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Contribution of voltage-dependent K⁺ channels to metabolic control of coronary blood flow

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Abstract

The purpose of this investigation was to test the hypothesis that K_V channels contribute to metabolic control of coronary blood flow and that decreases in K_V channel function and/or expression significantly attenuate myocardial oxygen supply-demand balance in the metabolic syndrome (MetS). Experiments were conducted in conscious, chronically instrumented Ossabaw swine fed either a normal maintenance diet or an excess calorie atherogenic diet that produces the clinical phenotype of early MetS. Data were obtained under resting conditions and during graded treadmill exercise before and after inhibition of K_V channels with 4-aminopyridine (4-AP, 0.3 mg/kg, i.v.). In lean-control swine, 4-AP reduced coronary blood flow ~15% at rest and ~20% during exercise. Inhibition of K_V channels also increased aortic pressure ($P < 0.01$) while reducing coronary venous P_{O₂} ($P < 0.01$) at a given level of myocardial oxygen consumption (MV_{O₂}). Administration of 4-AP had no effect on coronary blood flow, aortic pressure, or coronary venous P_{O₂} in swine with MetS. The lack of response to 4-AP in MetS swine was associated with a ~20% reduction in coronary K_V current ($P < 0.01$) and decreased expression of K_{V1.5} channels in coronary arteries ($P < 0.01$). Together, these data demonstrate that K_V channels play an important role in balancing myocardial oxygen delivery with metabolism at rest and during exercise-induced increases in MV_{O₂}. Our findings also indicate that decreases in K_V channel current and expression contribute to impaired control of coronary blood flow in the MetS.

Keywords

Coronary; exercise; K_V channels; metabolic syndrome; swine

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Disclosures

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1. Introduction

The myocardium is highly dependent on a continuous supply of oxygen and nutrients from the coronary circulation to meet its metabolic requirements and to maintain contractile performance [1;2]. Despite extensive investigation over the past half century, the primary mechanisms responsible for balancing myocardial oxygen delivery with myocardial energy demand have remained elusive. Metabolic control of coronary blood flow is hypothesized to occur via local production of vasoactive substances which regulate microvascular resistance via activation of downstream K^+ channels on vascular smooth muscle [3]. Although multiple types of K^+ channels are expressed in coronary smooth muscle, recent data from our investigative team indicate that voltage-dependent K^+ (K_V) channels represent a critical end effector mechanism that modulates coronary blood flow at rest [4;5], during cardiac pacing or catecholamine-induced increases in myocardial oxygen consumption (MV_{O_2}) [5], following brief periods of cardiac ischemia [4], and endothelial-dependent and independent vasodilation [4;6;7]. However, the functional contribution of K_V channels to metabolic control of coronary blood flow during physiologic increases in MV_{O_2} , as occur during exercise, has not been examined.

Earlier studies have demonstrated that disease states such as obesity and the metabolic syndrome (MetS) markedly impair the ability of the heart to adequately balance coronary blood flow with myocardial metabolism [8–10]. Coronary microvascular dysfunction in the MetS is evidenced by reductions in coronary venous P_{O_2} [9;11;12], diminished vasodilatory responses to pharmacologic agonists (i.e. coronary flow reserve) [13–17], and alterations in functional and reactive coronary hyperemia [18]. Decreases in K^+ channel function contribute to this impairment as MetS depresses outward K^+ current in coronary artery smooth muscle cells [14;19–21] and diminishes the role of specific K^+ channels in coronary vasodilatory responses [6;18]. In particular, decreases in K_V channel activity have been associated with key components of the MetS, including hypercholesterolemia [22;23], hypertension [24], and hyperglycemia [25–27]. We hypothesize that such reductions in the functional expression of K_V channels contribute to the impaired control of coronary blood flow in the setting of the MetS.

Accordingly, the primary goals of the present study were to: 1) examine the contribution of coronary K_V channels to regulation of coronary blood flow at rest and during exercise-induced increases in MV_{O_2} ; and 2) determine the effects of the MetS on coronary K_V channel activity and expression. Experiments were designed to test the hypothesis that decreases in K_V channel function and/or expression significantly attenuate myocardial oxygen supply-demand balance in MetS. This hypothesis was examined in chronically instrumented Ossabaw swine fed either a normal maintenance diet or an excess calorie, atherogenic diet that produces the common clinical phenotype of early MetS; i.e. obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, hypertension, and atherosclerosis [28;29]. Hemodynamic data and arterial/coronary venous blood samples were obtained before and during inhibition of K_V channels with 4-aminopyridine (4-AP, 0.3 mg/kg, iv) at rest and during graded treadmill exercise. In addition, whole cell K^+ currents were measured in freshly isolated coronary artery smooth muscle cells from lean and MetS swine and expression of coronary $K_V1.5$ and $K_V3.1$ channels determined by Western blot.

2. Materials and methods

2.1 Ossabaw swine model of metabolic syndrome

All experimental procedures and protocols used in this investigation were approved by the Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals*. Lean control swine were fed ~2200 kcal/day of standard

chow (5L80, Purina Test Diet, Richmond, IN) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. MetS swine were fed an excess ~8000 kcal/day high fat/fructose, atherogenic diet containing 16% kcal from protein, 41% kcal from complex carbohydrates, 19% kcal from fructose, and 43% kcal from fat (mixture of lard, hydrogenated soybean oil, and hydrogenated coconut oil), and supplemented with 2.0% cholesterol and 0.7% sodium cholate by weight (KT324, Purina Test Diet, Richmond, IN). Both lean (n = 7) and MetS (n = 5) castrated male swine were fed their respective diets for 16 weeks prior to surgical instrumentation.

2.2 Surgical instrumentation

Following an overnight fast, Ossabaw swine were sedated with telazol (5 mg/kg, sc) and xylazine (2.2 mg/kg, sc). After endotracheal intubation, a surgical plane of anesthesia was maintained by mechanical ventilation with 1–3% isoflurane gas, supplemented with oxygen. Utilizing sterile technique, a left lateral thoracotomy was performed in the fifth intercostal space. A 17 Ga pressure monitoring catheter (Edwards LifeSciences) was implanted in the descending thoracic aorta for blood pressure measurements and arterial blood sampling. A second catheter was placed in the coronary interventricular vein for coronary venous blood sampling and intravenous drug infusions. The left anterior descending coronary artery (LAD) was dissected free and a perivascular flow transducer (Transonic Systems Inc.) was placed around the artery. The pneumothorax was evacuated and the chest was closed in layers. Catheters and the flow transducer wire were tunneled subcutaneously and exteriorized between the scapulae. Antibiotics (cecece, 5 mg/kg, im), rimadyl (4mg/kg, im) and buprenorphine (0.015mg/kg, im) were administered to prevent infection and manage post-operative pain. Externalized wires/catheters were protected by a jacket and an elastomeric balloon pump (MILA International) was connected to the coronary venous catheter for continuous infusion of heparinized saline (5U/ml at 5ml/hr). The aortic catheter was flushed daily and filled with heparinized saline (5,000 U/ml).

2.3 Experimental protocol and blood sampling

Following recovery from surgery, experiments were conducted in lean (n = 7) and MetS (n = 5) Ossabaw swine under resting conditions and during graded treadmill exercise before and during inhibition of K_V channels with 4-AP (0.3 mg/kg, iv). Hemodynamics were continuously recorded at baseline and during two levels of treadmill exercise at ~ 2 mph and ~5 mph. Arterial and coronary venous blood samples were collected simultaneously in heparinized syringes when hemodynamic variables were stable at rest and at each level of exercise. Each exercise period was ~2 min in duration, and the animals were allowed to rest sufficiently between each level for hemodynamic variables to return to baseline.

Arterial and coronary venous blood samples were collected, immediately sealed and placed on ice. The samples were analyzed in duplicate for pH, P_{CO_2} , P_{O_2} , glucose, hematocrit, and oxygen content with an Instrumentation Laboratories automatic blood gas analyzer (GEM Premier 3000) and CO-oximeter (682) system. LAD perfusion territory was estimated to be 30% of total heart weight, as previously described by Feigl [30]. MV_{O_2} ($\mu l O_2/min/g$) was calculated by multiplying coronary blood flow by the arterial coronary venous difference in oxygen content.

2.4 Patch-clamp electrophysiology

Coronary smooth muscle cells were freshly isolated from proximal segments of the LAD as previously described [20]. Briefly, patch-clamp recordings were performed within 8 h of cell dispersion. Whole-cell K^+ currents were measured at room temperature with the conventional dialyzed configuration of the patch-clamp technique. Bath solution contained (in mM) 138 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, and 5 Tris (pH 7.4).

Pipettes had tip resistances of 2–4 M Ω when filled with solution containing (in mM) 140 KCl, 3 Mg-ATP, 0.1 Na-GTP, 0.1 EGTA, 10 HEPES, and 5 Tris (pH 7.1). After whole-cell access was established, series resistance and membrane capacitance were compensated. Current voltage relationships were assessed by 400-ms step pulses from –60 to +20 mV in 10-mV increments from a holding potential of –80 mV.

2.5 Western blot analysis

Following excision of hearts, coronary arteries from lean ($n = 5$) and MetS ($n = 5$) swine were quickly isolated, cleaned of adventitia, placed in liquid N₂ and stored at –80°C. Arteries were homogenized with lysis buffer and total protein collected and quantified by DC Protein Assay. Equivalent amounts of protein (40 μ g) were loaded onto 7.5% acrylamide gels and transferred overnight. Membranes were blocked for 1 h at ambient temperature prior to 24 h incubation at 4°C with rabbit polyclonal antibodies (Alomone Labs) directed against K_V 1.5 (1:100) and K_V 3.1 (1:500) in blocking buffer with 0.1% Tween 20 and mouse anti-actin antibody (MP Biomedicals, 1:15,000). Blots were washed and incubated for 1 h with IRDye 800 donkey anti-rabbit (1:10,000) and IRDye 700 donkey anti-mouse (1:20,000) secondary antibodies. Immunoreactivity for K_V channel subtypes was determined by the Li-Cor Odyssey system (Li-Cor Biosciences) and expressed relative to actin (loading control).

2.6 Statistical Analyses

Data are presented as mean \pm SE. Statistical comparisons were made by unpaired t-test (phenotype data in Table 1) or by two-way analysis of variance (ANOVA) for within group analysis (Factor A: drug treatment; Factor B: exercise level) and between group analysis (Factor A: diet with drug treatment; Factor B: exercise level) as appropriate. For all statistical comparisons, $P < 0.05$ was considered statistically significant. When significance was found with ANOVA, a Student-Newman-Keuls multiple comparison test was performed to identify differences between groups and treatment levels. Multiple linear regression analysis was used to compare slopes of response variables (aortic pressure, coronary venous P_{O₂}) plotted vs. MV_{O₂}. If the slopes of the regression lines were not significantly different, an analysis of covariance (ANCOVA) was used to adjust response variables for linear dependence on MV_{O₂}.

3. Results

3.1 Phenotype of Ossabaw swine

Phenotypic characteristics of lean and MetS swine are given in Table 1. Consistent with our recent studies [9;18;20;31], we found that the excess calorie, atherogenic diet induced classic features of early MetS in Ossabaw swine. In particular, relative to their lean counterparts MetS swine exhibited a significant 1.6-fold increase in body weight, a 5.6-fold increase in total cholesterol, a 3.7-fold increase in LDL/HDL ratio and a 1.5-fold increase in triglyceride levels. Blood samples obtained from swine at the time of exercise experiment (non-fasted) revealed modest increases in plasma glucose and insulin concentration ($P = 0.10$). Homeostatic model assessment (HOMA) index values were also ~2-fold higher in MetS swine ($P = 0.13$).

3.2 Coronary and cardiovascular response to exercise: lean vs. MetS swine

Hemodynamic and blood gas data for lean and MetS Ossabaw swine at rest and during exercise are summarized in Table 2. Although no changes in systolic blood pressure were observed in untreated MetS vs. lean swine under baseline-resting conditions, MetS swine tended to have higher diastolic blood pressure (Table 2; $P = 0.08$). Exercise-induced

increases in mean aortic pressure were significantly augmented in MetS swine while no differences in heart rate were noted between groups at rest or during exercise. Given these changes in blood pressure, coronary blood flow was reduced ~30–35% at rest and during exercise (Table 2). Normalizing coronary blood flow to aortic pressure revealed significant reductions in coronary conductance in MetS vs. lean swine at all levels (Table 2). MV_{O_2} was modestly reduced ~15% in MetS swine under baseline conditions ($P = 0.57$), but was significantly depressed ~35% at the highest level of exercise (Table 2). These changes in coronary blood flow and MV_{O_2} were associated with a significant decrease in coronary venous P_{O_2} (index of tissue P_{O_2}) at rest and during exercise. Importantly, the slope of the relationship between coronary venous P_{O_2} and MV_{O_2} (Fig. 1A vs. Fig. 1B) was significantly increased (lower P_{O_2} at given level of MV_{O_2}) in untreated MetS vs. lean swine ($P < 0.02$).

3.3 Role of K_V channels in coronary and cardiovascular response to exercise

Effects of K_V channel inhibition with 4-AP (0.3 mg/kg, iv) on hemodynamic and blood gas variables at rest and during exercise are also summarized in Table 2. Administration of 4-AP significantly increased mean aortic pressure at rest and during exercise in lean, but not MetS swine. Blockade of K_V channels also increased aortic pressure in lean animals to a level closer to that observed in MetS swine (Table 2). Heart rate was unaffected by 4-AP in either group. Despite increases in blood pressure in lean swine, 4-AP reduced coronary blood flow ~15% at rest ($P = 0.17$) and ~20% at the highest level of exercise ($P < 0.05$) (Table 2). Coronary blood flow was not significantly altered by the administration of 4-AP in MetS swine at rest or during exercise. Coronary conductance was significantly decreased by 4-AP at rest and during exercise in lean, but not MetS swine (Table 2). Increases in MV_{O_2} to exercise were also diminished ~30% by inhibition of K_V channels in lean swine. Regression analysis demonstrated that 4-AP produced a significant, parallel downward shift in the relationship between coronary venous P_{O_2} vs. MV_{O_2} in lean, but not MetS swine (Fig. 1).

3.4 Functional expression of coronary K_V channels in lean vs. MetS swine

Whole cell patch clamp recordings (Fig. 2A) demonstrate a ~20% reduction in coronary K^+ current at potentials greater than 0 mV, i.e. currents biophysically consistent with K_V channels (Fig. 2B, $P < 0.01$). Pharmacological characterization of K_V current, including separation from BK_{Ca} current can be found in the supplement. K_V channels produce characteristic tail currents upon repolarization of the membrane (inset Fig. 2A), the magnitude of which was reduced in cells from MetS pigs (1.4 ± 0.3 vs. 2.0 ± 0.2 pA/pF; $P < 0.05$). This observation supports the idea that the difference in outward current in Fig. 2B is a reduction in K_V current. Importantly, however, other characteristics of the tail currents were not different (voltage of half activation and slope factor of -6 ± 1 mV and 8 ± 1 vs. -8 ± 1 mV and 8 ± 1), suggesting the same types of K_V channels are expressed in cells from lean and MetS swine (Fig. 2C).

Several K_V channel proteins have been proposed to underlie the native current in smooth muscle, including $K_V1.5$ [32] and $K_V3.1$ [22]. Protein expression data of $K_V1.5$ and $K_V3.1$ channels in coronary arteries from lean and MetS swine are shown in Fig. 3. Bands for $K_V1.5$ and $K_V3.1$ were 68 and 98 kDa, respectively. Western analysis revealed a significant ~49% reduction in coronary $K_V1.5$ channel expression in arteries from MetS swine (Fig. 3A; $P < 0.05$). No significant difference in coronary $K_V 3.1$ channel expression was noted in lean vs. MetS swine (Fig. 3B; $P = 0.36$).

4. Discussion

4.1 Major findings of the present study

The primary goal of this investigation was to examine the hypothesis that coronary K_V channels contribute to local metabolic control of coronary blood flow and that reduced functional expression of these channels plays a role in microvascular dysfunction in the setting of the MetS. This hypothesis is supported by earlier studies indicating that K_V channels modulate coronary blood flow *in vivo* [4;5;18;33] and that specific components of the MetS decrease smooth muscle K_V current and their contribution to arteriolar vasodilatory responses [22–24;26;34–37]. The novel findings of this study are: 1) inhibition of K_V channels increases blood pressure at rest and during exercise in lean, but not MetS swine; 2) K_V channels contribute to the regulation of coronary blood flow at rest and during increases in MV_{O_2} in lean, but not MetS swine; 3) induction of MetS significantly decreases K_V channel current in coronary artery smooth muscle cells; 4) expression of $K_V 1.5$ channels is diminished in the coronary circulation of MetS swine. Taken together, these data demonstrate that K_V channels play a crucial role in balancing myocardial oxygen delivery with myocardial oxygen demand at rest and during exercise-induced increases in MV_{O_2} in normal lean swine. Our findings also indicate that decreases in K_V channel activity and expression contribute to impaired control of coronary blood flow in the MetS.

4.2 Role of K_V channels in control of blood pressure and coronary blood flow

K_V channels are widely expressed in both the systemic and coronary circulation [3]. Earlier investigations have established an active role for K_V channels in modulating smooth muscle membrane potential in isolated smooth muscle cells, arteries, and arterioles as well as vascular tone in anaesthetized preparations [3]. In particular, data from the present study demonstrate that inhibition of K_V channels with 4-AP significantly elevates mean aortic pressure at rest and during exercise in normal lean animals (Table 2). These data are consistent with previous findings from our laboratory [18] as well as others [38–40] and implicate a critical role for K_V channels in the control of systemic vascular resistance. We propose that the effects of 4-AP on blood pressure are mediated by effects on vascular smooth muscle and not by direct cardiac effects as intracoronary administration of 4-AP at concentrations ≤ 0.3 mM does not significantly alter arterial pressure [4]. In addition, 4-AP has also been shown to augment arterial pressure in the presence of adrenoceptor antagonists in anesthetized cats, arguing against direct sympathetic pressor effects [38].

Consistent with other recent studies [4;5;33;41], the present findings support a prominent role for K_V channels in regulating coronary blood flow. This effect is primarily evidenced by the ~15–20% reduction in coronary blood flow at rest and during exercise (Table 2) following 4-AP administration in lean swine. It is important to recognize that this decrease in coronary flow occurred in the presence of significant increases in blood pressure, i.e. 4-AP markedly reduced coronary conductance (Table 2). However, the parallel downward shift in the relationship between coronary venous P_{O_2} and MV_{O_2} supports more of a “tonic” role for K_V channels in the control of coronary blood flow; i.e. similar contribution to coronary vascular resistance at rest and during increases in MV_{O_2} (Fig. 1A). Together, these results indicate that vasodilator substances that converge on K_V channels are required for adequate myocardial oxygen supply-demand balance over a wide range of MV_{O_2} . Although we did not examine the identity of specific factor(s) that mediate coronary vasodilation via K_V channels in this study, previous studies from our investigative team implicate H_2O_2 as a feedforward dilator that couples coronary blood flow with myocardial metabolism, predominantly through 4-AP sensitive K^+ channels [5;33;41]. Other factors that have been shown to induce coronary vasodilation, at least in part, through K_V channels include adenosine, nitric oxide, prostacyclin, and EDHF [3]. However, a prominent role for these

factors in local metabolic control is unlikely as inhibition of these pathways has little, if any effect on coronary blood flow at rest or during increases in MV_{O_2} [2].

4.3 Effects of MetS on function and expression of coronary K_V channels

Although K_V channels regulate membrane potential, arteriolar diameter [3;22], and coronary blood flow [4;5] in normal lean animals, data from this study importantly demonstrate that the MetS markedly impairs the functional expression of K_V channels in vascular smooth muscle. In particular, while inhibition of K_V channels influenced blood pressure, coronary blood flow and the balance between coronary blood flow and MV_{O_2} in lean swine, 4-AP had no effect on any of these key variables in obese, MetS swine (Table 2). Interestingly, MV_{O_2} was significantly decreased in MetS vs. lean swine during exercise, despite a larger rate-pressure product in MetS swine (Table 2). The reason for this difference is not apparent but is not clearly associated with alterations in K_V channel function and suggests that the MetS independently abrogates the relationship between coronary blood flow and myocardial metabolism. The absence of any cardiovascular effect of 4-AP in MetS swine, along with the augmented pressor response (Table 2) and substantial imbalance between coronary blood flow and myocardial metabolism (Fig. 1B), indicates that microvascular dysfunction typically observed in the setting of the MetS [8] is directly related to the diminished contribution of K_V channels to overall vascular resistance. It is possible that the lack of an effect of 4-AP on the balance between coronary blood flow and MV_{O_2} is related to a generalized vasoconstriction of the coronary vasculature in MetS hearts. However, the absence of coronary effects of 4-AP in combination with the reduction in outward K_V current and expression of coronary K_V channels indicates that diminished functional expression of K_V channels contributes to the impairment in the control of coronary blood flow in the MetS. The overall degree to which decreased K_V channel function influences coronary microvascular dysfunction in MetS is unclear, but could be related to alterations in the release of specific vasoregulatory factors that converge on K_V channels, changes in K_V channel activity, specific channel subunit expression and/or a combination of these mechanisms.

To examine potential mechanisms by which MetS impairs the contribution of K_V channels to the control of coronary blood flow, we performed patch-clamp electrophysiology and Western blot studies in order to address functional and molecular expression of the channel proteins. Functional expression of K_V current was reduced in smooth muscle cells from MetS pigs (Fig. 2B). Importantly, our supplemental data show that the currents we recorded in porcine coronary smooth muscle cells possessed pharmacological properties consistent with those mediated by K_V channels (i.e. largely sensitive to inhibition by 4-AP) and were not contaminated by large conductance, Ca^{2+} -sensitive K^+ (BK_{Ca}) current (i.e. insensitive to penitrem A). This raises the possibility that MetS: a) reduces the expression of K_V channels and/or b) induces a phenotypic switch to other K_V channel types. The latter mechanism, however, seems unlikely, as intrinsic K_V current characteristics including the voltage-dependence of activation were not changed ($V_{1/2}$ and slope factor k ; Fig. 2C). Thus, we further investigated the possibility that MetS decreases K_V channel protein expression. It is unclear what K_V channel subtypes underlie the native K_V current in coronary smooth muscle, but candidates include $K_V1.5$ [32] and $K_V3.1$ [22]. In particular, $K_V1.5$ has been implicated as redox/oxygen sensing channels [32] while $K_V3.1$ has been interrogated in impaired adenosine-induced dilation in hypercholesterolemic swine [22]. Importantly, $K_V3.1b$ channels are also sensitive to oxygen [42] and auxiliary β subunits can confer oxygen sensitivity to subtypes not typically considered to be redox-sensitive [43]. We found that expression of $K_V1.5$ protein was reduced in coronary arteries from MetS pigs (Fig. 3A), while expression of $K_V3.1$ protein was not statistically affected ($P = 0.36$). These data indicate that $K_V1.5$ channels are a component of the native K_V current and that the MetS-induced

reduction in K_V current in coronary smooth muscle could be related to reduced molecular expression of $K_V1.5$ channels. Our interpretation is consistent with recent preliminary data indicating that metabolic coronary vasodilatation is reduced in $K_V1.5$ knockout mice [44]. The potential contribution of alternative K_V channels subtypes (e.g. $K_V1.2$, 1.3 , 1.5 , and/or 2.1) merits further investigation.

It remains unclear what component(s) of the MetS milieu alters K_V channel function and expression in coronary smooth muscle; however, several of the individual constituents have been investigated previously. These include hyperglycemia, hypercholesterolemia, and increased levels of circulating neurohumoral factors (endothelin, angiotensin II, and catecholamines). In particular, elevated glucose and an associated increase in reactive oxygen species production impaired K_V channel function in smooth muscle cells from rat coronary arteries [25;26]. Whether a similar mechanism is at play in MetS pigs with modestly elevated glucose, insulin, and HOMA scores remains to be determined. Hypercholesterolemia also impairs coronary arteriolar relaxation mediated by K_V channels and reduces K_V current in coronary vascular smooth muscle [22;23]. Our swine MetS model produces profound hypercholesterolemia; therefore, it is possible that this factor contributes to decreased molecular and functional expression of K_V channels. In addition, MetS is associated with an increase in circulating levels of endothelin [45–47] and sensitization of endothelin-mediated coronary vasoconstriction in dogs [48] and humans [49]. Endothelin also inhibits vascular smooth muscle K_V channels by a pathway involving protein kinase C [50], the activity of which we have recently reported to be increased in our MetS swine [51]. Thus, it is possible that elevated endothelin levels alter the functional and molecular expression of K_V channels in MetS swine. Similarly, related signal mechanisms activated by catecholamines [52] and angiotensin II [12] may contribute to impaired K_V channel function and expression in MetS pigs.

4.4 Limitations of the study

It is important to point out that systemic administration of 4-AP may confound interpretation of the present findings as increases in arterial pressure (~6 mmHg) influence both coronary blood flow and MV_{O_2} [1;2]. However, any effect of 4-AP on MV_{O_2} , the primary determinant of coronary blood flow, is accounted for by plotting key coronary response variables (coronary venous P_{O_2}) relative to MV_{O_2} (Fig. 1). Intravenous administration of 4-AP also tended to decrease hematocrit in both lean and MetS swine (Table 2). The mechanism underlying this effect is unclear but it would likely act to increase (not decrease) coronary blood flow secondary to a reduction in myocardial oxygen delivery. Given these effects, we speculate that the overall contribution of K_V channels to the control of coronary blood flow may be underestimated in this study. This hypothesis is supported by earlier data from our laboratory which showed a much greater reduction in coronary blood flow (~40–50% decrease) in response to intracoronary 4-AP in normal-lean canines [4]. Thus, future studies to examine the effects of intracoronary 4-AP on metabolic control of coronary blood flow are warranted.

We also acknowledge the use of conduit coronary arteries for patch-clamp studies and measurement of K_V channel expression as a limitation as changes in conduit K^+ channel current and protein expression may not directly reflect alterations at the microvascular level. Whether differences in macro vs. microcirculation account for the disparate ~20% reduction in K_V current (Fig. 2) relative to the complete loss of an effect of 4-AP on coronary blood flow (Table 2) is unclear. However, data obtained from the idealized conditions that allow for examination of K^+ current in isolated coronary vascular smooth muscle cells may not directly associate with overall K^+ channel function *in vivo*, where other compensatory mechanisms could also be at play [1]. Unfortunately we were unable to acquire sufficient

measures of whole cell K^+ current in the presence of 4-AP in smooth muscle cells from lean and MetS swine. Characterization of the currents is provided in the supplement.

5. Conclusions

In summary, data from this investigation support that vasodilatory factors that converge on K_V channels play a critical role in the control of systemic vascular resistance and the balance between coronary blood flow with myocardial metabolism at rest and during exercise in conscious, lean swine. In addition, our findings also demonstrate that diminished functional expression of K_V channels significantly contributes to coronary microvascular dysfunction and the imbalance between myocardial oxygen supply-demand observed in the setting of the MetS [8]. We hypothesize that therapeutic targeting of MetS components (e.g. hypercholesterolemia, angiotensin II) and/or signaling pathways (e.g. PKC) that are known to alter K_V channel activity and expression could improve cardiovascular outcomes in patients with the MetS.

RESEARCH HIGHLIGHTS

- K_V channels contribute to the control of coronary blood flow in lean swine.
- Metabolic syndrome attenuates coronary K_V channel current and expression.
- Coronary dysfunction in metabolic syndrome is related to impairment of K_V channels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

MetS	metabolic syndrome
K_V	voltage-activated potassium channels
4-AP	4-aminopyridine
MV_{O_2}	myocardial oxygen consumption

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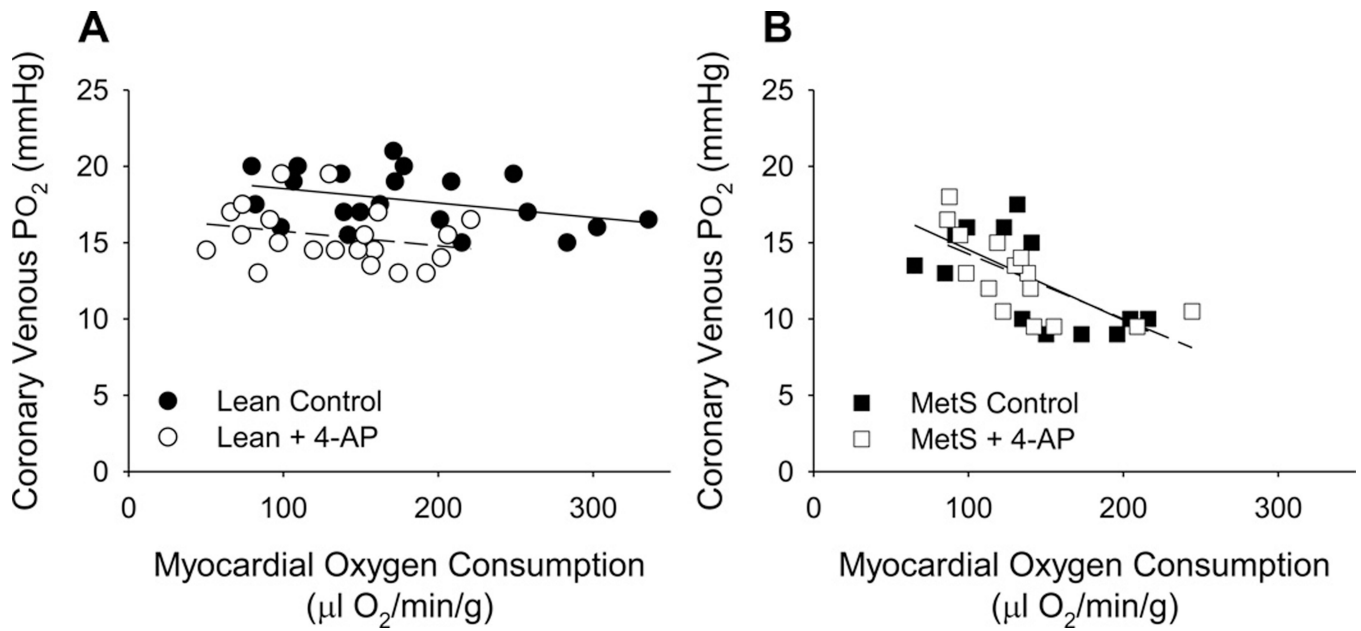


Figure 1.

Effect of K_V channel inhibition on the relationship between coronary venous P_{O_2} and myocardial oxygen consumption in lean (A) and MetS (B) swine. Inhibition of K_V channels with 4-aminopyridine (4-AP) significantly reduced coronary venous P_{O_2} at a given level of metabolism in lean ($P < 0.01$) but not MetS swine ($P = 0.84$). The slope of this relationship was also significantly decreased in untreated lean vs. MetS swine ($P < 0.02$).

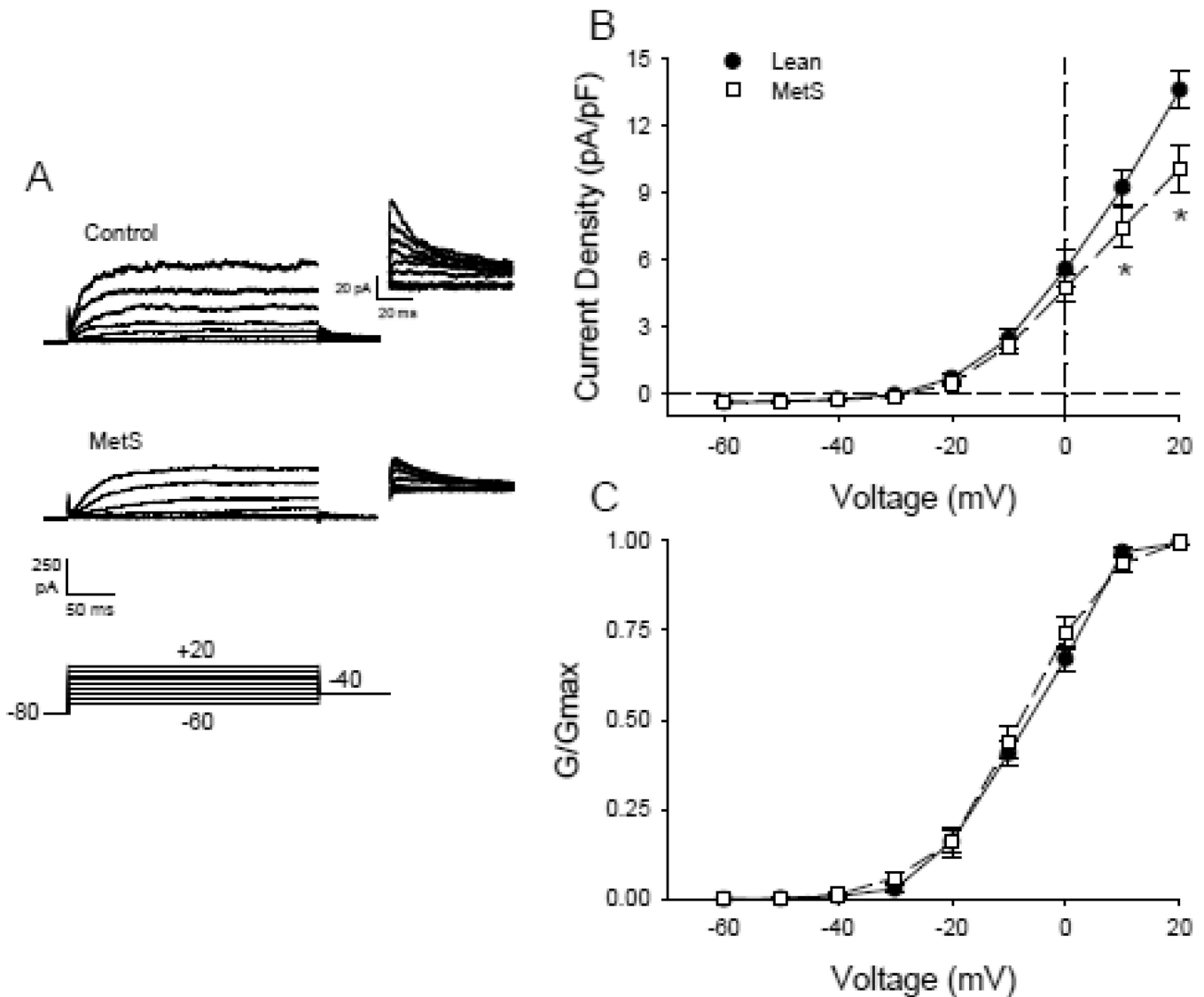


Figure 2.

Whole-cell voltage-dependent K^+ current in coronary smooth muscle of lean and MetS swine. (A) Families of current traces from representative cells of lean and MetS pigs. Voltage template is 400 ms long. K_V channels produce characteristic tail currents upon repolarization of the membrane (inset), the magnitude of which was reduced in cells from MetS pigs. (B) Group I-V data demonstrate a significant reduction in outward K^+ current at potentials greater than 0 mV, i.e. currents biophysically consistent with K_V channels. (C) Group G-V curves derived from tail currents at -40 mV. The voltage-sensitivity of the currents were not different (voltage of half activation and slope factor of -6 ± 1 mV and 8 ± 1 vs. -8 ± 1 mV and 8 ± 1 in control and MetS pigs, respectively. * $P < 0.01$ vs. lean, same voltage.

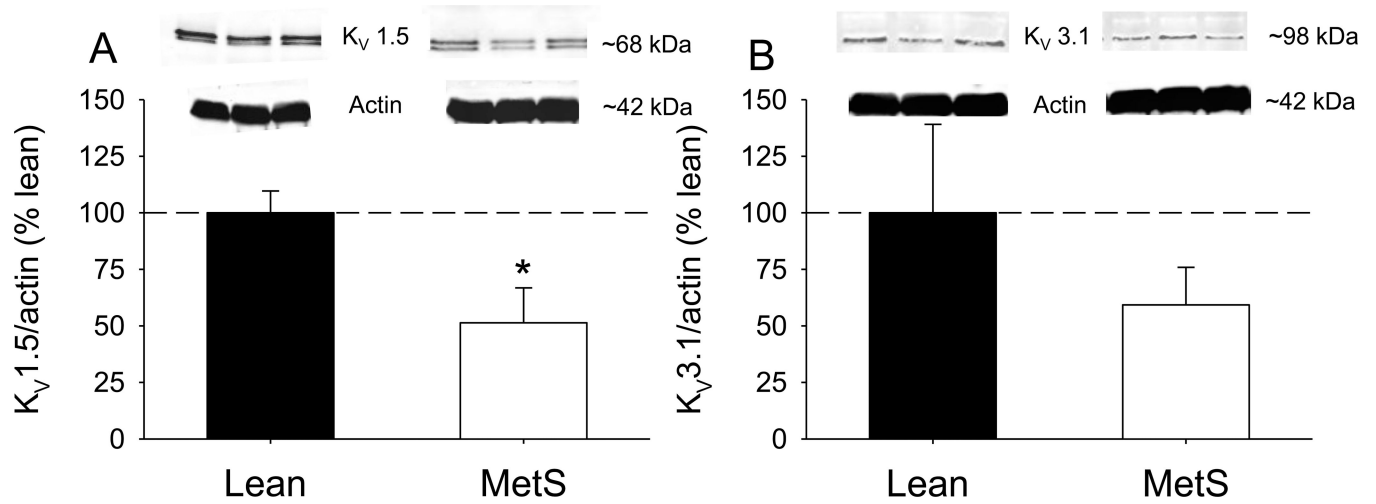


Figure 3.

Expression of KV1.5 and KV3.1 channels in coronary arteries of lean and MetS swine. (A) Western blot analysis demonstrated a significant reduction in KV1.5 channel protein expression in coronary arteries from MetS swine. (B) Expression of coronary KV3.1 channel protein was not significantly affected by induction of MetS ($P = 0.36$). * $P < 0.05$ vs. lean.

Table 1

Phenotypic characteristics of lean and metabolic syndrome Ossabaw swine.

	Lean	MetS
Body Weight (kg)	46 ± 3	72 ± 4*
Heart wt. / Body wt. (× 100)	0.36 ± 0.03	0.41 ± 0.03
Glucose (mg/dl)	74 ± 4	85 ± 5
Insulin (μU/ml)	16 ± 6	30 ± 9
HOMA index	2.9 ± 1.1	6.0 ± 1.5
Total cholesterol (mg/dl)	87 ± 5	486 ± 70*
LDL/HDL ratio	1.6 ± 0.1	5.9 ± 1.4*
Triglycerides (mg/dl)	43 ± 5	67 ± 18

Values are mean ± SE for lean (n = 7) and MetS (n = 5) swine.

* $P < 0.05$ vs lean.

Table 2

Hemodynamic and blood gas variables at rest and during graded treadmill exercise in lean and metabolic syndrome Ossabaw swine with and without 4AP (0.3mg/kg).

	Exercise		
	Rest	Level 1	Level 2
Systolic Blood Pressure (mmHg)			
Lean	113 ± 5	112 ± 4	121 ± 5
Lean + 4AP	119 ± 6	119 ± 4	125 ± 5
MetS	119 ± 6	131 ± 9 [†]	138 ± 9
MetS + 4AP	126 ± 7	125 ± 6	134 ± 4
Diastolic Blood Pressure (mmHg)			
Lean	73 ± 4	70 ± 4	75 ± 4
Lean + 4AP	79 ± 6	76 ± 4	86 ± 6 [*]
MetS	86 ± 3	89 ± 8 [†]	92 ± 6 [†]
MetS + 4AP	89 ± 5	81 ± 7	90 ± 5
Mean Aortic Pressure (mmHg)			
Lean	93 ± 4	92 ± 3	98 ± 4
Lean + 4AP	99 ± 6 [*]	99 ± 4 [*]	105 ± 4 [*]
MetS	103 ± 5	108 ± 8 [†]	115 ± 8 [†]
MetS + 4AP	100 ± 6	104 ± 7	114 ± 5
Heart Rate (beats/min)			
Lean	120 ± 8	162 ± 11	212 ± 13
Lean + 4AP	129 ± 10	172 ± 9	201 ± 10
MetS	134 ± 19	168 ± 20	183 ± 14
MetS + 4AP	125 ± 6	170 ± 7	184 ± 6
Coronary Blood Flow (ml/min/g)			
Lean	1.11 ± 0.09	1.46 ± 0.12	1.92 ± 0.12
Lean + 4AP	0.94 ± 0.13	1.24 ± 0.16 [*]	1.54 ± 0.17 [*]
MetS	0.80 ± 0.08	1.07 ± 0.14 [†]	1.21 ± 0.15 [†]
MetS + 4AP	0.95 ± 0.16	1.07 ± 0.16	1.25 ± 0.17
Coronary Conductance (μl/min/g/mmHg)			
Lean	11.9 ± 0.8	15.8 ± 0.8	19.5 ± 0.6
Lean + 4AP	9.3 ± 1.0 [*]	12.4 ± 1.3 [*]	14.6 ± 1.4 [*]
MetS	8.0 ± 1.1 [†]	10.2 ± 1.8 [†]	10.6 ± 1.3 [†]
MetS + 4AP	9.7 ± 1.9	10.3 ± 1.5	11.0 ± 1.4
Myocardial O₂ Consumption (μl O₂/min/g)			
Lean	117 ± 14	178 ± 23	244 ± 21
Lean + 4AP	102 ± 18	131 ± 19	166 ± 14 [*]
MetS	102 ± 7	143 ± 19	158 ± 22 [†]
MetS + 4AP	117 ± 23	132 ± 21	151 ± 21

	Exercise		
	Rest	Level 1	Level 2
Arterial pH			
Lean	7.55 ± 0.01	7.55 ± 0.01	7.54 ± 0.01
Lean + 4AP	7.60 ± 0.03	7.60 ± 0.03	7.56 ± 0.03
MetS	7.53 ± 0.02	7.52 ± 0.01 [†]	7.50 ± 0.02 [†]
MetS + 4AP	7.58 ± 0.02*	7.56 ± 0.01	7.54 ± 0.01
Coronary Venous pH			
Lean	7.47 ± 0.01	7.48 ± 0.01	7.47 ± 0.01
Lean + 4AP	7.50 ± 0.02	7.50 ± 0.02	7.49 ± 0.02
MetS	7.45 ± 0.02	7.45 ± 0.01	7.45 ± 0.01
MetS + 4AP	7.50 ± 0.02*	7.49 ± 0.01*	7.48 ± 0.01*
Arterial PCO₂ (mmHg)			
Lean	32 ± 1	31 ± 2	31 ± 1
Lean + 4AP	26 ± 2*	25 ± 2*	27 ± 2
MetS	33 ± 2	31 ± 2	30 ± 1
MetS + 4AP	29 ± 2	27 ± 1	29 ± 1
Coronary Venous PCO₂ (mmHg)			
Lean	49 ± 1	43 ± 3	45 ± 1
Lean + 4AP	41 ± 2*	42 ± 3	40 ± 3
MetS	49 ± 3	46 ± 2	44 ± 3
MetS + 4AP	42 ± 3*	42 ± 3*	44 ± 3
Arterial PO₂ (mmHg)			
Lean	94 ± 3	98 ± 3	95 ± 4
Lean + 4AP	107 ± 3*	108 ± 3	100 ± 8
MetS	89 ± 4	89 ± 3	94 ± 4
MetS + 4AP	98 ± 4	97 ± 2	96 ± 3
Coronary Venous PO₂ (mmHg)			
Lean	18 ± 0.6	18 ± 0.8	17 ± 0.7
Lean + 4AP	15 ± 0.6*	16 ± 0.9*	16 ± 0.8
MetS	14 ± 1.4 [†]	12 ± 1.2 [†]	12 ± 1.0 [†]
MetS + 4AP	13 ± 1.4	13 ± 1.5	13 ± 1.7
Coronary Venous O₂ Saturation (%)			
Lean	16 ± 2	13 ± 3	14 ± 2
Lean + 4AP	13 ± 3	13 ± 3	13 ± 2
MetS	14 ± 2	11 ± 1	10 ± 1
MetS + 4AP	13 ± 2	12 ± 2	12 ± 3
Arterial Hematocrit (%)			
Lean	34 ± 1	37 ± 1	37 ± 1
Lean + 4AP	30 ± 2	30 ± 1*	33 ± 2

	Exercise		
	Rest	Level 1	Level 2
MetS	36 ± 3	37 ± 2	37 ± 1
MetS + 4AP	32 ± 2	31 ± 2 [*]	33 ± 2

Values are mean ± SE for lean (n = 7) and MetS (n = 5) swine.

^{*} $P < 0.05$ vs. untreated control, same diet/condition;

[†] $P < 0.05$ vs. lean, same treatment.