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Kv1.3 Channels Facilitate the Connection Between Metabolism and Blood Flow in the Heart

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Abstract

Objective—The connection between metabolism and flow in the heart, metabolic dilation, is essential for cardiac function. We recently found redox-sensitive Kv1.5 channels play a role in coronary metabolic dilation; however, more than one ion channel likely plays a role in this process since animals null for these channels still showed limited coronary metabolic dilation. Accordingly, we examined the role of another Kv1 family channel, the energetically-linked Kv1.3 channel, in coronary metabolic dilation.

Methods—We measured myocardial blood flow (contrast echocardiography) during norepinephrine-induced increases in cardiac work (heart rate × mean arterial pressure) in wild type mice (WT), WT mice given correolide (preferential Kv1.3 antagonist), and Kv1.3 null mice (Kv1.3$^{-/-}$). We also measured relaxation of isolated small arteries mounted in a myograph.

Results—During increased cardiac work, myocardial blood flow was attenuated in Kv1.3$^{-/-}$ and in correolide-treated mice. In isolated vessels from Kv1.3$^{-/-}$ mice, relaxation to H_2O_2 was impaired (vs WT), but responses to adenosine and acetylcholine were equivalent to WT. Correolide reduced dilation to adenosine and acetylcholine in WT and Kv1.3$^{-/-}$, but had no effect on H_2O_2-dependent dilation in vessels from Kv1.3$^{-/-}$ mice.

Conclusion—Kv1.3 channels participate in the connection between myocardial blood flow and cardiac metabolism.

Introduction

The heart relies on aerobic metabolism to convert energetic substrates into chemical energy (ATP) to maintain normal cardiac pump function. The maintenance of cardiac function depends on a "balance" between supply of energetic substrates and oxygen, coupled to the demands for oxygen and energy. The principal components in this balance are the coronary circulation, which regulates supply, and the working cardiac myocytes, which dictate

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demand. The coupling between metabolism and flow is primarily dictated by the production of metabolic vasodilators that are by-products of metabolism[1]. The production of these vasoactive factors is essential because the primary manner by which the heart increases oxygen delivery is via increased blood flow as oxygen extraction is already of near maximum[2, 3]. Insufficient delivery of oxygen can impair contractile function of the heart within seconds[4]. Despite the importance of this connection there is a relative paucity of information regarding the key signaling proteins that transduce the metabolic signals into increases in vascular caliber.

Most previous efforts directed at understanding the links between metabolism and flow in the heart focused on specific metabolites, e.g., adenosine[5, 6], which signal through G-protein coupled receptors to produce vasodilation[7–10]. Although a large body of work supports the role of metabolites such as adenosine in coronary regulation, some observations have challenged this hypothesis[11, 12]. More recent work is directed at examining the role of ion channels as transducers of the metabolic information[9, 13, 14]. In this regard, tone of vascular smooth muscle is primarily controlled by the membrane potential[15–18], which regulates the open probability of voltage-gated calcium channels. Factors which induce hyperpolarization, such as opening of potassium channels, reduce calcium entry through the voltage-gated calcium channels, producing vasodilatation. In contrast, closure of K^+ channels leads to membrane depolarization and causes vasoconstriction[19–21].

Coronary blood flow is modulated by metabolic, myogenic and endothelium-dependent vasodilators and constrictors[22, 23]. These many signals are integrated at the level of the smooth cell with an overall effect on intracellular calcium levels mediated by changes in membrane potential and/or modulation of calcium release or uptake mechanisms in internal stores[15, 16, 24, 25]. A vasodilator will decrease sarcoplasmic calcium levels; thereby inducing vasodilation. In metabolic hyperemia, blood flow is largely controlled by the production of vasoactive metabolites, which induce vasodilation in order to maintain the match between myocardial metabolism or cardiac work and myocardial blood flow[1].

Our previous results have suggested the production of hydrogen peroxide by mitochondria is one of the metabolic mediators contributing to the coupling between metabolism and flow[26]. We also observed that the dilation produced by H_2O_2 is mediated by redox-dependent reactions via Kv channels[27, 28]. Using a murine model of coronary metabolic dilation, we observed that Kv1.5 channels play an important role in connecting flow to metabolism in the heart.[14] Mice null for Kv1.5 channels had blunted dilation (compared to wild types) during increases in cardiac work, but it is important to emphasize that some degree of dilation remained. Due to this residual dilation, we hypothesized that additional ion channels are likely involved in coronary metabolic dilation. Based on this speculation, we proposed that Kv1.3 channels play a role in coupling flow to metabolism in the heart, as these channels are thought to be regulated by cell metabolism[29–33].

Accordingly, we studied coronary metabolic dilation (changes in myocardial blood flow [MBF] in response to increases in cardiac work) in wild-type mice, mice null for Kv1.3 channels ($Kv1.3^{-/-}$), and mice acutely treated with the preferential Kv1.3 channel

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antagonist, correolide. Our results support the idea that Kv1.3 channels play a critical role in connecting myocardial blood flow to cardiac metabolism.

METHODS

Murine Models

Male and Female wild type mice (WT) on the C57Bl/6J background, and Kv 1.3 null mice on the same background (Kv1.3$^{-/-}$) were used in this study. Kv1.3$^{-/-}$ mice were obtained from Dr. Gary Desir (Yale University), who initially observed that mice null for these channels had altered energy homeostasis[34]. All procedures were conducted with the approval of the Institutional Animal Care and Use Committee of the Northeastern Ohio Medical University in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication no. 85–23, revised 1996). Mice were housed in a temperature-controlled room with a 12:12-h light-dark cycle and maintained with access to food and water *ad libitum*. Three groups of male and female mice (4–6 months of age) were studied to establish the role of Kv1.3 channel deletion or blockade on coronary vascular reactions: WT (N=35), Kv1.3$^{-/-}$ (N=20), and WT mice treated with two doses of correolide (80 and 800 ng/g) a preferential Kv1.3 channel antagonist[35]. Results from male and female mice were combined because no statistically significant differences between genders were observed.

Hemodynamic measurements and estimation of cardiac work

Anesthesia was induced in a small chamber equilibrated with 3% isoflurane with the balance of oxygen. After induction mice were situated on heated surgical table designed for murine surgical procedures and echocardiography. A nose cone was secured to the animal and anesthesia was maintained using isoflurane/oxygen. Body temperature was maintained at 37°C via a heated stage controlled by temperature measurement from a rectal probe. The right jugular vein was cannulated with PE-50 polyethylene tubing containing heparin (50 U/ml) in saline for intravenous drug infusions. The femoral artery was isolated and cannulated with a 1.2F pressure catheter (Scisense Inc.), then connected to a data acquisition system (PowerLab ML820; ADInstruments) through a pressure interface unit (SP200 Pressure System) to measure arterial blood pressure and heart rate (HR). The transducer was advanced into abdominal aorta. All measured variables were continuously recorded and stored on an iMac computer that used the PowerLab system (AD Instruments; Castle Hill, Australia). Blood pressures were collected and analyzed using AD Instruments Chart 7 software. All mice were euthanized following the experimental protocol by exsanguination following high dose of barbiturate or isoflurane.

The product of heart rate (HR) and mean arterial pressure (MAP) (the pressure rate product [PRP]) was used to estimate cardiac metabolic demands. Although this calculation does not account for alterations in stroke volume (SV), we have found that the product of HR × MAP correlates well to the index of triple product (HR × MAP × SV), which is an index of cardiac work.[14]

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Myocardial perfusion imaging by contrast echocardiography

Myocardial contrast echocardiography (MCE) was performed to measure myocardial blood flow (MBF). Contrast imaging was performed with a Sequoia 512 (Siemens Medical Systems) via infusion of contrast (microbubbles; 20 µl/min, 5×10^5 bubbles min^{-1}) into the right jugular vein through a PE-50 catheter. This catheter was also used for drug infusion. Long axis images of the left ventricle were obtained for perfusion imaging. After optimal visualization of the chamber and the ventricular wall, images were collected during a high-energy pulse sequence (used to destroy microbubbles) and for several seconds after destruction to establish refilling of the chamber and ventricular wall. Analysis was done off-line, in which regions of interest (ROI) were positioned within the anterolateral wall in the long axis view. A curve of signal intensity over time was obtained in ROI and fitted to an exponential function: $y = A(1 - e^{-\beta t})$, where y is the signal intensity at any given time, A is the signal intensity corresponding to the microvascular cross sectional volume, and β is the initial slope of the curve, which corresponds to the blood volume exchange frequency[36, 37]. Relative blood volume in the region of interest (RBV) was calculated as the ratio of myocardial to cavity signal intensity ($RBV=A/A_{LV}$); where, A_{LV} represents the signal intensity of the LV chamber. Myocardial blood flow (MBF) was estimated as the product RBV × β[38]. Flows were measured in 3–5 different images obtained under the same treatments/conditions. The image acquisition and analyses were performed by operators blinded to the genotype and treatment of the animals. Although measurements of myocardial blood flow using MCE are higher than what are obtained using microspheres, the relative magnitude of the differences would remain and the differences would still exist.

Measurements of myocardial blood flow, heart and arterial pressure were made under basal conditions, following administration of hexamethonium (5 mg/kg) (to eliminate reflexes during changes in arterial pressure), and i.v. doses of norepinephrine (0.5, 1.0, 2.5 and 5.0 $\mu g/kg.min^{-1}$) to increase myocardial oxygen demands. In mice treated with correolide, only the two highest doses of norepinephrine were used.

In vitro Relaxation of Small Arteries and Arterioles

Arterioles and small coronary arteries (100–150 µm, id; typically 3rd order branches from the left anterior descending artery) were dissected from the epicardial surface of the left ventricle. After euthanasia, the heart was removed from the mouse, and the left ventricle was visualized under a dissecting microscope in a temperature-controlled dissection dish (4°C) containing a physiological salt solution[39]. Vessel segments were dissected free from surrounding myocardial tissue and were used for isometric force generation experiments. Two small (30 µm diameter) wires were inserted into the vessel and connected to a force transducer (Living Instruments, Inc). After optimization of resting tension for highest developed force, experiments were conducted to determine constrictor or dilator responses to agonists. For either preparation, pharmacological agents were administered in the bath. Tension was recorded continuously during the course of particular intervention. Vessels were contracted with the thromboxane mimetic U46619 (1 µM) to establish basal tone, and then vasodilation to hydrogen peroxide, adenosine and acetylcholine was assessed. Between agonists, the bath was washed several times and the preparation was contracted to U46619 to develop active tension.

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Statistical analyses

All analyses were performed using GraphPad Prism version 4.0 for Windows (GraphPrism Software Inc.). Vasodilation to H_2O_2, adenosine and acetylcholine were analyzed by repeated measures ANOVA. The, relationships between MBF and Double Product (DP) were assessed by linear regression analysis, and r^2 and P values are reported for the regression analyses. A probability of $p<0.05$ was accepted as statistically significant.

RESULTS

Figure 1 summarizes the hemodynamics (heart rate, arterial pressure) under basal conditions, during administration of hexamethonium, and during infusion of norepinephrine in the various groups. Heart rate and arterial pressure were similar in all groups under basal conditions and during norepinephrine administration. However, the decrement in arterial pressure produced by ganglionic blockade with hexamethonium was greatest in the WT group compared to Kv1.3$^{-/-}$ mice ($P<0.05$). During administration of norepinephrine, heart rate and arterial pressures were comparable among the groups.

Figure 2 illustrates vasoactive reactions of small coronary arteries (internal diameters in the range of 100–150 μm) to hydrogen peroxide, adenosine, and acetylcholine. Arterioles from the WT and Kv1.3$^{-/-}$ mice exhibited similar vasodilation to adenosine and acetylcholine (NS). In contrast, the responses to H_2O_2 were decreased in arterioles isolated from Kv1.3$^{-/-}$ mice compared to WT. Administration of correolide blunted vasodilation to Ach and adenosine in vessels from WT and Kv1.3$^{-/-}$ mice. Interestingly, correolide decreased dilation to H_2O_2 in WT vessels, but not in those isolated from Kv1.3$^{-/-}$ mice.

The relationships between metabolic demands (as represented by the pressure rate product [PRP]) and myocardial blood flow for WT and Kv1.3$^{-/-}$ during norepinephrine infusion are shown in Figure 3. Note that the work-flow relationship was significantly reduced in the null mice compared to WT, i.e., MBF in Kv1.3$^{-/-}$ was significantly lower all levels of DP than in the WT group ($P<0.05$).

Figure 4 illustrates the percent changes in flow in WT and WT with correolide (WT-Corr80 and -Corr800). Moreover, treatment of WT with the lower dose of correolide (Corr80) reduced the percent increases in MBF to those comparable to Kv1.3$^{-/-}$ but reduced from WT mice. The higher dose of correolide (Corr800) significantly reduced the percent increases in blood flow compared to WT and Corr80.

DISCUSSION

Our observations support the conclusion that the voltage-gated potassium channel Kv1.3 plays a role in coupling myocardial blood flow to cardiac work. Our data also suggest that Kv1.3 channels contribute to H_2O_2-induced vasodilation, but these channels do not contribute to vasodilation produced by adenosine or acetylcholine. Our data also indicate that correolide has vasoactive effects beyond its reported "specificity" for Kv1.3 channels, as dilation to acetylcholine and adenosine was decreased even in vessels obtained from Kv1.3 null mice. Cogent to our conclusions are results in the literature that bear upon our findings

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and implications of our observations in understanding the control of coronary blood flow in health and disease.

Considerations from the literature

The role of potassium channels as metabolic transducers, i.e., connecting blood flow to metabolism has received some attention over the last several years. In the coronary circulation previous studies have focused on K_{ATP}[40] and BK channels[41], but the conclusions that these channels modulate coronary metabolic dilation has been challenged experimentally[42, 43] or conceptually because the conclusion drawn about BK channels was based on the use of a fairly non-specific potassium channel antagonist, tetra-ethyl-ammonium. A large hindrance in the *in vivo* interpretation of results using preferential ion channel antagonists is that the antagonists are preferential and can antagonize many more channels than the target. This invalidates the implicit assumption that the antagonist is targeting only the desired channel. For example, glibenclamide can antagonize the mitochondrial K_{ATP}[44] and Kv channels[45] rendering conclusions that an effect is due to inhibition of sarcolemmal K_{ATP} channels as suspect. Although in the present study we employed a reportedly preferential antagonist of Kv1.3 channels (correolide), we observed effects of correolide in small coronary arteries obtained from Kv1.3 null mice, so the drug must have other effects. To overcome the limitation of off-target effects, we also studied mice null for Kv1.3 channels. Importantly, the results from the Kv1.3 null mice and the WT mice treated with correolide suggested a role for Kv1.3 channels, as well as other mechanisms, in coronary metabolic hyperemia. Dick et al.[46] found that correolide slightly attenuated adenosine-induced dilation of isolated coronary arterioles. These investigators did not investigate an *in vivo* effect of the drug and did not examine the effects of H_2O_2-dependent vasodilation.

The role of Kv1.3 channels in vascular regulation is not well appreciated, and the functions of this channel are most noted in inflammatory cells[47, 48], the retina[49, 50], and the central nervous system[51–54]. In the vasculature, Kv1.3 channels have been found in endothelial cells[55] and appear to be involved in the phenotype of vascular smooth muscle[30, 56–58]. The Kv1.3 channel was reported in mitochondria and is proposed to interact with Bax and mediate apoptotic events in lymphocytes[59, 60]. Our results suggesting that Kv1.3 channels help facilitate the connection between metabolism and flow is new, and has not been previously suggested. However, we would like to offer two caveats that bear upon our interpretations. *First*, the use of the pharmacological inhibitor of Kv1.3 channels, correolide, must be interpreted in the context that the drug preferentially, but not exclusively blocks Kv1.3 channels. Other Kv1 family channels (e.g., Kv1.2 and Kv1.5) may be antagonized by correolide[61, 62] and it is important to note that we previously reported a role for Kv1.5 channels in connecting myocardial blood flow to cardiac work.[14] We also used two different doses of correolide *in vivo* and found the effects were greater with the higher dose. This implies that the higher dose blocked more than Kv1.3 channels, which is consistent with other observations about this antagonist[61, 63]; however, the lower dose may be more selective. Interestingly Figure 4 may bear upon the specific vs non-specific actions of correolide. Specifically, during the lower dose of correolide, arterial pressure was maintained during norepinephrine infusion; however, at the higher dose, arterial pressure fell

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during norepinephrine infusion. Previously we observed in Kv1.5 null mice that arterial pressure was not maintained during administration of the highest doses of norepinephrine, because flow was insufficient to match the higher metabolic demands resulting in pump dysfunction[64]. We believe this result suggests that the higher dose of correolide was blocking other Kv1 family channels in addition to the Kv1.3. Moreover, the higher dose of correolide also compromised the increase in myocardial perfusion to norepinephrine more than we observed at either the lower dose of the antagonist or in the Kv1.3 null mice (Figure 4) implying the higher dose of correolide blocked additional Kv1 family channels. *Second*, the experimental model, Kv1.3 null mice, has a global knockout of the channel in all tissues. Due to the expression of these channels in many cell types, we believe our conclusion that Kv1.3 channels facilitate coronary metabolic dilation is tenable, but we cannot state with conviction the cell type or types responsible for making this connection. Although it would seem unlikely that inflammatory cells or the central nervous system plays a key role in this connection (especially in view of our use of the ganglionic blocker hexamethonium to prevent autonomic influences), we cannot unequivocally eliminate effects of other cell types in affecting metabolic dilation.

Another caveat to our results regards our experimental approach in that we measured myocardial blood flow using contrast echocardiography. In our previous publication using this technique[64], we found that myocardial blood flow measured with this technique was about 40% higher than that using microspheres; however, the correlations between the two techniques were high (r^2 value of 0.98). Thus, our measurements of blood flow may be overestimated by contrast echocardiography, but the relative magnitude of the differences in flows would remain.

One puzzling aspect of our observations was that dilation to H_2O_2 was reduced (vs wild types) in vessels isolated from Kv1.3 null mice and in preparations treated with correolide. The reason we mention this is puzzling is because the Kv1.3 channel is not described as a redox-sensitive ion channel; as opposed to the Kv1.5 which is redox-sensitive[63, 65]. The reduction in dilation by correolide can be explained by actions of the drug blocking Kv1 family channels in addition to the Kv1.3 channel. However, we cannot readily explain why dilation to H_2O_2 was compromised in arterioles from the null mice. One possibility for this conundrum are observations of association between Kv1.3 and Kv1.5 channels forming heterotetrameric channels[66–68]. Although not explicitly tested in the studies, such heterotetrameric channels could have redox sensitivity due to the involvement of the Kv1.5 components, which would help explain why the null animals had reduced vasodilation to H_2O_2. Another possibility would relate to some actions of H_2O_2 on vasodilatory pathways that are not considered redox-mediated[69].

Implications in coronary microcirculatory physiology and pathophysiology

We previously proposed that H_2O_2 is a metabolic vasodilator, linking metabolism to flow in the heart[70]. In the scheme we proposed, the mitochondrial production of H_2O_2, which is directly linked to the rate of electron transfer by mitochondria, is a feed-forward signal, in that as cardiac metabolism increases, the production of H_2O_2 also rises. In that paper, we reported that the relationship between coronary blood flow and myocardial oxygen

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consumption was shifted by the Kv channel antagonist, 4-aminopyridine, resulting in less flow (compared to unblocked animals) for a given level of oxygen consumption. The key role that Kv channels play in this process was also reported by Berwick et al.[71] Moreover, Goodwill et al observed that correolide reduced basal coronary blood flow and attenuated both metabolic and ischemic vasodilation.[72] The present study builds upon these results by honing into a specific Kv channel, the Kv1.3 channel, in part responsible for the coupling of flow to metabolism. We do not view the present results suggesting a role for Kv1.3 channels as being discrepant from our recent results supporting the concept that Kv1.5 channels were important for coronary metabolic dilation[64]. With a process as important as coupling blood flow to metabolism, we project that likely there would be redundant mechanisms facilitating the connection.

One limitation in concluding that Kv1.3 channels links metabolism to flow is that we do not have insights into the oxygen balance in the myocardium. Previously we found that mice null for Kv1.5 channels show lower myocardial oxygen tensions at rest (compared to wild type).[14] Moreover, during a cardiac stress test with norepinephrine, myocardial oxygen tensions fell considerably in the null mice, but were maintained in wild types. This indicates that in the latter, the balance between oxygen supply and demand (consumption) is maintained in wild types, but the demands for oxygen exceed the supply in the Kv1.5 nulls leading to tissue hypoxia. However, we cannot draw such an unequivocal conclusion in the present study because we did not make the measurements.

We would also like to mention some caveats about interpretations of the results shown in Figure 3. We do not advocate the interpretation that the figure shows that the cardiac efficiency is greater in Kv1.3$^{-/-}$ mice than wild type mice. This interpretation could be drawn from the two relationships, where for a given level of work, flow is less in the null mice than in wild types. The reason we do not believe this to be occurring is due to the nature of the experimental protocol where flow during the stress test is measured during a 30–45 second period after the effects of norepinephrine are first observed, which is often followed by a period of acute cardiac failure (especially at the high doses of norepinephrine). This failure implies insufficient oxygen supply to meet the heightened oxygen needs during the stress test. Evidence for this paradigm is shown in Figure 4 with the reductions in arterial pressure at the two highest doses of norepinephrine (especially at the highest dose of correolide), where pressure starts to decline before the infusion of norepineprhine is stoppled. We interpret this as a insufficient flow to meet the oxygen demands of the heart, which leads to acute pump failure. Importantly, the flow measurements shown in Figure 3 are obtained at the early portions of the stress test when flow is maintained.

The role of the coronary microcirculation in ischemic heart disease has received more attention in the recent past. In part, this appreciation has been the result of large trials and clinical studies that have reported women, without large vessel disease, show symptoms consistent with myocardial ischemia when stressed[73–75]. In another clinical trial, 30.5% of women with unstable angina and 10.2% of women with STEMI had normal coronary arteries[76]. Interestingly, women showing symptoms of myocardial ischemia, in the absence of large vessel disease, also have impaired coronary vasodilator reserve[77]. Perhaps some

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inherent dysfunction in Kv1.3 channels contributes to the microvascular pathology in patients. At this point it is premature to draw any conclusion, or even speculate about a role of Kv1.3 channels in coronary microvascular disease in patients, because to date genetic studies have not shown any association of this channel with the disorder[78].

Conclusions

Our results are consistent with the concept that Kv1.3 channels play a critical role in coronary metabolic dilation, the process that links blood flow to metabolism in the heart.

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Perspectives

Despite the profound importance in the maintenance of normal cardiac pump function via aerobic metabolism, the link between cardiac work and myocardial blood flow remains incompletely understood. Our study has identified that Kv1.3 channels are an effector of this link. The significance of our observation may facilitate a better understanding of the cohort of patients with microvascular angina, that is to say, ischemic heart disease attributed to coronary microvascular pathology.

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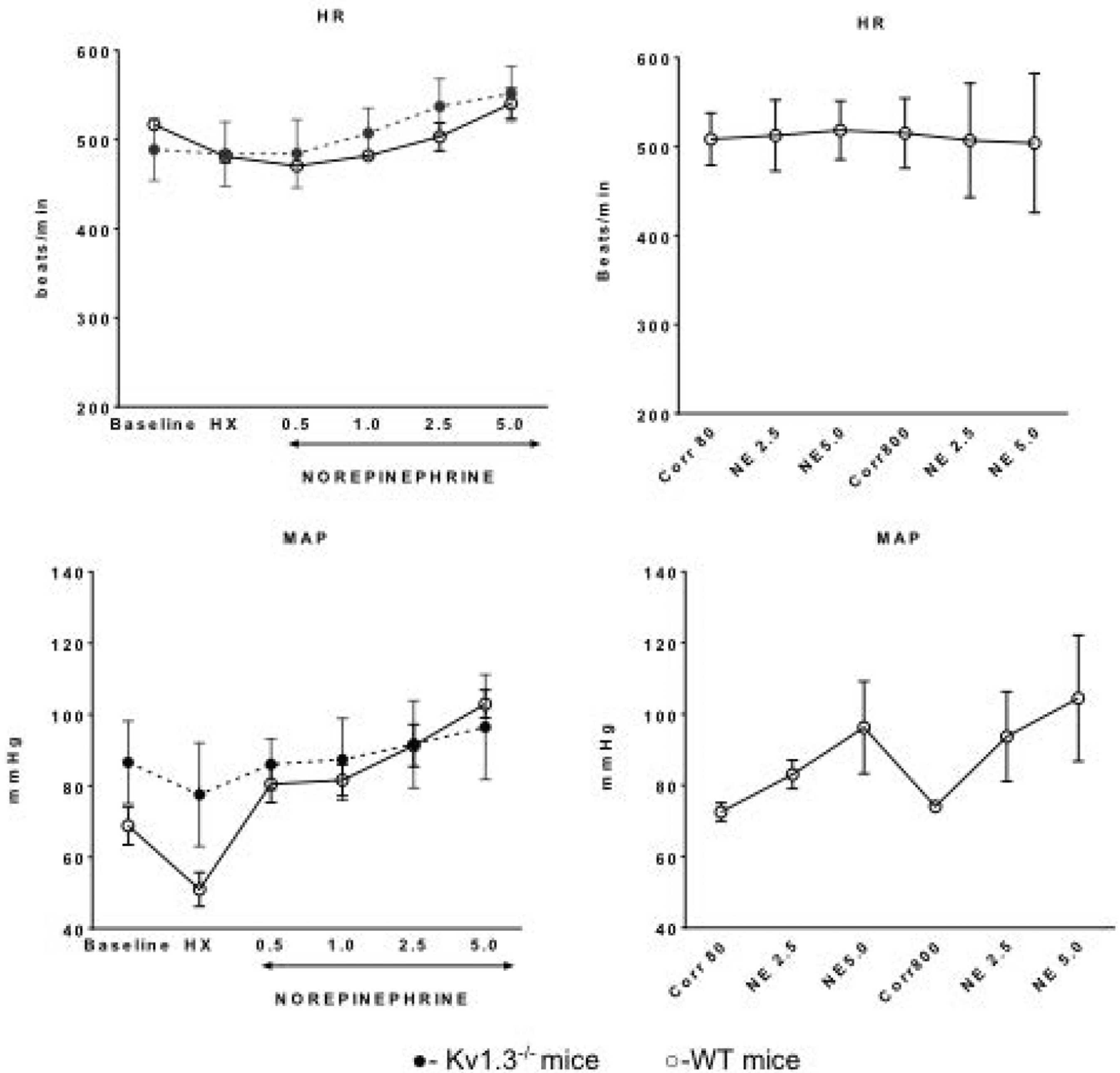

Figure 1.

Left: Hemodynamics (heart rate, mean arterial pressure) in WT (n=14) and Kv.1.3$^{-/-}$ (n=15) mice under basal conditions, during hexamethonium, and during norepinephrine infusion (with hexamethonium). Right: Hemodynamics of WT mice following correolide administration (Corr80 [80 ng/kg, n=6] and Corr800 [800 ng/kg, n=8]) before and during norepinephrine.

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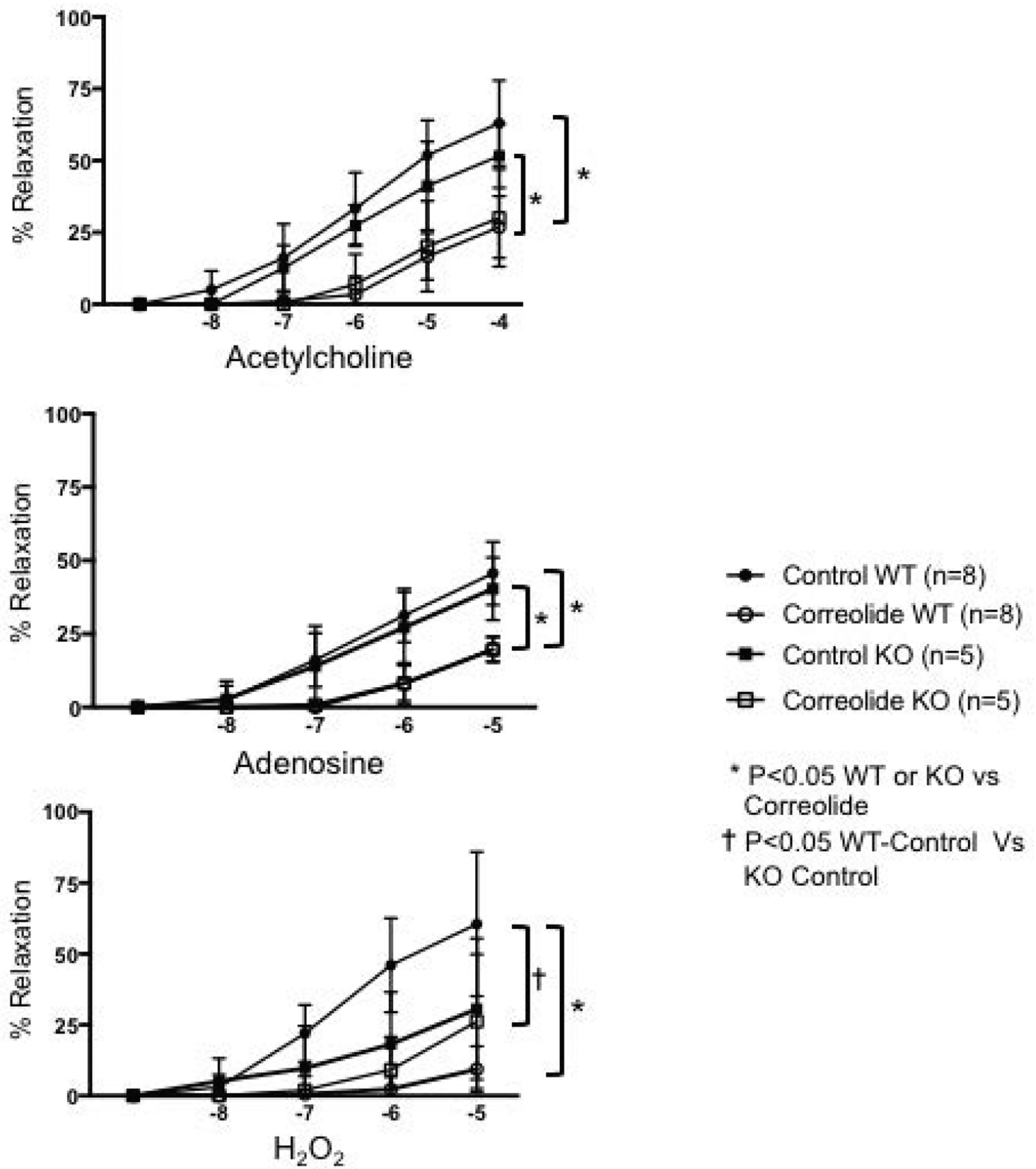

Figure 2.

Relaxation of isolated small coronary arteries and arterioles to acetylcholine, adenosine, and hydrogen peroxide (H_2O_2) from wild type (WT) and Kv1.$3^{-/-}$ (KO) mice. Relaxation was assessed under control conditions (without correolide) and after addition of correolide in the WT and KO mice.

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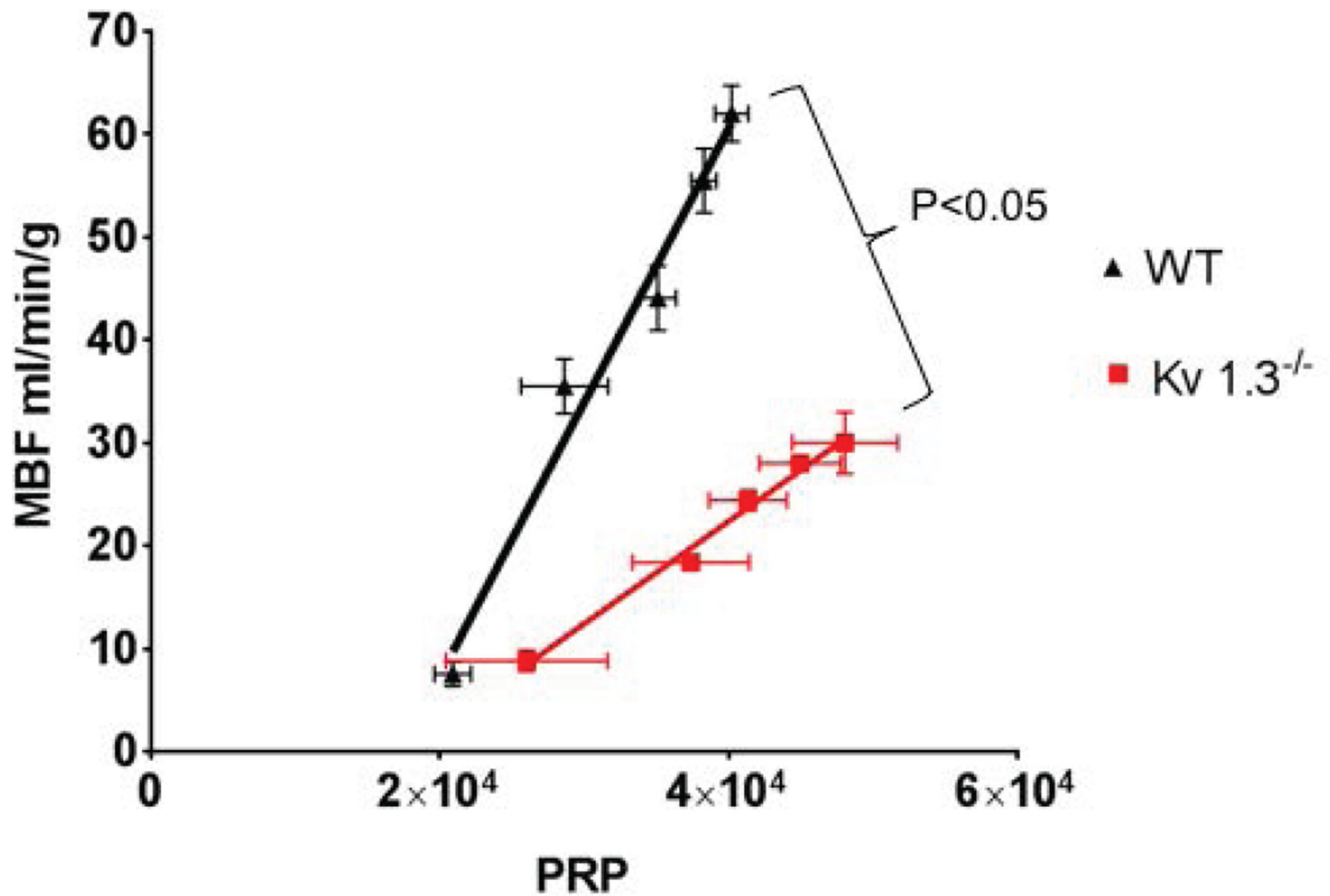

Figure 3.

Relationship between Pressure-Rate Product (PRP, mean arterial pressure X heart rate) and myocardial blood flow (MBF). MBF and DP were measured under basal conditions, hexamethonium and norepinephrine (0.5–5.0 μg/kg/min iv; in the presence of hexamethonium). In Kv $1.3^{-/-}$ mice (n=15) MBF was significantly lower compared to WT mice (n=14) at any level of the PRP.

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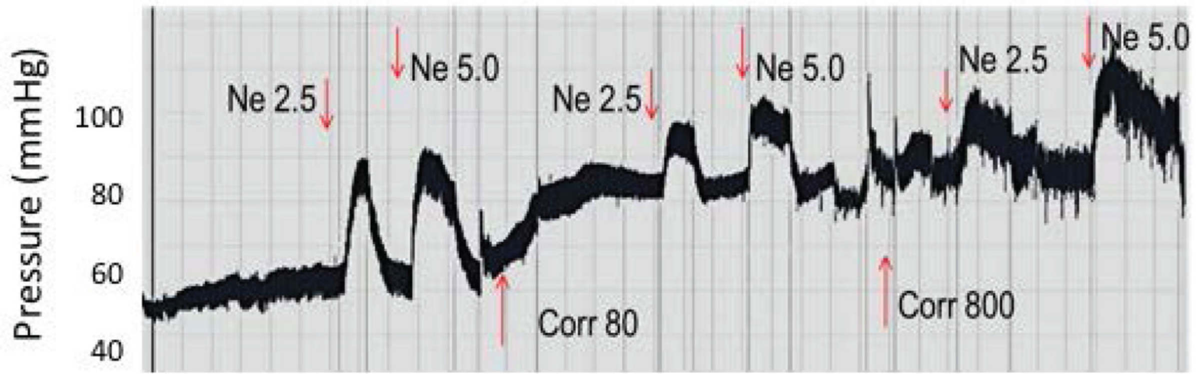

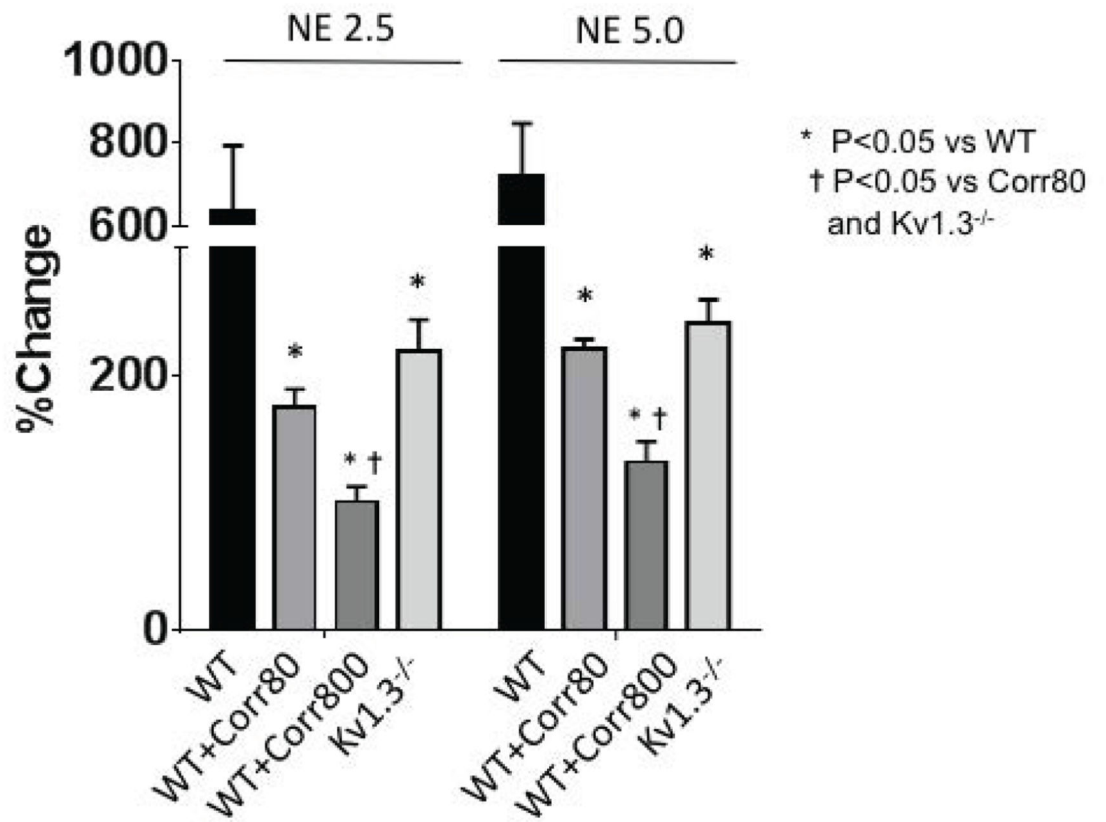

Figure 4.

Percent increases in myocardial blood flow during norepinephrine infusion in WT, correolide-treated WT (Corr80 [80 ng/g]; Corr800 [800 ng/g]) or Kv1.3$^{-/-}$ groups. Note the increases in blood flow were less (compared to WT) in both Corr and Kv1.3$^{-/-}$ groups. Corr800 suppressed the increase in flow more than Corr 80 and Kv1.3$^{-/-}$.