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Myocardial Blood Flow Control by Oxygen Sensing Vascular Kvβ Proteins

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Abstract

Rationale: Voltage-gated potassium (Kv) channels in vascular smooth muscle are essential for coupling myocardial blood flow (MBF) with the metabolic demand of the heart. These channels consist of a transmembrane pore domain that associates with auxiliary Kvβ1 and Kvβ2 proteins, which differentially regulate Kv function in excitable cells. Nonetheless, the physiological role of Kvβ proteins in regulating vascular tone and metabolic hyperemia in the heart remains unknown.

Objective: To test the hypothesis that Kvβ proteins confer oxygen sensitivity to vascular tone and are required for regulating blood flow in the heart.

Methods and Results: Mice lacking Kvβ2 subunits exhibited suppressed MBF, impaired cardiac contractile performance, and failed to maintain elevated arterial blood pressure in response to catecholamine-induced stress. In contrast, ablation of Kvβ1.1 reduced cardiac workload, modestly elevated MBF, and preserved cardiac function during stress compared with wild type mice. Coronary arteries isolated from Kvβ2$^{-/-}$, but not Kvβ1.1$^{-/-}$, mice, had severely blunted vasodilation to hypoxia when compared with arteries from wild type mice. Moreover, vasodilation of small diameter coronary and mesenteric arteries due to L-lactate, a biochemical marker of reduced tissue oxygenation and anaerobic metabolism, was significantly attenuated in vessels isolated from Kvβ2$^{-/-}$ mice. Inducible enhancement of the Kvβ1:Kvβ2 ratio in Kv1 channels of arterial smooth muscle abolished L-lactate-induced vasodilation and suppressed the relationship between MBF and cardiac workload.

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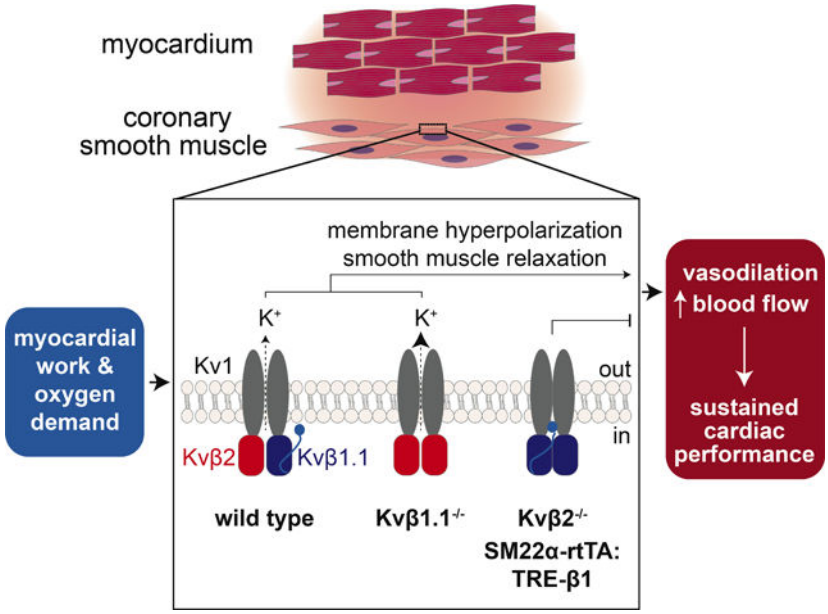
DISCLOSURES

None

Conclusions: The Kvβ proteins differentially regulate vascular tone and myocardial blood flow, whereby Kvβ2 promotes and Kvβ1.1 inhibits oxygen-dependent vasodilation and augments blood flow upon heightened metabolic demand.

Graphical Abstract



Keywords

Coronary Circulation; Ion Channels/Membrane Transport; Metabolism; Physiology; Vascular Biology; coronary arteries; myogenic tone; voltage-gated potassium channels; nicotinamide adenine dinucleotide; microcirculation; aldo-keto reductase; ion channels; ischemia

INTRODUCTION

An imbalance between myocardial oxygen supply and demand is a salient feature of heart disease, which remains the leading cause of death worldwide.[1] Impaired cardiac function associated with inadequate myocardial perfusion is commonly observed in patients with heart failure, hypertension, diabetes, and coronary artery disease.[2–5] Even in the absence of stenoses in large diameter conduit arteries, suppressed vasodilator capacity of small diameter coronary arteries and arterioles can lead to ischemia.[6, 7] Despite the vital importance of oxygen delivery to the preservation of cardiac structure and function, the fundamental mechanisms by which the coronary vasculature responds to fluctuations in myocardial metabolic demand remain poorly understood.

In the healthy heart, the coronary arteries and arterioles remain partially constricted, and they dilate or constrict further according to myocardial requirements for oxygen and nutrient delivery.[8, 9] As myocardial oxygen consumption increases (e.g., due to an increase in heart rate, myocardial contractility, or afterload), there is a corresponding demand for an increase in oxygen supply to sustain oxidative energy production. However, with little reserve for increased oxygen extraction, sustained cardiac function relies on the intimate link between

local and regional metabolic activity and vasodilation of the coronary vascular bed to deliver adequate blood flow to the myocardium (i.e., metabolic hyperemia).[10] In searching for molecular entities that couple vascular function to myocardial oxygen demand, recent studies from our group[11, 12] and others[13] have found that increased cardiac work promotes coronary vasodilation and hyperemia via the activation of Kv1 channels in smooth muscle cells. Nonetheless, how vascular Kv1 channels sense changes in oxygen demand to regulate blood flow to the heart is unclear.

In this study, we tested the hypothesis that regulation of myocardial blood flow (MBF) by Kv1 channels depends upon their auxiliary Kvβ subunits. The Kvβ proteins are functional aldo-keto reductases that bind NAD(P)(H) and differentially regulate channel gating in response to changes in cellular redox status.[14, 15] Hence, these proteins represent a plausible molecular link between metabolic activity, oxygen availability, and Kv activity that could regulate vasoreactivity.[16] The mammalian genome encodes three Kvβ proteins, which have been shown to control the voltage sensitivity, surface localization, and subcellular distribution of Kv1 channels in excitable cells of the cardiovascular and nervous systems.[17] Consistent with this, in our previous work, we reported that Kvβ proteins support the functional expression of Kv channels in cardiomyocytes and contribute to the metabolic regulation of cardiac repolarization.[18] The Kvβ proteins are expressed throughout the coronary vasculature of humans[19] and rodents,[20] and we have recently reported that native Kv1 channels of coronary arterial myocytes are heteromeric assemblies of Kvβ1.1 and Kvβ2 proteins.[20] Using a combination of genetically engineered mice with *ex vivo* and *in vivo* approaches, we now report that Kvβ1.1 and Kvβ2 have contrasting roles in regulating MBF and cardiac function under stress, and that they impart oxygen sensitivity to vascular tone.

METHODS

Data Availability.

Materials used for this study and supporting data are available from the corresponding authors upon reasonable request. Please see the Major Resources Table in the Supplemental Materials. All animal procedures used for this study were conducted in accordance with the Institutional Animal Care and Use Committee at the University of Louisville and Northeast Ohio Medical University, per the guidelines set forth by the National Institutes of Health. An expanded Materials and Methods can be found in the Online Supplement.

RESULTS

Kvβ2 is required for sustained cardiac pump function during stress.

Under conditions of heightened cardiac workload, sustained pump function is critically dependent on Kv1-mediated coronary vasodilation for sufficient oxygen delivery to meet myocardial metabolic demand.[11, 12, 21] We first tested whether loss of Kvβ proteins affects cardiac performance under stress. Figure 1A shows representative M mode echocardiographic images from wild type (WT) and Kvβ2$^{-/-}$ animals during intravenous infusion of norepinephrine (5 μg/kg·min^{-1}). Norepinephrine enhanced cardiac function, as indicated by an increase in ejection fraction. However, steady-state ejection fraction during infusion of 2.5 and 5 μg/kg·min^{-1} norepinephrine was significantly lower in Kvβ2$^{-/-}$

animals than in WT animals (Figure 1A, B). Specifically, ejection fraction after ~1 min of 5 μg/kg·min^{-1} norepinephrine infusion was 71 ± 1.7% in Kvβ2$^{-/-}$ mice versus 84 ± 2.2% in WT animals. Ejection fraction in Kvβ1.1$^{-/-}$ mice did not differ significantly from that in WT mice at any dose of norepinephrine (P = 0.093).

Figure 1C shows exemplary effects of norepinephrine infusion on arterial blood pressure in WT and Kvβ2$^{-/-}$ mice. Norepinephrine infusion increased steady state blood pressure in both groups. Consistent with our previous report,[12] norepinephrine led to an increase in arterial blood pressure in WT animals that was sustained for the duration of drug administration. However, in Kvβ2$^{-/-}$ mice, norepinephrine-induced elevation of pressure was not sustained, but declined after ~40 s of infusion. This inability to maintain elevated blood pressure during stress is reminiscent of effects in Kv1.5-null mice.[12] Therefore, as is the case with Kv1.5, Kvβ2 appears to play an essential role in supporting cardiac contractile performance under conditions of catecholamine stress and enhanced cardiac workload.

Relationship between myocardial blood flow and cardiac workload is disrupted in Kvβ2-null mice.

The inability of Kvβ2$^{-/-}$ mice to sustain cardiac performance may reflect insufficient oxygen delivery during stress. Thus, we postulated that Kvβ proteins may be integral to the relationship between myocardial blood flow (MBF) and cardiac workload. To test this, we used myocardial contrast echocardiography (MCE)[11, 12] to compare MBF in WT and Kvβ-null mice. MCE uses high-power ultrasound to destruct lipid-shelled echogenic microbubbles in circulation. Subsequent replenishment of signal intensity in a region of interest following disruption is used to calculate the tissue perfusion (Figure 2A, see Methods). Because MBF responds to changes in ventricular workload and myocardial metabolic activity, we used MCE to evaluate MBF as a function of cardiac workload (i.e., double product of mean arterial blood pressure x heart rate),[12] monitored at baseline and during intermittent intravenous infusions of norepinephrine (0.5 – 5 μg/kg·min^{-1}). Figure 2B shows representative contrast signal intensities plotted over a period of ~10 s after microbubble destruction and fit with a one-phase exponential function (see *inset*) in WT (129SvEv), Kvβ1.1$^{-/-}$, and Kvβ2$^{-/-}$ mice (5 μg/kg·min^{-1} norepinephrine). The relationship between MBF and double product shows a modest elevation of MBF, albeit across a lower workload range in Kvβ1.1$^{-/-}$ mice compared with WT mice (Figure 2C). However, consistent with impaired cardiac function under stress conditions described above (see Figure 1), levels of MBF recorded in Kvβ2$^{-/-}$ mice were markedly reduced. Specifically, linear regression analysis showed a significant reduction in the slope of the MBF-work relationship in Kvβ2$^{-/-}$ mice (Figure 2D). MAP, HR, and echocardiographic data at baseline and after acute norepinephrine infusion for each group are summarized in Online Figure I and Table I. Note that cardiac workload in Kvβ1.1$^{-/-}$ mice was reduced due to lower MAP relative to corresponding wild type mice in the presence of 1–5 μg/kg·min^{-1} norepinephrine (see Figures 2C and Online Figure I). However, MAP, HR, and double product were not significantly different between WT and Kvβ2$^{-/-}$ mice over the tested range of norepinephrine. Taken together, these data reflect differential roles for Kvβ1.1 and Kvβ2 proteins in regulating MBF, whereby loss of Kvβ2 suppresses MBF and impairs cardiac function as the heart is subjected to increased workloads.

Oxygen sensitivity of coronary arterial diameter is modified by Kvβ2.

Impaired Kv1-mediated coronary vasodilation results in a markedly reduced myocardial oxygen tension during increased metabolic demand.[22] We therefore posited that coronary vasodilation in response to metabolic stress may be impaired by loss of Kvβ2. Arteries of the systemic circulation exhibit robust dilation in response to metabolic stressors such as hypoxia and intracellular acidosis via a number of purported mechanisms, including activation of Kv channels.[23, 24] Hence, we examined the *ex vivo* vasoreactivity of coronary arteries isolated from WT and Kvβ2$^{-/-}$ mice in response to an acute reduction in oxygen. When subjected to physiological intravascular pressures, isolated coronary arteries developed myogenic tone (i.e., 8 ± 2% and 11 ± 2% at 60 and 80 mmHg, respectively). To evaluate vasodilatory capacity, arteries were pressurized (60 mmHg), pre-constricted with 100 nM U46619, and subjected to hypoxic bath conditions (physiological saline solution aerated with 95% N_2/5% CO_2 and containing 1 mM hydrosulfite).[25–27] Direct measurement of bath O_2 levels confirmed a significant reduction in O_2 from control levels during application of hypoxic conditions (Figure 3A). As shown in Figure 3B (***top***) and Online Figure II, coronary arteries isolated from WT mice responded to hypoxic perfusate with robust and reversible dilation. Vasodilation was not observed when 1 mM hydrosulfite was applied in the presence of 20% O_2 (Online Figure II). Consistent with the involvement of Kv1 channels, the selective Kv1 inhibitor psora-4 (500 nM) significantly attenuated (~58%) hypoxia-induced vasodilation (Online Figure II). Likewise, hypoxia-induced dilation was significantly reduced in arteries from Kvβ2$^{-/-}$ mice (19.6 ± 6.4%) compared with arteries from WT mice (56.9 ± 6.2%) (Figure 3B-D). Together, these data suggest that Kvβ2 proteins facilitate vasodilation to reduced PO_2 and support the notion that Kvβ proteins link tissue perfusion to local oxygen consumption.

L-lactate augments I_{Kv} in coronary arterial myocytes and induces coronary vasodilation via Kvβ2.

We tested whether Kv1 activity in coronary arterial myocytes is sensitive to acute changes in oxygen due to alterations in cellular redox potential via elevation of L-lactate. Our reasoning for examining the effects of L-lactate was two-fold: first, myocardial underperfusion leads to a rapid decline in tissue PO_2, increased anaerobic metabolism, and net accumulation of L-lactate that can promote feedback coronary vasodilation to increase MBF.[21, 28–31] Second, it is plausible that Kv1 channels, via association with Kvβ proteins, may be acutely responsive to changes in lactate secondary to modification of cellular NADH:NAD^+ ratio after uptake and interconversion to pyruvate via the lactate dehydrogenase reaction.[15, 17, 32–35] Consistent with this expectation, using the perforated whole cell configuration of the patch clamp technique, we observed a significant increase in outward K^+ current density (pA/pF) in isolated coronary arterial myocytes immediately following (1–3 min) application of 10 mM L-lactate in the bath (Figure 4A, C). However, this effect was abolished when L-lactate was applied in the presence of the Kv1 blocker psora-4 (500 nM, Figure 4B, D). The change in I_K induced by application of 10 mM L-lactate in coronary arterial myocytes in the absence and presence of psora-4 is shown in Figure 4E. These data indicate that L-lactate acutely potentiates I_{KV} in coronary arterial myocytes.

We next examined the vasodilatory response of preconstricted coronary arteries to increasing concentrations of extracellular L-lactate. As shown in Figure 4F and consistent with previous studies,[36, 37] isolated coronary arteries that were pre-constricted with 100 nM U46619 exhibited step-wise vasodilation in response to elevation of external L-lactate (5–20 mM). This effect was abolished when L-lactate was applied in the presence of 500 nM psora-4 (Figure 4G, I), consistent with involvement of I_{Kv} described above. Furthermore, L-lactate-induced vasodilation was also abolished in arteries isolated from Kvβ2$^{-/-}$ mice, indicating a key role for this subunit in L-lactate-induced vasodilation (Figure 4H, I). These data are consistent with the notion that the regulation of Kvβ2 via vascular intermediary metabolism controls coronary vasodilatory function upon acute changes in myocardial oxygen tension.

Functional role for Kvβ2 in L-lactate-induced vasodilation of resistance mesenteric arteries.

We next asked whether the role for Kvβ in redox-dependent vasoreactivity is confined to the coronary vasculature or is generally observed in peripheral resistance arterial beds where Kv1 prominently controls vascular tone. For this, we first compared Kvβ protein-protein interactions in arterial myocytes of coronary versus mesenteric (3rd and 4th order) arteries using *in situ* proximity ligation (PLA), as previously described.[18, 20, 38] The PLA method is based on dual labelling of proteins that are located within close proximity (<40 nm), and thus, is an approach used to identify protein-protein interactions in complexes with molecular resolution. We observed robust PLA-associated fluorescent signals in coronary arterial myocytes that were co-labelled with Kv1.5 and Kv1.2, Kv1.5 and Kvβ1, Kv1.5 and Kvβ2, or Kvβ1 and Kvβ2 (Figure 5A), consistent with heteromeric oligomerization of Shaker channels.[20, 39] The number of fluorescent sites assigned to these - α/α, α/β, and β/β interactions were similar between coronary and mesenteric arterial myocytes (Figure 5A, B). PLA-associated fluorescence in cells labeled for Kv1.5 alone was negligible for arterial myocytes of both beds. These data suggest that Kv α/β subunit expression patterns and interactions are similar in arterial myocytes of these two distinct vascular beds.

Next, we tested whether knockout of Kvβ1.1 or Kvβ2 alters the regulation of mesenteric arterial diameter. Note that ablation of either of these Kvβ proteins had no statistically significant effect on the active (i.e., myogenic tone) or passive responses to increases in intravascular pressure, nor did it impact vasoconstriction responses to direct membrane potential depolarization with 60 mM K^+ or the stable thromboxane A_2 receptor agonist U46619 (100 nM; Online Figure III). Similar to observations in isolated coronary arteries (see Figure 4F), application of L-lactate (5–20 mM) resulted in robust and reversible dilation of isolated mesenteric arteries (Figure 5C). L-lactate-mediated vasodilation was insensitive to endothelial denudation but was abolished when arteries were constricted with elevated external K^+, rather than U46619 (Online Figure IV). Consistent with observations in isolated coronary arteries, vasodilation in response to L-lactate was eliminated by the Kv1-selective inhibitor psora-4 and loss of Kvβ2 (Figure 5C-E). The dilatory response to L-lactate was not significantly different between arteries from Kvβ1.1$^{-/-}$ mice when compared with arteries from corresponding WT animals (Online Figure V). Moreover, in contrast to the disparate effects of L-lactate, vasodilation induced by adenosine (1 – 100 μM) was not significantly

different between Kvβ1.1$^{-/-}$ or Kvβ2$^{-/-}$ arteries, when compared with corresponding WT arterial preparations (Online Figure VI). Together with results shown in Figures 2–4, these data identify Kvβ2 as a functional regulatory constituent of Kv1 channels that imparts stimulus-dependent redox control of vascular tone.

Increasing the Kvβ1.1: Kvβ2 ratio suppresses redox-dependent vasodilation and MBF.

Native Kv1 channels are comprised of pore-forming subunits associated with more than one Kvβ subtype. This combinatorial variability may contribute to the diversity and cell-specific adaptability of channel function to a wide range of physiological and pathological stimuli. In coronary arterial myocytes, both Kvβ1.1 and Kvβ2 proteins are present in native Kv1 auxiliary subunit complexes;[20] however, our data suggest that these proteins may have divergent roles in the regulation of arterial diameter and myocardial perfusion. That is, in contrast to our observations made in Kvβ2$^{-/-}$ mice, deletion of Kvβ1.1 did not impede MBF. Structural comparison of the two subunits shows a clear difference in the N-termini of Kvβ1 and Kvβ2 subunits. The N-termini of Kvβ1 proteins form a ball-and-chain-like inactivation domain, a feature that is lacking in Kvβ2.[17] Thus, we hypothesized that the association of Kvβ1.1 with Kv1 channels may serve to counter the regulatory function imparted by Kvβ2. A testable prediction based on this hypothesis is that increasing the ratio of Kvβ1.1:Kvβ2 subunits in arterial myocytes would recapitulate the effects of Kvβ2 deletion. To examine this possibility, we generated transgenic mice with conditional doxycycline-inducible overexpression of Kvβ1.1 in smooth muscle cells (Figure 6A, see Methods). Briefly, this model consists of transgenic mice with a reverse tetracycline trans-activator driven by the SM22α promoter (SM22α-rtTA)[40] crossed to transgenic mice with Kcnab1 downstream of the tetracycline responsive element (TRE-Kvβ1) to yield double transgenic (SM22α-rtTA:TRE-Kvβ1) and single transgenic littermate control (SM22α-rtTA) mice. Western blot revealed elevated Kvβ1 protein abundance in arteries of SM22α-rtTA:TRE-Kvβ1 mice after doxycycline treatment, compared with arteries from doxycycline-treated SM22α-rtTA mice (Figure 6B,C). Consistent with a lack of doxycycline effects on Kvβ1 protein in peripheral tissues, no statistically significant differences were observed in Kvβ1-associated band intensities in brain lysates of SM22α-rtTA:TRE-Kvβ1 versus SM22α-rtTA mice.

We next measured the relative levels of Kv1α:Kvβ protein interactions in coronary arterial myocytes via PLA. We observed PLA-associated fluorescent punctae in coronary arterial myocytes from SM22α-rtTA that were either co-labelled with Kv1.5 and Kvβ1, or with Kv1.5 and Kvβ2. Consistent with results of Western blot experiments described above, we observed a significant increase in Kv1.5:Kvβ1-associated PLA signal in coronary arterial myocytes from SM22α-rtTA:TRE-Kvβ1 when compared with myocytes from SM22α-rtTA mice (Figure 6D, E). Notably, Kv1.5-Kvβ2-associated PLA signal was reduced in myocytes from SM22α-rtTA:TRE-Kvβ1 when compared with myocytes from SM22α-rtTA mice, suggesting that double transgenic mice express vascular Kv1 complexes with increased ratios of Kvβ1.1:Kvβ2 subunits. Functionally, enhanced Kvβ1.1:Kvβ2 subunit composition in arterial myocytes from SM22α-rtTA:TRE-Kvβ1 was associated with significantly blunted vasodilation of isolated mesenteric arteries in response to extracellular L-lactate when compared with arteries from single transgenic control mice (Figure 6F, G). Indeed, these

observations in SM22α-rtTA:TRE-Kvβ1 arteries were similar to those made in coronary and mesenteric arteries from Kvβ2$^{-/-}$ mice, as well as arteries from WT mice pre-treated with the Kv1-selective inhibitor psora-4 (see Figure 4F-I and and 5C-E). *In vivo* evaluation of the relationship between MBF and cardiac workload revealed significantly suppressed MBF in SM22α-rtTA:TRE-Kvβ1 mice when compared with SM22α-rtTA mice (Figure 6H). No differences in heart rate or MAP were observed between groups of mice (Online Figure VII). Together, these results indicate that Kvβ1.1 in arterial myocytes functions as an inhibitory regulator of vasodilation, and that the control of MBF is balanced on the juxtaposing functional influences of Kvβ1.1 and Kvβ2 proteins.

DISCUSSION

In this study we identify vascular Kvβ proteins as key regulators of myocardial blood flow. Our findings suggest that the auxiliary Kvβ subunits impart oxygen sensitivity to Kv1 channel function, enabling them to trigger vasodilation in response to an increase in oxygen demand. A functional role of Kvβ proteins in imparting oxygen-sensitivity to Kv1 channels and thereby regulating vasodilation is supported by the following key findings: 1) Kvβ2$^{-/-}$ mice exhibit acute cardiac failure during administration of norepinephrine; 2) MBF is significantly suppressed across the physiological range of cardiac workload in Kvβ2$^{-/-}$ mice, yet is moderately enhanced in Kvβ1.1$^{-/-}$ mice; 3) vasodilation of isolated coronary arteries in response to hypoxia and elevation of extracellular L-lactate is strongly attenuated by loss of Kvβ2; 4) whereas ablation of Kvβ proteins does not impact vasoconstriction of resistance caliber mesenteric arteries, vasodilation of these vessels in response to L-lactate is abolished by ablation of Kvβ2, comparable to effects of Kvβ2 deletion in coronary arteries; and 5) increasing the Kvβ1.1:Kvβ2 ratio in smooth muscle impairs L-lactate-induced vasodilation and suppresses MBF, similar to observations made in Kvβ2$^{-/-}$ arteries and mice. Collectively these results support the concept that Kvβ1.1 and Kvβ2 cooperatively control vascular function and regulate MBF upon changes in metabolic demand.

Kv1 channels belong to one of several Kv subfamilies that regulate membrane potential and $[Ca^{2+}]_i$ in arterial myocytes to control vessel diameter and blood flow.[41] Pharmacological blockade of Kv1 channels reduces whole-cell outward I_K by ≥50%,[42] whereas increased steady-state I_{Kv} results in membrane hyperpolarization and reduced Ca^{2+} influx via voltage-gated Ca^{2+} channels.[43] The resultant reduction in cytosolic $[Ca^{2+}]_i$ leading to myocyte relaxation, and vasodilation increases local tissue perfusion. Considering the relatively high resting input resistance (1–10 GΩ) of arterial smooth muscle cells, the opening or closure of few K^+ channels can generate substantial changes in membrane potential and vascular tone.[44, 45] Consequently, the functional expression of native Kv channels of arterial myocytes is dynamically controlled by multiple molecular processes, which include post-transcriptional regulation (e.g., phosphorylation, glycosylation), subcellular trafficking and recycling, redox modifications, as well as association with accessory subunits and regulatory proteins.[21, 31, 46–48] Adding to this complexity, our observation that deletion of Kvβ2 disrupts Kv1-dependent vasodilation is consistent with a functional role of this subunit in regulating the vasodilatory response to metabolic stress.

Kv channels in excitable cells assemble as either homomeric or heteromeric structures with varied $\alpha_4\beta_4$ configurations of pore-forming and auxiliary subunits.[49–52] This 'mix-and-match' capability of Kv channels contributes to the wide heterogeneity of K^+ currents that enables diverse physiological roles across different cell types. In our previous work we found that Kv1 channels in murine coronary arterial myocytes interact with Kvβ1.1/Kvβ2 heteromers,[20] and our present findings suggest a divergent functional regulation of vascular tone and blood flow by these proteins. These divergent roles are revealed by the observation that even though Kvβ2 ablation suppressed vasodilatory function and MBF, the loss of Kvβ1.1 had little impact on arterial diameter *ex vivo*, but elevated MBF *in vivo*. These findings suggest that Kvβ1 and β2 have somewhat divergent and potentially antagonist roles, which may relate to differences in their structures. The Kvβ1 has a ball-and-chain inactivation domain at the N-terminus, a feature that is lacking in Kvβ2. Potentially as a result of these differences, individual subunits have differential effects on the gating of non- and slowly-inactivating Kv1α channels.[53] Specifically, Kvβ1 induces N-type inactivation in non-inactivating Kv1α proteins whereas Kvβ2 increases current amplitude and shifts the voltage-dependence of activation towards more hyperpolarized potentials, with little impact on channel inactivation.[15, 32, 53] These effects are consistent with a greater steady-state activity of non-inactivating Kv1α channels (e.g., Kv1.5) when assembled with Kvβ2, as compared with those predominantly consisting of Kvβ1 proteins.

How the net competing influences of multiple Kvβ subtypes impact the function of native Kv1 channels remains to be resolved; however, it has been reported that within the same auxiliary complex, the N-terminal inactivation function of Kvβ1 is inhibited by Kvβ2 subunits,[54] an effect which may be due to competition between Kvβ subtypes for the intracellular domain of pore-forming Shaker subunits, or through modification of Kvβ1 function via β:β subunit interactions. We found that in arterial myocytes both Kvβ1.1 and Kvβ2 proteins are expressed in native Kv1 channels, and therefore, it is plausible that the greater abundance of Kvβ2 relative to Kvβ1.1 in Kv1 channels of coronary arterial myocytes underlies its functional dominance under physiological conditions. Consistent with this are the apparent differences in inactivation kinetics between slowly inactivating outward K^+ currents measured in coronary arterial myocytes in comparison with rapidly inactivating (i.e., A-type) currents recorded in retinal arteriolar myocytes, which predominantly express Kv1.5 + Kvβ1 proteins.[55–57] Indeed, our current data obtained from novel double transgenic mice overexpressing Kvβ1.1 in smooth muscle suggest that increased abundance of Kvβ1 proteins effectively diminishes the vasodilatory function attributed to Kvβ2. Thus, based on these findings, we speculate that Kvβ1 and β2 play antagonistic roles and that Kv channel remodeling which results in functional upregulation of Kvβ1.1 or downregulation of Kvβ2 (i.e., elevated Kvβ1.1:Kvβ2 ratio) could impair vasodilation and limit tissue perfusion.

The Kvβ proteins were discovered as functional AKRs, a group of enzymes that catalyze the reduction of carbonyl compounds by NAD(P)H.[58, 59] In our previous work, we found that the binding of oxidized and reduced pyridine nucleotides to Kvβ proteins differentially modifies channel gating,[15, 18, 32, 60, 61] thus, raising the possibility that the Kvβ subunits provide a molecular link between the metabolic state of a cell and Kv channel activity. Given the high affinity of Kvβ proteins for pyridine nucleotides,[14, 62] it is plausible that rapid changes in intracellular redox potential of pyridine nucleotides in arterial myocytes

may underlie Kv-mediated control of blood flow in the heart upon changes in metabolic demand. We recently reported that Kvβ2 subunits facilitate surface expression of Kv1 and Kv4 channels in cardiomyocytes and that they impart redox and metabolic sensitivity to cardiac Kv channels, thus coupling repolarization with intracellular pyridine nucleotide redox status;[18] however, to the best of our knowledge, the current study is the first to suggest a fundamental role for these subunits in controlling resistance vascular tone and blood flow.

Although our data show that Kvβ proteins regulate the diameter of resistance arteries subsequent to the modulation of NAD(H) redox via elevation of L-lactate, the precise identity of the factors responsible for coupling between myocardial oxygen consumption and coronary arterial tone remain unclear. Several myocardium-derived 'metabolites' (e.g., local O_2/CO_2 tensions, reactive oxygen species such as H_2O_2, lactate, endothelial-derived factors such as arachidonic acid metabolites)[9] could conceivably alter intracellular pyridine nucleotide redox potential and further work is required to identify specific metabolic processes that link intracellular redox changes to Kv activity. The function of coronary Kv1 channels could also be affected by other long-term biochemical processes. For example, the Kvβ proteins could plausibly alter patterns of basal post-transcriptional regulatory pathways (e.g., PKC-mediated channel phosphorylation)[63] or the surface density of functional channels. However, such differences would likely manifest as differences in myogenic tone development or differential responses to vasoconstrictor stimuli,[64] which were not seen in our study, suggesting that the vasoregulatory effects of Kvβ may reflect more dynamic modifications of channel function.

Even though our study has many strengths, some limitations should be considered. Our studies were performed in mice, which exhibit greater heart rates and MBF relative to larger mammals, including humans.[65] Nonetheless, the positive correlation between myocardial oxygen consumption and MBF is highly conserved across species, and the parallel importance of Kv1 channels in the regulation of MBF is established in small rodents and larger mammals (i.e., swine).[11–13] Thus, it is likely that regulation of MBF by Kvβ proteins, observed in our current study, extends to larger species. Additionally, we cannot exclude the possibility that deletion of Kvβ proteins in non-vascular cell types (e.g., cardiomyocytes, neurons) may contribute to effects on MBF in vivo. However, this is unlikely for several reasons. First, prior work from our group indicates that suppression of blood flow in animals lacking Kv1.5, a predominant Kv1α binding partner of Kvβ, is restored via its conditional reconstitution in smooth muscle.[12] Second, data from novel transgenic mice generated for the current study (SM22α-rtTA:TRE-Kvβ1) indicate that smooth muscle-selective overexpression of Kvβ1.1, which increases the ratio of Kvβ1.1:Kvβ2 subunits in native Kv1 channels in arterial myocytes, leads to robust suppression of vasodilation and MBF, similar to observations in global Kvβ2 knockout mice. Additional evidence from *ex vivo* arterial diameter measurements further supports the role for vascular Kvβ proteins in the regulation of vasoreactivity and is consistent with the notion that Kvβ subunits of native arterial Kv1 channels facilitate the metabolic hyperemia response.

In summary, we report a novel role for intracellular Kvβ subunits in the differential regulation of resistance artery diameter and control of myocardial blood flow. Our results indicate that proper coupling between coronary arterial diameter and myocardial oxygen

consumption relies on the molecular composition of Kv1 accessory subunit complexes such that the functional expression of Kvβ2 is essential for Kv1-mediated vasodilation. Moreover, the current study suggests that perturbations in Kvβ function or expression profile (i.e., Kvβ1.1:Kvβ2) may underlie the dysregulation of blood flow in disease states characterized by impaired microvascular function and ischemia-related cardiac dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms:

I_K	Potassium current
Kv	Voltage-gated potassium channel
Kvβ	Voltage-gated potassium channel beta protein
MBF	Myocardial blood flow
MCE	Myocardial contrast echocardiography
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
rtTA	Reverse tetracycline response element
SM22α	Smooth muscle actin 22 alpha
TRE	Tetracycline response element
WT	Wild type

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NOVELTY AND SIGNIFICANCE

What Is Known?

- Inadequate myocardial perfusion resulting from impaired coronary vasodilation contributes to cardiovascular morbidity and mortality.
- The coronary arteries and arterioles dilate in response to local increases in myocardial oxygen demand in a process that requires smooth muscle voltage-gated potassium (Kv) channels.
- The molecular components of native Kv channels that impart oxygen sensitivity to coronary vascular tone are unknown.

What New Information Does This Article Contribute?

- The intracellular Kv subunit complex (i.e., Kvβ proteins) in coronary arterial smooth muscle is an essential regulator of myocardial perfusion.
- Ablation of Kvβ2 proteins in mice suppresses the relationship between myocardial blood flow and cardiac workload in vivo, and attenuates the oxygen-sensitivity of vascular tone ex vivo.
- Driving an overabundance of Kvβ1.1 proteins in smooth muscle recapitulates the effects of Kvβ2 ablation, indicating the contrasting regulatory roles for Kvβ1 and Kvβ2 proteins within heteromeric vascular Kv1 complexes.

Dynamic changes in the diameter of coronary arteries and arterioles are required to closely match oxygen supply (i.e., blood flow) with the metabolic demands of the heart. Coronary vasodilation and elevated myocardial flow upon acute increases in cardiac workload (e.g., during exercise) requires Kv1 channels expressed in vascular smooth muscle cells. Nonetheless, how vascular Kv1 channels sense changes in oxygen demand to regulate blood flow to active myocardium is unresolved. In this study, we report that the regulation of myocardial blood flow by Kv1 channels is controlled by the intracellular Kvβ protein complex interacting with the membrane-bound pore of the channel. The Kvβ proteins bind cytosolic pyridine nucleotides and differentially regulate channel gating behavior in a redox-dependent manner. Our results support the concept that Kvβ1.1:Kvβ2 heteromeric complexes that interact with native Kv1 channels (e.g., Kv1.5) fine-tune channel responsiveness to changes in metabolic demand by contributing opposing regulatory roles, such that Kvβ2 enhances and Kvβ1.1 inhibits Kv-mediated oxygen-dependent coronary vasodilation. Our study has broad implications for future mechanistic investigations into how the metabolic hyperemia response is modified at the molecular level during disease development and progression.

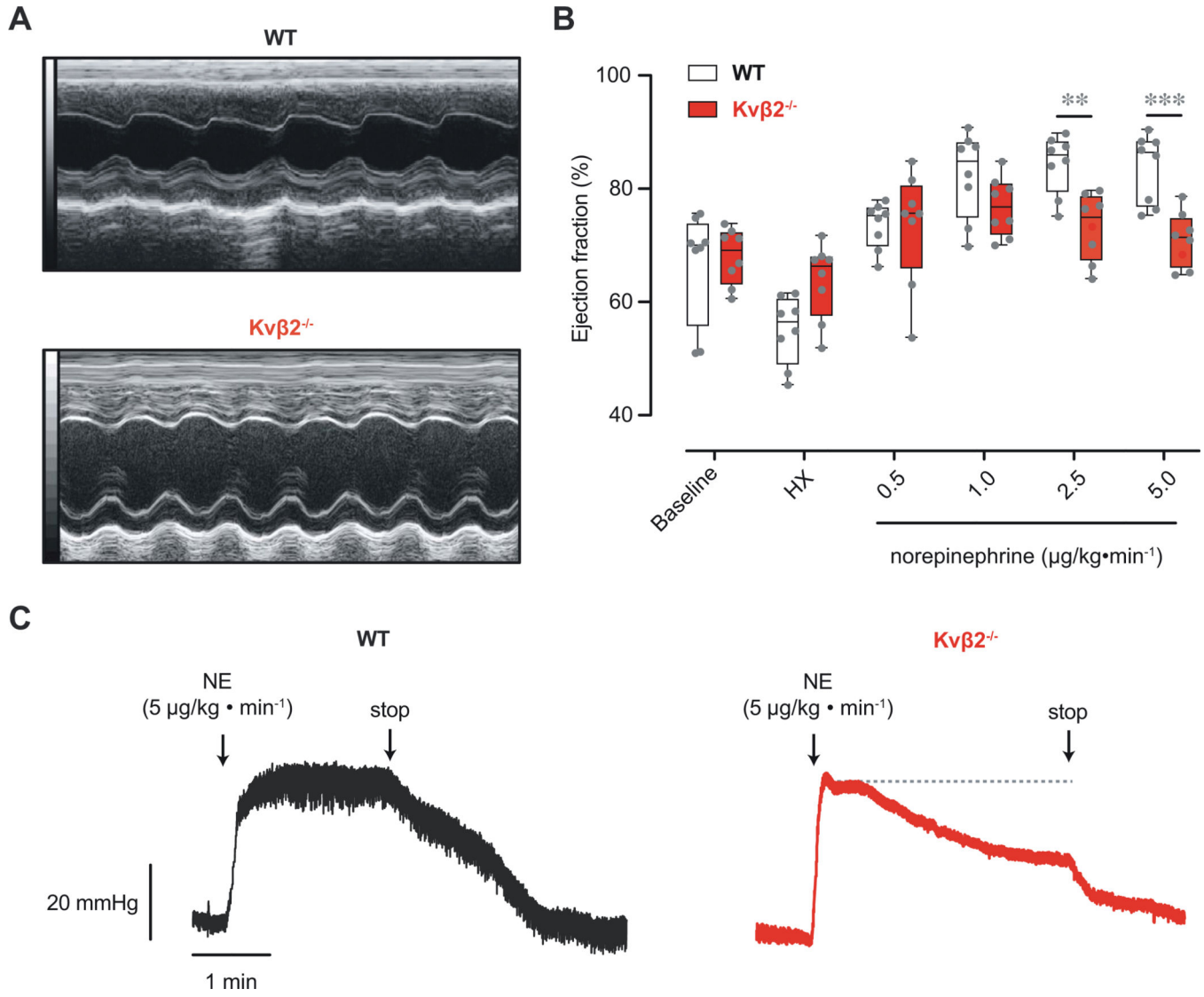

Figure 1: Loss of Kvβ2 impairs cardiac pump function during stress.

(**A**) Representative M-mode echocardiographic images obtained from wild type (WT; 129SvEv), and Kvβ2$^{-/-}$ mice during infusion of 5 μg/kg·min^{-1} norepinephrine. **(B)** Box-and-whisker plot of ejection fraction data for WT and Kvβ2$^{-/-}$ mice at baseline, after administration of hexamethonium (HX; 5 mg·kg^{-1}, i.v.), and during norepinephrine infusions (0.5 – 5 μg/kg·min^{-1}; 2–3 min duration). n = 8 each, **P<0.01, ***P<0.001 (two-way RM ANOVA). (**C**) Arterial blood pressure recordings obtained via femoral artery catheter in WT and Kvβ2$^{-/-}$ mice, before and after norepinephrine treatment (NE, 5 μg/kg·min^{-1}, indicated by arrows).

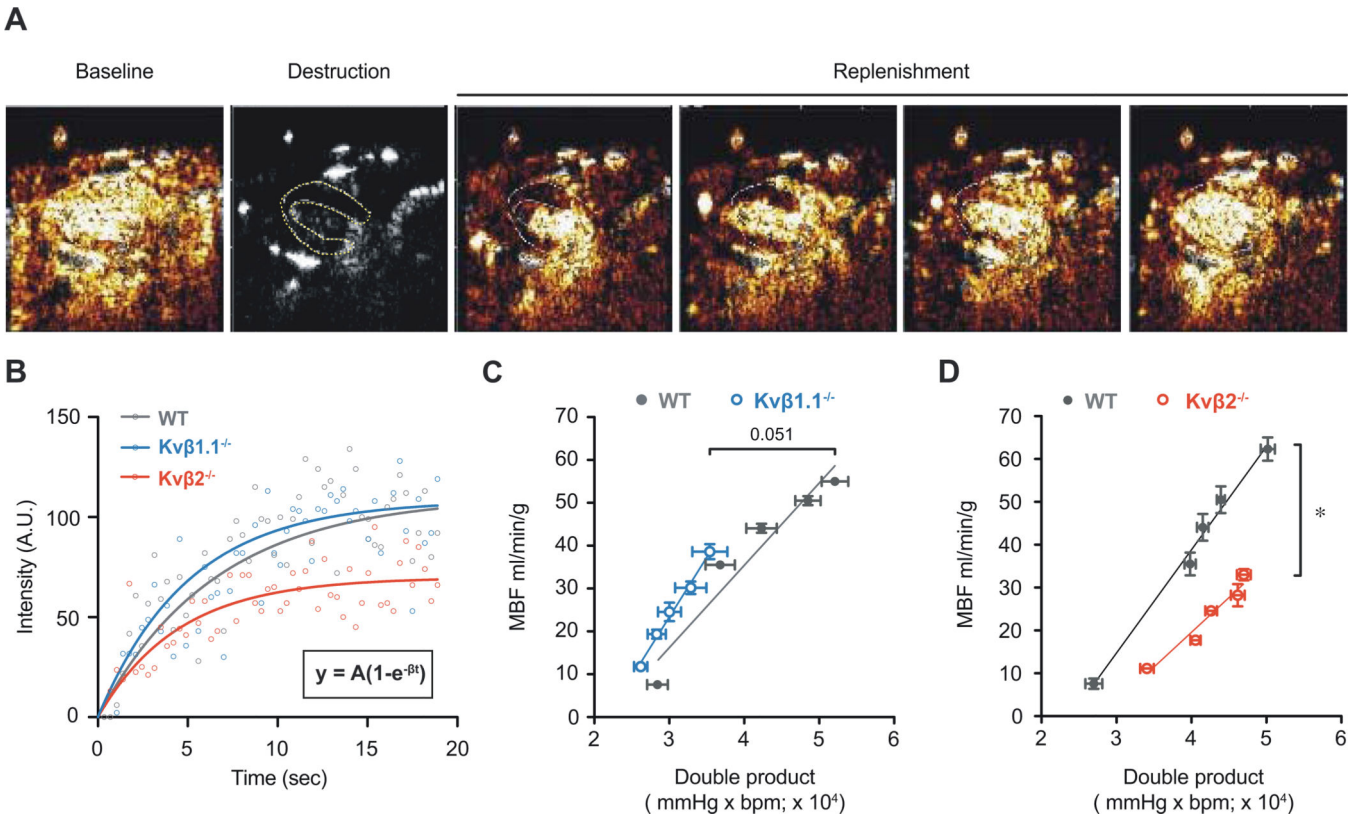

Figure 2: Relationship between myocardial blood flow and cardiac workload in Kvβ-null mice.

(**A**) Long axis MCE images showing signal intensity from myocardial tissue and cavity before destruction frame and during replenishment phase (~10 sec). The left ventricular wall is outlined with a yellow dashed line in the destruction frame. (**B**) Signal intensity versus time (following destruction frame) in region of interest in the anterior left ventricular myocardial wall of WT (129SvEv), Kvβ1.1$^{-/-}$, and Kvβ2$^{-/-}$ mice. Data were fit with exponential function (*see inset*). (**C,D**) Summary of MBF as a function of cardiac workload (double product; heart rate x mean arterial pressure) in Kvβ1.1$^{-/-}$ (*C*) and Kvβ2$^{-/-}$ (*D*) versus strain-matched wild type (WT) control mice. Data were fit with a simple linear regression model with slopes: WT (0.00192 ± 0.00031), Kvβ1.1$^{-/-}$ (0.00279 ± 0.00016); n = 6–8 mice; WT (0.00241 ± 0.00014), Kvβ2$^{-/-}$ (0.00162 ± 0.00022); n = 4–8 mice, *P<0.05, slope of Kvβ2$^{-/-}$ vs. WT.

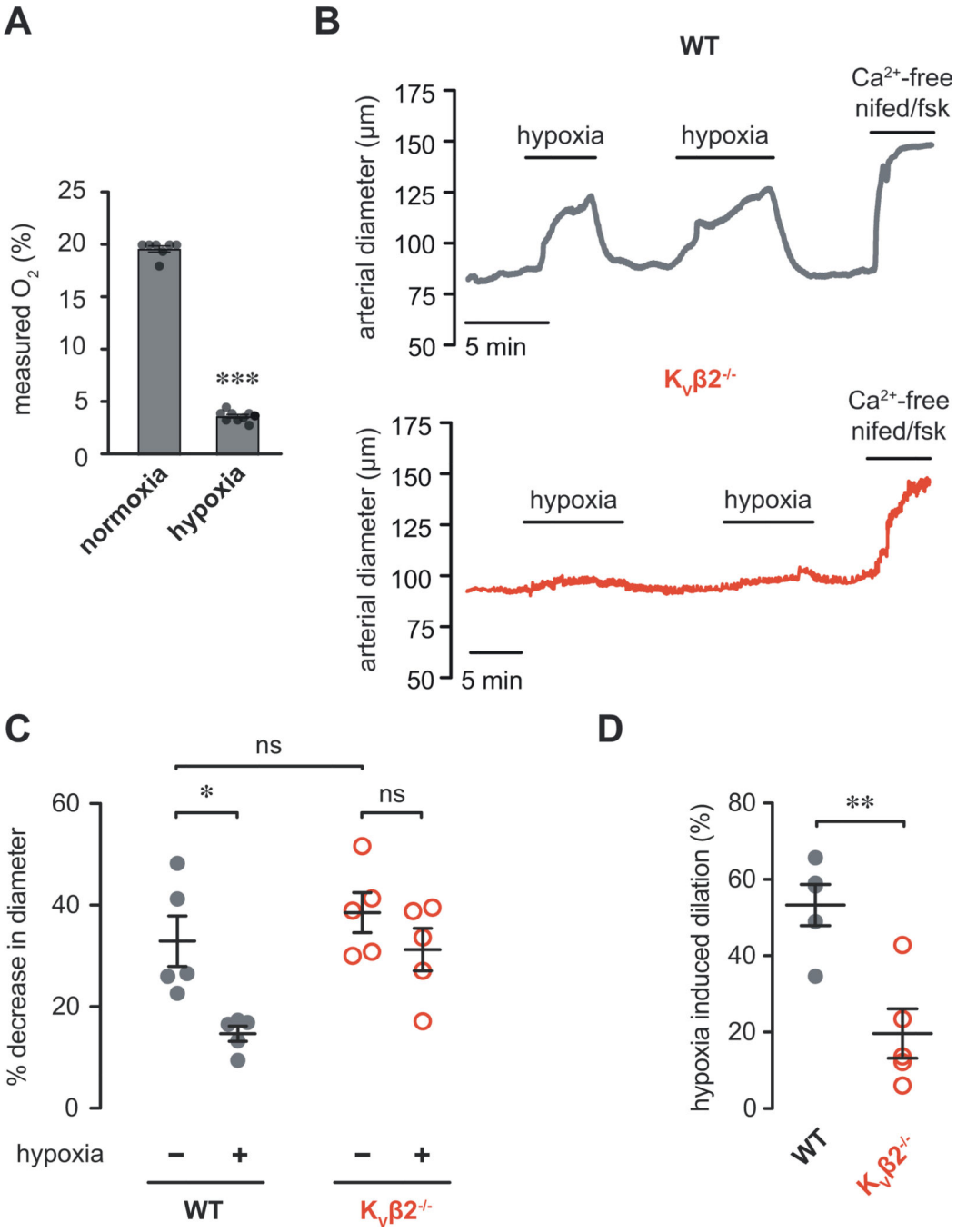

Figure 3: Ablation of Kvβ2 attenuates hypoxia-induced coronary vasodilation.

(**A**) Summarized bath O_2 (%) measured in normoxic and hypoxic conditions (perfusate aerated with 5% CO_2, balance N_2, + 1 mM $Na_2S_2O_4$); data are pooled from measurements obtained with wild type (129SvEv) and Kvβ2$^{-/-}$ coronary arteries. n = 7–9, ***P<0.001 (Mann Whitney U). (**B**) Representative arterial diameter recordings in isolated preconstricted (100 nM U46619) coronary arteries from wild type (WT; 129SvEv) and Kvβ2$^{-/-}$ mice in normoxic and hypoxic conditions. Ca^{2+}-free perfusate containing nifedipine (nifed; 1 µM) and forskolin (fsk; 0.5 µM) was introduced at the end of the

experiment to induce maximum dilation. (**C**) Scatter-plot and mean ± SEM showing percent decrease in diameter recorded under normoxic (- hypoxia) and hypoxic (+ hypoxia) conditions for arteries from WT and Kvβ2$^{-/-}$ mice. Normoxic and hypoxic conditions were both applied in continuous presence of U46619, see above (*B*). n = 5 arteries, 3–4 mice *P<0.05, ns: P≥0.05 (one-way ANOVA, Tukey). (**D**) Scatter-plot and mean ± SEM showing hypoxia-induced dilation (%) for arteries from WT and Kvβ2$^{-/-}$ mice. **P<0.01 (Mann-Whitney U test).

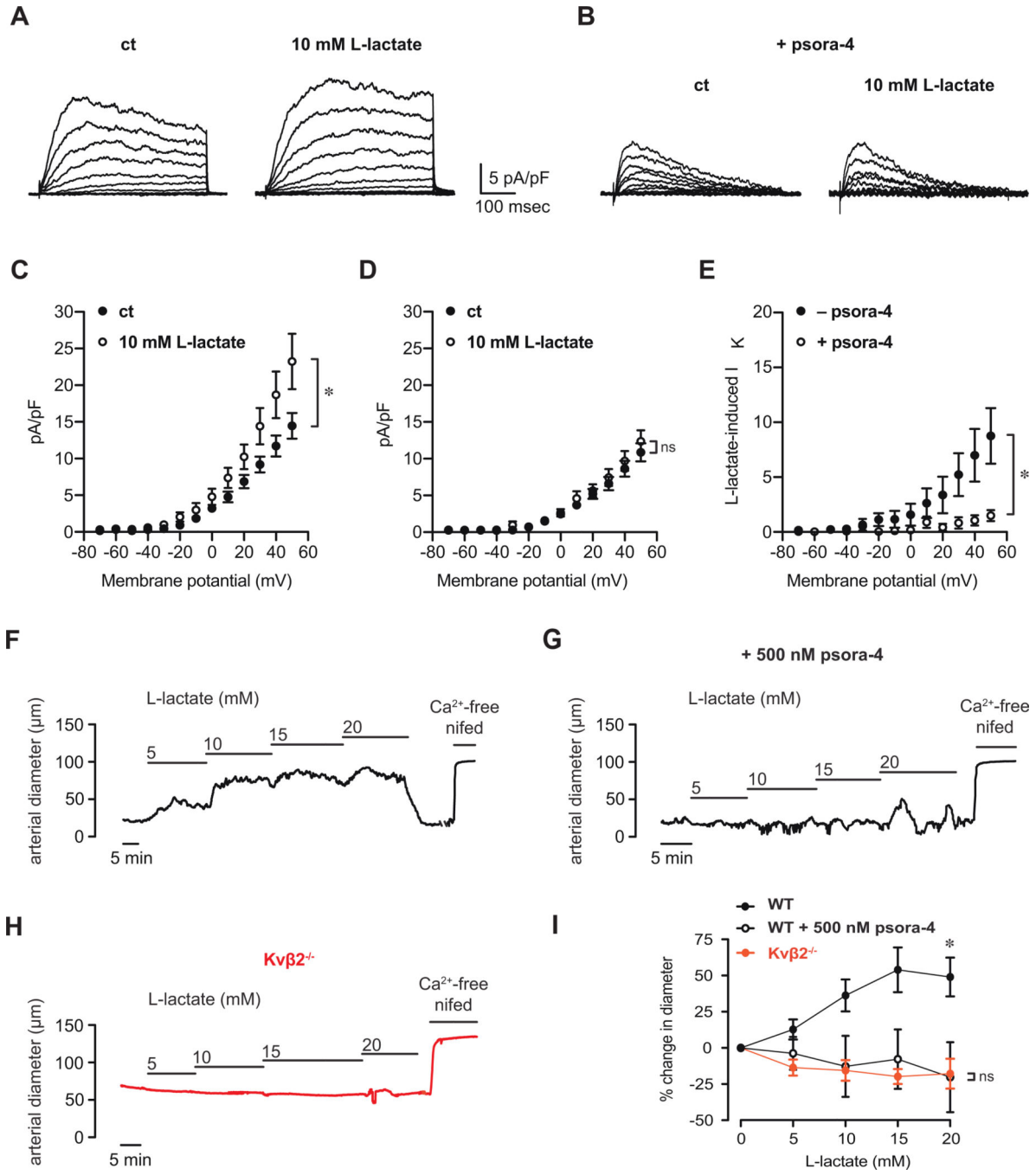

Figure 4: L-lactate enhances I_{Kv} in coronary arterial myocytes and promotes coronary vasodilation via Kvβ2.

(**A, B**) Representative outward K^+ current recordings normalized to cell capacitance (pA/pF) in response to step-wise (10 mV) depolarization to +50 mV from a holding potential of −70 mV in isolated coronary arterial myocytes. Currents were recorded before and after application of 10 mM L-lactate in bath solution lacking (*A*) or containing (*B*) 500 nM psora-4. (**C, D**) Summary current-voltage relationships obtained in coronary arterial myocytes before and after application of 10 mM L-lactate in bath solution lacking (*C*)

or containing (*D*) 500 nM psora-4. n = 5–7 cells from 4–7 mice. *P <0.05, ns: P≥0.05 (two-way RM ANOVA). (**E**) Summary of L-lactate-induced currents recorded in the absence and presence of 500 nM psora-4. n = 5–7 cells from 4–7 mice. *P <0.05 (mixed-effects). (**F-H**) Arterial diameter traces obtained from pressurized (80 mmHg) coronary arteries isolated from wild type (WT; 129SvEv; *F,G*) and Kvβ2$^{-/-}$ (*H*) mice in the absence and presence of L-lactate (5–20 mM, as indicated). Arteries were preconstricted with 100 nM U46619; for WT arteries, L-lactate was applied in the absence (*top*) and presence (*bottom*) of psora-4 (500 nM). Maximum passive diameter was recorded at the end of each experiment in Ca^{2+}-free saline solution with nifedipine (nifed; 1 μM) and forskolin (fsk; 0.5 μM). (**I**) Summary plot showing L-lactate-induced dilation, expressed as a percent change from baseline diameter relative to maximum passive diameter, for arteries isolated from WT (129SvEv; ± 500 nM psora-4) and Kvβ2$^{-/-}$ mice. n = 4 arteries from 4 mice for each. *P<0.001; ns: P≥0.05, lactate vs. baseline (Friedman).

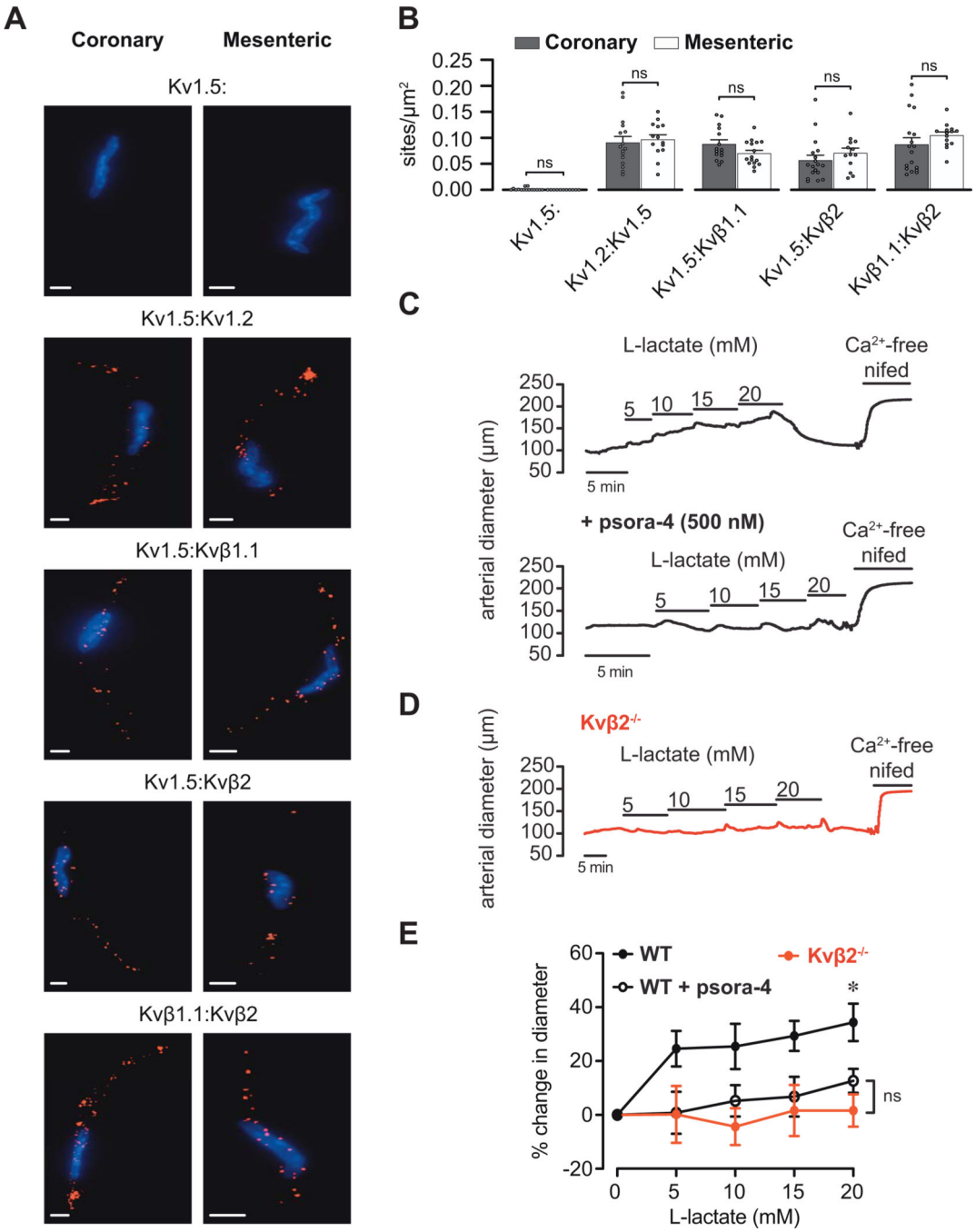

Figure 5: Kvβ2 controls redox-dependent vasoreactivity in resistance mesenteric arteries.

(**A**) Representative fluorescence images showing PLA-associated fluorescent punctae (red) in wild type coronary and mesenteric arterial myocytes. Cells were labelled for Kv1.5 alone, or co-labelled for Kv1.5 and Kv1.2, Kv1.5 and Kvβ1.1, Kv1.5 and Kvβ2, or Kvβ1.1 and Kvβ2 proteins. DAPI nuclear stain is shown for each condition (blue). Scale bars represent 5 μm. (**B**) Summary of PLA-associated punctate sites normalized to total cell footprint area for conditions and groups as in D. P values are shown for coronary versus mesenteric arteries (Mann Whitney U). (**C,D**) Arterial diameter traces obtained from pressurized (80 mmHg)

mesenteric arteries isolated from wild type (*C*; 129SvEv) and Kvβ2$^{-/-}$ (*D*) mice in the absence and presence of L-lactate (5–20 mM, as indicated). Arteries were preconstricted with 100 nM U46619 and L-lactate was applied in the absence (*top*) and presence (*bottom*) of the selective Kv1 channel inhibitor psora-4 (500 nM). Maximum passive diameters were recorded at the end of each experiment in Ca^{2+}-free saline solution with nifedipine (nifed; 1 μM) and forskolin (fsk; 0.5 μM). (**E**) Summary plot of L-lactate-induced dilation, expressed as the percent change from baseline diameter relative to maximum passive diameter, for arteries isolated from WT (129SvEv; ± psora-4) and Kvβ2$^{-/-}$ mice. n = 5 arteries from 4–5 mice for each. *P<0.05; ns: P≥0.05, lactate vs. baseline (Friedman).

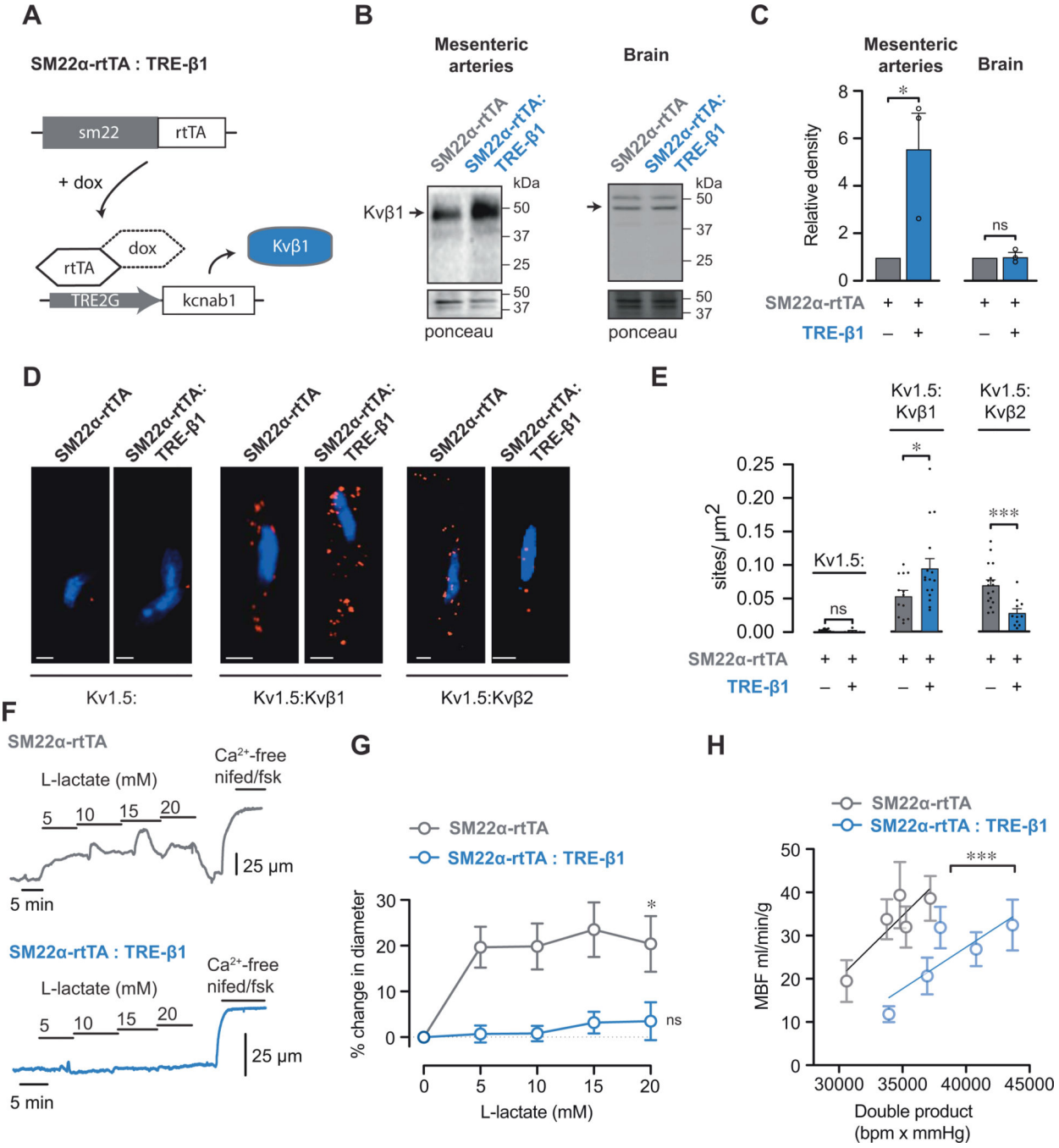

Figure 6: Increasing the ratio of Kvβ1.1:Kvβ2 subunits in smooth muscle inhibits L-lactate-induced vasodilation and suppresses myocardial blood flow.

(**A**) Schematic diagram describing the SM22α-rtTA:TRE-β1 model. Double transgenic animals (+dox) results in activation of the reverse tetracycline trans-activator (rtTA) in smooth muscle cells, and drives expression of Kvβ1.1. (**B**) Western blots showing immunoreactive bands for Kvβ1 in whole mesenteric artery and brain lysates from SM22α-rtTA (single transgenic control) and SM22α-rtTA:TRE-β1 (double transgenic) mice after doxycycline treatment. Ponceau-stained membrane (mol. Wt.: ~30–55 kDa) is shown as

an internal control for total loaded protein. (**C**) Summarized relative densities of Kvβ1.1-associated immunoreactive bands in mesenteric arteries and brains of SM22α-rtTA:TRE-β1 relative to SM22α-rtTA. n = 3 each. *P<0.05, ns: P≥0.05 (one sample t test). (**D**) Representative fluorescence images showing PLA-associated fluorescent punctae (red) in coronary arterial myocytes isolated from SM22α-rtTA and SM22α-rtTA:TRE-β1mice. Cells were labelled for Kv1.5 alone, or co-labelled for Kv1.5 and Kvβ1, or Kv1.5 and Kvβ2 proteins. DAPI nuclear stain is shown for each condition (blue). Scale bars represent 5 μm. (**E**) Summary of PLA-associated punctate sites normalized to total cell footprint area for conditions and groups as in D. n = 6–19 cells from 2–3 mice for each; *P<0.05, **P<0.001 (Mann Whitney U). (**F**) Representative arterial diameter recordings from 100 nM U46619-preconstricted mesenteric arteries isolated from SM22α-rtTA and SM22α-rtTA:TRE-β1 mice in the absence and presence of L-lactate (5–20 mM), as in Figure 5C,D. Passive dilation in the presence of Ca^{2+}-free solution + nifedipine (1 μM) and forskolin (fsk; 0.5 μM) is shown for each recording. (**G**) Summary plot of L-lactate-induced dilation for arteries isolated from SM22α-rtTA and SM22α-rtTA:TRE-β1 mice. n = 6–10 arteries from 5–6 mice; *P<0.05; ns: P≥0.05, lactate vs. baseline (Friedman). (**H**) Summary relationships between myocardial blood flow (MBF) and cardiac workload (double product; heart rate x mean arterial pressure) in SM22α-rtTA:TRE-β1 vs. SM22α-rtTA control mice. n = 5 each; ***P<0.001 (linear regression).