Video Article

Mesenteric Artery Contraction and Relaxation Studies Using Automated Wire Myography

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Abstract

Proximal resistance vessels, such as the mesenteric arteries, contribute substantially to the peripheral resistance. These small vessels of between 100-400 µm in diameter function primarily in directing blood flow to various organs according to the overall requirements of the body. The rat mesenteric artery has a diameter greater than 100 µm. The myography technique, first described by Mulvay and Halpern[1], was based on the method proposed by Bevan and Osher[2]. The technique provides information about small vessels under isometric conditions, where substantial shortening of the muscle preparation is prevented. Since force production and sensitivity of vessels to different agonists is dependent on the extent of stretch, according to active tension-length relation, it is essential to conduct contraction studies under isometric conditions to prevent compliance of the mounting wires. Stainless steel wires are preferred to tungsten wires because of oxidation of the latter, which affects recorded responses[3].The technique allows for the comparison of agonist-induced contractions of mounted vessels to obtain evidence for normal function of vascular smooth muscle cell receptors.

We have shown in several studies that isolated mesenteric arteries that are contracted with phenylyephrine relax upon addition of cumulative concentrations of extracellular calcium (Ca^{2+}_e). The findings led us to conclude that perivascular sensory nerves, which express the G protein-coupled Ca^{2+}-sensing receptor (CaR), mediate this vasorelaxation response. Using an automated wire myography method, we show here that mesenteric arteries from Wistar, Dahl salt-sensitive(DS) and Dahl salt-resistant (DR) rats respond differently to Ca^{2+}_e. Tissues from Wistar rats showed higher Ca^{2+}-sensitivity compared to those from DR and DS. Reduced CaR expression in mesenteric arteries from DS rats correlates with reduced Ca^{2+}_e-induced relaxation of isolated, pre-contracted arteries. The data suggest that the CaR is required for relaxation of mesenteric arteries under increased adrenergic tone, as occurs in hypertension, and indicate an inherent defect in the CaR signaling pathway in Dahl animals, which is much more severe in DS.

The method is useful in determining vascular reactivity *ex vivo* in mesenteric resistance arteries and similar small blood vessels and comparisons between different agonists and/or antagonists can be easily and consistently assessed side-by-side[6,7,8].

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=3119

Protocol

1. Isolation of rat mesenteric small artery

1. Anesthetize animal with isoflurane in a closed chamber and wipe the abdomen with alcohol.
2. Perform a mid-line laparotomy to expose mesenteric bed.
3. Using scissors, remove about 85 cm of intestine with feeding vasculature with the superior mesenteric artery. Cut the proximal end of intestinal section close to the pylorus and the distal end near the ileo-coecal junction. Segments of the intestine are isolated from rats that are deeply anesthetized with isoflurane and euthanized by open-chest cardiac puncture.
4. Placed excised section in a coated petri dish containing PSS and perform the dissection of the mesenteric artery at room temperature.
5. Pin down the proximal end of the intestine on the right hand side and pin out the remainder of the intestineina counter-clockwise direction (i.e.proximal end on the left feeding vasculature on the far side of the intestine).
6. Dissect out the branch II and III segments together with a piece of the proximal segment.
7. Dissect out the vein and isolate the artery (with V-shaped branch point) and clean it by removing the adipose and connective tissue. Avoid direct contact with the artery by pulling gently with forceps and cutting through the membrane of the connective tissue.

2. Mounting of vessel

1. Cut two short segments (≈ 4 cm) of a 40 µm tungsten-free stainless steel wire and, with a fine forceps,insert one into the lumen of each artery taking care not to damage the endothelium. Use the wire tip to open the lumen if necessary. Blood streaming out of the vessel is a good sign the lumen is opened.
2. Fill the myograph chamber with physiological salt solution (PSS; mM: 115 NaCl, 4.7 KCl, 1.4 $MgSO_4.7H_2O$, 5 $NaHCO_3$, 1.2 K_2HPO_4, 1.1 Na_2HPO_4, 1.0 $CaCl_2$, 20 HEPES and 5 glucose, pH 7.4) with ascorbic acid (100 µM) at 37 °C and using forceps carefully transfer the threaded vessel segment from the petri dish into the chamber at room temperature and transfer the excised proximal vessel segment to the myograph chamber and pull the end along the wire to feed it into the vessel. Avoid stretching the vessel.
3. Secure the near end of the wire counter-clockwise under the near fixing screw on the right-hand jaw connected to the micrometer. Catch the free end of the wire with forceps and secure it clockwise under the far fixing screw on the right-hand jaw. Ensure that the vessel segment along the wire is situated in the gap between the jaws without making any contact with the jaw itself.
4. Screw the jaws apart, and align the second wire parallel with the vessel and insert it into the far end of the lumen. Gently feed the wire through the lumen of the vessel segment in one motion using the already mounted wire as a guide. Hold the wire about 1 cm from the vessel to avoid stretching it during the maneuver, and avoid touching the endothelium.
5. Screw the jaws together and ensure that the second mounting wire moves underneath the first one secured on the right-hand jaw.
6. Secure the near end of the second wire in a clockwise direction under the near fixing screw of the left-hand jaw connected to the transducer.
7. Secure the far end of the wire under the fixing screw on the left-hand jaw and tighten to stretch the wire.
8. Once mounting is complete, reset the motor in the "Mounting Menu" and start normalization of the vessel.

3. Normalization

The Auto Dual Wire Myograph System- 510A has an automated normalization function, which is assessed from the "Normalization" menu and allows the vessel to be stretched to a normalized internal circumference by a standardized procedure according to the manufacturer's protocol after equilibration for 30 min at 37 °C. An exponential curve is then fitted to the internal circumference pressure data. The procedure defines the lumen diameter (*d*100) that the artery would have had *in vivo* when relaxed and under a transmural pressure of 100 mmHg[1].The normalization parameters for rat mesenteric artery are as follows:

1. Target transmural pressure = 13.3 kPa (100 mm Hg).
2. Time = 60 sec; Duration of each of the normalization steps.
3. IC_1/IC_{100} = 0.9 (IC_1 = normalized internal circumference, IC_{100} = internal circumference corresponding to target pressure.
4. Eyepiece calibration 2*Δ (mm/ocular division); 2 delta is for programming reasons.

Measure the length of the mounted mesenteric artery using the microscope eye-piece readings when the hairlines are over the far and near ends of the mounted vessel segment. Briefly, the length of the mounted vessel segment is measured at maximum magnification with a calibrated ocular eye-piece in the dissection microscope. The eye-piece reading with hair-line over the far end of the segment (a_1) and the near end of the segment (a_2) in ocular divisions are measured and recorded. These values, with the micrometer readings, before and after stretching the vessel are recorded and entered into the menus for the program to calculate the fitted curve and the internal diameter corresponding to the a target transmural pressure of 100 mm Hg

In our studies, the arteries were set to the lumen diameter of $d_1 = 0.9 \times d_{100}$, where active force development is maximal. Active force development of ≥ 10 mN in rat mesenteric arteries is considered optimum for experiments to proceed. Tissues with lower active force were discarded.

4. Measurement of responses

After normalization, the mechanical and functional properties of vessels are re-activated by performing a "standard start", which involves challenging vessels with repeated applications (normally 2 or 3) of 5 µM phenylephrine (PE) to obtain reproducible contractions. A typical "standard start" in our laboratory consists of a series of stimulations and washout periods as follows:

1. Replace myograph chamber cover and start aeration with 95% air and 5% CO_2.
2. Fill chamber with fresh PSS (containing 100 µM ascorbic acid)
3. Contract vessel with 5 µM PE for 5 min and wash 4 times with PSS buffer.
4. After the last wash, refill chamber with PSS and wait 3 min before repeating steps (ii) and (iii).

After the standard start, the artery is ready for the experiment. Relaxations of contracted vessels are assessed by cumulative addition of increasing concentrations of $CaCl_2$ (0.5 - 4 mM). When inhibitors are used vessels are pre-incubated with the compounds in the myograph chamber for 20 min and present during the assays.

5. Data analysis

The "Normalization" data for vessels and force tracings, directly converted to text data,were analyzed with the SigmaPlot 11.0 graphing program (Systat Software, Point Richmond, CA) and plotted as shown in Figure 1. Concentration-response data, calculated from the tracings, were analyzed by determining EC_{50} values from experimental data fitted to a four-parameter logistic function in the Pharmacology menu of the program. Comparisons between groups and within groups were carried out by One Way Analysis of Variance (*ANOVA*) and differences with $p < 0.05$ are considered significant.

6. Representative results:

L_{100} (µm)	83.26 ± 2.33	91.52 ± 4.67	117.45 ± 8.43*
X_1	604.21 ± 41.97	752.24 ± 85.25	745.84 ± 110.09
r^2	0.99 ± 0.003	0.97 ± 0.008	0.97 ± 0.007

**The difference between L_{100} values for Wistar and DR are statistically significant. Shapiro-Wilk Normality Test passed (p = 0.588); Equal Variance Test passed (p = 0.237).*

L_{100} = Internal diameter of the mounted segment corresponding to the target transmural pressure.

X_1 = The micro positioner setting required to stretch the mounted vessel to its normalized internal circumference (i.e. the value of the micrometer reading at which experiments were performed).

r^2 = Regression coefficient for fit of (X_i, Y_i) to an exponential curve.

NB: The detailed procedure is described in the manual for the 510A Myograph System[6].

Table 1. Sample Read-Out of normalization parameters for mesenteric artery segments from Wistar, DS and DR from Basic program shown in the "Normalization Menu". Values are means (± SEM) of 5 animals. *Differences between L_{100} values for Wistar and DS are statistically significant ($p < 0.05$).

14.2 ± 0.6	**25.8 ± 2.7***	**20.9 ± 1.3***

Table 2. Developed tensions(mN) in mesenteric arteries isolated from Wistar, DS and DR rats,following applications of 5 µM PE to each vessel. Values reported are means (± SEM) of 6-8 animals. *Significantly different from Wistar controls ($p < 0.05$).

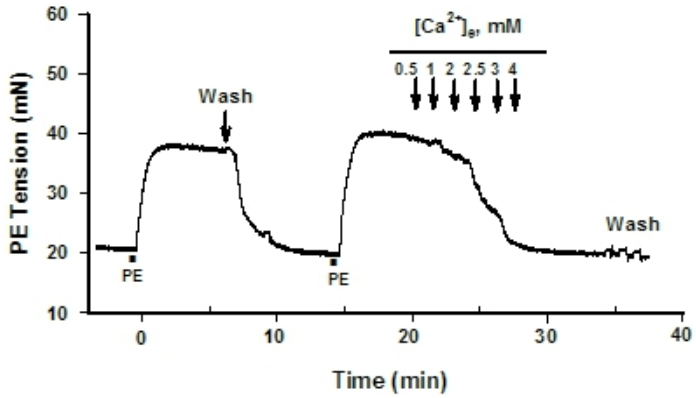

Figure 1. Ca^{2+}_e-induced relaxation of a PE-contracted mesenteric artery from a Wistar rat mounted in a wire myograph in physiological salt solution containing 1 mM Ca^{2+}. The artery was equilibrated for 30 min at 37 °C with constant aeration and responses to Ca^{2+}_e determined. A representative force tracing on addition of 5 µM PE followed by cumulative additions of Ca^{2+} is shown.

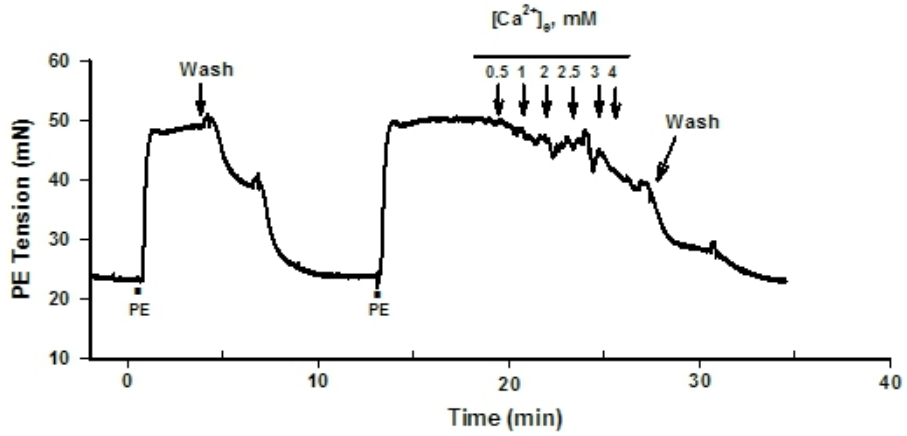

Figure 2. Ca^{2+}_e-induced relaxation of a PE-contracted mesenteric artery from a DS rat mounted in a wire myograph in physiological salt solution containing 1 mM Ca^{2+}. The artery was equilibrated for 30 min at 37 °C with constant aeration and responses to Ca^{2+}_e determined. A representative force tracing on addition of 5 µM PE followed by cumulative additions of Ca^{2+} is shown. Ca^{2+} relaxation was severely compromised in this tissue compared to those from Wistar and DR rats.

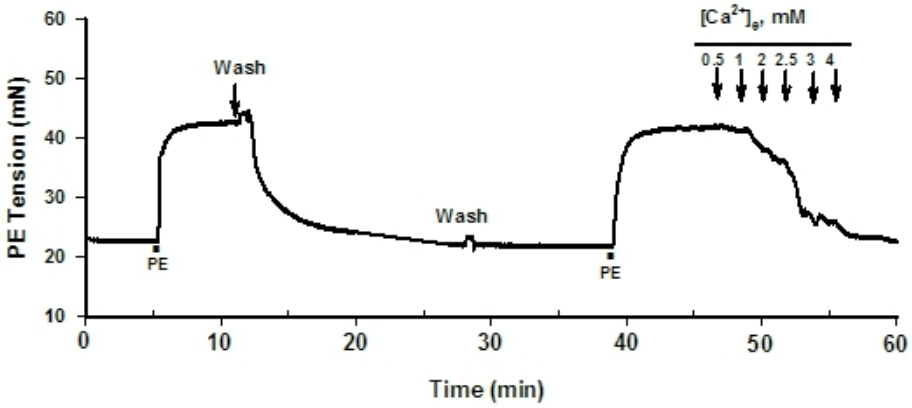

Figure 3. Ca^{2+}_e-induced relaxation of a PE-contracted mesenteric artery from a DR rat mounted in a wire myograph in physiological salt solution containing 1 mM Ca^{2+}. The artery was equilibrated for 30 min at 37 °C with constant aeration and responses to Ca^{2+}_e determined. A representative force tracing on addition of 5 μM PE followed by cumulative additions of Ca^{2+} is shown.

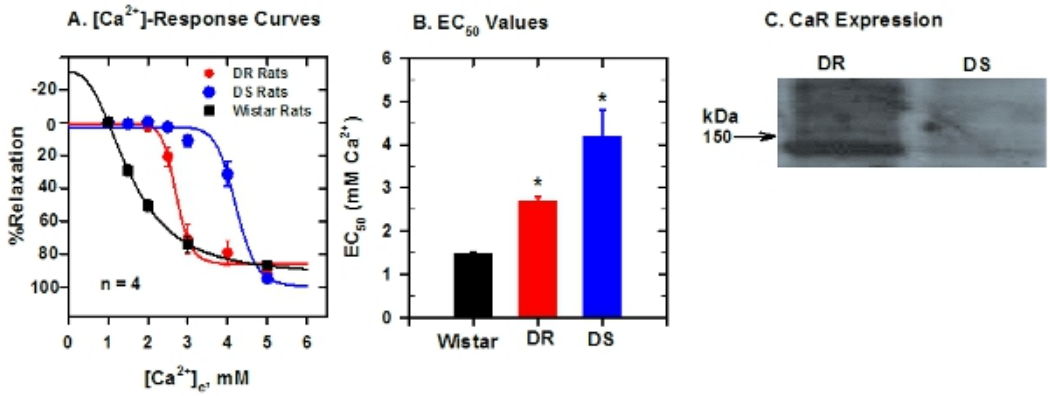

Figure 4. A. $[Ca^{2+}]_e$-response curves. **B.** Bar chart showing EC_{50} values for relaxation determined by fitting the data to a four parameter logistic curve. **C.** CaR expression in mesenteric arcades isolated from DR and DS rats.

Discussion

Hypertension is a leading cause of cardiovascular, cerebral and renal morbidity/mortality. The occurrence of hypertension is high in the population and salt-sensitive hypertension is particularly high in the aging population, and more prevalent in blacks than whites. This is believed to be due to the tendency of blacks to retain sodium in their kidneys[9]. Salt-sensitivity is a major contributing factor to kidney disease and is associated with endothelial dysfunction, but the mechanism is not fully understood. Recent studies in our laboratory have revealed that high salt diets reduce interstitial fluid Ca^{2+} concentration ($[Ca^{2+}]_{IF}$), and increase systolic pressure in salt-sensitive rats[10,11]. These effects may be attributed to reduced expression of CaRs in mesenteric arteries and kidneys.Finding similar reductions in CaR levels in salt-sensitive hypertensive patients could provide a target for development of new therapies. We have been using the automated wire myography with isolated mesenteric arteries from a number of animal models of hypertension to study vascular reactivity to Ca^{2+}_e to understand the mechanism of CaR signaling in the vasculature.

$[Ca^{2+}]_e$ is maintained within a narrow range (1.1 - 1.4 mM) in humans[12], therefore the CaR must be able to detect small changes in $[Ca^{2+}]_e$ for receptor activation to be possible. In fact, the CaR has been shown to detect small changes in Ca^{2+}_e[13], and small increases in $[Ca^{2+}]_{IF}$ that are within the physiological range[14]. *In situ* microdialysis studies measuring $[Ca^{2+}]_{IF}$ in the duodenal submucosa and kidney cortex, two tissues that are essential for the regulation of peripheral resistance and blood pressure (BP), showed dynamic changes as a function of the gut lumen $[Ca^{2+}]$. Increasing gut $[Ca^{2+}]$ from 0 to 6 mM, increased the $[Ca^{2+}]_{IF}$ from 1.1 to 1.9 mM[4,5,15], which is in the range observed to activate the perivascular nerve CaR[16], and relax isolated mesenteric arteries[17]. We have also shown that the CaR detects decreases in $[Ca^{2+}]_e$ and increase the action potential duration through activation of the non-selective cation channel, which in turn attenuates the impact on release probability at neocortical terminals[18]. This suggests that the CaR provides pre-synaptic feedback to alter brain excitability in response to Ca^{2+}_e. Furthermore, desensitization of the PvN CaR occurs with repeated, prolonged stimulation[16], suggesting that an understanding of the regulation of this receptor will clarify its role under both normal and hypertensive conditions. Bukoski and colleagues have shown that small changes in $[Ca^{2+}]_{IF}$ are enough to activate the CaR and contribute to vasodilator synthesis under normal conditions[3,4,19],. Based on these observations, we postulate that this response will also occur under conditions of increased vascular tone as seen in hypertension and can therefore be exploited to control blood pressure. The study of CaR activation and how this leads to phosphorylation of signaling intermediates is, therefore, required to probe its role in normal and hypertensive physiology. The CaR is G protein-coupled receptor (GPCR) and is regulated via similar mechanisms as other GPCRs, which are common therapeutic targets. Therefore, understanding the mechanisms of its regulation in blood vessels will provide useful data to determine its potential as a target for anti-hypertensive therapy.

Disclosures

No conflicts of interest declared.

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