CONTRIBUTION OF K^+ CHANNELS TO CORONARY DYSFUNCTION IN METABOLIC SYNDROME

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ABSTRACT

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Coronary microvascular function is markedly impaired by the onset of the metabolic syndrome and may be an important contributor to the increased cardiovascular events associated with this mutlifactorial disorder. Despite increasing appreciation for the role of coronary K^+ channels in regulation of coronary microvascular function, the contribution of K^+ channels to the deleterious influence of metabolic syndrome has not been determined. Accordingly, the overall goal of this investigation was to delineate the mechanistic contribution of K^+ channels to coronary microvascular dysfunction in metabolic syndrome. Experiments were performed on Ossabaw miniature swine fed a normal maintenance diet or an excess calorie atherogenic diet that induces the classical clinical features of metabolic syndrome including obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, hyperleptinemia, and atherosclerosis. Experiments involved *in vivo* studies of coronary blood flow in open-chest anesthetized swine as well as conscious, chronically instrumented swine and *in vitro* studies in isolated coronary arteries, arterioles, and vascular smooth muscle cells. We found that coronary microvascular dysfunction in the metabolic syndrome significantly impairs coronary vasodilation in response to metabolic as well as ischemic stimuli. This impairment was directly related to decreased membrane trafficking and functional expression of BK_{Ca} channels in vascular smooth muscle cells that was accompanied by augmented L-type Ca^{2+} channel activity and increased intracellular Ca^{2+} concentration. In addition, we discovered that impairment of coronary vasodilation in the metabolic

syndrome is mediated by reductions in the functional contribution of voltage-dependent K^+ channels to the dilator response. Taken together, findings from this investigation demonstrate that the metabolic syndrome markedly attenuates coronary microvascular function via the diminished contribution of K^+ channels to the overall control of coronary blood flow. Our data implicate impaired functional expression of coronary K^+ channels as a critical mechanism underlying the increased incidence of cardiac arrhythmias, infarction and sudden cardiac death in obese patients with the metabolic syndrome.

Johnathan D. Tune, Ph.D., Chair

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CHAPTER 1: INTRODUCTION

The epidemic of obesity and metabolic syndrome

Obesity in Western society has reached epidemic proportions, as an estimated 100 million Americans are overweight or obese (66). In addition, recent estimates indicate that there are approximately 1 billion persons worldwide who are overweight (body mass index 25 – 30 kg/m^2) (161). Many of these individuals are affected for years by the so called *"metabolic syndrome,"* the combined disorder of obesity, insulin resistance, hypertension and dyslipidemia before therapeutic measures are initiated or the development of overt type II diabetes mellitus occurs. Presently, an estimated 30% of the U.S. adult population exhibits characteristics of the pre-diabetic metabolic syndrome (3; 57; 116). According to the commonly used diagnostic definition of the National Cholesterol Education Program's Adult Treatment Panel-III, a patient is diagnosed with metabolic syndrome when three or more of the following clinical criteria are present in one individual: elevated waist circumference (≥ 40 in for men, 35 in for women), elevated triglycerides (≥ 150 mg/dL), reduced HDL cholesterol (< 40 mg/dL for men, 50 mg/dl for women), elevated blood pressure (≥ 130/85 mmHg), and elevated fasting glucose (≥110 mg/dL) (101). These contribute to the cluster of metabolic risk factors including abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance and/or glucose intolerance, prothrombotic state, and proinflammatory state that comprise the syndrome (66).

Earlier studies have established that each component of metabolic syndrome is an independent risk factor for cardiovascular disease (66). Recent estimates suggest that individuals with metabolic syndrome have a 61% increased risk of cardiovascular disease compared to those without metabolic syndrome (59). Follow-up data of the 1948 Framingham Heart Study, which sought to identify common factors that contribute to

cardiovascular disease, demonstrated significantly increased incidence of coronary atherosclerotic disease, cardiomyopathies, myocardial infarction, sudden death, congestive heart failure, and atherothrombotic stroke in obese subjects relative to lean (59; 66; 70; 75; 76; 96; 129). Obese subjects were also found to be at twice the risk of coronary disease (75). Despite the known link between obesity and cardiovascular disease the pathophysiologic mechanisms underlying obesity- and metabolic syndrome-induced cardiovascular diseases remain poorly understood. Accordingly, the long-term goal our research is to delineate mechanisms of obesity-related coronary vascular disease and thereby elucidate pathways and novel therapeutic targets to reduce the incidence of cardiovascular complications in this patient population. The central premise of our studies is that impaired coronary microvascular function is an important contributor to increased cardiovascular morbidity and mortality in obese patients with the metabolic syndrome.

Metabolic syndrome and the coronary circulation

Due to the limited anaerobic capacity of the myocardium, the heart depends on a continuous supply of oxygen from the coronary circulation to meet its metabolic requirements (43; 151; 154). To ensure adequate balance between coronary blood flow and myocardial metabolism, powerful regulatory mechanisms exist to maintain nutritive blood flow to the heart to protect the myocardium from ischemia. If this need for oxygen is not met, the resulting ischemia substantially diminishes cardiac function within seconds (25; 68; 69; 132). Thus, under normal physiological conditions myocardial oxygen delivery is closely matched with the rate of myocardial oxidative metabolism.

There is mounting evidence that this ability to match oxygen delivery to myocardial demand is diminished in metabolic syndrome. Data from human patients demonstrate diminished coronary flow reserve (the difference between maximal and baseline coronary blood flow) with obesity and metabolic syndrome (27; 33; 87; 92; 137), indicating that the capacity to vasodilate is greatly reduced in obesity. For instance, Kiviniemi *et al.* reported a negative correlation between coronary flow reserve and waist to hip ratio (Figure 1.1) such that maximal flow capacity diminished in proportion to the degree of obesity (87). In addition to diminished flow reserve, recent investigations provide evidence for impaired insulin-mediated capillary recruitment, altered obesity-related endocrine signaling, and increased arterial stiffening in metabolic syndrome (125; 139). Remodeling of the microcirculation is considered a hallmark of established vascular disease, and reduced perfusion has been linked to reduced wall compliance and increased wall thickness in peripheral arterioles of obese Zucker rat (144). Therefore, microvascular defects play an important role in the end-organ damage associated with this combined disorder.

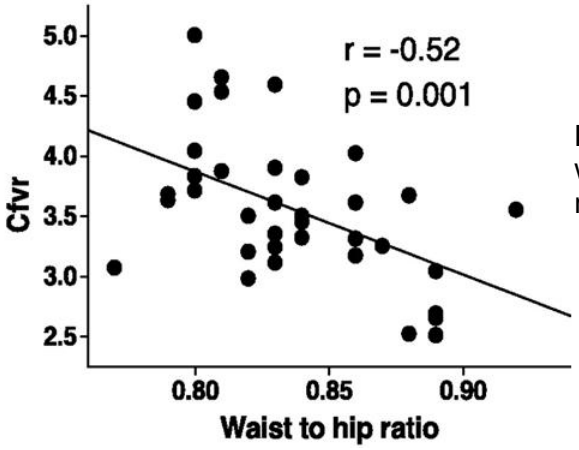

Figure 1.1 Correlation of waist-to-hip ratio with coronary flow velocity reserve (CFVR; n = 36). Taken from Kiviniemi et al. (73)

To examine the effects of the metabolic syndrome on myocardial oxygen supply demand balance, our laboratory explored the control of coronary blood flow in conscious, instrumented control and chronically high-fat-fed dogs at rest and during

graded treadmill exercise (141). We found that the metabolic syndrome significantly attenuated exercise-induced coronary hyperemia. Specifically, slopes of coronary blood flow vs. MVO_2 and coronary conductance (coronary blood flow normalized to mean aortic pressure) vs. MVO_2 relationships were significantly reduced in metabolic syndrome dogs relative to lean controls (Figure 1.2A), indicating that metabolic syndrome impairs the ability of the coronary circulation to match myocardial oxygen delivery to metabolism. In addition, there was a significant parallel downward shift in the relationship between coronary venous oxygen tension and MVO_2 (a sensitive measure of tissue oxygenation that reflects whether changes in coronary blood flow adequately match changes in MVO_2) in the high-fat-fed dogs (Figure 1.2B), indicating that in these animals, oxygen delivery was not sufficient to meet myocardial metabolic demand such that these animals had to increase extraction in order to meet their requirements for oxygen. Further, this finding suggests a loss of a tonic vasodilator mechanism and/or activation of a tonic vasoconstrictor mechanism (151; 154) that was present at rest as well as during exercise. These data are consistent with attenuated coronary flow responses to

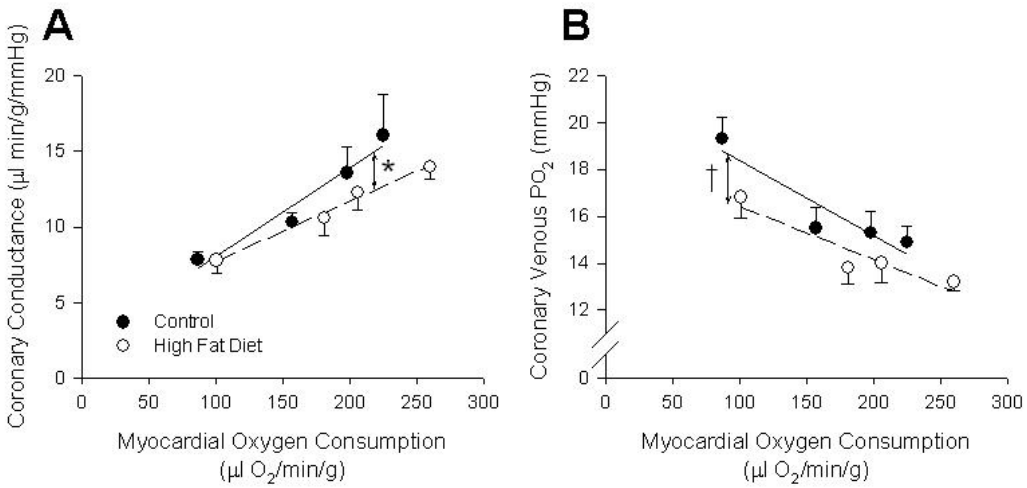

Figure 1.2 Metabolic syndrome impairs the ability of the coronary circulation to match myocardial oxygen delivery to myocardial metabolism. Taken from Setty *et al.* (118)

cardiac pacing in obese patients (27) and indicate that metabolic syndrome significantly impairs regulation of coronary microvascular function to the extent that the balance between coronary blood flow and myocardial metabolism is compromised.

The mechanisms responsible for coronary microvascular dysfunction in metabolic syndrome have not been fully elucidated. Recent data from our laboratory indicate that coronary vasomotor dysfunction in the metabolic syndrome is related to sensitization of key coronary vasoconstrictor pathways (39; 88; 89; 141; 173). In particular, metabolic syndrome is associated with elevated basal plasma epinephrine and norepinephrine levels and sensitization of α_1- and α_2-adrenoceptor signaling in metabolic syndrome dogs that limits control of coronary blood flow in response to sympathetic activation (39). In addition, metabolic syndrome was associated with elevated plasma renin activity and angiotensin II levels that act via increased angiotensin II type 1 receptors (173). These data indicate that coronary vasomotor dysfunction in metabolic syndrome is related to chronic activation of the renin-angiotensin and sympathetic nervous system that leads to augmented AT_1 and α_1-adrenoceptor mediated coronary vasoconstriction. Further, coronary vasoconstriction in canine isolated arterioles to endothelin-1 was similar in control and metabolic syndrome despite significantly decreased ET_A-receptor transcript levels and protein expression, indicating that ET_A-receptor signaling is also sensitized by induction of metabolic syndrome (90). These pathways converge on smooth muscle L-type Ca^{2+} channels to increase intracellular Ca^{2+} concentrations and depolarize smooth muscle cells, thereby inducing vasoconstriction (88). In addition, the sensitization of the angiotensin signaling pathway directly contributes to microvascular dysfunction as inhibition of angiotensin II type 1 (AT_1) receptors significantly improved the balance between coronary blood flow and myocardial metabolism in dogs with the metabolic syndrome (173).

Several recent studies suggest that alterations in these mechanisms, especially angiotensin II, could directly inhibit K^+ channels, in particular large conductance Ca^{2+}-activated (BK_{Ca}) K^+ channels. Agonist-induced vasoconstriction by angiotensin II has been found to involve inhibition of BK_{Ca} channels by c-Src tyrosine kinase via direct phosphorylation of the channel protein (4). Further, administration of angiotensin II-activated calcineurin/NFATc3 signaling in murine arterial smooth muscle significantly diminished BK_{Ca} channel function via down-regulation of the BK_{Ca} channel β_1 subunit expression (114). Consistent with these findings, patch-clamp studies of coronary vascular smooth muscle cells have demonstrated that angiotensin II inhibits BK_{Ca} channel function by altering the open and closed states of the channel thereby prolonging the closed confirmation (150). These findings suggest that sustained angiotensin II signaling, such as seen in hypertension or metabolic syndrome, impair K^+ channel-mediated dilatory mechanisms. This can in turn be linked to depolarization of vascular smooth muscle membrane potential (E_m) and induction of arterial dysfunction (4; 8; 21; 23; 88; 114; 150). These previous findings indicate that in addition to enhanced vasoconstrictor pathways, depressed vasodilator mechanisms could also contribute to impaired microvascular function in metabolic syndrome.

Coronary K^+ channels and metabolic syndrome

Vascular smooth muscle cells express a variety of ion channels involved in a wide number of physiological and pathophysiological mechanisms. K^+ channel activity is an important factor in the determination and regulation of membrane potential and vascular tone (81; 113). The opening of K^+ channels in smooth muscle cells leads to diffusion of K^+ ions out of the cell, causing membrane hyperpolarization and closure of voltage-gated Ca^{2+} channels (Figure 1.3). This in turn results in a decreased concentration of intracellular Ca^{2+} and induces vasorelaxation. The closure of K^+

channels has the opposite effect, leading to the opening of L-type Ca^{2+} channels and membrane depolarization, inducing an increase in intracellular Ca^{2+} levels, and ultimately resulting in vasoconstriction. Many cellular metabolites, such as endothelial-dependent factors (e.g. nitric oxide (NO), prostacyclin, endothelial-derived hyperpolarizing factors (EDHF)) as well as endogenous cardiomyocyte-derived metabolites (e.g. adenosine, hydrogen peroxide) are released in response to increased myocardial metabolism and/or decreased tissue oxygenation to induce vasodilation through smooth muscle K^+-channel mediated pathways (82).

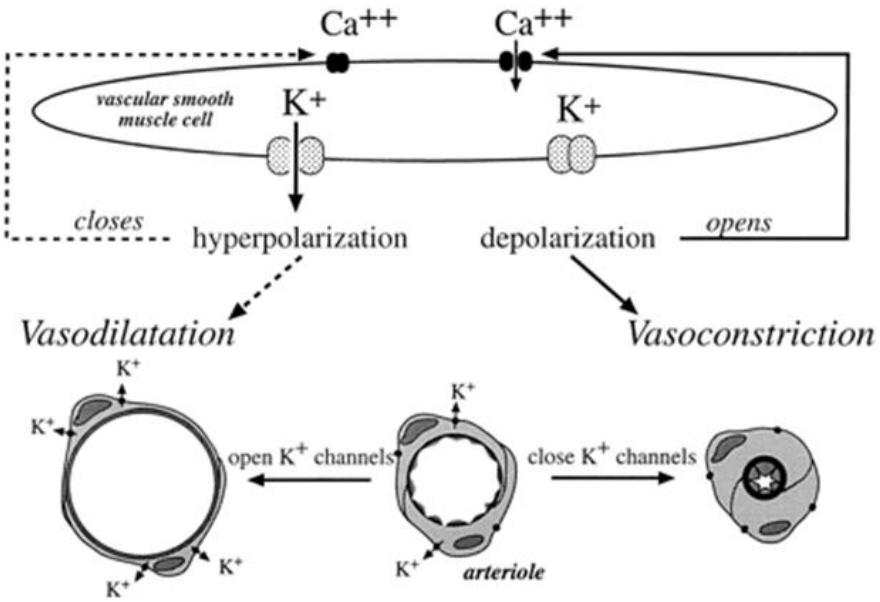

Figure 1.3 Schematic diagram of electromechanical coupling in vascular smooth muscle. Taken from Jackson (68).

Importantly, recent studies have linked metabolic syndrome with diminished functional expression of vascular smooth muscle K^+ channels (26; 38; 73; 108). BK_{Ca} channels of insulin resistant rat mesenteric arterial myocytes were found to have reduced current density relative to lean while single channel activity and channel protein expression remained similar (38). In arterial myocytes of diabetic fatty rat models of type II diabetes mellitus, BK_{Ca} channel current was diminished despite absence of changes in

channel expression (26). Alternatively, diminished BK_{Ca} channel function could be related to reduced activation of the channels by factors such as prostacyclin (101). Further, decreased coupling of sarcoplasmic reticulum-mediated Ca^{2+} sparks to spontaneous transient outward K^+ currents were demonstrated in coronary microvessels of alloxan-diabetic dyslipidemic swine (108). These changes are not limited to BK_{Ca} channels, as studies also report impaired functional dilation of K_{ATP} channels in obese Zucker rat models without changes in protein expression, linking impaired dilation to K_{ATP} channel sensitivity (73). Therefore, decreases in K^+ channel function could represent an important component of coronary vascular dysfunction in disease states such as metabolic syndrome. However, despite decades of research, the contribution of K^+ channels to the regulation of coronary blood flow has not been fully elucidated, especially in the setting of the metabolic syndrome.

K_{Ca} channels

There are three major classifications of K^+ channels regulated by intracellular Ca^{2+} levels based according to the biophysical property of conductance (i.e. by the slope of their single channel current-voltage relationships): small (SK_{Ca}), intermediate (IK_{Ca}) and large/big (BK_{Ca}) conductance K^+ channels. Very little is known about the role of SK_{Ca} and IK_{Ca} channels in the regulation of coronary blood flow. No patch-clamp data are available regarding SK_{Ca} channels in coronary vascular smooth muscle. IK_{Ca} channels are expressed in cultured coronary smooth muscle cells and may contribute to phenotypic modulation (148), but their role in coronary vascular reactivity remains unknown. The few studies conducted of SK_{Ca} and IK_{Ca} channel involvement in coronary vasodilation seem to suggest an involvement of these channels in mediating EDHF-induced dilation (31; 58). Smooth muscle cell function of SK_{Ca} and IK_{Ca} channels and its contribution to coronary blood flow will be an interesting area of future research.

More is known about the molecular and biophysical properties of coronary vascular smooth muscle BK_{Ca} channels (32; 146). BK_{Ca} channels are highly expressed in the coronary vascular smooth muscle (20; 108; 143) and have been implicated in coronary endothelial-dependent dilation under normal-lean conditions (71; 105; 106). In particular, bradykinin-induced endothelial-dependent relaxation in coronary arteries is mediated, in part, by the activation of BK_{Ca} channels (71; 105; 106). Studies in swine implicate endothelial NO release, hydroxyl radicals, or cytochrome-P450-independent endothelial hyperpolarizing factors (71), whereas studies in human coronary artery do not support a role for NO (105). Flow-induced vasodilation of human coronary arteries has also been reported to involve BK_{Ca} channels but via an NO-mediated pathway that is lost in patients with coronary artery disease (106). Despite the evidence for BK_{Ca} channel contribution to endothelial-dependent dilation, earlier studies failed to show a significant effect of BK_{Ca} channel blockade on resting coronary blood flow, though evidence suggests a role in exercise-induced and ischemic vasodilation (103; 115). Merkus *et al.* found that administration of the BK_{Ca} channel inhibitor tetraethylammonium resulted in a significant decrease in the relationship between coronary venous P_{O_2} and MV_{O_2} (Figure 1.4A) in normal-lean swine both at rest and during exercise (103). Animal and human studies indicate that this relationship under control conditions is similar in pigs, dogs, and in humans patients (42; 50; 103; 155). In addition, Node *et al.* reported that the BK_{Ca} channel antagonist iberiotoxin significantly decreased coronary blood flow during ischemia (Figure 1.4B), suggesting that ischemic vasodilators may activate BK_{Ca}-channel mediated dilation (115). Therefore, previous studies implicate BK_{Ca} channels in the regulation of endothelial-dependent dilation, metabolic control of coronary blood flow during increases in MV_{O_2} and also during episodes of cardiac ischemia.

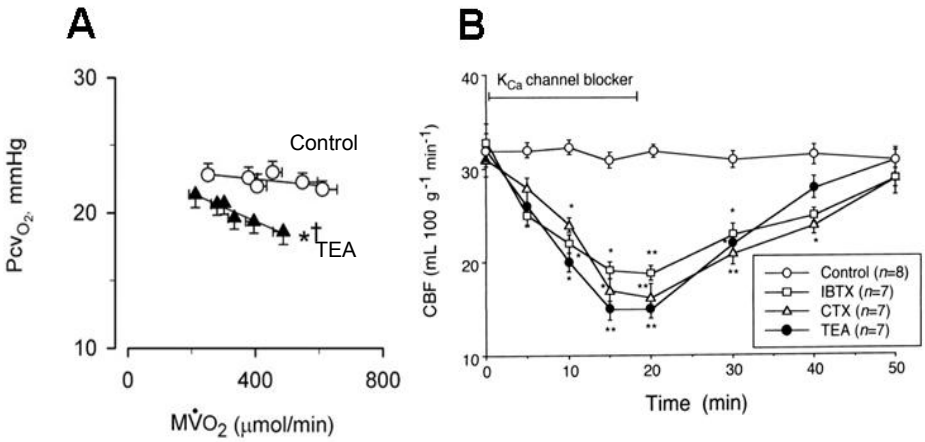

Figure 1.4 Role of BK_{Ca} channels in coronary vasodilation during exercise (A; Merkus *et al.* (84)) and myocardial ischemia (B; Node *et al.* (94)).

Even fewer studies have examined a role for BK_{Ca} channels in obesity and metabolic syndrome. Interpretation of the role for BK_{Ca} channels in vascular disease is confounded by the presence of conflicting results among the different components of metabolic syndrome, as summarized in Table 1.1. Evidence for diminished BK_{Ca} channel function has been observed in models of insulin resistance (38) and high-fat feeding (168). Numerous studies investigating BK_{Ca} channel role in hypertension alone observed evidence for increased BK_{Ca} channel function (98-100; 174) whereas others found diminished function (7; 21; 23). Findings in models of overt type I and type II diabetes

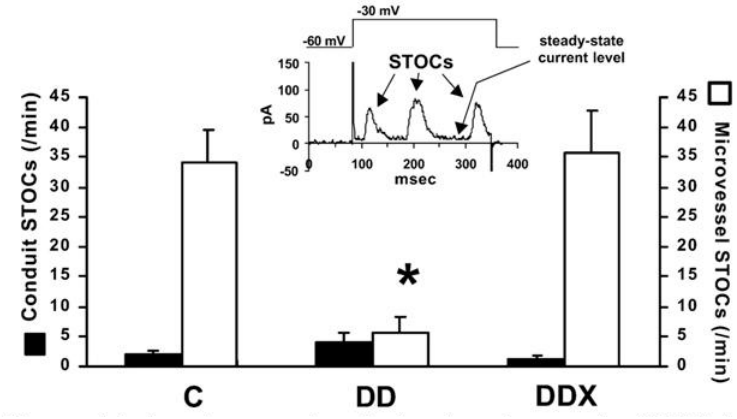

Figure 1.5 Spontaneous transient outward currents (STOCS) are attenuated in smooth muscle cells from microvessels obtained from diabetic dyslipidemic animals but not in cells from conduit arteries. Taken from Mokelke *et al.* (88)

mellitus are less clear, as there is evidence for decreased BK_{Ca} channel function (102), no change (169), as well as increased function (109). These may be due to species, vascular bed examined, or artery size (108; 109). Recent data from the Sturek laboratory indicates that diabetic dyslipidemia increases the functional coupling of BK_{Ca} channels to sarcoplasmic reticulum Ca^{2+} release in vascular smooth muscle cells from large-conduit coronary arteries (108). This increase was attributed to a compensatory change in response to the increase in Ca^{2+} influx (or a vasoconstrictor influence) as previously noted in conduit arteries of aldosterone-salt hypertensive rats (99). However, additional studies in coronary microvessels revealed a significant decrease in the coupling of sarcoplasmic reticulum-mediated Ca^{2+} spark events (which activate BK_{Ca} channels) with spontaneous transient outward K^+ current (STOC) frequency (Figure 1.5) (108). These findings indicate that diabetic dyslipidemia impairs microvascular BK_{Ca} channel function, however, the contribution of BK_{Ca} channel defects to the control of coronary blood flow and vascular dysfunction in metabolic syndrome has not been directly examined.

Table 1.1. Summary of the role of BK_{Ca} channels in vascular disease.

Investigators	Species	Model	Vascular bed	Role of BK_{Ca} channels
Dimitropoulou *et al.* 2002 (38)	Rats	Insulin resistance	Mesenteric microvessels	↓ current — protein expression — unitary conductance — Ca2+/voltage sensitivity
Yang *et al.* 2007 (168)	Pig	High fat diet	Coronary artery	↓ current
Du *et al.* 2006 (41)	Rabbit	High cholesterol diet	Sphincter of Oddi	↓ protein expression
Godlewski *et al.* 2009 (62)	HEK cells	High cholesterol	Mesenteric artery	↓ current
Jeremy-McCarron 2000 (83)	Rabbit	High cholesterol	Femoral artery	↓ dilation
Ye *et al.* 2004 (169)	Mouse	Streptozotocin type I diabetes mellitus	Aorta	↓ unitary conductance ↑ open probability
McGahon *et*	Rat	Streptozotocin	Retinal	↓ current

al. 2007 (102)		type I diabetes mellitus	arterioles	↓ STOCs ↑ activation (Ca2+ sparks) ↓ protein expression ↓ mRNA expression
Mokelke *et al.* 2005 (108)	Pig	Alloxan-induced diabetic dyslipidemia	Coronary conduit arteries & microvessels	↓ activation (Ca2+ sparks) ↓ STOCs, microvessels — STOCs, conduit ↓ current, microvessels — current, conduit
Mokelke *et al.* 2003 (109)	Pig	Alloxan-induced diabetic dyslipidemia; High cholesterol	Coronary artery	↑ current — protein expression
Burnham *et al.* 2006 (26)	Rat	ZDF type II diabetes mellitus	Mesenteric artery	↓ current — protein expression — mRNA expression
Lu T *et al.* 2005 (101)	Rat	ZDF type II diabetes mellitus	Coronary artery	— current ↓ activation (PGI) — protein expression
Liu *et al.* 1995 (99)	Rat	Aldosterone-salt hypertension	Aorta	↑ STOCs — unitary conductance
Liu *et al.*1997 (100)	Rat	Spontaneous hypertension	Aorta	↑ current ↑ protein expression — mRNA expression
Liu *et al.* 1998 (98)	Rat	Spontaneous hypertension	Cerebral arterioles	↑ current ↑ protein expression — unitary conductance — Ca2+/voltage sensitivity
Zhang Y *et al.* 2005 (174)	Rat	Spontaneous hypertension	Mesenteric artery	↑ STOCs ↑ current ↑ protein expression
Amberg *et al.* 2003 (7)	Rat	Ang II-induced hypertension	Cerebral artery	↓ current ↑ activation (Ca2+ sparks) ↓ protein expression
Bratz *et al.* 2005 (23)	Rat	l-NNA-induced hypertension	Mesenteric artery	↓ current — unitary conductance — Ca2+/voltage sensitivity
Bratz *et al.* 2005 (21)	Rat	l-NNA-induced hypertension	Mesenteric artery	↓ protein expression

K_V channels

Voltage-gated, delayed rectifier K^+ (K_V) channels are prominently expressed in coronary artery smooth muscle cells (63). These channels conduct outward, hyperpolarizing K^+ current that is important for the regulation of cell membrane potential (80; 126) and determining coronary vascular resistance and blood flow (131). Recent studies implicate K_V channels as important end-effectors in the regulation of local metabolic control of coronary blood flow as well as in ischemic coronary vasodilation. More specifically, Saitoh *et al*. demonstrated that coronary infusion of the K_V channel antagonist 4-aminopyridine in dogs significantly impaired the vasodilatory response to pacing and norepinephrine-induced increases in MVO_2 (135). Importantly, they observed a significant difference in the slope of the relationship between coronary venous oxygen tension and MVO_2 (Figure 1.6A), indicating a mismatch in the balance between myocardial oxygen delivery and myocardial metabolism with K_V channel inhibition. In addition, Dick *et al.* recently found that K_V channels play an important role in the regulation of baseline coronary blood flow and the reactive hyperemic response. This effect was evidenced by marked reductions in baseline coronary blood flow following administration of 4-aminopyridine; to the extent that myocardial ischemia was evident

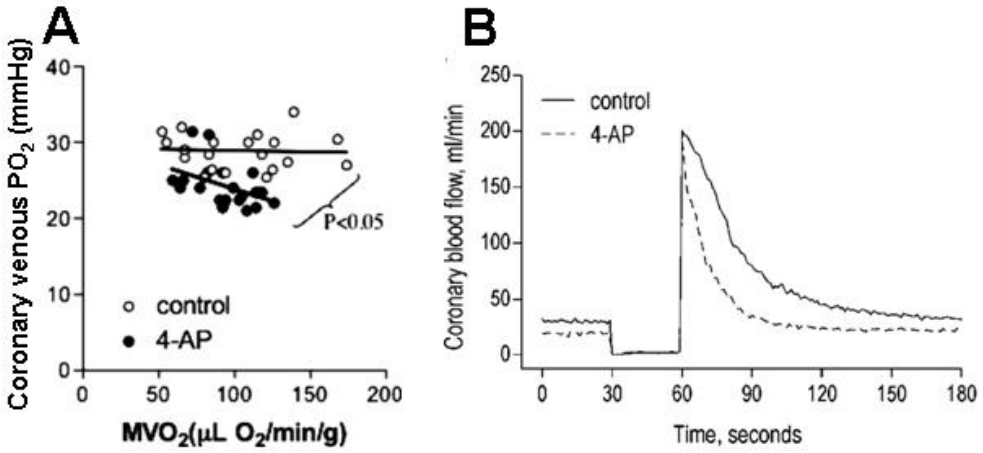

Figure 1.6 Role for K_V channels in coronary vasodilation in response to increases in cardiac metabolism (A) and ischemia (B). Taken from Saito *et al.* (112) and Dick *et al.* (33)

(ST segment depression). In addition, 4-aminopyridine significantly decreased the coronary debt to repayment ratio (Figure 1.6B) thus implicating a role for K_V channels in mediating vasodilation in response to cardiac ischemia (36).

Despite mounting evidence for K_V channel contribution in mediating local metabolic and ischemic vasodilation under normal physiological conditions, the effect of obesity and metabolic syndrome on K_V channel-mediated vasodilation remains unknown. Recent studies have linked components of metabolic syndrome, in particular hypertension, to diminished K_V channel current and expression in other vascular beds (21; 23). However, the effect of obesity and metabolic syndrome on K_V channel-mediated vasodilation warrants further investigation.

K_{ATP} channels

K_{ATP} channels are comprised of pore-forming $K_{IR}6$ family subunits combined with sulfonylurea receptor subunits. They are regulated by the ATP:ADP ratio and the channels close as ATP concentrations increase. The majority of evidence indicates that K_{ATP} channels are not obligatory for exercise-induced dilation, but rather contribute to the tonic regulation of coronary blood flow. In the presence of the selective K_{ATP} inhibitor glibenclamide, Merkus *et al*. found that K_{ATP} channel inhibition in a swine diminished coronary venous oxygen tension at any given rate of MVO_2 (103). This was especially prominent under resting conditions and its effect diminished with increasing MVO_2, which suggests that these channels are important for the regulation of coronary blood flow under baseline-resting conditions. These findings are consistent with previous data from our laboratory which found that K_{ATP} channel inhibition with glibenclamide in dogs resulted in a parallel downward shift of the relationship between coronary venous oxygen tension and MVO_2, again indicating that K_{ATP} channels are not required for local metabolic coronary dilation (45; 127; 128; 156). Although one study in humans found that glibenclamide attenuated metabolic dilation in response to cardiac pacing (53),

experiments in dogs and pigs, as well as a recent study in human forearm (138), demonstrate only a role for tonic regulation of coronary blood flow and no role for K_{ATP} channels in mediating local metabolic dilation to exercise under physiological conditions. Alternatively, in alloxan-diabetic canines glibenclamide made the slope of the relationship between coronary venous oxygen tension and MVO_2 more negative, indicating a more prominent role for K_{ATP} channels in local metabolic dilation in type I diabetes mellitus (156). These data suggest that K_{ATP} channel function and activity is possibly altered under pathophysiological conditions. However, this effect has not been shown in other animal models, and the contribution of K_{ATP} channels to the regulation of coronary blood flow and vascular tone in obesity and metabolic syndrome remains unexplored.

Based on these earlier studies, we suggest that BK_{Ca}, K_V, and K_{ATP} channels merit investigation as potential end-effectors of metabolic vasodilators. Elucidating the role of these important channels in the regulation of coronary blood flow, further attention can be focused to uncover the relative contribution of coronary K^+ channels to the coronary vascular complications of obesity and metabolic syndrome.

Hypothesis and aims of the investigation

Earlier studies have demonstrated that coronary microvascular function is markedly impaired by the onset of the metabolic syndrome (27; 39; 90; 137; 141; 173) and indicate that this may be an important contributor to increased cardiovascular events in obese patients with this combined disorder (66; 70; 96). Although recent investigations have made important strides in elucidation of key mechanisms underlying this dysfunction, much remains unclear. Although there is mounting evidence of coronary K^+ channel dysfunction in the metabolic syndrome (26; 38; 73; 108), there are no conclusive data to directly address the contribution of K^+ channels to impaired coronary

microvascular responsiveness in the setting of the metabolic syndrome. Accordingly, the overall goal of this investigation was to delineate the mechanistic contribution of K^+ channels to coronary microvascular dysfunction in metabolic syndrome (Figure 1.7).

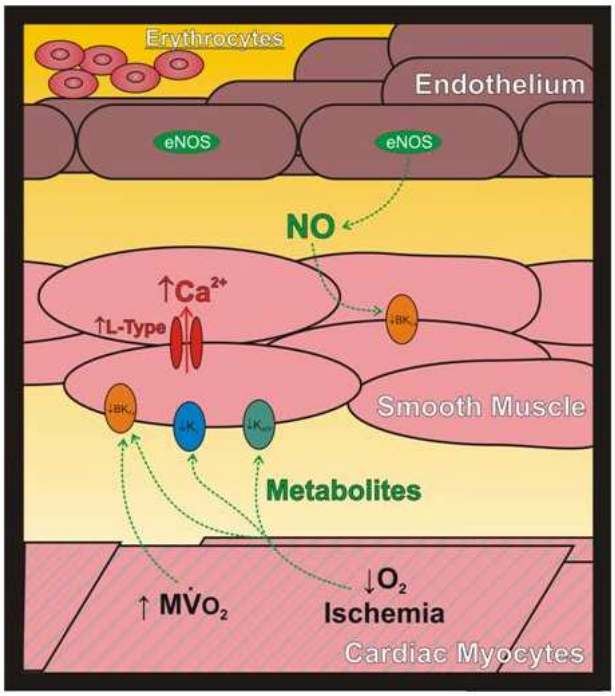

Figure 1.7 Schematic diagram of proposed studies to examine the contribution of K^+ channels to coronary microvascular dysfunction in metabolic syndrome.

Aim 1 was designed to examine the molecular and functional expression of BK_{Ca} channels that regulate coronary vascular function and to elucidate mechanisms underlying the deleterious influence of metabolic syndrome. The rationale for this study came from recent investigations which found that BK_{Ca} channel activity was significantly impaired in coronary microvascular smooth muscle of alloxan-diabetic dyslipidemic swine (108), mesenteric microvessels of insulin resistant rats (38), and coronary arterial myocytes of diabetic fatty rats (26; 101). In addition, BK_{Ca} channels are highly expressed in the coronary vascular smooth muscle cells (20; 108; 143) and have been implicated as end-effectors in the regulation of coronary vasodilation in response to key endothelial and metabolic metabolites (71; 105; 106). However, the

contribution of BK_{Ca} channel defects to the control of coronary blood flow and vascular dysfunction in metabolic syndrome has not been examined.

Aim 2 was designed to examine whether impaired functional expression of coronary microvascular BK_{Ca} channels in metabolic syndrome significantly attenuates the balance between myocardial oxygen delivery and metabolism at rest and during exercise-induced increases in MVO_2. The rationale for this aim stems from studies which have shown that BK_{Ca} channels contribute to coronary endothelial-dependent and exercise-induced dilation under normal-lean conditions (16; 103; 105; 106). To date, no study has examined the contribution of BK_{Ca} channels to metabolic coronary vasodilation in the setting of the metabolic syndrome. We propose that decreases in BK_{Ca} channel function could be an underlying mechanism of impaired metabolic control of coronary blood flow in the metabolic

Aim 3 was designed to elucidate the relative contribution of specific K^+ channels to coronary reactive hyperemia and test the hypothesis that metabolic syndrome impairs coronary vasodilation to cardiac ischemia via decreases in the relative contribution of BK_{Ca}, K_V, and K_{ATP} channels to the reactive hyperemic response. The rationale for this set of experiments is supported by previous investigations which documented a role for BK_{Ca} channels (115), K_V channels (36), as well as K_{ATP} channels (10; 28; 172) in coronary vasodilation in response to a brief episode of myocardial ischemia, i.e. reactive hyperemia. However, the relative contribution of these specific K^+ channels to ischemic coronary vasodilation has not been delineated in lean or obese subjects with the metabolic syndrome.

This study integrated molecular, cellular, and systems approaches to directly examine these three aims. Experiments were performed on Ossabaw miniature swine fed a normal maintenance diet or an excess calorie atherogenic diet that induces many common features of metabolic syndrome, including: obesity, insulin resistance, impaired

glucose tolerance, and dyslipidemia (22; 49; 145). Experiments involved *in vivo* studies of coronary blood flow in open-chest anesthetized swine as well as conscious, chronically instrumented swine and *in vitro* studies in isolated coronary arteries, arterioles, and vascular smooth muscle cells. Results from this investigation stand to offer novel mechanistic insight into the role and contribution of K^+ channels in metabolic syndrome-induced coronary vascular disease.

CHAPTER 2

Impaired Functional Expression of Coronary BK_{Ca} Channels in Metabolic Syndrome

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Abstract

The role of large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels in regulation of coronary microvascular function is widely appreciated, but the molecular and functional expression underlying the deleterious influence of metabolic syndrome has not been determined. Studies were conducted in Ossabaw miniature swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (45% kcal from fat, 2% cholesterol, 20% kcal from fructose) that induces metabolic syndrome. Metabolic syndrome significantly impaired BK_{Ca} channel-mediated coronary vasodilation to NS1619 *in vivo* (30 – 100 μg) and the contribution of these channels to adenosine-mediated microvascular vasodilation *in vitro* (1 – 100 μM). Metabolic syndrome reduced whole-cell penitrem A (1 μM) sensitive K^+ current and NS1619-activated (10 μM) current in isolated coronary vascular smooth muscle cells. These changes were associated with marked increases in coronary vasoconstriction to the L-type Ca^{2+} channel agonist BayK 8644 (1 pM – 10 nM) and intracellular Ca^{2+} concentration. BK_{Ca} channel α and β1 subunit expression was increased in coronary arteries from metabolic syndrome swine; however, fewer channels were present in the plasma membrane, as confocal imaging revealed diffuse intracellular localization in coronary smooth muscle cells of metabolic syndrome swine. Coronary vascular dysfunction in metabolic syndrome is related to decreased membrane trafficking and functional expression of BK_{Ca} channels that is accompanied by significant increases in L-type Ca^{2+} channel-mediated coronary vasoconstriction and increased intracellular Ca^{2+} concentration.

Keywords: blood flow, circulation, ion channels, obesity

Introduction

Obesity is associated with cardiovascular and metabolic risk factors such as insulin resistance, impaired glucose tolerance, hypertension, and dyslipidemia, i.e., metabolic syndrome (59; 96). As each component of metabolic syndrome is an independent risk factor for cardiovascular disease, it is not surprising that metabolic syndrome patients have elevated morbidity and mortality to many cardiovascular-related diseases, including stroke, coronary artery disease, cardiomyopathies, myocardial infarction, congestive heart failure, and sudden cardiac death (66; 70; 96). However, the mechanisms underlying metabolic syndrome-induced cardiovascular disease remain poorly understood.

Evidence suggests that coronary microvascular dysfunction may be an important contributor to the increased cardiovascular events associated with metabolic syndrome (88; 139). Recent investigations provide evidence for impaired insulin-mediated capillary recruitment, altered obesity-related endocrine signaling, increased arterial stiffening, and diminished flow reserve in metabolic syndrome (125; 139). These microvascular defects may play an important role in the end-organ damage associated with this combined disorder. Importantly, metabolic syndrome is characterized by an imbalance between coronary blood flow and myocardial metabolism that is related to sensitization of angiotensin II, endothelin-1, and α-adrenoceptor mediated vasoconstriction pathways (88). Alterations in these mechanisms could significantly inhibit large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (4; 114; 150). BK_{Ca} channels are highly expressed in the coronary vascular smooth muscle (20; 108; 143) and have been implicated in exercise- and ischemia-induced coronary vasodilation in lean/control animals (20; 108; 115). In addition, BK_{Ca} channel activity was recently shown to be significantly impaired in coronary microvascular smooth muscle of alloxan-diabetic dyslipidemic swine (108), mesenteric microvessels of insulin resistant rats (38) and coronary arterial myocytes of

diabetic fatty rats (26; 101). A lesser studied mechanism of BK_{Ca} channel regulation is the trafficking of channels from intracellular membranes to the plasma membrane to yield functional ion channel activity. Excellent evidence exists in overexpression systems in cultured cells (149) but there are no reports of this novel BK_{Ca} channel trafficking mechanism in native vascular smooth muscle cells from *in vivo* models of vascular disease. Thus, the contribution of BK_{Ca} channel defects to the control of coronary blood flow and vascular dysfunction in metabolic syndrome has not been examined.

The goal of this investigation was to examine the molecular and functional expression of BK_{Ca} channels that regulate coronary microvascular function and to elucidate mechanisms underlying the deleterious influence of metabolic syndrome. Studies were conducted in Ossabaw miniature swine fed a normal maintenance diet or an excess calorie atherogenic diet that induces many common features of metabolic syndrome, including: obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia (22; 49; 145). Experiments involved *in vivo* studies of coronary blood flow in open-chest anesthetized swine and *in vitro* studies in isolated coronary arteries, arterioles, and vascular smooth muscle cells.

Methods

Swine Model of Metabolic Syndrome

All procedures were approved by the Institutional Animal Care and Use Committee in accordance with the *Guide for the Care and Use of Laboratory Animals*. Lean swine were fed ~2200 kcal/day of standard chow (5L80, Purina TestDiet, Richmond, IN) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. Metabolic syndrome swine were fed an excess ~8000 kcal/day high fat/fructose, atherogenic diet containing 17% kcal from protein, 20% kcal from complex carbohydrates, 20% kcal from fructose, and 43% kcal from fat (lard and hydrogenated soybean and coconut oils), and supplemented with 2.0% cholesterol and 0.7% sodium cholate by weight (5B4L, Purina TestDiet, Richmond, IN). Prior to sacrifice, blood was drawn for glucose, insulin, and lipid assays (22; 49; 145) and *in vivo* studies were conducted. Pigs were then euthanized and tissue was harvested for subsequent *in vitro* analyses.

Surgical Preparation and *In Vivo* Coronary Dose-Response Experiments

Lean (n = 6) and metabolic syndrome (n = 5) Ossabaw swine were sedated with telazol (5 mg/kg, sc) and xylazine (2.2 mg/kg, sc). Animals were intubated and ventilated with O_2-supplemented air. Anesthesia was maintained with morphine sulfate (3 mg/kg, im) and α-chloralose (100 mg/kg, iv). The left anterior descending coronary artery (LAD) was isolated and a perivascular Transonics flow transducer (2.5 mm) was placed around the artery. A 24 gauge angiocatheter was inserted into the LAD for infusion of the BK_{Ca} channel agonist NS1619 (3 – 100 μg bolus) before and after inhibition of BK_{Ca} channels with penitrem A (10 μg/kg, iv, Biomol). In a separate experiment (n = 4), endothelial-dependent coronary flow reserve was assessed by intracoronary infusion of bradykinin

(30 μg/min) before and after penitrem A. Following *in vivo* experiments, hearts were electrically fibrillated, excised, and immediately immersed in 4°C saline to dissect tissues for *in vitro* and molecular experiments.

Isolation and Functional Assessment of Coronary Arterioles

Subepicardial coronary arterioles (50 – 150 μm in diameter) were isolated, cannulated, and pressurized to 60 cmH_2O as previously described (89). Intraluminal diameter was measured continuously with videomicrometers and recorded on a MacLab workstation. Arterioles that were free from leaks were allowed to equilibrate for approximately 1 hr at 37°C with the bathing physiological salt solution (PSS) solution changed every fifteen minutes. Coronary arterioles (Lean n = 6; Metabolic syndrome n = 5) were pre-constricted to 50 – 70% tone with endothelin-1 (2 nM) then washed, and dose-response studies conducted with the stable adenosine analog 2-chloroadenosine (2-CADO; 0.1 nM – 0.1 mM) before and after inhibition of BK_{Ca} channels with iberiotoxin (100 nM; 30 min incubation).

Functional Assessment of Isolated Epicardial Coronary Artery Rings

Isolated coronary artery studies were performed as previously described (22; 89). Left circumflex coronary arteries from lean (n = 4) and metabolic syndrome (n = 4) swine were isolated, cleaned of surrounding tissue and cut into 3 mm ring segments. Arterial rings were then mounted in organ baths and optimal length was determined by assessing contraction to 60 mM KCl. Arterial contractile responses to L-type Ca^{2+} channel activation were assessed by the addition of graded concentrations of BayK 8644 (0.1 pM – 10 nM).

Smooth Muscle Cell Isolation and Electrophysiology Studies

Coronary vascular smooth muscle cells were isolated as previously described (22; 36). Patch-clamp recordings and immunocytochemistry/confocal studies (see below) were performed within 8 h of cell dispersion. Whole cell K^+ currents were measured at room temperature using the conventional dialyzed configuration of the patch-clamp technique (36). 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 MΩ 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 -100 to +100 mV in 10-mV increments from -80 mV holding potential. Currents were measured in the absence and presence the BK_{Ca} channel agonist NS1619 (10 μM) as well as with and without the BK_{Ca} channel antagonist penitrem A (1 μM).

Fura-2 Microfluorometry

Experiments to assess intracellular Ca^{2+} concentration were performed at room temperature using an InCyt Basic IM Calcium Imaging System (Intracellular Imaging, Cincinnati, OH) as previously described (22). Briefly, freshly dispersed cells were incubated with 2.5 mM fura-2 AM (Molecular Probes) in a shaking water bath at 37°C for 20 min. Cells were spun down and washed in a solution containing horse serum in order to cleave extracellular dye. An aliquot of fura 2-loaded cells was placed on a coverslip in a superfusion chamber mounted atop an inverted epifluorescence microscope. Light from a 300-W xenon arc lamp was passed through 360 ± 10- and 380 ± 10-nm band-pass filters. Emitted light (510 nm) was collected using a monochrome charge-coupled

device camera (COHU) attached to a 100-MHz Pentium data acquisition computer. Data are presented as F360/F380 ratio which is indicative of intracellular Ca^{2+} concentration.

Western Blot Analysis

Western blotting was performed as previously described (22; 39). Coronary arteries from lean (n = 3) and Metabolic syndrome (n = 3) swine were isolated and immediately placed in liquid N_2 and stored at -80°C. Arteries were homogenized, centrifuged, and the supernatants containing total membrane protein were collected for analysis. Equivalent amounts of protein were loaded onto 12% (for BK_{Ca} channel α subunit, ~110 kDa) and 7% (for BK_{Ca} channel β1 subunit, ~28 kDa) acrylamide gels for electrophoresis and blotting. After membranes were blocked for 1 h at ambient temperature with 5% nonfat milk, membranes were incubated overnight at 4°C with polyclonal antibodies directed against BK_{Ca} α subunit or BK_{Ca} β1 subunits (both 1:1000 dilution; Affinity BioReagents). Blots were washed and incubated with donkey anti-rabbit IgG-HRP secondary antibody (1:5000 dilution; Santa Cruz Biotechnology) for 2 h at ambient temperature. The same blots were stripped and reblotted with β-actin antiserum (1:3000 dilution; Santa Cruz Biotechnology) as the internal control. Immunoreactivity was visualized using an ECL Western blotting detection kit and quantified by scanning densitometry.

Confocal Microscopy

Confocal microscopy was performed similar to that previously described (85). Cells were fixed for 10 min in 4% paraformaldehyde/PBS. Cells were washed in PBS, permeabilized in 0.2% Triton X-100/PBS for 10 min, rinsed again and incubated overnight at 4°C in 2.5% BSA in PBS to block non-specific binding. Cells were next incubated for 1 h at 37°C with polyclonal antibodies directed against BK_{Ca} α (diluted

1:100 in 2.5% BSA in PBS; Affinity BioReagents) and/or BK_{Ca} β1 subunits (diluted 1:100 in 2.5% BSA in PBS; Santa Cruz Biotechnology). The primary antibodies were detected by incubating cells for 45 min with affinity-purified IgG conjugated to Cy5 or FITC (Jackson ImmunoResearch) at 1:200 dilution. After final washes, the cells were mounted with fluoromount-G (Southern Biotechnology) and examined by laser scanning confocal microscopy (Zeiss). For detection of Cy5 and FITC fluorescence, the excitation was at 488 nm and emission at 620 – 680 nm and 505 – 570 nm, respectively. Metamorph software was used for analysis. Staining at the periphery of the cell was compared to that of the cytoplasmic portion to obtain a plasma membrane/cytoplasm ratio. The ratios of 7 – 10 randomly chosen cells from each lean (n = 9) and Metabolic syndrome (n = 7) pig were averaged and corrected to background.

Statistical Analyses

Data are presented as mean ± SE and n represents number of subjects. Statistical comparisons were made with unpaired and paired *t*-tests and one- or two-way repeated measures analysis of variance (ANOVA) as appropriate. In all statistical tests, $P < 0.05$ was considered statistically significant. When significance was found with ANOVA, a Student-Newman-Keuls multiple comparison test was performed to identify differences between treatment levels.

Results

Phenotype of Ossabaw Swine

Phenotypic characteristics of lean and metabolic syndrome are listed in Table 2.1. Compared to their lean counterparts, metabolic syndrome swine exhibited a 33% increase in body mass, 75% increase in fasting glucose, 210% increase in fasting insulin, 483% increase in total cholesterol, 313% increase in LDL/HDL ratio, and 105% increase in triglyceride levels. Using the homeostatic model assessment method, percent β cell function and percent insulin sensitivity diminished from 218.1% and 84.9% in lean swine to 158.3% and 24.5% respectively in swine with metabolic syndrome. Meanwhile, insulin resistance increased from 1.2 in lean to 4.1 in metabolic syndrome swine.

Table 2.1 Phenotypic Characteristics of Lean and Metabolic Syndrome Ossabaw Swine

	Lean	**Metabolic syndrome**
Body Weight (kg)	45 ± 4	60 ± 6*
Heart wt. / Body wt. * 100	0.38 ± 0.03	0.37 ± 0.03
Fasting glucose (mg/dl)	65 ± 5	114 ± 8*
Fasting insulin (μU/ml)	10 ± 1	31 ± 4*
Total cholesterol (mg/dl)	60 ± 3	350 ± 31*
LDL/HDL ratio	0.8 ± 0.1	3.3 ± 0.3*
Triglycerides (mg/dl)	22 ± 1	45 ± 7*
Mean arterial pressure (mmHg)	95 ± 5	87 ± 6
Heart rate (beats/min)	95 ± 11	108 ± 6

Values are mean ± SE for lean (n = 15) and metabolic syndrome (n = 13) swine. * $P<0.05$ vs lean.

Contribution of BK_{Ca} Channels to Coronary Microvascular Vasodilation

Coronary arteriolar endothelium-independent vasodilation *in vitro* to 2-CADO (0.1 nM – 0.1 mM), a stable adenosine analog was similar between lean and metabolic syndrome swine (Figure 2.1). However, iberiotoxin (100 nM) diminished relaxation to 2-CADO in arterioles from lean swine (1 μM – 100 μM; $P < 0.01$) but had no effect on 2-CADO-induced relaxation in arterioles from metabolic syndrome swine (Figure 2.1). In addition, endothelial-dependent vasodilation to bradykinin (30 μg/min) was significantly decreased from 1.6 ± 0.3 ml/min/g to 1.0 ± 0.3 ml/min/g by inhibition of BK_{Ca} channels with penitrem A (10 μg/kg, iv) *in vivo* in lean swine ($P = 0.01$).

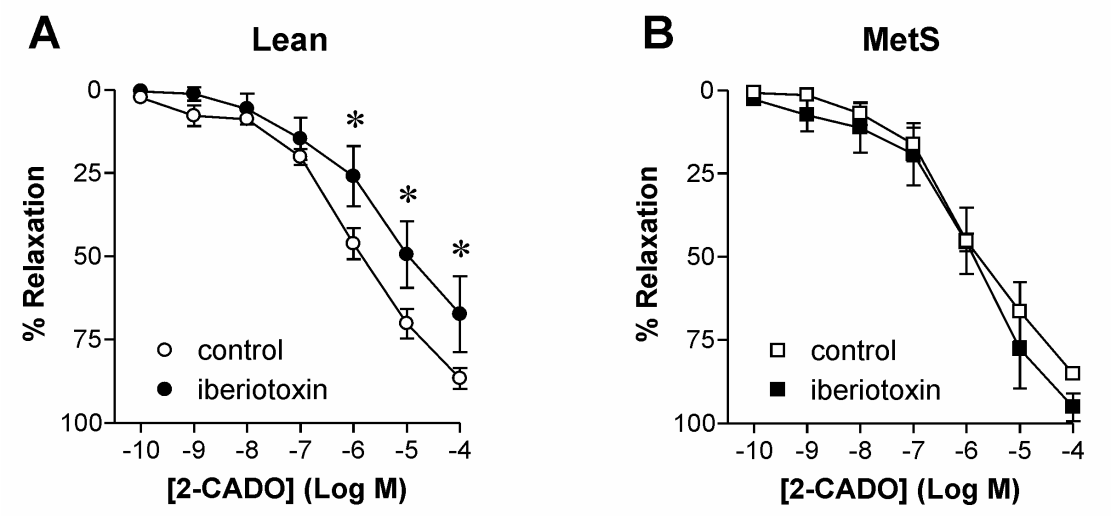

Figure 2.1 Concentration-response curves of isolated pressurized coronary arterioles from lean and metabolic syndrome swine to adenosine analog 2-CADO with and without BK_{Ca} channel blockade by iberiotoxin (IBTX; 100 nM). Relaxation to 2-CADO was not different between lean (A, n = 6) and metabolic syndrome (B, n = 5) swine under control conditions. However, iberiotoxin diminished relaxation to 2-CADO in lean swine (* $P < 0.01$ vs control) but not in metabolic syndrome.

Effect of Metabolic Syndrome on Coronary BK_{Ca} Channels *In Vivo* and *In Vitro*

Figure 2.2 shows the coronary blood flow response to the BK_{Ca} channel agonist NS1619 in lean and metabolic syndrome swine. NS1619 dose-dependently increased coronary blood flow in lean swine. However, coronary vasodilation to NS1619 was significantly

attenuated in metabolic syndrome swine at 30 – 100μg ($P < 0.01$). NS1619 vehicle (ethanol) had no effect on coronary blood flow (data not shown). The decrease of NS1619-mediated dilation in metabolic syndrome swine was similar to that observed in lean swine following blockade of BK_{Ca} channels with penitrem A (Figure 2.2).

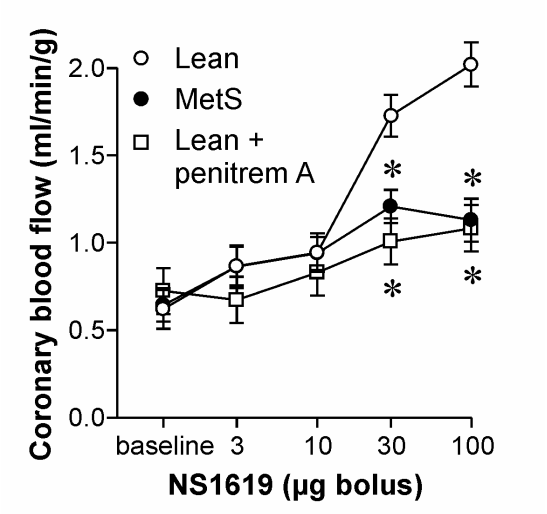

Figure 2.2 BK_{Ca} channel agonist NS1619 produced dose-dependent increases in coronary blood flow that were attenuated in metabolic syndrome swine (n = 4) relative to lean (n = 5). Blockade of BK_{Ca} channels with penitrem A (10 μg/kg, iv) decreased NS1619-induced vasodilation in lean swine to a similar level as observed in swine with metabolic syndrome. * $P <$ 0.01 vs. lean at same dose.

Average normalized whole cell steady-state K^+ current was attenuated ~20% in Metabolic syndrome swine (n = 7) vs. lean (n = 6) at potentials > +50 mV, i.e. currents biophysically consistent with BK_{Ca} channels ($P < 0.05$). Outward current generated in response to the BK_{Ca} channel agonist NS1619 (10 μM) was markedly depressed in coronary vascular smooth muscle cells from metabolic syndrome swine (Figure 2.3A; $P < 0.001$). Further, Penitrem A (1 μM) significantly attenuated outward K^+ current at potentials > +50mV and abolished the difference in outward current between lean and metabolic syndrome swine (Figure 2.3A; $P < 0.001$). Vasoconstriction to the L-type Ca^{2+} channel agonist BayK 8644 was significantly elevated (1 pM – 10 nM; $P < 0.01$) in isolated coronary conduit arteries from metabolic syndrome relative to lean swine (Figure 2.3B). These data are consistent with findings from fura-2 studies that showed a

significant increase in intracellular Ca^{2+} concentrations in metabolic syndrome (F360/F380 ratio = 2.97 ± 0.04) vs. lean (F360/F380 ratio = 1.36 ± 0.04) swine.

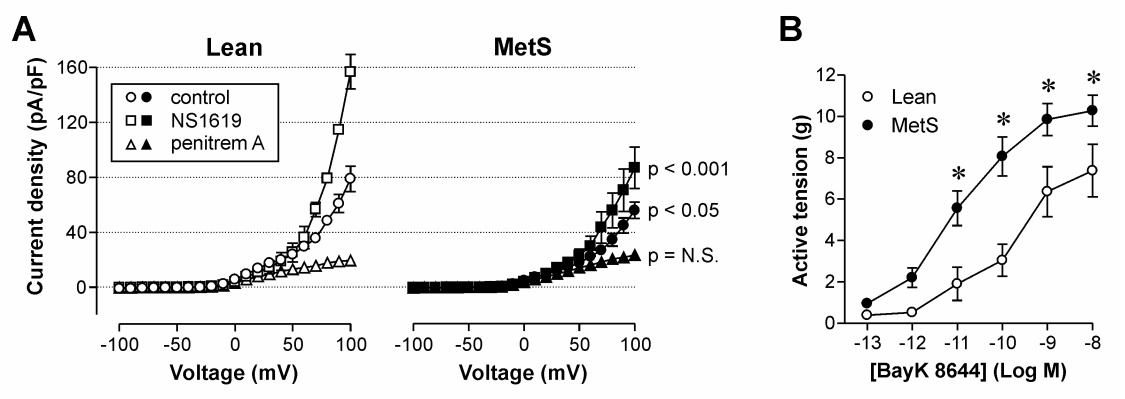

Figure 2.3 A: Whole cell patch-clamp recordings demonstrated reduced current in coronary artery smooth muscle cells from metabolic syndrome (closed symbols, n = 7) relative to lean swine (open symbols, n = 6). This difference was abolished and current was significantly attenuated in the presence of penitrem A (0.1 μM). Activation of BK_{Ca} channel current with NS1619 (1 μM) was significantly diminished in metabolic syndrome swine. **B:** Concentration-response studies in isometric arterial rings demonstrated vasoconstriction to the L-type Ca^{2+} channel agonist BayK 8644 was significantly elevated in coronary arteries from metabolic syndrome (n = 4) vs. lean swine (n = 4). * $P < 0.01$ vs. lean control at same dose.

Coronary BK_{Ca} Channel Protein Expression and Membrane Trafficking

Paradoxically, Western blot analysis revealed a dramatic up-regulation in the coronary expression of the BK_{Ca} channels in metabolic syndrome swine as expression of the pore-forming α and regulatory β1 subunits were increased 57 ± 13% and 74 ± 15% ($P < 0.05$), respectively (Figure 2.4). However, confocal microscopy revealed distinctly different patterns of BK_{Ca} channel localization in coronary vascular smooth muscle cells from lean and metabolic syndrome swine. BK_{Ca} channel α and β1 subunit expression was largely confined to the plasma membrane region in lean swine. In contrast, the BK_{Ca} channel subunit expression in metabolic syndrome swine was detected diffusely throughout the cytoplasm (Figure 2.5A). Quantitative analysis of confocal images

revealed a ~67% reduction ($P < 0.001$) in the membrane/cytoplasm ratio for both α and β1 subunits in metabolic syndrome vs. lean smooth muscle cells (Figure 2.5B).

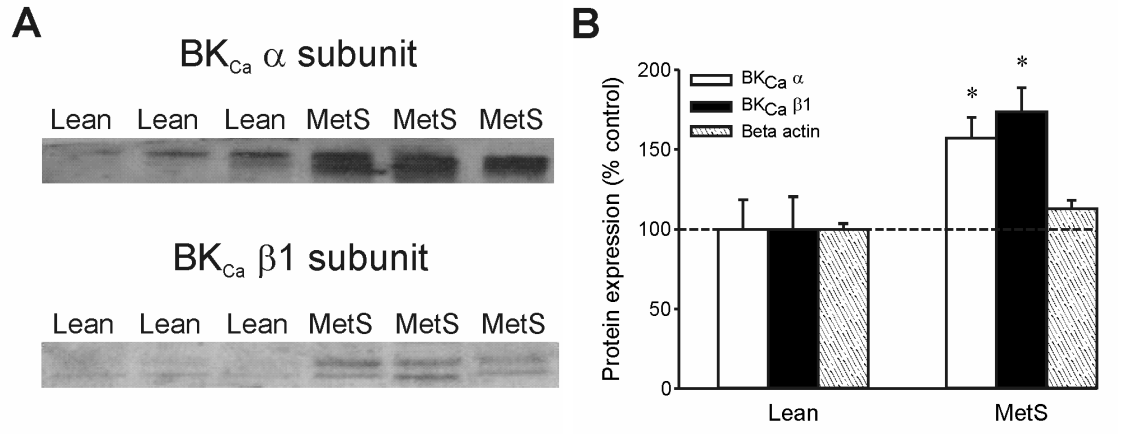

Figure 2.4 A: Western blot analysis of BK_{Ca} channel α and β1 subunits in lean and metabolic syndrome swine. **B:** Averaged data demonstrating a significant increase in expression of both BK_{Ca} channel α and β1 subunits in metabolic syndrome (n = 3) relative to lean (n = 3) swine. * $P < 0.05$ vs lean.

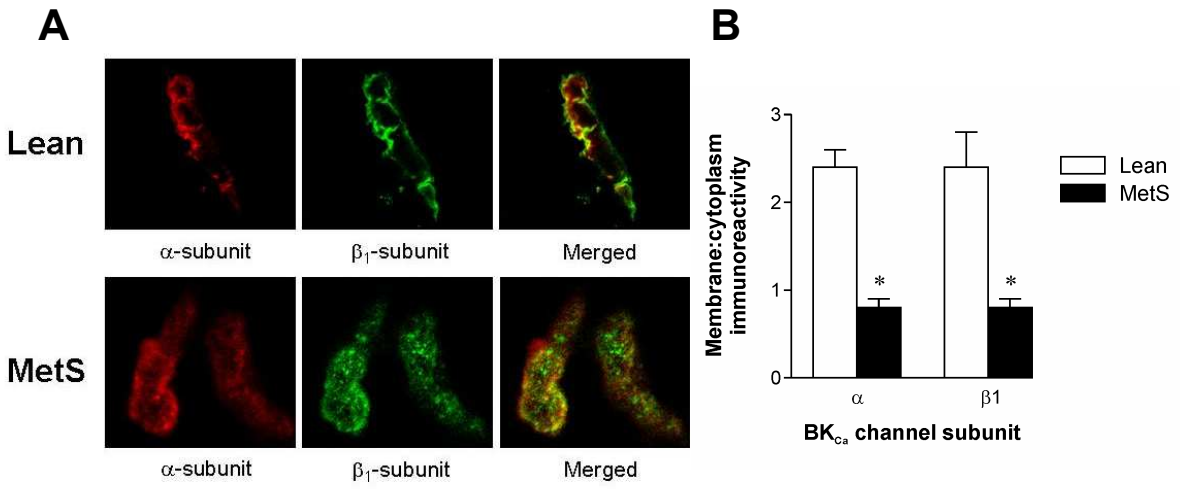

Figure 2.5 A: Representative confocal images of BK_{Ca} channel α and β1 subunit expression in coronary vascular smooth muscle cells from lean and metabolic syndrome swine. **B:** Averaged data demonstrating metabolic syndrome significantly attenuated membrane expression of both BK_{Ca} channel α and β1 subunits. * $P < 0.001$ vs. lean.

Discussion

The present investigation was designed to examine the molecular and functional expression of BK_{Ca} channels and elucidate the mechanisms underlying the deleterious influence of metabolic syndrome. The primary findings of this study are: 1) BK_{Ca} channels contribute to adenosine-and bradykinin-induced coronary vasodilation in lean swine; 2) Metabolic syndrome significantly impairs BK_{Ca} channel-mediated coronary vasodilation *in vivo* and the contribution of these channels to adenosine-mediated microvascular vasodilation *in vitro*; 3) coronary smooth muscle BK_{Ca} channel current is diminished in metabolic syndrome; 4) Impaired coronary BK_{Ca} channel current in metabolic syndrome is associated with augmented L-type Ca^{2+} channel-mediated vasoconstriction and an increase in intracellular Ca^{2+} concentration; 5) Coronary expression of the pore-forming BK_{Ca} channel α-subunit and modulatory β1-subunit are up-regulated in Metabolic syndrome; but 6) membrane trafficking of BK_{Ca} channel subunits is defective in metabolic syndrome. Taken together, these data indicate that coronary vascular dysfunction in metabolic syndrome (88) is related, at least in part, to diminished functional expression of vascular smooth muscle BK_{Ca} channels. Further, this is the first report of impaired membrane trafficking of BK_{Ca} channels associated with coronary vascular dysfunction.

BK_{Ca} channels are abundantly expressed in coronary vascular smooth muscle cells (20; 108) and data from this study demonstrate that these channels play a functional role in mediating endothelium-independent coronary vasodilation to the adenosine analog 2-CADO (Figure 2.1) and the endothelium-dependent vasodilator bradykinin. Our findings are consistent with earlier studies which documented that K_{Ca} channels contribute to endothelial-dependent relaxation (71; 105; 106; 115), exercise-mediated dilation (103) and ischemic dilation in response to brief coronary artery occlusions (115). Therefore, decreases in coronary BK_{Ca} channel function could be

detrimental to the regulation of coronary microvascular function under a variety of physiologic/pathophysiologic stimuli.

Findings from this investigation indicate coronary vascular smooth muscle BK_{Ca} channel function is markedly depressed by metabolic syndrome. Diminished BK_{Ca} channel function is evidenced by the impaired contribution of BK_{Ca} channels to 2-CADO-mediated coronary arteriolar dilation in coronary arterioles from metabolic syndrome swine (Figure 2.1) and the reduction in BK_{Ca} channel current in isolated coronary vascular smooth muscle cells from metabolic syndrome swine (Figure 2.3). Since we did not repeat this experiment in denuded arterioles, it is not possible to state with certainty that this is a smooth muscle effect, especially since studies have demonstrated endothelium-dependent adenosine-induced relaxation (1; 93) in addition to its endothelium-independent effects. This observed impairment is consistent with other studies which found decreased vascular BK_{Ca} channel current in insulin resistant (38) and diabetic fatty rats (26; 101). Decreases in coronary total K^+ current and spontaneous transient outward K^+ currents, which are elicited by Ca^{2+} sparks and indicative of BK_{Ca} channel activation (123; 175), have also been observed in dyslipidemic (168) and diabetic-dyslipidemic Yucatan swine (108). Importantly, our data demonstrate that this decrease in coronary vascular smooth muscle BK_{Ca} channel function is phenotypically evident *in vivo* by the significant decrease in coronary vasodilation to NS1619 in open-chest anesthetized metabolic syndrome swine (Figure 2.2). Although also used in cultured endothelial cell lines, recent data from Feng et al indicates that NS1619 is largely smooth muscle-mediated, as endothelial denudation had no effect on NS1619-induced relaxation of human atrial microvessels (56). Additionally, we found that coronary vasoconstriction to L-type Ca^{2+} channel activation was markedly elevated in metabolic syndrome swine (Figure 2.3B) which is consistent with earlier data from our laboratory documenting increased functional expression of L-type Ca^{2+} channels in dogs

with metabolic syndrome (88). These changes in coronary electromechanical coupling were accompanied by significant increases in intracellular Ca^{2+} concentration in coronary smooth muscle cells from metabolic syndrome swine. The increased functional expression of L-type Ca^{2+} channels in coronary smooth muscle *in vivo* in metabolic syndrome Ossabaw swine is opposite of that in hyperlipidemia (18) and the combination of gross hyperglycemia and hyperlipidemia (diabetic dyslipidemia) (160). Collectively, these data strongly suggest that the entire metabolic syndrome milieu is critical in determining ion channel functional expression and results cannot be over-generalized from one disease state to another.

The mechanisms underlying impaired functional expression of vascular BK_{Ca} channels in metabolic syndrome could be related to modifications in BK_{Ca} channel protein expression and/or membrane trafficking (102; 114) as well as alterations in channel activation kinetics and open probability (169). In experiments to address these possible mechanisms we found a paradoxical increase in the expression of both the pore-forming α-subunit and the modulatory β1- subunit in coronary arteries from metabolic syndrome swine (Figure 2.4). While our data are consistent with other investigations that have reported diminished current and channel activity in the presence of cardiovascular risk factor (26; 38; 101; 108; 114), they are at odds with other studies which documented a downregulation of either one or both BK_{Ca} channel subunits (41; 102). However, it is important to recognize that ours is not the first example of a situation where BK_{Ca} channel function and protein expression are inversely related. For example, Benkusky *et al*. found that functional and molecular expression of BK_{Ca} channels in myometrial smooth muscle cells is regulated by gestation in a similar opposing manner such that BK_{Ca} current decreases while BK_{Ca} channel protein expression increases (12). Additionally, in collaboration with the Toro laboratory our group showed that increased BK_{Ca} channel current in conduit coronary arteries in lean hyperlipidemic and diabetic

dyslipidemic pigs is accompanied by no change in BK_{Ca} channel protein expression (109). We hypothesize that the increase in BK_{Ca} channel expression represents a compensatory increase in response to the impaired membrane trafficking of BK_{Ca} channel subunits in metabolic syndrome (Figure 2.5). Whether this diminished membrane trafficking is mediated by alternative mRNA splicing (165; 166) and/or the formation of channels with accessory subunits that inhibit membrane expression (164) merits further study.

Another possible explanation is improper folding of proteins in the endoplasmic reticulum (ER) and subsequent entrapment therein due to ER stress. The ER is a central organelle and is the site for protein folding and maturation. Plasma membrane proteins, such as BK_{Ca} channels, are folded into their tertiary and quaternary structure in the ER, and conditions that interfere with the function of the ER, i.e. ER stress, can be detrimental to signal transduction and homeostatis functions of a cell. ER stress can be caused by the accumulation of unfolded/malfolded protein aggregates or excessive protein traffic and can result in decreased rate of protein translation (67). This can be crudely tested by incubating isolated smooth muscle cells of metabolic syndrome swine with chemical or pharmacological chaperones (such as 10% glycerol) which can rescue intracellularly-trapped proteins via protein stabilization and examining whether cell surface expression of BK_{Ca} channels is restored. Accumulating evidence suggests that ER stress may play a role in the pathogenesis of diabetes mellitus, contributing to pancreatic β-cell loss and insulin resistance (52) and may also link obesity and insulin resistance in type II diabetes mellitus (112; 121). Recent studies have identified ER stress as a plausible mechanism in the pathogenesis of type I diabetes mellitus (120) and progressive decrease in β-cell mass (120; 157). Further, chronic ER stress has also been found to cause a disruption of Ca^{2+} release (74) and similar findings have been

observed in the sarcoplasmic reticulum (SR), in particular upregulation of genes encoding SR Ca^{2+}-handling proteins (72; 77). Whether ER stress presents in prediabetic metabolic syndrome and whether they contribute to the impaired trafficking of vascular smooth muscle cell BK_{Ca} channels would be an interesting area of further investigation.

In summary, findings from this investigation support the hypothesis that coronary vascular dysfunction in metabolic syndrome is related to decreased membrane trafficking and functional expression of BK_{Ca} channels in vascular smooth muscle cells that is accompanied by significant increases in L-type Ca^{2+} channel-mediated coronary vasoconstriction and increased intracellular Ca^{2+} concentration (88). We propose that these alterations in electromechanical coupling contribute to coronary endothelial dysfunction (15; 29; 60; 124), sensitization of key vasoconstrictor pathways (39; 90; 173) and diminished functional hyperemia (27; 141) typically observed in obese subjects with metabolic syndrome.

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CHAPTER 3

Role of BK_{Ca} Channels in Local Metabolic Coronary Vasodilation in Ossabaw Swine with Metabolic Syndrome

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Abstract

This investigation was designed to examine the hypothesis that impaired functional expression of coronary microvascular BK_{Ca} channels in metabolic syndrome significantly attenuates the balance between myocardial oxygen delivery and metabolism at rest and during exercise-induced increases in myocardial oxygen consumption (MVO_2). Studies were conducted in conscious, chronically instrumented Ossabaw swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (45% kcal from fat, 2% cholesterol, 20% kcal from fructose) that induces many common features of metabolic syndrome. Data were collected under baseline/resting conditions and during graded treadmill exercise before and after blockade of BK_{Ca} channels with penitrem A (10 μg/kg, iv). We found that the exercise-induced increases in blood pressure were significantly elevated in metabolic syndrome swine. No differences in heart rate were noted. Induction of metabolic syndrome produced a parallel downward shift in the relationship between coronary venous PO_2 and MVO_2 ($P < 0.001$) that was accompanied by a marked release of lactate (negative lactate uptake) as MVO_2 was increased with exercise ($P < 0.005$). Inhibition of BK_{Ca} channels with penitrem A did not significantly affect blood pressure, heart rate, or the relationship between coronary venous PO_2 and MVO_2 in lean or metabolic syndrome swine. These data indicate that BK_{Ca} channels are not required for local metabolic control of coronary blood flow under physiological (lean) or pathophysiological (metabolic syndrome) conditions. Therefore, diminished functional expression of BK_{Ca} channels does not contribute to the impairment of myocardial oxygen-supply demand balance in metabolic syndrome.

Keywords: coronary blood flow, myocardial oxygen consumption, exercise, penitrem A

Introduction

Due to the limited anaerobic capacity of the myocardium, the heart depends on a continuous supply of oxygen from the coronary circulation to meet its metabolic requirements (151). Thus, under normal physiological conditions myocardial oxygen delivery is closely matched with the rate of myocardial oxidative metabolism. To ensure adequate balance between coronary blood flow and myocardial metabolism, powerful regulatory mechanisms exist to increase nutritive blood flow to the heart whenever myocardial oxygen consumption (MVO_2) is elevated (43; 151). However, despite decades of research, the exact mechanisms responsible for local metabolic control of coronary blood flow have yet to be clearly defined (151).

Previous studies have demonstrated that disease states such as obesity and metabolic syndrome significantly impair control of coronary blood flow at rest and during increases in MVO_2 (27; 39; 90; 137; 141; 173). Coronary dysfunction in the metabolic syndrome is characterized by an imbalance between coronary blood flow and myocardial metabolism (141) which has been attributed to sensitization of key vasoconstrictor pathways such as angiotensin II and α_1-adrenoceptors (88). Recently, data from our laboratory, as well as others, also established that obesity, insulin resistance and type II diabetes mellitus diminishes end-effector mechanisms that regulate coronary vasodilation (16; 26; 38; 101; 108). In particular, we found that the functional expression of coronary large conductance Ca^{2+} activated K^+ channels (BK_{Ca}) channels is markedly depressed in Ossabaw swine with metabolic syndrome (16; 108). Since BK_{Ca} channels have been shown to contribute to coronary endothelial-dependent and exercise-induced dilation under normal-lean conditions (16; 20; 71; 103; 105; 106; 108), we propose that decreases in BK_{Ca} channel function could be an underlying mechanism of impaired metabolic control of coronary blood flow in metabolic syndrome.

However, no study has examined the contribution of BK_{Ca} channels to metabolic coronary vasodilation in the setting of metabolic syndrome.

Accordingly, the goal of this investigation was to examine the hypothesis that impaired functional expression of coronary microvascular BK_{Ca} channels in metabolic syndrome (16; 108) significantly attenuates the balance between myocardial oxygen delivery and metabolism at rest and during exercise-induced increases in MVO_2. Studies were conducted in conscious, chronically instrumented Ossabaw swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (45% kcal from fat, 2% cholesterol, 20% kcal from fructose) that induces many common features of metabolic syndrome, including: obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia (16; 22; 49; 145). Data were recorded and arterial and coronary venous blood samples collected before and after blockade of BK_{Ca} channels with penitrem A (10 μg/kg, iv) under baseline/resting conditions and during graded treadmill exercise up to ~75% of maximum whole body VO_2 (heart rate > 200/min).

Methods

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, Richmond, IN) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. Metabolic syndrome swine were fed an excess ~8000 kcal/day high fat/fructose, atherogenic diet containing 17% kcal from protein, 20% kcal from complex carbohydrates, 20% 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 (H46T-F20-5L80, Purina, Richmond, IN). Both lean and metabolic syndrome swine were fed their respective diets for 20 weeks.

Surgical Instrumentation and Intravascular Ultrasound

Ossabaw swine were fasted overnight prior to surgery. The animals were initially 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 with supplemental oxygen. Utilizing sterile technique, a 7F vascular introducer sheath (Boston Scientific) was inserted into the right femoral artery and a guiding catheter (Amplatz L, sizes 0.75 – 2.0, Boston Scientific) was advanced to engage the left main coronary ostium. A 3.2F, 30-MHz Intravascular Ultrasound (IVUS) catheter (Boston Scientific) was advanced over a guide wire and positioned in the coronary artery. Automated IVUS pullbacks were performed at 0.5 mm/s to obtain artery diameters. Video images were analyzed off-line (Sonos Intravascular Imaging System, Hewlett Packard) (51).

Following the IVUS procedure, a left lateral thoracotomy was performed in the fifth intercostal space. A catheter (17 Ga. Pressure monitoring catheter, Edwards LifeSciences) was implanted in the descending thoracic aorta to measure aortic blood pressure and to obtain arterial blood samples. 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 was dissected free and a Transonics perivascular flow transducer was placed around the artery. A chest tube was placed to evacuate the pneumothorax and the chest was closed in layers. The catheters and the flow transducer wire were tunneled subcutaneously and exteriorized between the scapulae. Antibiotics (cephalexin) and aspirin (81 mg) were administered twice daily for 7 days. A jacket was placed on the animals to protect the catheters and the flow transducer wire. An elastomeric balloon pump (Access Technologies) was connected to the coronary venous catheter so heparinized saline (5 U/ml) could be continuously infused at 0.5 ml/hr. The aortic catheter was flushed daily and filled with heparinized saline (5,000 U/ml) (140; 141; 173).

Experimental Protocol

Following recovery from surgery, experiments were conducted in lean (n = 6) and metabolic syndrome (n = 5) Ossabaw swine before and after inhibition of BK_{Ca} channels with penitrem A (10 μg/kg, iv) under baseline/resting conditions and during graded treadmill exercise up to ~75% of maximum whole body V_{O_2} (heart rate > 200/min). We previously demonstrated that this intravenous dose of penitrem A essentially abolished coronary vasodilation to the BK_{Ca} channel agonist NS1619 in anesthetized, open-chest lean Ossabaw swine (16). Coronary blood flow, aortic pressure, and heart rate were continuously recorded while the pigs were resting upright on the treadmill and then during two levels of treadmill exercise: 1) ~2 mph – 0% grade and 2) ~4 mph – 5%

grade. Both lean and metabolic syndrome swine exercised at similar levels. 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.

Blood Sampling

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, hematrocrit, and oxygen content with an Instrumentation Laboratories automatic blood gas analyzer (GEM Premier 3000) and CO-oximeter (682) systems. Coronary blood flow was normalized to an estimated weight of the LAD perfusion territory as previously described by Feigl (55). Myocardial oxygen consumption (MV_{O_2}, μl O_2/min/g) was calculated by multiplying coronary blood flow by the coronary arterial-venous difference in oxygen content. Lactate uptake (μmol/min/g) was calculated by multiplying coronary blood flow by the coronary arterial-venous difference in lactate concentration.

Statistical Analyses

Data are presented as mean ± SE. Statistical comparisons were made with *t*-tests and three-way repeated measures analysis of variance (ANOVA) (Factor A: Diet; Factor B: Drug Treatment; Factor C: Exercise Level) as appropriate. In all statistical tests, $P < 0.05$ 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. Linear regression analysis was used to compare slopes of response variables (aortic pressure, heart rate, coronary venous P_{O_2}, lactate

uptake) plotted vs. MV_{O_2}. 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_{O_2}.

Results

Phenotype of Ossabaw Swine

Phenotypic characteristics of lean and metabolic syndrome swine are given in Table 3.1. We found that 20 weeks of an excess calorie atherogenic diet induced classic features of metabolic syndrome in Ossabaw swine. In particular, relative to their lean counterparts Metabolic syndrome swine exhibited a 1.3-fold increase in body weight, 1.4-fold increase in fasting glucose, 3.1-fold increase in fasting insulin, 6.5-fold increase in total cholesterol and a 3-fold increase in triglyceride levels. Resting mean aortic pressure was not different between lean and metabolic syndrome swine (Table 3.2). Metabolic syndrome induced a 4-fold increase in coronary atherosclerosis wall coverage of the LAD as well as a non-flow limiting but significant 3-fold increase in percent stenosis relative to lean (Figure 3.1). Using the homeostatic model assessment method, percent β-cell function and percent increased from 182.6% in lean swine to 217.6% in swine with metabolic syndrome likely as compensation for the diminished reduction in percent insulin sensitivity from 82.6% in lean to 25.5% in metabolic syndrome swine. Meanwhile, insulin resistance increased from 1.2 in lean to 3.9 in metabolic syndrome swine.

Table 3.1 Phenotypic Characteristics of Lean and Metabolic syndrome Ossabaw Swine

	Lean	**Metabolic syndrome**
Body Weight (kg)	58 ± 6	77 ± 5*
Heart wt. / Body wt. * 100	0.41 ± 0.01	0.39 ± 0.05
Fasting glucose (mg/dl)	71 ± 4	96 ± 7*
Fasting insulin (ng/dl)	10 ± 1	31 ± 4*
Total cholesterol (mg/dl)	59 ± 4	383 ± 44*
LDL/HDL ratio	0.8 ± 0.1	3.4 ± 0.4*
Triglycerides (mg/dl)	22 ± 2	67 ± 6*

Values are mean ± SE for lean (n = 6) and metabolic syndrome (n = 5) swine. * $P<0.05$ vs. lean.

Table 3.2 Hemodynamic and blood gas variables at rest and during graded treadmill exercise in lean and metabolic syndrome Ossabaw swine with and without penitrem A (10 μg/kg, iv).

	Exercise		
	Rest	**Level 1**	**Level 2**
Systolic Blood Pressure (mmHg)			
Lean	101 ± 7	109 ± 7	119 ± 8*
Lean + Penitrem A	108 ± 8	107 ± 8	118 ± 9*
Metabolic syndrome	108 ± 9	114 ± 7	141 ± 5*†
Metabolic syndrome + Penitrem	109 ± 8	134 ± 11*	148 ± 9*
Diastolic Blood Pressure (mmHg)			
Lean	64 ± 4	67 ± 5	74 ± 6
Lean + Penitrem A	71 ± 5	68 ± 6	73 ± 7
Metabolic syndrome	70 ± 8	79 ± 10	92 ± 10*†
Metabolic syndrome + Penitrem	73 ± 6	90 ± 8*	97 ± 8*
Mean Aortic Pressure (mmHg)			
Lean	84 ± 5	88 ± 6	97 ± 6
Lean + Penitrem A	90 ± 6	88 ± 7	96 ± 7
Metabolic syndrome	90 ± 8	97 ± 8	117 ± 7*†
Metabolic syndrome + Penitrem	92 ± 7	112 ± 9*	122 ± 8*
Heart Rate (beats/min)			
Lean	105 ± 7	169 ± 14*	207 ± 10*
Lean + Penitrem A	113 ± 8	178 ± 6*	218 ± 9*
Metabolic syndrome	118 ± 9	178 ± 11*	212 ± 18*
Metabolic syndrome + Penitrem	120 ± 8	187 ± 14*	204 ± 17*
Coronary Blood Flow (ml/min/g)			
Lean	0.86 ± 0.09	1.40 ± 0.20*	1.77 ± 0.19*
Lean + Penitrem A	0.92 ± 0.13	1.42 ± 0.19*	1.96 ± 0.26*
Metabolic syndrome	0.97 ± 0.07	1.58 ± 0.15*	1.84 ± 0.10*
Metabolic syndrome + Penitrem	1.06 ± 0.11	1.83 ± 0.27*	2.17 ± 0.35*
Coronary Conductance (μl/min/g/mmHg)			
Lean	10.3 ± 1.1	16.1 ± 2.4*	18.5 ± 1.9*
Lean + Penitrem A	9.9 ± 1.0	16.2 ± 2.1*	20.4 ± 2.2*
Metabolic syndrome	11.1 ± 1.3	16.8 ± 1.9*	15.8 ± 1.5*
Metabolic syndrome + Penitrem	11.6 ± 1.4	16.7 ± 2.2*	17.8 ± 2.7*
Myocardial O_2 Consumption (μl O_2/min/g)			
Lean	103 ± 12	192 ± 39*	240 ± 34*
Lean + Penitrem A	107 ± 23	182 ± 32*	257 ± 42*
Metabolic syndrome	108 ± 8	213 ± 31*	253 ± 16*

Metabolic syndrome + Penitrem	122 ± 10	243 ± 36*	281 ± 45*
Arterial pH			
Lean	7.55 ± 0.02	7.54 ± 0.01	7.55 ± 0.02
Lean + Penitrem A	7.54 ± 0.01	7.52 ± 0.03	7.53 ± 0.02
Metabolic syndrome	7.53 ± 0.01	7.57 ± 0.02	7.54 ± 0.03
Metabolic syndrome + Penitrem	7.55 ± 0.01	7.55 ± 0.04	7.50 ± 0.05
Arterial P_{CO_2} (mmHg)			
Lean	34 ± 1	33 ± 1	33 ± 1
Lean + Penitrem A	34 ± 1	34 ± 1	33 ± 1
Metabolic syndrome	35 ± 1	30 ± 1*	30 ± 2*
Metabolic syndrome + Penitrem	31 ± 1	32 ± 2	30 ± 2
Arterial P_{O_2} (mmHg)			
Lean	89 ± 3	83 ± 3	84 ± 4
Lean + Penitrem A	89 ± 4	84 ± 3	77 ± 3
Metabolic syndrome	83 ± 1	94 ± 2*†	85 ± 4
Metabolic syndrome + Penitrem	90 ± 3	80 ± 3	82 ± 4
Arterial Hematocrit (%)			
Lean	32 ± 2	37 ± 3	37 ± 2
Lean + Penitrem A	33 ± 3	35 ± 3	37 ± 3
Metabolic syndrome	31 ± 3	35 ± 2	34 ± 1
Metabolic syndrome + Penitrem	30 ± 2	33 ± 3	34 ± 1

Values are mean ± SE for lean (n = 6) and metabolic syndrome (n = 5) swine. * $P < 0.05$ vs. respective baseline (rest); † $P < 0.05$ vs. lean same level.

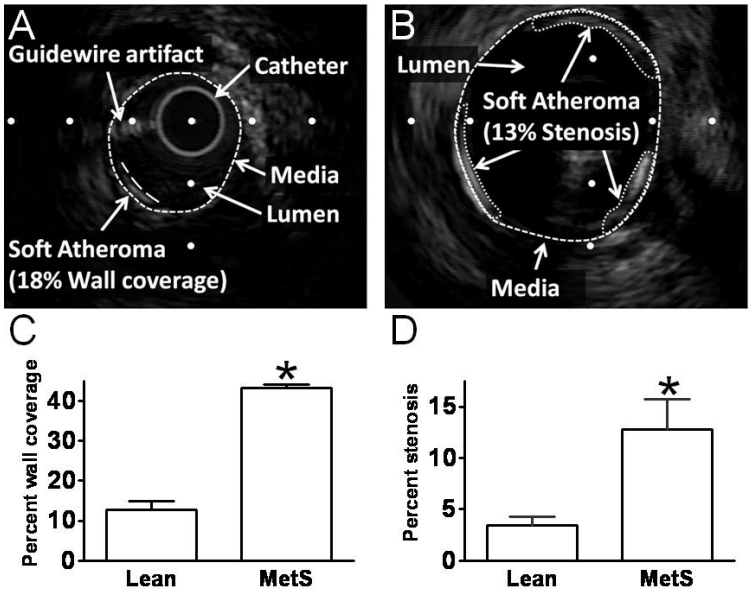

Figure 3.1 Metabolic syndrome atherosclerosis is not flow-limiting. **A:** Representative IVUS image of Lean LAD demonstrating method of determining percent wall coverage. **B:** Representative IVUS image of metabolic syndrome LAD demonstrating method of determining percent stenosis. The 13% stenosis corresponds to 56% wall coverage in this example. **C:** Atherosclerosis is increased in metabolic syndrome LAD compared to Lean. **D:** Percent stenosis at the site of maximum atherosclerosis in the LAD demonstrates atherosclerosis in metabolic syndrome is not flow-limiting. * $P < 0.05$ vs lean.

Effects of Metabolic syndrome on Coronary and Cardiovascular Responses to Exercise

Hemodynamic and blood gas data at rest and during exercise for the lean and metabolic syndrome Ossabaw swine before and after inhibition of BK_{Ca} channels are summarized in Table 3.2. We found that the exercise-induced increases in blood pressure were significantly elevated in metabolic syndrome swine. This effect is also evidenced in the relationship between mean aortic pressure and MVO_2 shown in Figure 3.2A (P = 0.06). No differences in heart rate were noted between groups at rest or during exercise (Figure 3.2B).

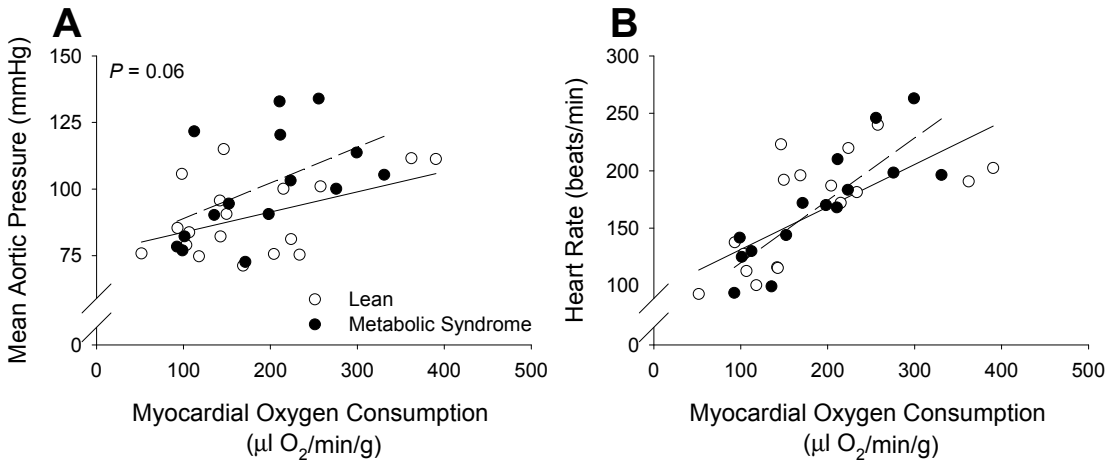

Figure 3.2 No effect of metabolic syndrome on mean aortic pressure (A) and heart rate (B) at rest and during exercise-induced increases in myocardial oxygen consumption. Exercise-induced increases in blood pressure were elevated in metabolic syndrome swine as evidenced in the relationship between mean aortic pressure and MVO_2. No differences in heart rate were noted between lean or metabolic syndrome swine at rest or during exercise.

Coronary blood flow and MVO_2 were elevated ~2-fold in both lean and metabolic syndrome swine at the highest level of exercise. The relationship between coronary venous PO_2 and MVO_2 (Figure 3.3A), a sensitive index of myocardial tissue oxygenation that reflects whether changes in myocardial oxygen delivery adequately match increases

in myocardial metabolism (151), revealed a parallel downward shift in coronary venous P_{O_2} at a given level of MV_{O_2} in metabolic syndrome swine ($P < 0.001$). The impairment of myocardial oxygen supply-demand balance in metabolic syndrome swine was accompanied by significant decreases in myocardial lactate uptake with exercise-induced increases in MV_{O_2} ($P < 0.005$; Figure 3.3B). The net release of lactate across the coronary circulation of metabolic syndrome animals, which indicates the onset of anaerobic glycolysis and cardiac ischemia, is in stark contrast to the significant increases in myocardial lactate uptake (i.e. metabolic consumption) observed during exercise in lean animals.

Role of BK_{Ca} channels in Local Metabolic Coronary Vasodilation

Inhibition of BK_{Ca} channels with penitrem A (10 μg/kg, iv) did not significantly affect blood pressure, heart rate, coronary blood flow or MV_{O_2} in lean or metabolic syndrome swine

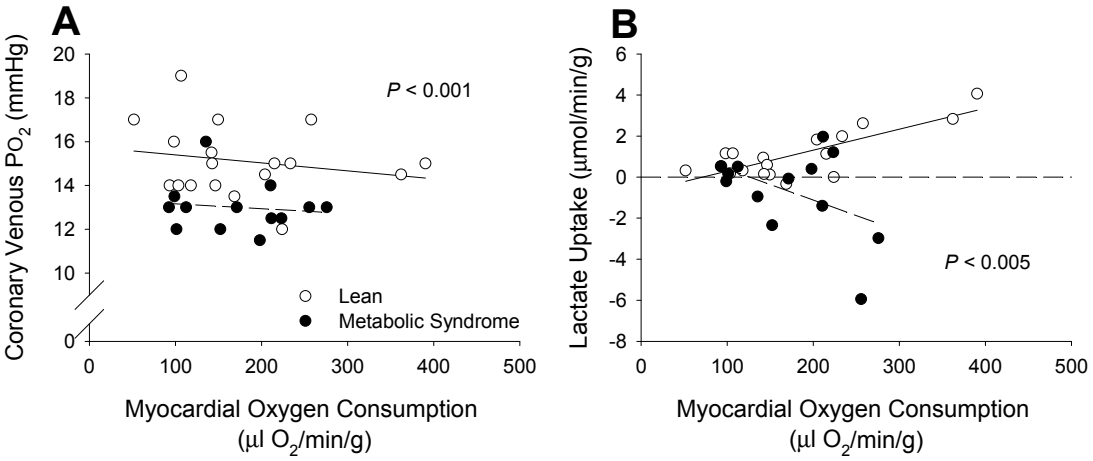

Figure 3.3 Effect of metabolic syndrome on the relationship between myocardial oxygen consumption and coronary venous P_{O_2} (A) and myocardial lactate uptake (B). The relationship between coronary venous P_{O_2} and myocardial oxygen consumption revealed a parallel downward shift in coronary venous P_{O_2} at a given level of myocardial oxygen consumption in metabolic syndrome swine. This impairment of myocardial oxygen supply-demand balance was accompanied by a marked release of lactate (negative lactate uptake) in metabolic syndrome swine as myocardial oxygen consumption was elevated.

at rest or during exercise (Table 3.2). Additionally, Figure 4 demonstrates that the relationship between coronary venous P_{O_2} and MV_{O_2} was not significantly affected by inhibition of BK_{Ca} channels in either lean (Figure 3.4A) or metabolic syndrome (Figure 3.4B) swine. In fact, administration of penitrem A tended to increase coronary venous P_{O_2} at a given level of MV_{O_2}, however this effect failed to reach statistical significance (P = 0.10). It is important to point out that we previously demonstrated that this intravenous dose of penitrem A is effective in inhibiting coronary vasodilation to the BK_{Ca} channel agonist NS1619 in anesthetized, lean Ossabaw swine (16). Therefore, these data indicate that BK_{Ca} channels are not required for local metabolic coronary vasodilation during exercise.

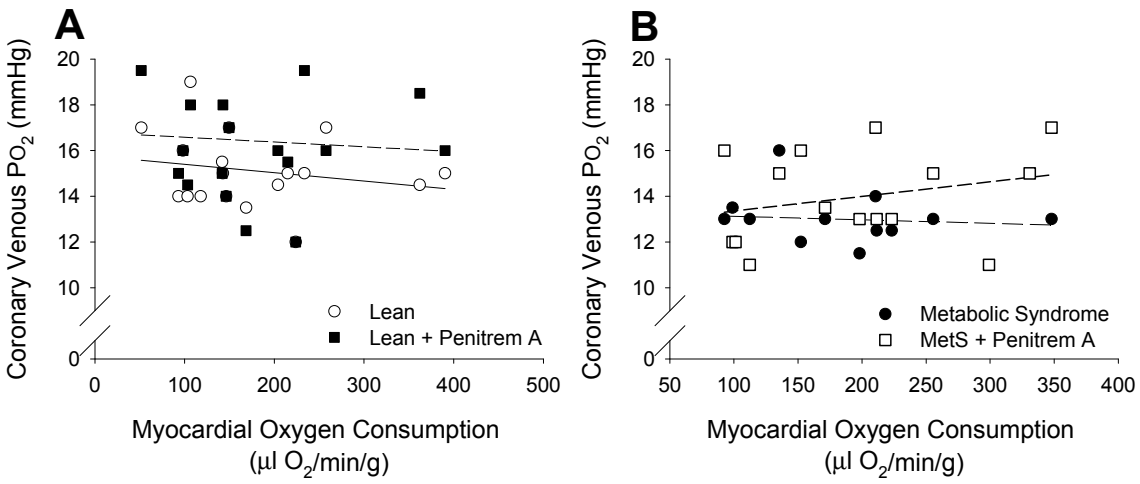

Figure 3.4 Effect of BK_{Ca} channel inhibition on the relationship between coronary venous P_{O_2} and myocardial oxygen consumption in lean (A) and metabolic syndrome (B) swine. Inhibition of BK_{Ca} channels with penitrem A did not significantly affect the relationship between coronary venous P_{O_2} and myocardial oxygen consumption in either lean or metabolic syndrome swine.

Discussion

Data from this investigation demonstrate that metabolic syndrome significantly impairs the ability of the coronary circulation to adequately balance myocardial oxygen delivery with myocardial metabolism at rest and during exercise-induced increases in MVO_2. This supply-demand imbalance forces the myocardium to utilize its limited oxygen extraction reserve in effort to meet the tissue requirements for oxygen (Figure 3.3A). The marked release of lactate (negative lactate uptake) observed in metabolic syndrome swine as MVO_2 was elevated indicates that this increase in oxygen extraction was inadequate to the extent that myocardial underperfusion and ischemia was evidenced by the onset of anaerobic glycolytic metabolism (Figure 3.3B). It is important to recognize that the initiation of cardiac ischemia was not related to the presence of a significant flow limiting stenosis as metabolic syndrome Ossabaw swine had only ~13% luminal narrowing of the coronary circulation (Figure 3.1). These data suggest that coronary microvascular dysfunction precipitates the onset of *"demand ischemia"* as MVO_2 is elevated in metabolic syndrome hearts that could contribute, at least in part, to the increased incidence of myocardial ischemia, infarction and sudden cardiac death observed in obese patients with metabolic syndrome (66; 96). Thus, understanding the mechanisms responsible the impairment of coronary flow regulation in metabolic syndrome is critical to the treatment and possible prevention of these complications.

The present study was designed to examine the hypothesis that impaired functional expression of coronary microvascular BK_{Ca} channels (16; 108) significantly contributes to the imbalance between myocardial oxygen supply and demand in metabolic syndrome. This hypothesis was based on recent data from our laboratory which demonstrated impaired membrane trafficking of BK_{Ca} channel α and $\beta 1$ subunits in coronary vascular smooth muscle cells from metabolic syndrome Ossabaw swine that was accompanied by diminished BK_{Ca} channel-mediated current generation and

microvascular vasodilation both *in vitro* and *in vivo* (16). Given the abundant expression of BK_{Ca} channels in coronary vascular smooth muscle cells (20; 24; 61; 63; 108; 109) and the importance of K^+ channels to the regulation of coronary vascular resistance (45; 47; 156), we predicted that decreases in BK_{Ca} channel function would likely contribute to diminished local metabolic coronary vasodilation in metabolic syndrome. This assertion is supported by a recent study from Merkus *et al.* who found that administration of the BK_{Ca} channel inhibitor tetraethylammonium (TEA) resulted in a significant decrease in the relationship between coronary venous PO_2 and MVO_2 in normal-lean swine both at rest and during exercise (103). In addition, BK_{Ca} channels have also been shown to contribute to coronary endothelial-dependent dilation (16; 71; 105; 106) and to the regulation of coronary microvascular tone in ischemic dog hearts (115).

In contrast to our hypothesis we found that the impaired balance between myocardial oxygen delivery and MVO_2 in metabolic syndrome was not related to the diminished contribution of BK_{Ca} channels to local metabolic control of coronary blood flow. In fact, our data fail to support a functional role for BK_{Ca} channels in the regulation of coronary microvascular tone at rest or during exercise as the inhibition of BK_{Ca} channels with penitrem A did not significantly decrease the relationship between coronary venous PO_2 and MVO_2 in lean (Figure 3.4A) or metabolic syndrome (Figure 3.4B) swine. Although this finding is directly at odds with the early study of Merkus *et al.* (103), it is important to recognize that TEA is also an autonomic ganglionic blocker that inhibits sympathetic output (40; 122). Therefore, TEA is a non-selective inhibitor that would not only affect the balance between coronary blood flow and MVO_2 by inhibiting BK_{Ca} channels but also by decreasing β-adrenoceptor mediated coronary vasodilation, a prominent, well accepted mechanism of exercise-induced coronary vasodilation (44; 54; 64; 65; 107). In addition, even though BK_{Ca} channels are known to contribute to adenosine-induced and endothelial (NO)-mediated dilation (16; 71; 105; 106), the lack of

an effect of BK_{Ca} channel inhibition on myocardial oxygen supply-demand balance is not surprising given that numerous earlier studies have failed to establish a role for adenosine or NO in exercise-induced coronary vasodilation (6; 14; 46; 78; 142; 152; 153; 155; 158). Whether increases in the activity of other K^+ channels (i.e. K_{ATP} and/or K_v) compensate to regulate coronary flow when BK_{Ca} channels are inhibited is unknown and merits further study.

In summary, our data indicate that factors released from the vascular endothelium and/or myocardium do not regulate local metabolic control of coronary blood flow through a BK_{Ca} channel-dependent mechanism under physiological (lean) or pathophysiological (metabolic syndrome) conditions. Thus, diminished functional expression of BK_{Ca} channels (16; 26; 38; 101; 108) does not significantly contribute to the impairment of myocardial oxygen-supply demand balance at rest or during increases in MVO_2 in metabolic syndrome. However, our findings do not contradict a role for decreases in BK_{Ca} channel function contributing to coronary endothelial dysfunction that is typically observed in obesity, insulin resistance and metabolic syndrome (22; 88).

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CHAPTER 4

Contribution of K^+ Channels to Ischemic Coronary Vasodilation in Metabolic Syndrome

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Abstract

The mechanisms by which the metabolic syndrome impairs control of coronary blood flow have not been clearly defined. This study tested the hypothesis that metabolic syndrome reduces coronary vasodilation to cardiac ischemia via decreases in the relative contribution of specific K^+ channels to the reactive hyperemic response. Studies were conducted in anesthetized miniature Ossabaw swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (45% kcal from fat, 2% cholesterol, 20% kcal from fructose) for 9 (MetS-9) or 20 (MetS-20) weeks. Ischemic vasodilation was determined by the coronary flow response to a 15 sec transient coronary occlusion before and after cumulative administration of the BK_{Ca} channel antagonist penitrem A (10 μg/kg, iv), the K_V channel inhibitor 4-aminopyridine (0.3 mM), and the K_{ATP} channel antagonist glibenclamide (1 mg/kg). We found that the coronary reactive hyperemic response was progressively diminished in MetS-9 and MetS-20 swine. Inhibition of BK_{Ca} channels had no effect on the reactive hyperemic response in either lean or MetS-20 swine. Subsequent inhibition of K_V channels significantly reduced the peak hyperemic response and attenuated the overall volume of repayment in lean swine. Additional blockade of K_{ATP} channels tended to further diminish the peak coronary reactive hyperemic response. Inhibition of BK_{Ca}, K_V and/or K_{ATP} channels had no effect on the coronary reactive hyperemic response in MetS-20 swine. These data indicate that metabolic syndrome reduces coronary vasodilation to cardiac ischemia via decreases in the relative contribution of K_V channels to the reactive hyperemic response.

Keywords: coronary blood flow, reactive hyperemia, ischemic vasodilation, K^+ channels, Ossabaw miniature swine

Introduction

Coronary vasodilation in response to myocardial ischemia is an important mechanism that acts to maintain adequate oxygen delivery to the myocardium in order to mitigate ischemic injury and infarction (117; 133). Although the exact mechanisms of ischemic dilation are not completely understood, earlier studies support that the putative vasodilator metabolites adenosine and nitric oxide (NO) significantly contribute to this response via activation of vascular smooth muscle K^+ channels (5; 9; 36; 47; 117). K^+ channels have been implicated in mediating ischemic dilation via their purported role as end-effectors in vascular smooth muscle. Specifically, it is well known that K^+ channels mediate vasodilation in response to key endothelial and metabolic metabolites, e.g. adenosine (36; 47; 78; 93) and NO (16; 36; 105; 106), as these channels determine vascular smooth muscle membrane potential and thereby regulate electromechanical control of vascular tone (81; 113). This hypothesis is directly supported by previous investigations which documented a role for large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels (115), voltage-gated K^+ (K_V) channels (36), as well as ATP-dependent K^+ (K_{ATP}) channels (10; 28; 173) in coronary vasodilation in response to a brief episode of myocardial ischemia, i.e. reactive hyperemia. However, the relative contribution of these specific K^+ channels to ischemic coronary vasodilation has not been delineated.

Earlier studies have demonstrated that disease states such as obesity and the metabolic syndrome significantly impair control of coronary blood flow (27; 39; 90; 137; 141; 173). In particular, recent evidence indicates that peripheral (forearm, calf) vasodilation in response to ischemia is significantly impaired in obese and type II diabetic human patients (2; 34; 79). Although coronary flow reserve has been shown to be reduced by obesity and metabolic syndrome (27; 33; 87; 92; 137), no study has examined the effects of metabolic syndrome on ischemic coronary vasodilation. Based on recent data indicating that metabolic syndrome impairs the functional expression of

vascular smooth muscle K^+ channels (16; 26; 38; 73; 108) we propose that metabolic syndrome attenuates coronary reactive hyperemia via alterations in the contribution of specific K^+ channels to ischemic vasodilation. Examining the mechanisms by which metabolic syndrome affects the coronary response to myocardial ischemia stands to improve our understanding of the increased incidence of cardiac arrhythmias, infarction and sudden cardiac death observed in obese patients with metabolic syndrome (59; 66; 70; 75; 76; 96; 129).

The goal of this investigation was to elucidate the relative contribution of specific K^+ channels to coronary reactive hyperemia. In particular, we tested the hypothesis that metabolic syndrome impairs coronary vasodilation to cardiac ischemia via decreases in the relative contribution of BK_{Ca}, K_V, and/or K_{ATP} channels to the reactive hyperemic response. To examine this hypothesis, experiments were conducted in acute anesthetized miniature Ossabaw swine fed a normal maintenance diet (11% kcal from fat) or an excess calorie atherogenic diet (45% kcal from fat, 2% cholesterol, 20% kcal from fructose) that induces many common features of metabolic syndrome, including: obesity, insulin resistance, impaired glucose tolerance, and dyslipidemia (16; 22; 49; 145). The contribution of K^+ channels to ischemic vasodilation was determined by assessing the coronary microvascular response to a 15 sec transient occlusion before and after the cumulative administration of selective K^+ channel antagonist: 1) BK_{Ca} channel inhibitor penitrem A (10 μg/kg, iv); 2) K_V channel inhibitor 4-aminopyridine (0.3 mM); plus the 3) K_{ATP} channel inhibitor glibenclamide (1 mg/kg).

Methods

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 Ossabaw swine were fed ~2200 kcal/day of standard chow (5L80, Purina TestDiet) containing 18% kcal from protein, 71% kcal from complex carbohydrates, and 11% kcal from fat. Metabolic syndrome Ossabaw swine were fed an excess ~8000 kcal/day high fat/fructose, atherogenic diet containing 17% kcal from protein, 20% kcal from complex carbohydrates, 20% 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 (5B4L, Purina TestDiet). Swine were fed their respective diets for 9 or 20 weeks.

Surgical Preparation and *In Vivo* Coronary Blood Flow Studies

Lean, metabolic syndrome swine following 9-weeks of diet (MetS-9) and metabolic syndrome swine following 20-weeks of diet (MetS-20) were sedated with telazol (5 mg/kg, sc) and xylazine (2.2 mg/kg, sc). Animals were intubated and ventilated with O_2-supplemented air. Anesthesia was maintained with morphine sulfate (3 mg/kg, im) and α-chloralose (100 mg/kg, iv). Catheters were placed in the right femoral artery and vein as well as the left femoral artery. Blood pressure was measured from the right femoral artery catheter. Further, arterial blood was drawn from this catheter to analyze blood gases parameters every 15 – 30 min. Ventilatory adjustments and/or sodium bicarbonate administration was performed as required to arterial blood gases within normal physiological limits. A left thoracotomy was performed at the fifth intercostal space. The left lung was restrained in gauze and the pericardium was opened. A

proximal portion of the left anterior descending coronary artery (LAD) was isolated and a perivascular Transonics flow transducer (2.5 mm) was placed around the artery for measurement of coronary blood flow as well as a snare occluder for transient LAD occlusion.

Hemodynamic variables were allowed to stabilize 15 – 30 min before initiation of the reactive hyperemia protocol. Following baseline measurements, the LAD was clamped using the snare occluder for 15 sec and then released to measure the reactive hyperemia. The reactive hyperemia protocol was performed under control conditions (n = 7) and in MetS-9 (n = 7) and MetS-20 (n = 7) swine. Additional studies were also performed to examine the relative contribution of K^+ channels to ischemic coronary vasodilation in lean (n = 5) and MetS-20 (n = 5) swine by the cumulative administration of 1) BK_{Ca} channel antagonist penitrem A (10 μg/kg, iv); 2) K_V channel antagonist 4-aminopyridine (4-AP, 0.3 mM, iv); 3) K_{ATP} channel blocker glibenclamide (1 mg/kg, iv). Since sulfonylureas stimulate insulin secretion, glibenclamide was prepared in a glucose solution. LAD perfusion territory was estimated as previously described by Feigl *et al.* (55).

Statistical Analyses

Data are presented as mean ± SE from *n* pigs. Hyperemic volume was calculated as area under the curve with Prism software (GraphPad, San Diego, CA). Statistical comparisons were made with *t*-tests and two-way repeated measures analysis of variance (ANOVA) (Factor A: Diet; Factor B: Drug Treatment) as appropriate. In all statistical tests, $P < 0.05$ 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.

Results

Phenotype of Ossabaw Swine

Phenotypic characteristics of lean and metabolic syndrome swine are given in Table 4.1. No differences in key phenotypic parameters were noted between lean swine at 9 vs. 20 weeks of diet. Data presented in Table 4.1 are from lean swine following 9 weeks of diet (body weight of 20 week lean swine averaged 55 ± 7 kg). As previously documented by our laboratory (16; 17; 22; 49), we found that 9 and 20 weeks of an excess calorie atherogenic diet induced increases in body weight, fasting glucose, total cholesterol as well as triglyceride levels; i.e. classic clinical features of metabolic syndrome. Intravascular ultrasound revealed a progressive increase in the amount of coronary atherosclerosis with the duration of metabolic syndrome. The elevated fasting glucose relative to prior studies from our group is likely due to the use of isoflurane anesthesia.

Table 4.1. Phenotypic Characteristics of Lean and Metabolic syndrome Ossabaw Swine

	Lean	**MetS-9**	**MetS-20**
Body Weight (kg)	38 ± 3	54 ± 3*	83 ± 7*†
Fasting glucose (mg/dl)	60 ± 8	126 ± 7*	129 ± 7*
Total cholesterol (mg/dl)	61 ± 6	316 ± 42*	503 ± 41*†
Triglycerides (mg/dl)	21 ± 2	24 ± 3	48 ± 5*†

Values are mean ± SE for lean-9 weeks of diet (n = 7), metabolic syndrome-9 weeks of diet (MetS-9; n = 7) and metabolic syndrome-20 weeks of diet (MetS-20; n = 7) swine. * $P<0.05$ vs. lean. † $P < 0.05$ vs. MetS-9.

Effects of Metabolic Syndrome on Coronary Reactive Hyperemia

The effect of metabolic syndrome on coronary vasodilation in response to a 15 sec occlusion was determined in lean (n = 7; 9 weeks diet), MetS-9 (n = 7) and MetS-20 (n = 7) swine. For all *in vivo* experiments, blood gas parameters were maintained within

normal physiological limits throughout the experimental protocol in both lean and metabolic syndrome swine (arterial values: pH = 7.43 ± 0.02; P_{CO_2} = 41 ± 1 mmHg; P_{O_2} = 150 ± 9 mmHg). As depicted in Figure 4.1, we found that the average coronary reactive hyperemic response was progressively diminished in MetS-9 and MetS-20 swine. More specifically, the peak reactive hyperemic response trended downward with the duration of metabolic syndrome (Figure 4.1D) and the duration of the response (defined as time until return to baseline flow for 5 sec) was significantly reduced in metabolic syndrome-20 vs. lean swine (Figure 4.1E). The repayment to debt ratio was also significantly reduced in both MetS-9 and MetS-20 swine (Figure 4.1F).

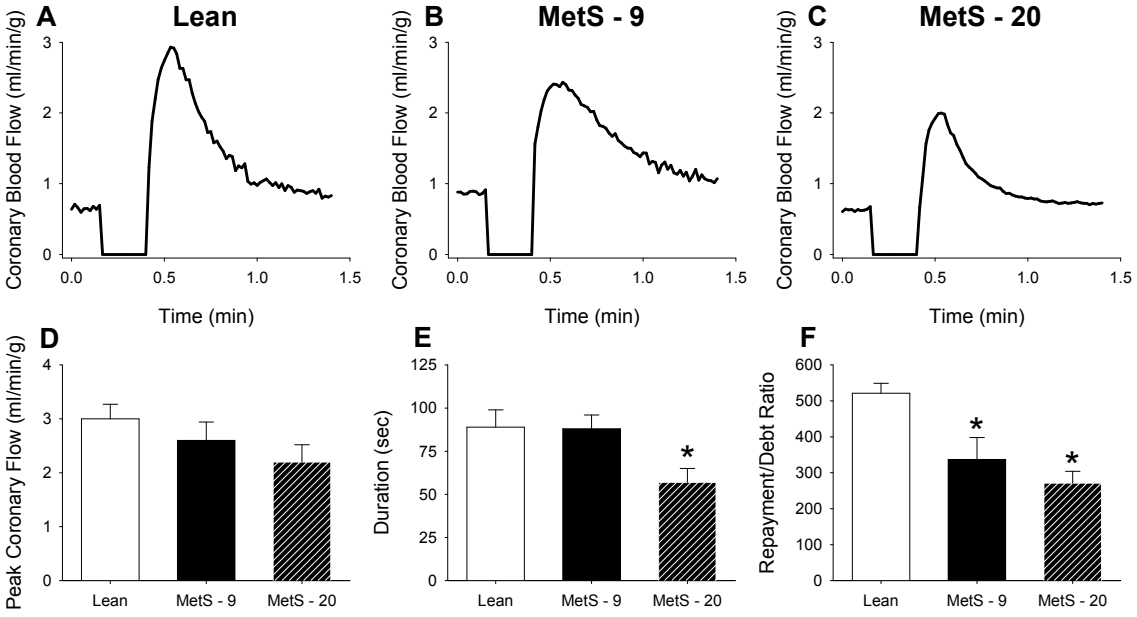

Figure 4.1 Coronary reactive hyperemia and progression of metabolic syndrome. Group average traces of the coronary reactive hyperemia response in lean (A, n = 7), 9 week-fed metabolic syndrome (B, n = 7) and 20 week-fed Metabolic syndrome (C, n = 7) swine. The peak hyperemic response (D) tended to decrease with the degree of metabolic syndrome. The duration (E) of reactive hyperemic response was significantly attenuated in MetS-20 swine. The repayment to debt ratio (F) was reduced in both MetS-9 and MetS-20 swine. *P < 0.05 vs. lean-control swine.

Table 4.2 Effect of K^+ Channel Blockade on Baseline Systemic Hemodynamics & Coronary Blood Flow

Groups	Mean Arterial Pressure (mmHg)	Heart Rate (bpm)	Coronary Blood Flow (ml/min/g)	Coronary Conductance (μl/min/g/mmHg)
Lean	99 ± 8	66 ± 10	0.52 ± 0.08	5.3 ± 0.5
Lean + PenA	101 ± 6	64 ± 12	0.55 ± 0.12	5.1 ± 0.8
Lean + PenA + 4AP	131 ± 11*	49 ± 8*	0.56 ± 0.10	3.9 ± 0.7*
Lean + PenA + 4AP + Glib	144 ± 7*	49 ± 8*	0.47 ± 0.10	3.1 ± 0.6*
MetS-20	90 ± 5	68 ± 6	0.60 ± 0.04	6.7 ± 0.4
MetS-20 + PenA	90 ± 3	65 ± 9	0.70 ± 0.04	7.6 ± 0.5
MetS-20 + PenA + 4AP	106 ± 6*	55 ± 2	0.78 ± 0.07	7.2 ± 0.8
MetS-20 + PenA + 4AP + Glib	122 ± 6*	58 ± 2	0.69 ± 0.07	5.7 ± 0.5

Values are mean ± SE for lean 20-weeks of diet (n = 5) and 20-week Metabolic syndrome (MetS-20; n = 5) swine. Pen A = penitrem A; 4-AP = 4-aminopyridine; Glib = glibenclamide. * $P < 0.05$ vs. respective untreated control.

Effect of K^+ Channel Blockade on Baseline Hemodynamics and Coronary Blood Flow

To investigate the relative contribution of BK_{Ca}, K_V, and K_{ATP} channels to ischemic coronary vasodilation, experiments were conducted in MetS-20 and aged matched lean (20 weeks of diet) swine before and after cumulative administration of specific K^+ channel blockers. Table 4.2 illustrates the effect of intravenous infusion of these K^+ channel blockers on baseline systemic hemodynamic and coronary blood flow. Inhibition of BK_{Ca} channels with penitrem A (10 μg/kg) did not significantly affect mean aortic pressure, heart rate or coronary blood flow in lean or MetS-20 swine. Subsequent administration of the K_V channel antagonist 4-AP (0.3 mM) markedly increased blood pressure > 30 mmHg in lean swine, and 16 mmHg in MetS-20 swine, which resulted in a reflex-mediated decrease in heart rate. Successive addition of the K_{ATP} channel antagonist glibenclamide (1 mg/kg) caused a further elevation of blood pressure in both lean and MetS-20 swine. Baseline coronary blood flow was not significantly altered by K^+ channel inhibition in either group. This lack of an effect on coronary flow was directly

related to the marked elevation in arterial pressure as normalization of coronary blood flow to aortic pressure revealed a significant reduction in coronary conductance following administration of 4-AP and glibenclamide. However, this effect of K^+ channel blockade was only observed in lean swine.

Contribution of K^+ Channels to Coronary Reactive Hyperemia

The effect of K^+ channel inhibition on the coronary reactive hyperemic response is shown in Figure 4.2 for lean swine and in Figure 4.3 for MetS-20 swine. Given the marked increases in mean aortic pressure following administration of 4-AP and glibenclamide (Table 4.2), coronary conductance data were used to assess the relative contribution of K^+ channels to ischemic coronary vasodilation. We found that blockade of BK_{Ca} channels with penitrem A had no effect on the reactive hyperemic response in either lean (Figures 4.2A, 4.2D, and 4.2E) or MetS-20 (Figures 4.3A, 4.3D, and 4.3E) swine. Consistent with our recent findings (36), subsequent inhibition of K_v channels with 4-AP reduced the peak hyperemic response (Figure 4.2D; $P = 0.07$) and significantly decreased the overall volume of repayment (Figure 4.2E) in lean swine. Additional blockade of K_{ATP} channels tended to further diminish (~15%) the peak coronary reactive hyperemic response ($P = 0.08$). Combined inhibition of BK_{Ca}, K_V and K_{ATP} channels significantly diminished the degree of coronary reactive hyperemia, i.e. the volume of repayment was reduced ~50% in lean swine (Figure 4.2E). In contrast, inhibition K^+ channels alone or in combination had no effect on the coronary reactive hyperemic response in MetS-20 swine (Figure 4.3A-E).

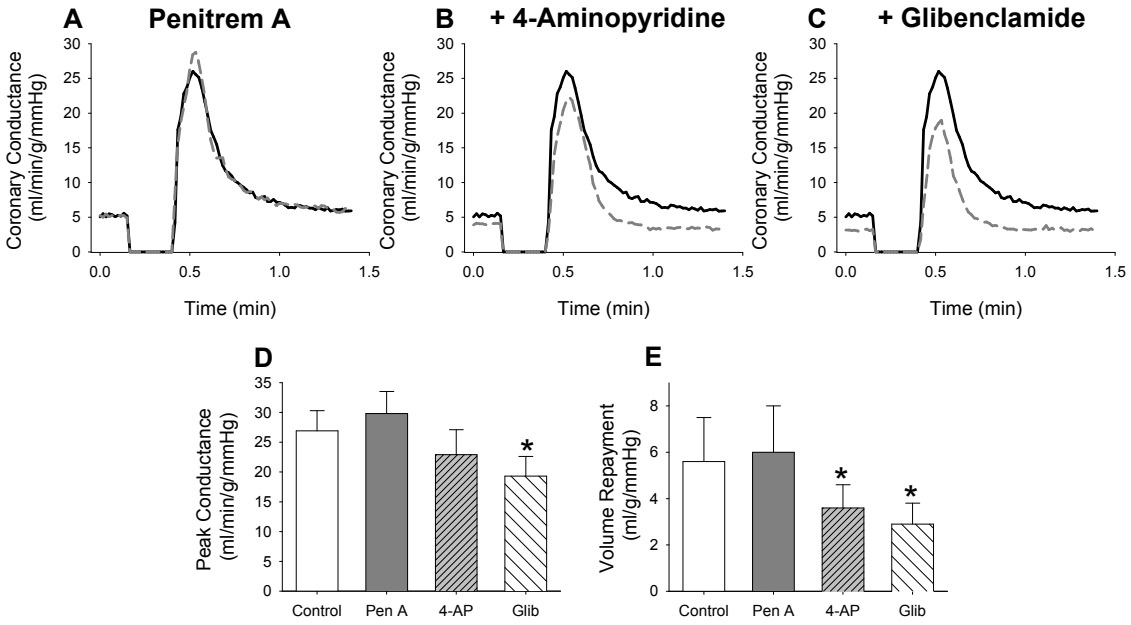

Figure 4.2 Effect of K^+ channel inhibition on coronary reactive hyperemia in lean swine (n = 5). The average control reactive hyperemia response is plotted (black line) relative to the cumulative administration of the BK_{Ca} channel antagonist penitrem A (A; Pen A), the K_V channel antagonist 4-aminopyridine (B; 4-AP), and the K_{ATP} channel antagonist glibenclamide (C; Glib) (K^+ channel blockade shown by dashed grey line). Peak hyperemic conductance (D) and the volume of repayment (E) were diminished following the administration of 4-AP and Glib. *P < 0.05 vs. control; † P = 0.07 vs. control.

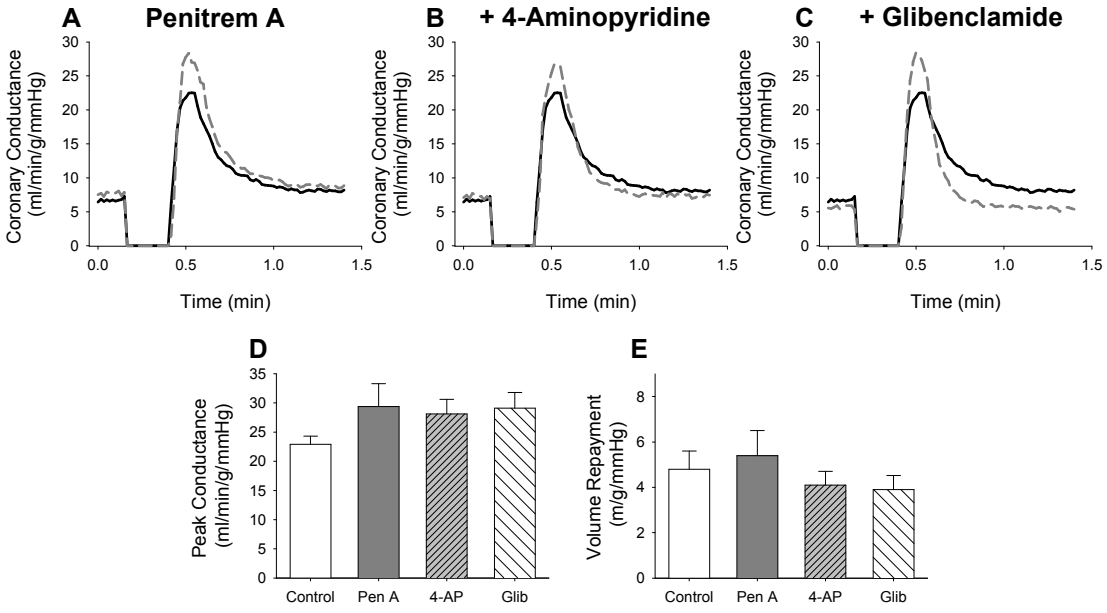

Figure 4.3 Effect of K^+ channel inhibition on coronary reactive hyperemia in metabolic syndrome swine (n = 5). The average control reactive hyperemia response is plotted (black line) relative to the cumulative administration of the BK_{Ca} channel antagonist penitrem A (A; Pen A), the K_V channel antagonist 4-aminopyridine (B; 4-AP), and the K_{ATP} channel antagonist glibenclamide (C; Glib) (K^+ channel blockade shown by dashed grey line). K^+ channel inhibition had no effect on the peak hyperemic conductance (D) or the volume of repayment (E) relative to baseline control.

Discussion

The present investigation was designed to elucidate the relative contribution of K^+ channels to coronary reactive hyperemia in lean and metabolic syndrome Ossabaw swine. In particular, we tested the hypothesis that metabolic syndrome impairs coronary vasodilation to cardiac ischemia via decreases in the relative contribution of BK_{Ca}, K_V, and/or K_{ATP} channels to the reactive hyperemic response. The primary findings of this study are: 1) Coronary reactive hyperemia is progressively diminished in relation to the duration and degree of metabolic syndrome; 2) BK_{Ca} channels do not contribute to the reactive hyperemic response in lean or metabolic syndrome swine; 3) K_V channels significantly contribute to ischemic coronary vasodilation in lean but not metabolic syndrome swine; and 4) K_{ATP} channels play a modest role in the coronary reactive hyperemic response in the presence of BK_{Ca} and K_V channel inhibition in lean swine. Taken together, these data indicate that the metabolic syndrome significantly impairs coronary vasodilation to cardiac ischemia via reductions in the relative contribution of K_V channels to the dilator response.

Our data demonstrate that the ability of the coronary circulation to vasodilate in response to a brief episode of myocardial ischemia is markedly depressed by induction of metabolic syndrome (Figure 4.1). It is important to recognize that this impairment was not related to the presence of a significant flow limiting stenosis as we have previously demonstrated that similarly-fed metabolic syndrome Ossabaw swine had only ~13% luminal narrowing of the coronary circulation (17). Our finding is consistent with earlier studies that found decreases in coronary flow reserve (27; 33; 87; 92; 137) and impaired ischemic peripheral (forearm, calf) vasodilation (2; 34; 79) in obese human patients. In addition, these data nicely extend our earlier observation of impaired metabolic control of coronary blood flow at rest and during exercise-induced increases in MVO_2 (17; 141; 173). Taken together, these findings importantly establish that coronary microvascular

dysfunction in metabolic syndrome impairs the ability of the coronary circulation to respond to both physiologic (exercise) and pathophysiologic (ischemia) stimuli.

We hypothesized that metabolic syndrome impairs coronary reactive hyperemia via the diminished contribution of specific K^+ channels to the ischemic vasodilatory response. This hypothesis is based on earlier studies which documented a role for BK_{Ca} (115), K_V (36), and K_{ATP} (10; 28; 172) channels in ischemic vasodilation in normal-lean subjects as well as recent data indicating that metabolic syndrome impairs the functional expression of these K^+ channels in vascular smooth muscle cells (16; 26; 38; 73; 108). However, to date no study has examined the relative contribution of K^+ channels to the coronary reactive hyperemic response in the context of metabolic syndrome. We found that BK_{Ca} channels do not contribute to the regulation of ischemic vasodilation in either lean (Figure 4.2) or MetS-20 (Figure 4.3) swine as BK_{Ca} channel inhibition with penitrem A (10 μg/kg, iv) had no effect on the peak hyperemic response or the overall volume of repayment. These data are in contrast with an earlier study by Node *et al.* which showed that inhibition of BK_{Ca} channels with iberiotoxin reduced both the peak coronary reactive hyperemic response and the repayment/debt ratio by ~20% in anesthetized dogs (115). Aside from differences in species, anesthesia and BK_{Ca} channel antagonist used, the reason for these discrepant results is unclear. However, it is important to point out that we previously demonstrated that the dose of penitrem A used in this study is effective as it essentially abolished coronary vasodilation in response to the BK_{Ca} channel agonist NS1619 in anesthetized, open-chest lean Ossabaw swine (16). Therefore, we are confident that BK_{Ca} channels play little, if any role in coronary vasodilation at rest or in response to cardiac ischemia in lean or metabolic syndrome Ossabaw swine.

Consistent with a recent study from our laboratory (36), we found that K_V channels play an important role in the regulation of baseline coronary blood flow and the reactive hyperemic response in lean-control swine. This effect was evidenced by the

decrease in coronary conductance at rest (Table 4.2) and the reduction of the peak hyperemic response and the volume of repayment (Figure 4.2) following the administration of the K_V channel antagonist 4-AP (0.3 mM) to swine pre-treated with penitrem A, i.e. combined blockade of BK_{Ca} and K_V channels. Since the inhibition of BK_{Ca} channels alone had no effect on key coronary response variables we attribute these changes to 4-AP sensitive K_V channels; i.e. not to compensatory increases in K_V channel activity. This assumption is supported by the previous findings of Dick *et al.* which documented a similar ~35% reduction in volume of repayment (Figure 4.2E) following inhibition of K_V channels alone (36). We failed to find any evidence for a role of K_V channels in mediating either baseline coronary flow or the reactive hyperemic response in metabolic syndrome-20 swine (Figure 4.3). The decreased contribution of K_V channels to reactive hyperemia in metabolic syndrome could be related to alterations in channel function and/or vasoactive factors and their respective signaling pathways that result in the activation of K_V channels. Based on recent whole-cell patch-clamp data from our laboratory which found no difference in K_V channel current in isolated coronary vascular smooth muscle cells from lean vs. metabolic syndrome Ossabaw swine (16), we propose that the diminished contribution of K_V channels is mediated by specific changes in channel activation. For example, earlier studies indicate that NO contributes to the coronary reactive hyperemic response (5; 9; 36; 47; 117) largely via a K_V channel-dependent pathway (36). Therefore, diminished coronary endothelial NO production, which is typically observed in metabolic syndrome (22; 88), would be expected to diminish the activation of K_V channels and thus their contribution to the overall hyperemic response. Alternatively, alterations in the production and/or signaling of the cardiac metabolite H_2O_2 could also play a role (36; 130; 131; 135). Further studies are needed to elucidate the exact mechanisms underlying the diminished contribution of K_V channels to coronary vasodilation in response to cardiac ischemia.

Our data do not support a significant role for K_{ATP} channels in coronary reactive hyperemia, at least in the presence of combined BK_{Ca} and K_V channel blockade. Although, we did find that additional blockade of K_{ATP} channels tended to further diminish the peak coronary reactive hyperemic response in lean swine (~15%; $P = 0.08$). Since we did not conduct studies with glibenclamide alone and earlier data have shown this drug reduces the peak hyperemic response by ~30% in normal dogs (48), we cannot exclude a more prominent role for K_{ATP} channels in the absence of BK_{Ca} and K_V channel inhibition. However, our data do show that combined inhibition of BK_{Ca}, K_V and K_{ATP} channels significantly diminishes the degree of coronary reactive hyperemia, i.e. the volume of repayment by ~50% (Figure 4.2E). Therefore, vasoactive mediators that converge on these K^+ channels, in particular K_V channels, comprise a significant proportion of the coronary vasodilatory response to ischemia. Furthermore, we submit that the lack of an affect of combined K^+ channel blockade on the coronary reactive hyperemic response in metabolic syndrome swine (Figure 4.3E) indicates that diminished contribution of K^+ channels to ischemic dilation represents an important mechanism of this coronary microvascular dysfunction in metabolic syndrome.

In summary, findings from this investigation demonstrate that metabolic syndrome significantly impairs coronary vasodilation in response to cardiac ischemia. The decrease in coronary reactive hyperemia in metabolic syndrome is not related to decreases in coronary BK_{Ca} channel function (16; 108) but more directly to a diminished contribution of K_V channels to the ischemic dilator response. We propose that this decrease in the functional contribution of K_V channels to the control of coronary blood flow in metabolic syndrome likely represents a critical mechanism underlying the increased incidence of cardiac arrhythmias, infarction and sudden cardiac death in obese patients with the metabolic syndrome that should be further explored (59; 66; 70; 75; 76; 96; 129).

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CHAPTER 5: DISCUSSION

Major findings of this investigation

Earlier studies have demonstrated that coronary microvascular function is markedly impaired by the onset of metabolic syndrome (27; 39; 90; 137; 141; 173) and indicate that this may be an important contributor to increased cardiovascular events in obese patients with this combined disorder (59; 66; 70; 75; 76; 96; 129). There is mounting evidence that implicate coronary K^+ channel dysfunction in metabolic syndrome as key mechanisms underlying the deleterious effects of metabolic syndrome. Recent investigations have identified impaired BK_{Ca} channel activity in coronary microvascular smooth muscle of alloxan-diabetic dyslipidemic swine (108), mesenteric microvessels of insulin resistant rats (38), and coronary arterial myocytes of diabetic fatty rats (26; 101). In addition, BK_{Ca} channels are highly expressed in the coronary vascular smooth muscle cells (20; 108; 143) and have been implicated as end-effectors in the regulation of coronary vasodilation in response to key endothelial and metabolic metabolites (71; 105; 106). However, the contribution of BK_{Ca} channel defects to the control of coronary blood flow and vascular dysfunction in metabolic syndrome has not been examined. Further, although studies have shown that BK_{Ca} channels contribute to coronary endothelial-dependent and exercise-induced dilation under normal-lean conditions (16; 103; 105; 106), no study has examined the contribution of BK_{Ca} channels to metabolic coronary vasodilation in the setting of metabolic syndrome. Recent literature also documented a role for BK_{Ca} channels (115), K_V channels (36), as well as K_{ATP} channels (10; 28; 172) in coronary vasodilation in response to a brief episode of myocardial ischemia, i.e. reactive hyperemia. Yet, the relative contribution of these specific K^+ channels to ischemic coronary vasodilation has not been delineated in lean or obese subjects with metabolic syndrome. Accordingly, the purpose of this

investigation was to examine the contribution of K^+ channels to coronary vascular disease in metabolic syndrome. The major findings of this investigation are as follows:

1) Coronary microvascular dysfunction in metabolic syndrome significantly impairs coronary vasodilation in response to metabolic and ischemic stimuli. Our data showed that the metabolic syndrome significantly depressed the relationship between coronary venous P_{O_2} and MV_{O_2} at rest and during exercise. Furthermore, we found a marked release of lactate (negative lactate uptake) in metabolic syndrome swine during exercise which indicates an imbalance between myocardial oxygen delivery and consumption to the point of overt cardiac ischemia. In additional studies we demonstrated that the ability of the coronary circulation to vasodilate in response to a brief episode of myocardial ischemia is progressively diminished in relation to the duration and degree of metabolic syndrome. This effect was evidenced by a decrease in the coronary reactive hyperemia repayment to debt ratio. It is important to recognize that the impairment of coronary vasodilation in response to both physiologic (exercise) and pathophysiologic (ischemia) stimuli was not related to the presence of a significant flow limiting stenosis; i.e. the impaired responses are directly related to coronary microvascular dysfunction and not the presence of significant atherosclerotic disease. We propose that the impairment of coronary microvascular function could contribute to the greater incidence of arrhythmias, sudden cardiac death, and other cardiovascular-related events exhibited in metabolic syndrome.

2) Coronary microvascular dysfunction in metabolic syndrome is related to decreased membrane trafficking and functional expression of BK_{Ca} channels that is accompanied by significant increases in L-type Ca^{2+} channel-mediated coronary vasoconstriction and increased intracellular Ca^{2+} concentration. Experiments revealed that coronary vasodilation in response to BK_{Ca} channel activation was attenuated in metabolic syndrome swine that was related to diminished current and

reduced membrane trafficking of these channels. Despite this, we found that BK_{Ca} channels do not significantly contribute to coronary vasodilation in response to local metabolic (exercise) or ischemic stimuli. We did however observe that inhibition of BK_{Ca} channels diminished coronary vasodilation to bradykinin in anesthetized open-chest lean Ossabaw swine, thus indicating BK_{Ca} channel involvement in endothelial-dependent. This observation is consistent with our isometric tension studies on freshly isolated circumflex coronary arteries (Figure 5.1) which showed significant attenuation of endothelial-dependent vasodilation to bradykinin in metabolic syndrome swine (EC_{50} 10.0 ± 1.3 nM) relative to lean (EC_{50} 0.8 ± 0.8 nM). Furthermore, endothelial-independent dilation to sodium nitroprusside was significantly increased in arteries from metabolic syndrome (EC_{50} 0.4 ± 1.2 μM) compared to lean (EC_{50} 2.9 ± 1.0 nM) swine. These data suggest that metabolic syndrome-induced coronary endothelial dysfunction is accompanied by a heightened response to NO in vascular smooth muscle, consistent with hypersensitivity of vascular smooth muscle to NO previously documented by Moncada *et al.* (110). However, at present we have not examined the extent to which BK_{Ca} channel dysfunction contributes to coronary microvascular endothelial dysfunction in metabolic syndrome. Based on these studies we hypothesize that alterations in electromechanical coupling contribute to coronary endothelial dysfunction (71; 105; 106) and the sensitization of key vasoconstrictor pathways (39; 88; 90; 141; 173) typically observed in obese subjects with metabolic syndrome.

Although we did not uncover a functional role for BK_{Ca} channels in exercise or ischemic dilation, the abundance of coronary vascular smooth muscle BK_{Ca} channels ((20)) and their capacity to be activated strongly suggest a role for these channels in mediating vascular tone. Numerous endogenous modulators of vascular BK_{Ca} channels have been identified. Putative EDHFs such as epoxyeicosatrienoic and

dihydroxyeicosatrienoic acids (97) as well as hydrogen peroxide (104) and shear stress (106) have been implicated in flow-induced vasodilation. BK_{Ca} channels are also regulated by kinase activity, in particular cyclic AMP- and cyclic GMP-dependent protein kinases, as activation of these kinases resulted in stimulation of rat cerebral artery smooth muscle BK_{Ca} channels (163). Other modulators such as endocannabinoids (62) and estrogenic compounds (35; 37) have also been identified as activators of BK_{Ca} channels.

In contrast, known endogenous inhibitory modulators of BK_{Ca} channels include ROS (147) and angiotensin II (126, 93). BK_{Ca} channel activity has also been found to be affected by membrane cholesterol. Cholesterol is necessary for proper BK_{Ca} channel

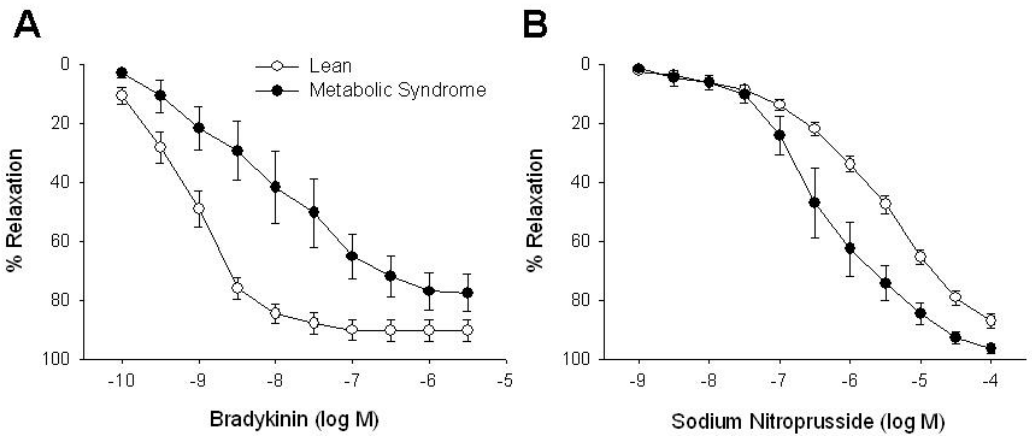

Figure 5.1 Metabolic syndrome markedly impaired coronary endothelial function in coronary arteries from Ossabaw swine (A). This was accompanied by a vascular smooth muscle hypersensitivity to NO (B).

function as they reside in lipid rafts for optimal function. However, the affect of high cholesterol on BK_{Ca} channels is still unknown as hypercholesterolemia has been shown to both impair BK_{Ca} channel activation by impairing signaling of factors that activate these channels (62) and by impeding BK_{Ca} channel subunit trafficking to the membrane (83), whereas other studies have demonstrated increased BK_{Ca} channel current in the presence of high cholesterol (109). Further studies are needed to elucidate the effects of cholesterol on BK_{Ca} channel activity.

Little is known about modulation of vascular smooth muscle BK_{Ca} channels by perivascular adipose, neural signaling, or platelet products. Given the abundant expression and diversity of smooth muscle BK_{Ca} channel tetramers, these various modulators likely permit site specific regulation of vascular tone that may be useful as therapeutic targets. More research is needed, however, to elucidate the functional role of BK_{Ca} channel in the regulation of vascular resistance.

3) Metabolic syndrome significantly impairs coronary microvascular vasodilation to cardiac ischemia via reductions in the relative contribution of K_V channels to the dilator response. Consistent with a recent study from our laboratory (36), we found that K_V channels play an important role in the regulation of baseline coronary blood flow and the reactive hyperemic response in lean-control swine. This effect was evidenced by a decrease in coronary conductance at rest and a reduction of the peak hyperemic response and the volume of repayment following the administration of the K_V channel antagonist 4-AP. We failed to find any evidence for a role of K_V channels in mediating either baseline coronary flow or the reactive hyperemic response in the setting of metabolic syndrome. Given that our whole-cell patch clamp data did not show any evidence for altered K_V channel function in vascular smooth muscle cells from swine with metabolic syndrome (Figure 5.2), we purpose that the loss of K_V channel involvement in ischemic vasodilation is not due to altered channel function, but rather due to alterations in factors that

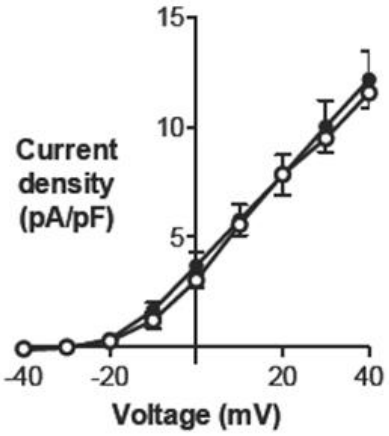

Figure 5.2 K_V channel current in coronary artery smooth muscle cells from lean (open circle) vs. metabolic syndrome (closed circle) Ossabaw swine in the presence of penitrem A.

activate the channel. We submit that this decrease in the functional contribution of K_V channels to the control of coronary blood flow in metabolic syndrome likely represents a critical mechanism underlying the increased incidence of cardiac arrhythmias, infarction and sudden cardiac death in obese patients with metabolic syndrome that should be further explored (59; 66; 70; 75; 76; 96; 129).

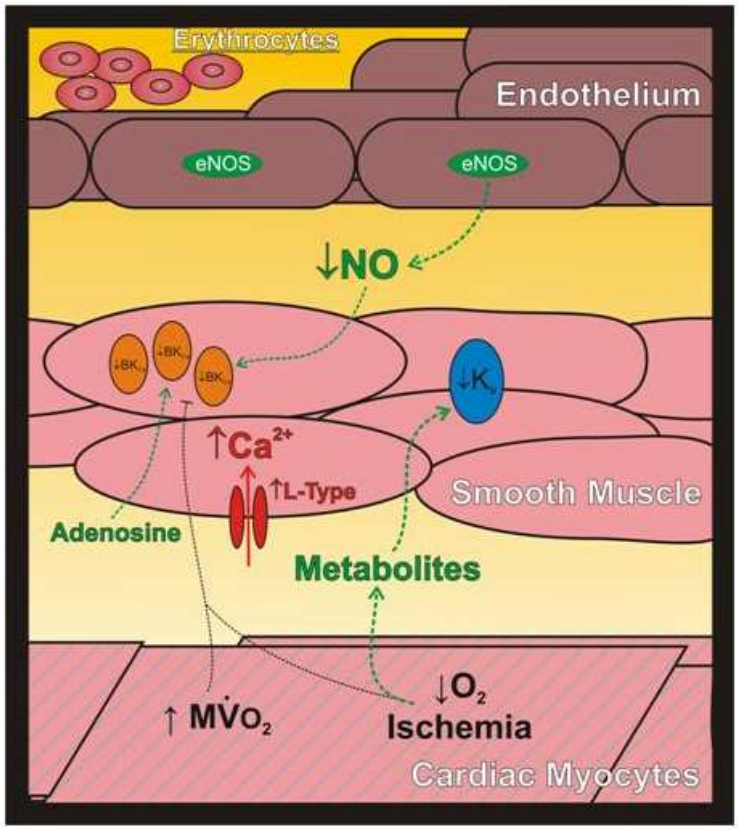

Figure 5.3 Schematic diagram of the contribution of K^+ channels to coronary microvascular dysfunction in metabolic syndrome.

Future directions

Mechanisms of BK_{Ca} Channel Trafficking

Findings from this investigation indicate that coronary vascular endothelial dysfunction in metabolic syndrome is related, at least in part, to diminished functional expression of vascular smooth muscle BK_{Ca} channels via impaired membrane trafficking. However, the mechanisms underlying BK_{Ca} channel trafficking remain unknown. One

possibility is alternative mRNA splicing that yields BK_{Ca} channels that are not trafficked to the cell membrane. Alternative splicing refers to the generation of several different mature mRNA transcript isoforms from a single gene due to variations in the splicing reaction of pre-mRNA, the primary gene transcript. The inclusion of exclusion of exons in the final product, or the recognition of different splice sites by the spliceosome that carries out the reaction, can alter the resultant final mRNA transcript. This phenomenon allows for a greater diversity of protein products to be generated from a single gene. The BK_{Ca} channel is comprised of a tetramer of pore-forming α subunits encoded by the single KCNMA1 (aka Slo1) gene. This gene is highly alternatively spliced, and it is the assembly of these channel variants that yield functional BK_{Ca} channels with different properties despite its ubiquitous expression throughout various tissues in the body. Alternative mRNA splicing has been linked to alterations in cell surface expression. For example, alternative splicing that skips exon 23 are dominant-negative for surface expression and when combined with other a subunits, prevents membrane, but not total, BK_{Ca} channel expression as these channels are trapped intracellularly (30). Motifs in the large cytoplasmic C-terminal are particularly important in determining the functional properties of BK_{Ca} channels, and alternative splicing in these sites can drastically alter expression on the plasma membrane (86; 95). An N-terminal insert has also been identified that prevents membrane trafficking of BK_{Ca} α-subunits and leads to intracellular accumulation of the channels (170; 171).

Yet another possibility is formation of BK_{Ca} channels with accessory subunits that inhibit membrane expression. In addition to modulation Ca^{2+}/voltage sensitivity and the biophysical and pharmacological properties of BK_{Ca} channels, the accessory β_1-subunit has recently been shown to alter channel trafficking. Toro *et al.* has found that β_1-subunit decreases the surface expression of BK_{Ca} channels via an endocytic targeting

mechanism (149). This suggests that increases in β_1 subunit expression could reduce the functional expression of BK_{Ca} channels at the membrane.

Diminished BK_{Ca} channel activity

Alternative splicing can also produce channels with reduced Ca^{2+}- and/or voltage-sensitivity. Alterations in the major exon that affects Ca^{2+}/voltage-sensitivity of BK_{Ca} α-subunits, in particular the STREX exon, can influence membrane expression and reduction of this key exon was found to produce a phenotype of functional impairment (167) regardless of increased total BK_{Ca} channel expression (166). Further, BK_{Ca} channel sensitivity to Ca^{2+} has been found to be significantly enhanced by the tissue-specific expression of auxiliary β_1 subunit (11). The BK_{Ca} channel β_1 subunit also amplifies the functional effects of α-subunit oxidation leading to increase in channel open probability and slowed channel deactivation (136). Further, this enhanced sensitivity of BK_{Ca} channels due to the accessory β_1 subunit could be diminished in disease. Sensitivity to Ca^{2+} was diminished in hypercholesterolemia that was related to reduced BK_{Ca} channel β_1 subunit expression in rabbit sphincter of Oddi (136). Thus a reduction in the Ca^{2+}/voltage-sensitivity of BK_{Ca} channels could decrease current generated at a given voltage and Ca^{2+} concentration, simultaneous with an increase in total BK_{Ca} channel protein expression.

K_V Channels in Metabolic Syndrome

Ours was the first investigation to examine the contribution of K_V channels to the coronary reactive hyperemic response in the context of metabolic syndrome. As outlined above, we propose that the diminished contribution of K_V channels to coronary ischemic vasodilation is mediated by specific changes in channel activation and not changes in

channel activity per se (see Figure 5.2 above). For example, earlier studies indicate that NO contributes to the coronary reactive hyperemic response (136) largely via a K_V channel-dependent pathway (36). Therefore, diminished coronary endothelial NO production, which is typically observed in metabolic syndrome (22; 88), would be expected to diminish the activation of K_V channels and thus their contribution to the overall hyperemic response. Alternatively, alterations in the production and/or signaling of the cardiac metabolite H_2O_2 could also play a role (36; 130; 131; 135). Alterations in the availability and/or signaling of adenosine, which has been found to be an important mediator in hypoxic vasodilation (13) and reactive hyperemia (118; 119; 134), could also influence this response. However, we found no change in adenosine dilation in isolated coronary arterioles between lean and metabolic syndrome swine. This may be due to the fact that these experiments were conducted in 9 week high fat-fed swine and thus metabolic syndrome had not progressed enough to obtain significant results.

Additional studies are also needed to more specifically study coronary vascular smooth muscle K_V channel function and activity as well as examine possible changes in membrane trafficking that could also diminish functional expression of these important vasoregulatory channels. Further studies are also needed to elucidate the specific Kv channel subtype(s) involved. Our earlier study indicates that K_V1 channels serve as end-effectors that integrate multiple physiological, pathophysiological, and pharmacological stimuli to regulate coronary microvascular resistance (36). However, the effect of metabolic syndrome on K_V1 channels has not been examined. The contribution of other K_V channel subtypes to the regulation of coronary vascular resistance, especially in the context of metabolic syndrome, merits further study.

K^+ Channel Electromechanical Coupling to L-type Ca^{2+} Channels

While K^+ channels are critical determinants of membrane potential and importantly contribute to the regulation of vasomotor tone, Ca^{2+} channels also influence vascular resistance by controlling levels of intracellular Ca^{2+} and subsequent activation of Ca^{2+}-dependent biochemical machinery. Voltage-gated L-type Ca^{2+} channels are the major source of Ca^{2+} entry into smooth muscle cells (84; 111). As their activity depends on membrane potential set by K^+ channels (162), K^+ channel impairment, as described in this investigation, leads to subsequent depolarization of the smooth muscle and results in the opening of L-type Ca^{2+} channels, increased intracellular Ca^{2+} levels, and resultant vasoconstriction. Consistently, data from this investigation demonstrated that vasoconstriction to the L-type Ca^{2+} channel agonist BayK 8644 was significantly elevated in isolated coronary conduit arteries from metabolic syndrome swine relative to lean. Further, increased Ca^{2+} current was reported in cerebrovascular smooth muscle cells of high fat-fed rats (159). This suggests that coronary vascular defects of metabolic syndrome could be attributed to augmented expression or activity of smooth muscle L-type Ca^{2+} channels. More studies are needed to address the function of coronary smooth muscle L-type Ca^{2+} channels in the context of obesity and metabolic syndrome.

Recent data from the Sturek laboratory indicates that diabetic dyslipidemia increases the functional coupling of BK_{Ca} channels to sarcoplasmic reticulum Ca^{2+} release in vascular smooth muscle cells from large-conduit coronary arteries (108). This increase was attributed to a compensatory change in response to the decrease in Ca^{2+} influx from L-type Ca^{2+} channels and augmented vasoconstrictor influence as previously noted in conduit arteries of aldosterone-salt hypertensive rats (99). Several vascular complications in diabetes mellitus has been linked to altered regulation of myoplasmic Ca^{2+} (72), including alterations in Ca^{2+} influx, smooth muscle cell intracellular Ca^{2+} levels, and Ca^{2+} signaling. Hill *et al.* previously observed an increase in Ca^{2+} buffering by the

plasma membrane Ca^{2+} pump and an increase in the activity and expression of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (72). This is consistent with other studies identifying impaired Ca^{2+} transporter function in diabetes mellitus (91) and high glucose (94). However, additional studies in coronary microvessels revealed a significant decrease in the coupling of sarcoplasmic reticulum-mediated Ca^{2+} spark events with STOC frequency (108). Meanwhile, data from high fat-fed dogs showed augmented functional expression of L-type Ca^{2+} channels in both arteries and arterioles (88). Specifically, Knudson *et al.* demonstrated increased coronary vasoconstriction to the L-type Ca^{2+} channel agonist BayK 8644 in isolated coronary arterioles, enhanced nicardipine-induced coronary vasodilation in vivo, and increased L-type Ca^{2+} channel current under basal conditions and in the presence of BayK 8644 in isolated conduit arterial smooth muscle cells (88). Since the density of L-type Ca^{2+} channel current is inversely related to arterial diameter (19), L-type Ca^{2+} channels may be differentially regulated in the macro- versus microcirculation. Thus, further studies are needed to examine the functional expression of coronary vascular L-type Ca^{2+} channels in metabolic syndrome.

Closing remarks

Metabolic syndrome is associated with a state of coronary microvascular dysfunction that significantly impairs the ability of the coronary circulation to match myocardial oxygen delivery with myocardial metabolism. Recent investigations implicated coronary K^+ channel dysfunction as an important contributor to increased cardiovascular events in patients with metabolic syndrome, but this is the first study to demonstrate that metabolic syndrome significantly impairs microvascular function to exercise-induced and ischemic vasodilation. Though we failed to find evidence to support a role of BK_{Ca} channels in mediating these processes, we did find that the

functional expression of BK_{Ca} channel is depressed in metabolic syndrome and may contribute to endothelial dysfunction that is classically associated with this syndrome. Further, we demonstrated that impaired ischemic dilation is related at least in part to diminished K_V channel response to ischemic metabolites. Collectively, these findings demonstrate that decreased functional expression of K^+ channels could be a critical mechanism underlying increased morbidity and mortality to coronary and cardiac events in patients with metabolic syndrome. Future studies should examine mechanisms underlying metabolic syndrome-induced impairment of BK_{Ca} channel membrane trafficking as well as alterations in K_V and L-type Ca^{2+} channels.

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Bratz IN, Payne GA, **Borbouse L**, Bohlen HG, Dick GM, Tune JD. Perivascular adipose tissue alters coronary arterial smooth muscle and endothelial function. FASEB J. 2007

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