle tone, for example as associated with impaired NO-cGMP signaling [10,13], can directly contribute to the development of systemic hypertension [14].

In conclusion, the pressure myograph technique is a very powerful tool in the hands of the vascular physiologist or pharmacologist and can be used to analyze micro vessel function. Our study demonstrates the applicability of pressure myography in detecting underlying vascular abnormalities in a model of systemic hypertension.

Disclosures

The authors have nothing to disclose.

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