Reduced vascular smooth muscle BK channel current underlies heart failure-induced vasoconstriction in mice

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ABSTRACT Excessively increased peripheral vasoconstriction is a hallmark of heart failure (HF). Here, we show that in mice with systolic HF post–myocardial infarction, the myogenic tone of third-order mesenteric resistance vessels is increased, the vascular smooth muscle (VSM) membrane potential is depolarized by ~20 mV, and vessel wall intracellular [Ca2+] is elevated relative to that in sham-operated control mice. Despite the increased [Ca2+], the frequency and amplitude of spontaneous transient outward currents (STOCs), mediated by large conductance, Ca2+-activated BK channels, were reduced by nearly 80% (P<0.01) and 25% (P<0.05), respectively, in HF. The expression of the BK α and β1 subunits was reduced in HF mice compared to controls (65 and 82% lower, respectively, P<0.01). Consistent with the importance of a reduction in BK channel expression and function in mediating the HF-induced increase in myogenic tone are two further findings: a blunting of paxilline-induced increase in myogenic tone in HF mice compared to controls (0.9 vs. 10.9%, respectively), and that HF does not alter the increased myogenic tone of BK β1-null mice. These findings identify electrical dysregulation within VSM, specifically the reduction of BK currents, as a key molecular mechanism sensitizing resistance vessels to pressure-induced vasoconstriction in systolic HF.—Wan, E., Kushner, J. S., Zakharov, S., Nui, X.-W., Chudasama, N., Kelly, C., Waase, M., Doshi, D., Liu, G., Iwata, S., Shiomi, T., Katchman, A., D’Armiento, J., Homma, S., Marx, S. O. Reduced vascular smooth muscle BK channel current underlies heart failure-induced vasoconstriction in mice. FASEBJ. 27, 000–000 (2013). www.fasebj.org

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Patients with diabetes, hypertension, and heart failure (HF) have increased vascular tone, which adversely affects their cardiovascular outcomes. Increased vascular tone in peripheral vessels increases the workload of the heart. In patients with HF, reduction in myocardial blood flow (1–5) and increased cardiac workload (6–8) together impair cardiac output and ventricular remodeling, accelerating the progression to decompensated HF. Thus, HF begets worsening HF. Decreasing vascular tone, thereby unloading the heart and improving coronary perfusion, is an important therapeutic approach in patients with diseases associated with increased vasoconstriction, especially acute and chronic HF. Many of the currently available vasodilators (e.g., dobutamine, milrinone), however, have either negative inotropic effects, which preclude their use, or increase cardiomyocyte contractility at the expense of increased heart rate, arrhythmias, and mortality. Current therapies for HF are therefore insufficient and do not directly target the molecular mechanisms responsible for elevated vascular tone in HF.

The diameter of resistance vessels, which fundamentally regulates blood flow within an organ, as well as the systemic vascular resistance and arterial pressure, is primarily determined by the contraction of vascular smooth muscle (VSM) cells. Depolarization of the VSM cell membrane potential and elevated cytosolic [Ca2+] increase VSM contractility, causing vasoconstriction, whereas hyperpolarization and decreased [Ca2+] reduce VSM contractility, causing vasodilation. VSM cell depolarization renders the vessel less sensitive to vaso-
to vasodilators that rely on hyperpolarization, and more sensitive to vasoconstrictors (9, 10).

Myogenic tone is a major contributor to vascular resistance, because it underlies the local autoregulation of microvascular blood flow and sets the vascular diameter on which vasodilators and vasoconstrictors act (9, 11). VSM cell depolarization is a key mediator of myogenic tone because it leads to the opening of the voltage-gated L-type Ca\(^{2+}\) channel and Ca\(^{2+}\) influx. Pressure-induced VSM cell depolarization, however, does not require Ca\(^{2+}\) entry (12–14). Changes in ion flux, membrane potential, and cytosolic [Ca\(^{2+}\)] are principal events that regulate VSM contraction via control of myosin light-chain (MLC) phosphorylation.

Strong hyperpolarizing currents are required to prevent excessive VSM cell depolarization and vascular contractility in response to intraluminal pressure and vasoconstrictors. They are also required to enable VSM cell hyperpolarization and vascular relaxation in response to vasodilators. VSM cells express several types of K\(^+\) channels, including large conductance Ca\(^{2+}\)-activated potassium (BK), voltage-dependent K\(_{V, i}\), inward rectifier (K\(_{IR}\)), and K\(_{ATP}\) channels (9, 11). BK channels are especially important contributors to the hyperpolarizing currents by virtue of their large conductance and Ca\(^{2+}\) sensitivity (14).

We hypothesized that the increased myogenic tone observed in systolic HF (15–19) was due to changes in the electrical properties of VSM cells, disrupting the balance between depolarizing and hyperpolarizing ionic currents. We discovered that in resistance vessels of the splanchnic circulation of wild-type (WT) mice with systolic HF, 6 wk post–myocardial infarction (MI), the VSM cell membrane potential is depolarized, cytosolic [Ca\(^{2+}\)] is elevated, and the expression and activity of vascular BK channels are markedly reduced relative to sham-operated controls. Our findings offer the first evidence that systolic HF alters the electrical properties of VSM cells, reducing hyperpolarizing currents responsible for preventing VSM cell depolarization.

**MATERIALS AND METHODS**

**Reagents**

All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) with the following exceptions: Fura-2AM (Molecular Probes; Life Technologies, Grand Island, NY, USA), pluronic acid (Life Technologies), collagenase (Worthington Biochemical Corp, Lakewood, NJ, USA), ethylene glycol-bis(2-aminoethyl)-N,N,N,N'-tetraacetic acid (EGTA; Research Organics, Sigma Aldrich), papilline (Tocris Bioscience, Bristol, UK), and iberiotoxin (Tocris Biosciences).

**Animals**

Experiments were performed in accordance with protocols approved by the Columbia University Institutional Animal Care and Use Committee. WT male C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Homozygous BK \(\beta\)-null mice were provided by Dr. Robert Brenner (Department of Physiology, University of Texas Health Science Center at San Antonio, USA). BK \(\beta\)-null mice are congenic, and are maintained as homozygous lines.

**Surgical procedures**

Male WT or BK \(\beta\)-null mice, aged 11–12 wk, underwent a proximal left anterior descending (LAD) artery ligation (20). The left coronary artery was identified, and a permanent LAD artery ligation was made with a 8-0 nylon suture, which was confirmed by blanching of the myocardium. For sham operations, the suture needle was passed under the artery, but the artery was not ligated.

**Transthoracic echocardiography**

The mice were anesthetized with isoflurane and placed on a warming pad. Echocardiography was performed using a Visual Sonics 30-MHz transducer (Sonosite, Toronto, ON, Canada) applied parasternally to the chemically depilated chest wall. The operator was blinded to the genotype of the mouse and the type of procedure (sham vs. LAD artery ligation).

**Cardiac pathology**

Hearts were fixed in 10% formalin and embedded in paraffin. The sections were stained with Masson trichrome stain. The MI was confirmed by a pathologist blinded to procedure type and genotype.

**Functional assessment of mesenteric artery tone and contractility**

Six weeks after sham or LAD artery ligation surgery, third-order mesenteric arteries (~150 \(\mu\)m) were dissected and placed in a Petri dish with ice-cold physiological saline solution (142 mM NaCl, 4.7 mM KCl, 3.5 mM MgSO\(_4\), 1.2 mM KH\(_2\)PO\(_4\), 2.5 mM CaCl\(_2\), 10 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH at 10°C). The vessels were mounted onto two glass cannulas in a vessel chamber (Living Systems Instrumentation, St. Albans, VT, USA) in a Krebs-HEPES solution (118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO\(_3\), 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgCl\(_2\), 2.5 mM CaCl\(_2\), 10 mM glucose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH at room temperature). The vessels were equilibrated at 50 mmHg for 20 min in a Krebs buffer (118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO\(_3\), 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgCl\(_2\), 2.5 mM CaCl\(_2\), and 10 mM glucose, saturated with 95% O\(_2\) and 5% CO\(_2\), pH 7.35) at 37°C. The viability of the vessel preparations was then briefly assessed using 20–40 mM KCl followed by a thorough washout (8–10 vol changes). Vessels that failed to constrict were deemed nonviable and were discarded. Diameter changes in pressurized segments were measured at 40, 80, and 120 mmHg using automatic edge detection (Living Systems or Ionoptix, Milton, MA, USA), in the absence of intraluminal flow. Vessels were first subjected to a stepwise increase in intraluminal pressure from 40 to 120 mmHg, followed by a stepwise decrease in intraluminal pressure from 120 to 40 mmHg. We used the diameter measured during the step-down as the determinant of contractile response, since more consistent and greater myogenic contraction was observed in control vessels during the step-down than the step-up in intraluminal pressure. The vessel diameter was measured continuously for \(\geq\)5 min, to ensure a steady state was reached, at each pressure step. The arteries were then incubated in a Ca\(^{2+}\)-free Krebs solution (same as above though with 0 mM CaCl\(_2\) + 2 mM EGTA) in order to prevent contraction, and the pressure-diameter measure-
ments were repeated. The degree of myogenic tone was calculated as \((\text{Ca}^{2+}\text{-free diameter} - \text{Ca}^{2+}\text{-containing diameter}) / \text{Ca}^{2+}\text{-free diameter}) \times 100\). The effect of paxilline (1 \(\mu\)M) on myogenic tone was determined in vessels pressurized to 120 mmHg.

**Wall thickness and distensibility measurements**

After the vessels were cannulated onto the perfusion myograph system, wall thickness was calculated as (vessel outer diameter – vessel inner diameter)/2 in the Ca\(^{2+}\) free solution at various intramural pressures (21). Distensibility was evaluated in the Ca\(^{2+}\) free solution by lowering the intramural pressure until the vessel began to collapse, usually at 1 mmHg, and then increasing the pressure to determine the diameter of the vessel under inflated but unstressed conditions at 2 mmHg (21). Intramural pressure was then increased in a stepwise fashion up to 100 mmHg. Lumen diameter was allowed to stabilize for 1 min after each increase in pressure. Distensibility (%) was calculated as described previously (21).

**Membrane potential measurements in pressurized vessels**

The membrane potential was measured in intact vessels using an electrometer (WPI Electro 705; World Precision Instruments, Sarasota, FL, USA) by impaling VSM cells through the adventitia with borosilicate glass microelectrodes (22). The analog output from the amplifier was sampled at 1 ms. The primary criteria for a successful cellular impalement were a sharp drop in voltage from baseline on entry of the microelectrode tip into the cell, a sharp return to 0 on exit, and a stable membrane potential in between. From each vessel and for each pressure tested (40, 80, and 120 mmHg), multiple data points were obtained and averaged for each vessel.

**Measurement of vascular wall [Ca\(^{2+}\)], in pressurized arteries**

After cannulation and measurement of background fluorescence, the pressurized arteries (50 mmHg; at 22–24°C) were loaded in the dark for 40–50 min with 3–5 \(\mu\)M Fura-2AM in the presence 0.05% pluronic acid (22). Ratiometric measurements of Fura-2 fluorescence were performed with a photomultiplier system (IonOptix, Milton, MA, USA). Background-corrected ratios of 510 nm emission were obtained at a sampling rate of 5 Hz from arteries alternately excited at 340 and 380 nm. All experimental protocols were started after an additional 20 min equilibration period at 50 mmHg to allow for intracellular deesterification of Fura-2AM and equilibration at 37°C.

**Measurement of spontaneous transient outward currents (STOCs)**

Third-order mesenteric arteries (100–150 \(\mu\)m) were digested using standard enzymatic dissociation methods (23). After trituration with fire-polished Pasteur pipettes, [Ca\(^{2+}\)], was gradually increased in increments, from 0.05 to 1 mM over 30 min. Cells were stored at 4°C and were used within 3–4 h of isolation. Transient BK currents were measured using the whole-cell patch-clamp technique (Axopatch 200B; Molecular Devices, Sunnyvale, CA, USA) in the amphotericin B (250 \(\mu\)g/ml) perforated-patch configuration. Cells were superfused with a solution containing 140 mM NaCl, 4.7 mM KCl, 1 mM MgCl\(_2\), 2 mM CaCl\(_2\), 10 mM glucose, and 10 mM HEPES (pH 7.4). Patch pipettes were filled with an internal solution containing 64 mM K\(^+\) aspartate, 75 mM KCl, 10 mM NaCl, 1 mM MgCl\(_2\), 0.05 mM EGTA, and 10 mM HEPES (pH 7.2). Recordings were analyzed using pClamp10 (Molecular Devices) to determine the transient BK current amplitude and frequency. STOCs were identified by setting a current threshold at 3 times the mean BK single-channel amplitude at \(-20\) and 0 mV, and all events that were greater than this threshold were included (24).

**Immunohistochemistry**

The third-order mesenteric arteries were fixed with 4% paraformaldehyde fixative for 24 h and embedded in paraffin wax. The sections (4 \(\mu\)m) were deparaffinized, autoclaved with Tris-buffer (pH 9.0) at 121°C for 15 min for antigen retrieval, and then treated with 0.3% \(\text{H}_2\text{O}_2\) to block endogenous peroxidases. The sections were incubated with 1:100 anti-BK \(\alpha\) antibody (cat. no. APC-107; Alomone Labs, Jerusalem, Israel), 1:500 anti-BK \(\beta\)1 antibody (PA1-924; Thermo Scientific, Rockford, IL, USA) or the same dilutions of nonimmune rabbit polyclonal IgG (AbCam Inc., Cambridge, MA, USA) at 4°C overnight. After washing the slides, the sections were exposed to peroxidase-labeled polymer reagents against IgG (ImmPRESS polymerized reporter enzyme staining system; Vector Laboratories, Inc., Burlingame, CA, USA) and DAB solution (ImmPRESS DAB Peroxidase Substrate; Vector Laboratories). After the reaction, the sections were counterstained with hematoxylin and observed and photographed using a light microscope. DAB staining was quantified using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA). The staining and analysis were performed in a blinded fashion.

**Real-time PCR**

Mesenteric resistance vessels were homogenized and RNA purified (RNaseasy 74104; Qiagen, Valencia, CA, USA). cDNA was synthesized (4387406; Applied Biosystems, Foster City, CA, USA), and 20 ng of template was used for each 20-\(\mu\)l reaction. Real-time PCR was performed using a StepOne Plus Real-Time PCR system with StepOne 2.0 software and inventoried primers (all from Applied Biosystems). PCR was performed for 40 cycles with automated detection of crossing threshold. PCR reactions were performed with duplicate wells with GAPDH as a reference.

**Statistical analysis**

All values are expressed as means ± se. An unpaired Student’s \(t\) test was utilized for comparison of 2 groups. When appropriate, a paired Student’s \(t\) test was used to compare pre- vs. postintervention. For multiple comparisons, a 2-way ANOVA with Bonferroni correction or generalized estimating equations were used. Differences were considered statistically significant at values of \(P < 0.05\). Generalized estimating equations were performed in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). All other statistical programming was performed using GraphPad Prism 5 (GraphPad, La Jolla, CA, USA).

**RESULTS**

**Myogenic tone is increased in mesenteric arteries of HF mice**

Male C57BL/6 mice underwent surgical ligation of the proximal LAD coronary artery to cause a large MI and
progressive cardiac dysfunction leading to HF (20). Surgery was considered successful if the anterior left ventricular (LV) wall visibly blanched immediately after the ligation of the LAD artery. Sham-treated mice also underwent thoracotomy, though without LAD artery ligation. After 6 wk, Masson’s trichrome staining confirmed large anterior MIs (Fig. 1A, B). After 6 wk, mice that underwent LAD artery ligation had systolic HF, manifested by greater heart weight to body weight ratio (Supplemental Fig. S1), and LV end-diastolic and endsystolic dimensions (LVEDD and LVESD; Fig. 1E, F), as well as reduced mean LV systolic ejection fraction (Fig. 1C) and fractional shortening (Fig. 1D) compared to the sham-treated mice.

We compared the myogenic responses of third-order mesenteric arteries (≈150 μm) freshly isolated from sham-treated controls and LAD artery-ligated HF mice, 6 wk post-MI. Diameter changes in pressurized segments of sham-treated control mice (no HF) and mice with HF, in the absence of intraluminal flow, were measured at 40, 80, and 120 mmHg (Fig. 2A, B). Over a physiological range of pressures, arterioles normally respond to acute increases in pressure by constricting and acute decreases in pressure by dilating. We measured the vessel diameter of control and HF vessels in response to both increases and decreases in pressure (Fig. 2A, B). The arteries were then incubated in Ca²⁺-free saline solution to prevent Ca²⁺ influx and pressure-induced vasoconstriction, and the measurements were repeated. The difference in diameter in the presence and absence of Ca²⁺ (Fig. 2A, B), representing the active, myogenic contractile response, was significantly increased in mesenteric arteries from HF mice compared to sham-treated mice (Fig. 2C). This increase in myogenic tone was not caused by vessel remodeling or changes in extracellular matrix constituents, as measured by the endpoints, distensibility (Fig. 2D), wall thickness (Fig. 2E), and thickness of the tunica media layer (Supplemental Fig. S2). Taken together, our results show that myogenic constriction is markedly increased in resistance vessels isolated from HF mice, in the absence of systemic factors such as short-acting circulating neurohormones and neural inputs, which are not present in this ex vivo experimental system.

Reduced BK currents are responsible for enhanced pressure-induced myogenic constriction observed in HF vessels

In VSM cells, strong hyperpolarizing currents modulate myogenic tone by preventing regenerative Ca²⁺ influx and action potential initiation. Such feedback permits incremental changes in arterial diameter that finely modulate organ-specific blood flow and perfusion pressure. BK channels are especially important contributors to these hyperpolarizing currents by virtue of their very large conductance and Ca²⁺ sensitivity (14).

Inhibiting BK channels using pharmacological inhibitors of BK channels, such as iberiotoxin or paxilline, increases the myogenic tone of mesenteric arteries. The effects of iberiotoxin and paxilline on myogenic tone are significantly reduced in BK β1-null mice (25, 26). We reasoned that if HF alters myogenic tone by reducing BK channel expression and currents, then the effect of paxilline on myogenic tone should be reduced in the HF vessels. The effect of paxilline was determined in no-HF and HF third-order mesenteric arteries pressurized to 120 mmHg. In mesenteric arteries isolated from mice without HF, paxilline increased constriction by 10.9% (relative increase of 51.9% in myogenic tone), whereas paxilline increased constriction by 0.9% (relative increase of 3% in myogenic tone) in mesenteric arteries isolated from HF mice (Fig. 3A). Inhibiting BK channels with paxilline in vessels isolated from sham-treated control mice caused an increase in myogenic tone, approximating the increased myogenic tone of vessels isolated from HF mice (Fig. 3B).

VSM membrane potential and [Ca²⁺] are increased in mesenteric arteries of HF mice

Taken together, the lack of an increase in myogenic tone after paxilline in HF vessels (Fig. 3A) and the equivalent amount of myogenic tone in paxilline-treated no-HF and HF vessels (Fig. 3B) suggest that the increased myogenic tone in HF vessels was due to reduced hyperpolarizing currents, leading to mem-
brane depolarization. The membrane potential of VSM cells was directly measured in intact vessels by impaling VSM cells through the adventitia with glass microelectrodes (22). Vessels from HF and sham-treated mice both exhibited progressive VSM cell depolarization as intraluminal pressure increased (Fig. 4A), as expected, but the degree of depolarization was significantly greater in vessels from HF mice at all three pressures.

Membrane depolarization leads to opening of L-type voltage gated Ca$^{2+}$/H$^{+}$ channels and Ca$^{2+}$/H$^{+}$ influx. To test whether HF is associated with elevated global [Ca$^{2+}$/H$^{+}$]i in these mesenteric vessels, we measured cytosolic Ca$^{2+}$/H$^{+}$ within the vessel wall of pressurized vessels using ratio-metric measurements of Fura-2 fluorescence. Background-corrected ratios of 510-nm emission were obtained from arteries alternatively excited at 340 and 380 nm. Pressurized vessels from HF mice exhibited a higher VSM [Ca$^{2+}$/H$^{+}$]i than those from sham-treated mice (Fig. 4B). Thus, HF causes both a depolarized membrane potential of VSM cells and increased cytosolic [Ca$^{2+}$/H$^{+}$] within VSM cells.

**Decreased expression and function of BK channel in VSM cells of HF mice**

The simultaneous opening of several BK channels in response to localized Ca$^{2+}$ sparks, from ryanodine receptors in the sarcoplasmic reticulum, is known as a STOC. The amplitude and frequency of STOCs are markedly reduced by paxilline and iberiotoxin (Supplemental Fig. S3), which are inhibitors of BK channels, and by ryanodine (Supplemental Fig. S4), which is an inhibitor of ryanodine receptors (14). We hypothesized that the increased VSM cell depolarization and the reduced responsiveness of vessels isolated from HF mice to paxilline reflected a loss of BK currents that would be manifested as a reduced STOC frequency and amplitude.

We measured STOCs at −20 and 0 mV in freshly isolated VSM cells from third-order mesenteric arteries of sham-treated control and HF mice using perforated whole-cell configuration of the patch-clamp technique (Fig. 5A). At these voltages, arterial voltage-dependent Ca$^{2+}$/H$^{+}$ channels exhibit maximal steady-state currents and partial voltage-dependent inactivation. We selected these voltages because in HF, the VSM membrane potential is ~−20 mV at 80 mmHg (Fig. 4A). Depolarization of the membrane potential increased the fre-
frequency and amplitude of STOCs in VSM cells from both sham-treated control and HF mice (Fig. 5A), as expected, but both amplitude (Fig. 5B) and frequency (Fig. 5C), at −20 and 0 mV, were significantly reduced in the VSM cells from HF mice. At −20 mV, the STOC frequency was reduced by nearly 80% (Fig. 5C), and the amplitude was reduced by 60% (Fig. 5B). At 0 mV, the STOC frequency was reduced by nearly 60% (Fig. 5C), and the amplitude was reduced by ~40% (Fig. 5B). Cell size, as indexed by cell capacitance, did not differ between VSM cells from HF mice and sham-treated control mice (16.1±0.6 vs. 14.8±0.1 pF, respectively; P=NS by t test).

We hypothesized that the reduced amplitude and frequency of STOCs were due to a decrease in the expression of BK α subunits and/or a decrease in the sensitivity of BK channels to activation by Ca$^{2+}$. The regulatory β1 subunit is required for BK α to sense physiological [Ca$^{2+}$]; (27). BK α and β1 subunit expression, which is limited to the tunica media layer, was markedly decreased in mesenteric arteries from HF mice compared to mesenteric arteries from sham-treated control mice (Fig. 6A, B). Semiquantitative analysis of the mean area of BK α-DAB-staining showed that BK α expression was 65% lower in mesenteric vessels from HF mice compared to those from sham-treated control vessels (Fig. 6C). Likewise, semiquantitative analysis of the mean area of BK β1-DAB-staining demonstrated that BK β1 expression was 82% lower in mesenteric vessels from HF mice compared to those from sham-treated control mice (Fig. 6D). Similar reductions in the mRNA expression of BK α and β1 were observed (Supplemental Fig. S5). The mRNA expression of Kv1.5, in contrast, was only reduced by ~20%. The reduced expression of BK α and β1 subunits is likely secondary to the increased systemic levels of neurohormones and inflammatory mediators. VSM BK β1 mRNA expression is decreased by angiotensin II-induced activation of calcineurin and nuclear factor of activated T cells (28–30), as well as by aldosterone, which also reduces BK α mRNA expression (31). The reduced frequency and amplitude of STOCs and the reduced expression of BK α and β1 subunits in HF vessels correlate with the diminished responsiveness of the mesenteric vessels to paxilline (Fig. 3).

Reduced BK currents are responsible for enhanced pressure-induced myogenic constriction observed in HF vessels

If the increased myogenic tone of HF was due to the diminished BK currents secondary to reduced expression of BK channel subunits, we hypothesized that HF should have a minimal effect on myogenic tone in the BK β1-null mice. At physiological membrane potentials, BK channel activity and, thus, STOCs are reduced in BK β1-null mice (26). To test this premise, we compared the myogenic tone of sham-operated and LAD artery-ligated BK β1-null mice. After 6 wk, BK β1-null mice that underwent LAD artery ligation had systolic HF, manifested by a greater heart weight to body weight ratio (Supplemental Fig. S1) and a re-

Figure 5. Decreased STOCs in HF mice. A) Representative traces of transient BK currents at −20 and 0 mV, recorded from freshly isolated mesenteric VSM cells of sham-treated and LAD artery-ligated HF mice. Asterisks indicate time points of traces on right. B, C) STOC amplitude (B) and frequency (C). Values are means ± s.e. No HF: n = 7; HF: n = 12. *P < 0.05, **P < 0.01; Student’s t test.
Ca^{2+} influx, leading to activation of MLC kinase, MLC phosphorylation, and contraction (34). In VSM cells, membrane depolarization and Ca^{2+} influx also activate Rho-kinase, which phosphorylates and inhibits MLC phosphatase, potentiating contraction (35–37). We found that in mice with HF, myogenic tone is increased, and VSM cells are more depolarized and have greater cytosolic [Ca^{2+}] compared to mice without HF. Although Ca^{2+} entry and VSM [Ca^{2+}]i correlate with greater MLC phosphorylation and VSM cell contraction, Ca^{2+} entry is not required for pressure-induced depolarization of the VSM cell membrane potential. Thus, the elevated global [Ca^{2+}] does not cause depolarization of the VSM cell membrane potential, but rather is the consequence of the VSM cell depolarization. The myogenic constriction was abolished by removing Ca^{2+} from the extracellular bath (Fig. 2), implicating Ca^{2+} influx through the L-type Ca^{2+} channel as the primary pathway responsible for the myogenic constriction. Our studies implicate increased depolarization of the VSM cell membrane potential as the basis for the increased myogenic tone in HF.

The membrane potential is a balance between depolarizing and repolarizing ionic currents. We demonstrate that the expression of both α and β1 subunits of the BK channel are decreased during HF, and this is coupled with a reduction of STOCs. BK channels play an important role in limiting myogenic constriction (26, 38, 39) by enhancing hyperpolarization of the membrane potential and limiting Ca^{2+} influx. Pharmacological inhibition of BK channels or genetic ablation of BK channel subunits markedly increases myogenic tone. Notably, vessels from WT mice with HF were relatively resistant to paxilline, a specific inhibitor of BK channels, compared to vessels isolated from WT mice without HF (Fig. 3). In addition, HF did not further increase the myogenic tone of mesenteric arteries of BK β1-null mice (Fig. 7), suggesting that decreased BK channel function is therefore sufficient to mediate the HF-induced increase in myogenic tone.

Recent studies have attributed the increased myo-

DISCUSSION

The contractility of small blood vessels, typified by mesenteric resistance arteries within the splanchnic circulation, is the prime determinant of systemic vascular resistance (32). Initially compensatory in HF, vasoconstriction of small resistance vessels, especially of the nonessential circulations, becomes excessive in order to maintain blood pressure at normal levels. As the HF syndrome progresses, the elevated vascular tone increases cardiac workload and reduces myocardial perfusion, detrimentally affecting cardiac remodeling and leading to a perpetuating, decompensatory cycle.

The diameter of resistance vessels is determined by the intrinsic contractile properties of VSM, referred to as myogenic tone, and by neighboring endothelial cells and circulating hormones, which modulate VSM contractility. Myogenic tone requires pressure and stretch activation of transient receptor potential (TRP) channels, leading to VSM cell depolarization (33); depolarization-induced opening of voltage-gated Ca^{2+} chan-

**Figure 6.** Decreased BK channel expression in HF mice. A, B) DAB-immunohistochemical staining of transverse sections of third-order mesenteric arteries from no-HF and HF mice. BK α (A) and BK β1 (B) were detected using anti-BK α and anti-BK β1 antibodies. Scale bars = 50 μm (images); 20 μm (insets). C, D) Quantification of area of BK α (C) and BK β1 (D) DAB-positive staining as fraction of total vessel area. **P < 0.01; Student’s t test; n = 3.**

**Figure 7.** HF does not increase myogenic constriction in BK β1-null mice. Effect of intraluminal pressure on myogenic tone in mesenteric arteries from BK β1-null sham-treated, no-HF mice and BK β1 LAD artery-ligated, HF mice. Values are means ± se. No HF: n = 6; HF: n = 8 mice. NS, not significant (P > 0.05) for HF vs. no HF by repeated-measures 2-way ANOVA and generalized estimating equations.

Reduced vascular BK currents in heart failure

REDUCED VASCULAR BK CURRENTS IN HEART FAILURE
genic tone in HF to sphingosine-1-phosphate induced changes in MLC phosphorylation, constitutive activation of the angiotensin 1 (AT1) receptor, or reduced caveolae (15–19). Lacking in these studies were measurements of VSM cell membrane potential, \([\text{Ca}^{2+}]_{v}\), and ion channel function, which are the key regulators of myogenic tone and are potential therapeutic targets.

Increased myogenic tone and reduced expression and function of BK channels are observed in hypertension, diabetes, and metabolic syndrome, diseases associated with an increased incidence and more rapid progression of HF (28, 29, 40–49). Reduced BK currents have also been proposed as a mechanism for the reduced coronary reserve in LV hypertrophy (50, 51). The reduced BK currents in LV hypertrophy, however, are unrelated to the level of expression of BK \(\alpha\) and \(\beta1\) subunits (51).

The loss of protection from depolarizing influences in the vasculature of HF animals is analogous to altered expression and function of K\(^{+}\) channels in cardiomyocytes occurring in conditions such as HF and cardiac hypertrophy (52). Although other molecular mechanisms are undoubtedly involved in the abnormal vascular tone in HF, our findings suggest that reduced BK channel-mediated repolarizing currents are one of the final common mechanisms.

Blocking \(\text{Ca}^{2+}\) influx into VSM cells is one of the most efficacious approaches to reduce vascular contractility and treat hypertension. In patients with HF after MI, however, \(\text{Ca}^{2+}\) channel antagonists are not well tolerated and are frequently contraindicated because of their negative inotropic effects. Since BK channels do not affect \(\text{Ca}^{2+}\) handling in the heart and may in fact serve a cardioprotective role (53), they may be ideal targets through which to indirectly reduce \(\text{Ca}^{2+}\) influx and vascular contractility, without affecting cardiac inotropy in patients with HF.

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