Published in final edited form as:

Basic Res Cardiol. 2016 September ; 111(5): 56. doi:10.1007/s00395-016-0575-0.

Critical contribution of K_V1 channels to the regulation of coronary blood flow

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Abstract

Ion channels in smooth muscle control coronary vascular tone, but the mechanisms require further investigation. The purpose of this study was to evaluate the functional role of K_V1 channels on porcine coronary blood flow by using the selective antagonist correolide. K_V1 channel gene transcripts were found in porcine coronary arteries, with KCNA5 (encoding $K_V1.5$) being most abundant ($P<0.001$). Immunohistochemical staining demonstrated $K_V1.5$ protein in the vascular smooth muscle layer of both porcine and human coronary arteries, including microvessels. Whole-cell patch clamp experiments demonstrated significant correolide-sensitive (1–10 μM) current in coronary smooth muscle. *In vivo* studies included direct intracoronary infusion of vehicle or correolide into a pressure-clamped left anterior descending artery of healthy swine (n=5 in each group) with simultaneous measurement of coronary blood flow. Intracoronary correolide (~0.3–3 μM targeted plasma concentration) had no effect on heart rate or systemic pressure, but reduced coronary blood flow in a dose-dependent manner ($P<0.05$). Dobutamine (0.3–10 μg/kg/min) elicited coronary metabolic vasodilation and intracoronary correolide (3 μM) significantly reduced coronary blood flow at any given level of myocardial oxygen consumption ($P<0.001$). Coronary artery occlusions (15 s) elicited reactive hyperemia and correolide (3 μM) reduced the flow volume repayment by approximately 30% ($P<0.05$). Taken together, these data support a major role for K_V1 channels in modulating baseline coronary vascular tone and perhaps vasodilation in response to increased metabolism and transient ischemia.

Keywords

coronary circulation; metabolic vasodilation; KCNA5; $K_V1.5$; correolide

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Introduction

The limited anaerobic capacity and oxygen extraction reserve of the heart renders the myocardium highly dependent on a continuous supply of oxygen to maintain normal cardiac function. The ability of the coronary circulation to provide adequate perfusion may be challenged by a variety of physiologic factors that modulate microvascular resistance and balance oxygen delivery with the underlying myocardial requirements. A number of regulatory mechanisms participate in the control of coronary blood flow [7], many of which may be affected by disease [37]. Despite decades of research, understanding of the mechanisms responsible for regulation of coronary blood flow remains incomplete and there are currently more hypotheses than accepted pathways [8].

Significant evidence supports the idea that K^+ channels function as critical downstream effectors on which multiple signaling pathways converge to modulate coronary vasomotor tone [27]. Although multiple types of K^+ channels are expressed in coronary vascular smooth muscle, voltage-dependent K^+ (K_V) channels appear to play a predominant role, as relatively nonselective inhibition of these channels with 4-aminopyrindine (4-AP) reduces baseline coronary blood flow and inhibits local metabolic and ischemic vasodilation [3, 4, 9]. The precise K_V channel subtypes involved have not, however, been clearly defined. Given emerging data regarding the role of H_2O_2 in endothelial and metabolic coronary vasodilation [16, 22, 36], recent studies have focused on redox-sensitive K_V channels with varying conclusions. While data do not support a prominent role for K_V7 channels in coronary metabolic dilation of rodents or swine [15, 23], genetic deletion of $K_V1.5$ channels was found to significantly inhibit the tight coupling of coronary blood flow to myocardial metabolism in mice [31]. These findings support an evolving paradigm in which K_V1 channels play requisite roles in controlling coronary blood flow responses to physiological and pathophysiological stimuli.

This study tested the hypothesis that K_V1 channels are functionally expressed in coronary smooth muscle and regulate coronary vascular tone at rest, in response to increases in myocardial metabolism, and following transient ischemic episodes (reactive hyperemia). We used swine as experimental models and performed quantitative polymerase chain reaction (qPCR) to investigate the expression of KCNA genes and immunohistochemistry to determine the presence of K_V1 channel protein in coronary vascular smooth muscle. The contribution of K_V1 channels to outward K^+ current in isolated coronary smooth muscle cells was assessed by patch-clamp electrophysiology with correolide, a selective K_V1 channel inhibitor (1–10 μM). The role of K_V1 channels in regulating coronary vascular resistance *in vivo* was examined with intracoronary administration of correolide (10 swine were divided into two groups; 5 received vehicle only, while 5 were treated with correolide). We determined the effects of correolide on: a) resting coronary blood flow (infused directly into the coronary artery to reach 0.3–3 μM in the plasma); b) metabolic dilation evoked by dobutamine challenge (0.3–10 μg/kg/min); and c) ischemic dilation in response to a 15 s coronary artery occlusion (reactive hyperemia). Correolide has been tested against more than 100 ion channels and receptors; it blocks all members of the K_V1 family, but has no known inhibitory effects on other targets [12, 18, 21]. Unique effects of correolide on K_V1 channels

are due to the presence of a conserved, high specificity binding site in the S5 and S6 segments of these channels [17].

Methods

All protocols were approved by an Institutional Animal Care and Use Committee and performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85-23, Revised 2011). Human coronary artery samples were obtained with the oversight of an Institutional Review Board (protocol # 1306011568).

qPCR analysis of KCNA expression

The left anterior descending (LAD) coronary artery was dissected from the heart and cleaned of adipose and connective tissue. Total RNA was isolated using the RNeasy fibrous tissue kit (QIAGEN) and stored at −80°C. cDNA synthesis was performed using 1 μg of total RNA and the iScript cDNA synthesis kit (Bio-Rad). qRT-PCR was performed using SYBR Green Supermix (Bio-Rad). The specificity of PCR amplification products was validated by melting-curve analysis. KCNA and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) primers were designed using Premier Primer 5 software (PREMIER Biosoft). Primer sequences and amplicon sizes are given in Table 1. KCNA gene expression was normalized to GAPDH and analyzed with Bio-Rad CFX Manager software.

K_V1.5 immunohistochemical staining, immunofluorescent localization, and immunoblot

These experiments were performed using an antibody with established reactivity against K_V1.5 in both swine and humans (APC-150; Alomone Labs; [3]). For conventional immunohistochemistry, formalin-fixed, paraffin-embedded sections of swine and human coronary arteries were stained in conjunction with the Indiana University Immunohistochemistry Laboratory Core (Indiana University Health, Department of Pathology). A separate section of human coronary artery was additionally exposed to appropriate isotype control to demonstrate specificity of the primary antibody. Slides were imaged on an inverted microscope (model 3032, Accu-Scope Inc.) and images captured with a digital camera and associated software (DMC2900 and LAS V4.6, Leica Microsystems).

Immunofluorescent staining of the swine coronary microvasculature was performed on myocardial samples. Tissue was fixed in 4% paraformaldehyde for 2 hrs on ice, washed in phosphate buffered saline, and frozen in Tissue-Tek O.C.T Compound (Sakura Finete) on a bed of dry ice and isopentane. Cryosections (8 μm) were cut and O.C.T. was removed by a 5 min wash in Tris buffered saline (TBS). Tissues were permeabilized in 0.2% Triton X-100 in TBS for 5 min, washed, and blocked in 5% horse serum in TBS at room temperature for 1 hr. Sections were incubated at 37° with the K_V1.5 antibody (1:200) and an anti-smooth muscle actin antibody conjugated to Cy3 (1:250; C1698, Sigma). Sections without the K_V1.5 primary antibody served as a negative control. To further verify specificity of the K_V1.5 immunoreactivity, some sections were incubated with anti-K_V1.5 and blocking peptide (i.e., control antigen; ~1 μg of peptide/μg of antibody). K_V1.5 immunoreactivity was detected with anti-rabbit Alexa Fluor 647 (1:4,000; Jackson ImmunoResearch). Slides were

mounted in ProLong Gold with DAPI (Invitrogen) and visualized by confocal microscopy (Olympus Fluoview FV1000).

Western blots with the $K_V1.5$ antibody were performed on lysates prepared from coronary arteries. Arterial segments were homogenized in lysis and extraction buffer containing (mM) 50 β-glycerophosphate, 0.1 Na_3VO_4, 2 $MgCl_2$, 1 EGTA, 1 DTT, 0.02 pepstatin, 0.02 leupeptin, and 1 PMSF, as well as 0.5% Triton X-100 and 0.1 U/ml aprotinin. Lysate (100 μg of protein by Bradford assay; Bio-Rad) was loaded on a 4–12% Bis-Tris gel (Life Technologies) and electrophoresed. Protein was transferred to nitrocellulose membranes overnight at 4 °C and then blocked 1 hr at room temperature with 5% bovine serum albumin. Membranes were incubated in primary antibody (1:200) at 4 °C overnight. Membranes were washed and placed for 1 hr at room temperature in horseradish peroxidase-linked secondary antibody (1:2000 of goat anti-rabbit; Santa Cruz Biotechnology). $K_V1.5$ immunoreactivity was visualized with ECL Prime (Amersham).

Patch-clamp electrophysiology

Segments of the swine LAD were enzymatically digested to isolate coronary smooth muscle as previously described [9]. Currents were measured at room temperature in conventional dialyzed patches. The bath solution contained (mM): 135 NaCl, 5 KCl, 2 $MnCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, and 5 Tris; pH 7.4. The pipette solution contained (mM): 140 KCl, 3 Mg-ATP, 1 Na-GTP, 1 EGTA; 10 HEPES, and 5 Tris; pH 7.1. After whole-cell access was established, series resistance and membrane capacitance were compensated as fully as possible (200B amplifier and pCLAMP software, Axon Instruments).

Surgical preparation

Adult male domestic swine (n = 10) were sedated with telazol, xylazine, and ketamine (5, 2.5, and 2.5 mg/kg, s.c.) prior to anesthesia with morphine (3 mg/kg, i.m.) and α-chloralose (100 mg/kg, i.v.). Once a surgical plane of anesthesia was established, pigs were intubated and ventilated with O_2-supplemented room air. Catheters were placed into the thoracic aorta via the right femoral artery (for blood pressure and heart rate monitoring), into the left femoral artery (to draw a supply of blood for the extracorporeal perfusion system), and into the right femoral vein (for supplemental anesthetic, heparin, and sodium bicarbonate). The heart was accessed via thoracotomy so that the LAD could be isolated and cannulated. The animal was heparinized (500 U/kg, iv) and acute *in vivo* experiments were conducted using an extracorporeal pressure-clamped perfusion system. Coronary perfusion pressure was maintained at 100 mm Hg by a servo-controlled peristaltic pump throughout the study and blood flow was continuously measured by an in-line flow transducer (Transonic Systems). A catheter was inserted into the interventricular coronary vein to allow sampling of venous blood from the LAD perfusion territory. Blood gas parameters were kept within normal physiologic limits through periodic blood gas analyses and appropriate adjustments to breathing rate, tidal volume, and/or bicarbonate supplementation. Data were continuously recorded using IOX data acquisition software (EMKA Technologies).

Experimental Protocol

Following coronary cannulation, hemodynamic variables were allowed to stabilize for 15–30 min before initiation of the protocol. Swine were then randomly assigned to the correolide-treated or vehicle only group (n = 5 each). Three different protocols were performed to examine the role of K_V1 channels in the control of coronary blood flow. 1) To examine the capacity of K_V1 channels to modulate coronary vascular resistance at rest, vehicle (DMSO diluted to 10%) or correolide (in 10% DMSO) was continuously infused into the coronary perfusion circuit via a syringe pump. In order to obtain the target plasma concentrations of correolide (0.3–3 μM), pump infusion rates were calculated based on hematocrit and coronary blood flow. 2) The contribution of K_V1 channels to metabolic coronary vasodilation was assessed with infusion of dobutamine (0.3–10 μg/kg/min, iv) with or without intracoronary correolide (3 μM in coronary plasma). 3) The role of K_V1 channels in ischemic coronary vasodilation was investigated by occluding the coronary perfusion circuit for 15 s and then measuring reactive hyperemia with or without intracoronary infusion of correolide (3 μM in coronary plasma). Hemodynamic measurements were continuously acquired and analyzed when variables stabilized (e.g., 3–5 minutes after an intravenous administration of dobutamine).

Statistical analyses

Data are expressed as mean ± standard error for a given number of swine or smooth muscle cells. Statistical comparisons were made with t-tests and one or two-way analysis of variance (ANOVA) as appropriate. In all statistical test, $P < 0.05$ was considered statistically significant. When significance was detected with ANOVA, a Student-Newman-Keuls multiple comparison test was performed to identify differences between groups and treatment levels. Data regarding the effects of correolide on the balance between coronary blood flow and myocardial metabolism were analyzed by linear regression and analysis of covariance (ANCOVA). Volume payment of reactive hyperemia was calculated as area under the curve using Prism software (GraphPad Inc.).

Results

PCR analysis of KCNA expression in coronary arteries

KCNA genes (KNCA1…KCNA10) encode K_V1 channels ($K_V1.1$…$K_V1.8$). We performed qPCR analyses to establish the relative abundance of KCNA transcripts in coronary arteries from swine (n = 3; Fig. 1). Primer sequences are provided in Table 1. KCNA expression was normalized to a reference gene, GAPDH. We detected the expression of all 8 known KCNA gene transcripts, with KCNA5 being most abundant (KCNA5 expression was significantly higher than all other KCNA transcripts). KCNA2 was also expressed prominently (KCNA2 expression was significantly higher than all other KCNA transcripts, except for KCNA5). The abundance of all other KCNA genes were lower and statistically indistinguishable (no statistically significant differences between the levels of KCNA1, KCNA3, KCNA4, KCNA6, KCNA7, or KCNA10).

Immunohistochemical detection $K_V1.5$ protein in coronary arteries, including resistance-sized microvessels

Building on the observation that KCNA5 was the most abundant of all K_V1 transcripts in epicardial coronary samples, we assessed coronary artery expression of $K_V1.5$ protein with immunohistochemical techniques. This analysis was performed using a commercially available antibody directed against rat $K_V1.5$ with demonstrated reactivity for human $K_V1.5$ in immunohistochemistry and immunoblots. $K_V1.5$ immunoreactivity was abundant in both swine (Fig. 2A) and human (Fig. 2B) coronary artery sections with marked staining of the vascular smooth muscle layer. Isotype controls performed with human coronary artery samples demonstrated that positive staining was not an artifact (Fig. 2C). Moreover, we confirmed that this antibody has appropriate reactivity in swine using Western blot techniques (Fig. 2D), as done previously [3].

Since regulation of coronary blood flow is largely controlled by vessels with diameters less than 200 μm, we further examined the localization of $K_V1.5$ protein in myocardial sections containing resistance-sized microvessels (Fig. 3). Sections of swine myocardium were treated with antibodies against $K_V1.5$ and smooth muscle actin (SMA), as well as a nuclear stain. Immunoreactivity of the antibodies was detected by fluorescence in separate color channels (red and green). Overlaying the color images revealed prominent $K_V1.5$ immunoreactivity in SMA-positive microvessels (Fig. 3A–C). Pre-adsorbing the $K_V1.5$ antibody with the antigenic peptide eliminated the detection of $K_V1.5$ immunoreactivity in SMA-positive microvessels (Fig. 3D–F). These data indicate that $K_V1.5$ channels are expressed in vessels of a caliber appropriate for regulating coronary vascular resistance.

Correolide-sensitive K_V1 current in coronary vascular smooth muscle

Based on the presence of KCNA transcripts and $K_V1.5$ protein in epicardial coronaries, patch clamp electrophysiology was used to investigate the functional expression of K_V1 channels in coronary smooth muscle cells. Correolide inhibits all members of the K_V1 family, but does not affect other K^+ channel types [12, 17, 18, 21]. We measured K_V currents in coronary smooth muscle cells under conditions that minimize the contribution of other K^+ channel types (e.g., we lessened the influence of $Ca2^+$-activated and ATP-dependent K^+ channels by adding a $Ca2^+$ chelator and ATP to the pipette solution). Under these conditions, the K_V or delayed rectifier K^+ current was prominent (i.e., there was time- and voltage-dependent current that saturated at positive potentials [14, 39]). Correolide inhibited K_V current in smooth muscle cells from the porcine coronary artery in a concentration-dependent manner (Fig. 4), indicating that K_V1 subunits likely comprise a major portion of the channels active under these conditions. Current in the presence of 1 μM correolide was reduced >30% at voltages between +20 and +100 mV ($P < 0.05$; Fig. 4C; n = 4). Current in the presence of 10 μM correolide was reduced >80% at voltages between −20 and +100 mV ($P < 0.05$; Fig. 4F; n = 5).

K_V1 channels regulate coronary blood flow at the resting heart rate

Our first *in vivo* experiment was to determine the effect of correolide on coronary blood flow at the resting heart rate. Concentration-response experiments showing the effects of direct intracoronary infusion of correolide (to targeted plasma concentrations between 0.3

and 3 μM; n = 5 correolide-treated swine) are in Fig. 5. Correolide is completely insoluble in water and, thus 1:10 DMSO in saline was used as the vehicle. It is our experience that DMSO infusions, even very dilute solutions, can elicit dilator responses in the coronary circulation; therefore, a second set of swine (n = 5) received intracoronary infusions of this vehicle only. Data are presented as a change in flow relative to the baseline within a group (vehicle vs. correolide). Vehicle infusions rates were matched to those necessary for correolide. Vehicle alone significantly increased coronary blood flow (0.18 ± 0.07 ml/min/g; n = 5) at the infusion rates needed to approach concentrations of 1 and 3 μM correolide (Fig. 5). Initial vehicle studies with infusion rates necessary to obtain coronary plasma concentrations of 10 μM correolide increased baseline coronary blood flow > 2-fold. Thus, we were unable to perform *in vivo* studies with concentrations of correolide exceeding 3 μM. In contrast to vehicle administration, intracoronary infusion of correolide concentration-dependently decreased coronary blood flow (−0.12 ± 0.03 ml/min/g; Fig. 5). Correolide administration significantly reduced coronary blood flow ~25% (i.e., reduced from ~1.0 to 0.72 ml/min/g with 3 μM correolide; $P = 0.01$). Because DMSO increased flow and is the vehicle for correolide, our experiment likely underestimates the vasoconstriction caused by inhibition of K_V1 channels. A caveat for the experiment, however, is that coronary blood flow decreased as we infused correolide at a constant rate and hence the target concentration in coronary plasma was exceeded. To determine the degree to which the target and actual plasma concentrations differed, we recalculated and determined that reduced coronary plasma flow raised the effective plasma concentrations of correolide to 0.32 ± 0.01, 1.01 ± 0.03, 3.27 ± 0.10 μM. Coronary blood flow data in Fig. 5 are plotted against these calculated correolide concentrations. Despite changes in coronary blood flow relative to MVO_2 (Fig 5), neither vehicle nor correolide infusion significantly altered coronary venous PO_2 when myocardial oxygen demand was at a resting level (P = 0.16).

Role of K_V1 channels in metabolic vasodilation

Our second *in vivo* experiment was designed to determine the contribution of K_V1 channels to coronary vasodilation in response to increases in myocardial metabolism (e.g., the functional hyperemia that accompanies an increase in heart rate). Hemodynamic data in response to dobutamine (0.3–10 μg/kg/min, iv) in the absence and presence of intracoronary correolide administration (3 μM in coronary plasma) are presented in Table 2. Dobutamine dose-dependently increased systolic blood pressure and heart rate in vehicle-treated swine (n = 4; a technical problem with blood samples prevented calculation of myocardial oxygen consumption in 1 of the 5 animals intended for this group). Dobutamine increased heart rate, systolic, diastolic, and mean blood pressure in correolidetreated swine (n = 5), however, blood pressures were significantly lower than the vehicle only group (despite randomization of subjects obtained from a common vendor). Regardless of the differences in blood pressure, the dobutamine-induced increase myocardial oxygen consumption was not different in vehicle- vs. correolide-treated swine (49 ± 8 to 63 ± 9 vs. 44 ± 7 to 73 ± 7 μl O_2/min/g; $P = 0.97$). This is likely due to the fact that heart rate is a more important determinant of myocardial oxygen consumption than afterload [10]. The effect of K_V1 channel inhibition on metabolic coronary vasodilation was examined by analyzing the relationship between coronary blood flow and myocardial oxygen consumption (Fig. 6A). Linear regression determined that the slope of this relationship was not different in the

vehicle and correolide groups ($P = 0.34$). ANCOVA revealed that correolide markedly reduced coronary blood flow relative to myocardial oxygen consumption across the dobutamine concentration range ($P < 0.001$; i.e., correolide shifts the relationship down in a parallel manner). Examining coronary venous PO_2 revealed interesting effects of correolide on the balance between myocardial oxygen supply and demand (Fig. 6B). In the vehicle only group, coronary venous PO_2 declined as myocardial oxygen consumption increased. In contrast, in the correolide-treated group, coronary venous PO_2 was lower at rest and did not decline further as myocardial oxygen consumption increased ($P < 0.05$).

K_V1 channels function in ischemic vasodilation

Our final *in vivo* experiment was designed to determine whether K_V1 channels play a role in coronary reactive hyperemia (i.e., vasodilation in response to a transient myocardial ischemia). Hyperemic responses were elicited by occluding the arterial perfusion cannula for 15 s. Coronary blood flow responses were measured under control conditions (in swine treated with vehicle only; n = 5) and during infusion of correolide at a plasma concentration of 3 μM (n = 5 different swine). Average reactive hyperemia responses are shown in Fig. 7. Correolide reduced baseline coronary blood flow by ~25% (1.06 ± 0.28 to 0.79 ± 0.19 ml/min/g; $P < 0.05$). Inhibition of K_V1 channels did not affect peak hyperemia (~3.5 ml/min/g; $P = 0.29$), but correolide significantly decreased the overall volume of repayment by ~35% (1.86 ± 0.39 to 1.21 ± 0.28 ml/g; $P < 0.05$).

Discussion

We used an integrative and complementary set of *in vitro* and *in vivo* approaches to evaluate the role of K_V1 channels in the regulation of coronary blood flow in swine. We tested the hypothesis that K_V1 channels are functionally expressed in coronary smooth muscle and regulate coronary vascular tone at rest, in response to increases in myocardial metabolism, and following transient ischemic episodes (reactive hyperemia). All 8 known KCNA transcripts were expressed in swine coronary arteries, with KCNA5 (encoding $K_V1.5$) being most abundant. Immunohistochemistry experiments showed that $K_V1.5$ channel protein was expressed in the medial (smooth muscle) layer of human and swine coronary arteries – including resistance vessels, which regulate flow. In porcine coronary smooth muscle, the K_V1-selective inhibitor correolide blocked the majority of K^+ current, demonstrating that K_V1 subunits are functionally expressed in abundance. K_V1 channels maintain baseline coronary vasomotor tone, as selective inhibition of these channels with correolide reduced coronary blood flow ~25% at the resting heart rate. Correolide significantly depressed the relationship between coronary blood flow and myocardial oxygen consumption, indicating that K_V1 channels may participate in functional hyperemia. K_V1 channels may also function in ischemic dilation, as correolide reduced the overall volume of flow repayment in response to a 15 s coronary occlusion (i.e., K_V1 channels regulate the duration of coronary reactive hyperemia). Alternative interpretations could be that K_V1 channels have a tonic role in the regulation of coronary vascular tone and that inhibiting them results in a parallel shift in the relationship between coronary blood flow and cardiac metabolism and no impairment in peak ischemic vasodilation. These findings support the idea that K_V1 channels are important end-effectors regulating coronary microvascular resistance.

Molecular and functional expression of K_V1 channels in coronary arteries

KCNA gene and K_V1 channel expression in swine coronary arteries confirm and extend prior studies demonstrating the presence of multiple KCNA transcripts and K_V1 channel proteins in coronary arteries from mice, rats, dogs, and swine [3, 9, 13, 30, 31]. Expression of other K_V channel isoforms (e.g., K_V3 and K_V7 families) has also been documented in coronary arteries of mice, rats, and swine [3, 15, 19, 20, 23, 24]. Correolide blocked a majority of the K^+ current in smooth muscle cells; therefore, it is reasonable to conclude that channels composed of K_V1 channel subunits produce a significant portion of the delayed rectifier K^+ current. The substantial inhibitory effect of correolide agrees well with what we observed previously in canine coronary smooth muscle cells [9]. Further, the delayed rectifier K^+ currents of smooth muscle cells from the coronary arteries of dogs, pigs, and humans have many biophysical and pharmacological similarities [9, 14], suggesting that K_V1 subunits form at least a portion of the channels. This idea is supported by the detection of $K_V1.5$ protein expression in the coronary smooth muscle of swine and humans, including resistance vessels.

Role of K_V1 channels in metabolic coronary vasodilation

Identifying the mechanisms coupling coronary blood flow to myocardial metabolism has been a central quest in coronary physiology for over 50 years. There is a growing body of evidence supporting K_V channels as critical modulators in the local metabolic control of coronary blood flow [3, 31, 36]. Other K^+ channels, such as inwardly rectifying K^+ channels (Kir) may also be important [11], but were not studied here. Non-selective inhibition of K_V channels with 4-AP dose-dependently reduces resting coronary blood flow and decreases the balance between myocardial oxygen delivery and metabolism in response to cardiac pacing, norepinephrine, and exercise [3, 36]. Data from this study may support a role for K_V1 channels in coronary metabolic vasodilation [31], but the mostly parallel downward shift in the relationship between coronary blood flow and myocardial oxygen consumption caused by correolide may be indicative of a tonic role. Intuitively, one would expect a similar parallel downward shift in the relationship between coronary venous PO_2 and myocardial oxygen consumption; however, no such shift was detectable statistically. This may be due factors such as the elevated coronary venous PO_2 typically observed in cannulated preparations and the effect of DMSO to elicit coronary vasodilation. The role of K_V1 channels in regulating the balance between coronary flow and myocardial metabolism is supported by the modest reduction in coronary venous PO_2 at lower levels of myocardial demand (Fig 6B). Coronary venous PO_2 was higher in vehicle-treated animals relative to correolide-treated counterparts at the lowest infusion rates (42 ± 4 vs. 35 ± 2 mmHg). This vehicle-induced overperfusion became less significant with increasing metabolic demand. However, owing to lower coronary venous PO_2 values relative to myocardial oxygen consumption at low infusion rates, the slope of the relationship is much flatter in correolide-treated animals than is found in the vehicle only controls (Fig 6B).

A recent study demonstrated a much more profound reduction in the slope of the relationship between coronary flow and cardiac double product (index of myocardial metabolism) in $K_V1.5$ knockout mice during norepinephrine administration [31]. Importantly, this phenotype was associated with significant myocardial hypoxia and was

rescued by smooth muscle-specific expression of $K_V1.5$ in the global knockouts. It should be recognized that while the knockout mouse model provides compelling evidence with genetic targeting of specific channels, compensatory upregulation of other K_V channel subtypes and marked differences in the underlying cardiovascular phenotype of mice (heart rate >500 beats/min; coronary blood flow >10 fold higher than large animal models or humans) must be taken into account. We were unable to use higher concentrations of correolide *in vivo* to further investigate coronary metabolic vasodilation, as effects of vehicle made higher dose rates of impractical. Together, these findings collectively support a prominent role for K_V1 channels as crucial end-effectors in the coronary circulation of mice, dogs, and swine.

We did not focus on identifying specific factors that converge on Kv1 channels in this investigation. However, prior studies have demonstrated that putative dilators such as adenosine, nitric oxide, prostacyclin, and H_2O_2 mediate coronary vasodilation, at least in part, via 4-AP-sensitive K_V channels. Our prior study also established that correolide significantly reduces dilation of isolated coronary arterioles to adenosine and sodium nitroprusside [9], indicating that K_V1 channels are the targets of known coronary vasodilators. There are likely numerous endothelial and metabolic factors that converge on K_V1 channel subtypes to modulate coronary vascular tone, including H_2O_2 [34–36]. Alternatively, it is well established that direct β-adrenoceptor activation contributes to the control of coronary blood flow during increases in myocardial metabolism [29]. Thus, it is possible that increases in coronary flow in response to catecholamine administration (or elevated sympathetic tone and plasma catecholamine concentrations with exercise) induce smooth muscle relaxation via a cAMP-protein kinase dependent activation of K_V1 channels [1, 2]. Numerous additional studies will be needed to examine the precise K_V channel subtypes responsible for eliciting dilation to these, as well as other, putative factors.

Role of K_V1 channels in ischemic coronary vasodilation

Coronary dilation in response to myocardial ischemia is a critical mechanism that sustains oxygen delivery to the heart to mitigate ischemic injury and infarction. A well accepted experimental approach to examine ischemic coronary vasodilation is to measure coronary blood flow responses following a brief coronary artery occlusion. This reactive hyperemia has been attributed to a variety of mechanisms including myogenic, endothelial, and local metabolic factors [38]. Prior work from our laboratory support relatively modest roles for adenosine and nitric oxide, with a more pronounced contribution of K_V and K_{ATP} channels [5, 9]. The extent to which K_V1 channels contribute to coronary reactive hyperemia, however, has not been previously investigated.

Inhibition of K_V1 channels with correolide significantly decreased overall repayment of flow debt. This suggests that factors produced during acute myocardial ischemia elicit vasodilation, in part, via K_V1 channels. This overall effect of correolide is similar to our earlier findings in dogs with 4-AP, which diminished baseline flow and the overall volume of repayment [9]. In contrast, we recently documented that inhibition of K_V7 channels with linopirdine had no effect on coronary reactive hyperemic responses in swine [15]. Although correolide diminished the duration of reactive hyperemia, it is important to point out that

peak hyperemia was unaffected by the correolide, which could be interpreted as no role for K_V1 channels. Peak responses increase with the duration of the occlusion up to ~15–30 s, indicating that maximal vasodilator capacity is reached within 30 s of ischemia. Thus, one could conclude that K_V1 channels do not contribute to ischemic coronary vasodilation. However, we argue that the reduction in volume of repayment (duration of ischemic vasodilation) supports a role for these channels, as the hyperemic response is estimated to provide only ~80% of the necessary oxygen repayment incurred during the flow deficit.

Implications and conclusions

K_V1 channels are functionally expressed and contribute to the regulation of coronary microvascular resistance at rest, during β-adrenoceptor activation, and myocardial ischemia. We propose that $K_V1.5$ channels are likely to be the predominant subtype, however, the lack of specific pharmacologic agents to activate or inhibit $K_V1.5$ channels currently prevents direct examination of this hypothesis in our large animal preparation. While these studies were performed in normal healthy swine, prior evidence from our laboratory [3] and others [25, 26, 28] indicates that K_V channel function is impaired by obesity and/or diabetes mellitus. Furthermore, coronary microvascular dysfunction has been linked with several other pathologic conditions including hypertension, Takotsubo cardiomyopathy, heart failure with preserved ejection fraction, and no re-flow phenomenon [6, 32, 33]. Thus, further examination of the role of coronary K_V1 channels is likely to provide much needed insight in to physiologic and pathophysiologic mechanisms of coronary flow regulation.

Acknowledgments

This study was supported by U01HL118738 (G. Kassab) and R01HL11760 (J. Tune). Dr. Goodwill was supported by American Heart Association 13POST1681001813. Ms. Noblet and Mr. Sassoon were supported by TL1 TR000162 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award.

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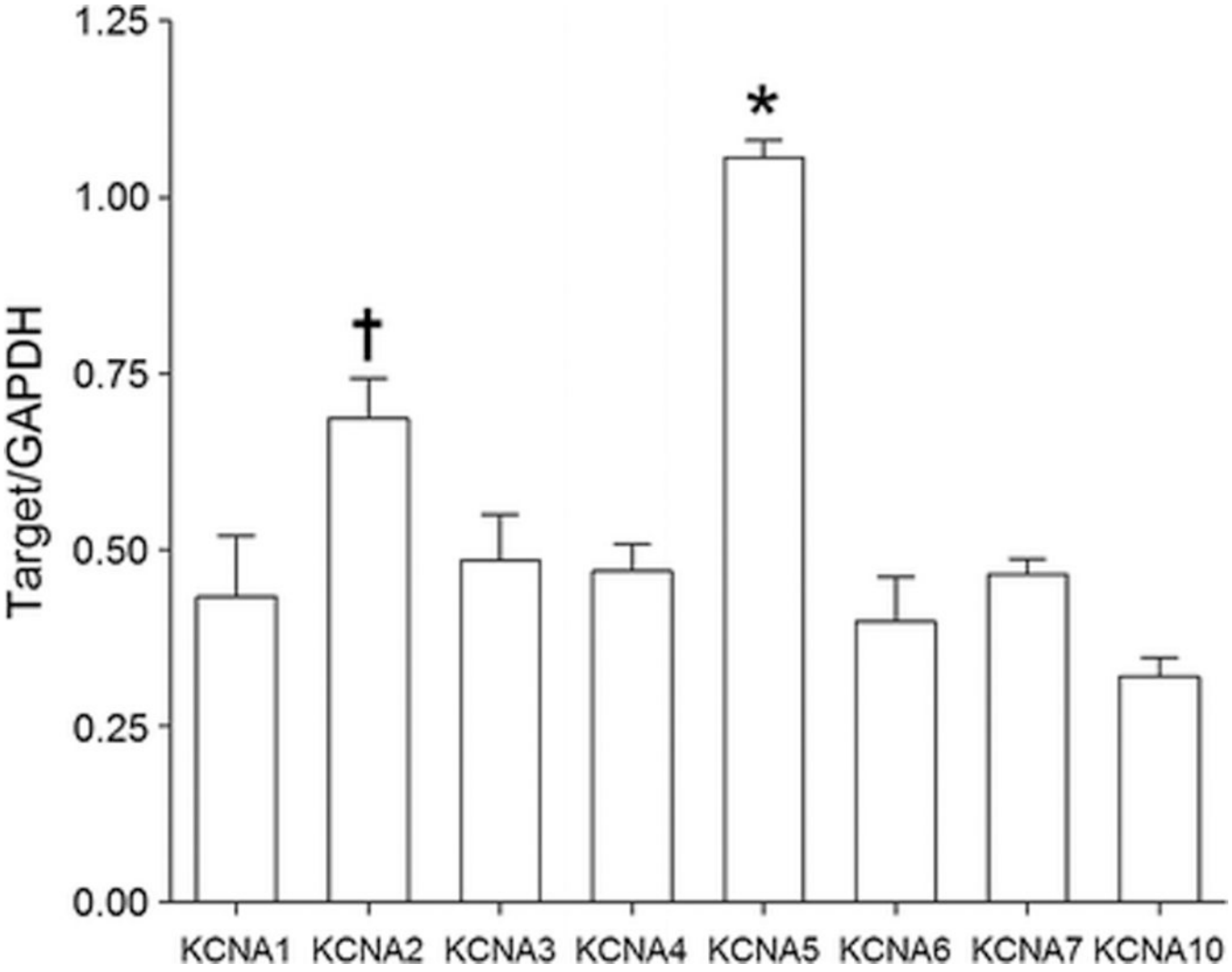

Fig. 1. KCNA gene transcripts are expressed in swine coronary arteries

Group data (samples from n = 3 pigs) for qPCR are shown. KCNA transcripts are normalized to the abundance of a reference gene, GAPDH. KCNA2 and KCNA5 were the most abundant transcripts. Asterisk indicates that the KCNA5 value is higher than that for all other KCNA isoforms, including KCNA2. Dagger indicates that the KCNA2 value is higher than all other KCNA isoforms, excluding KCNA5.

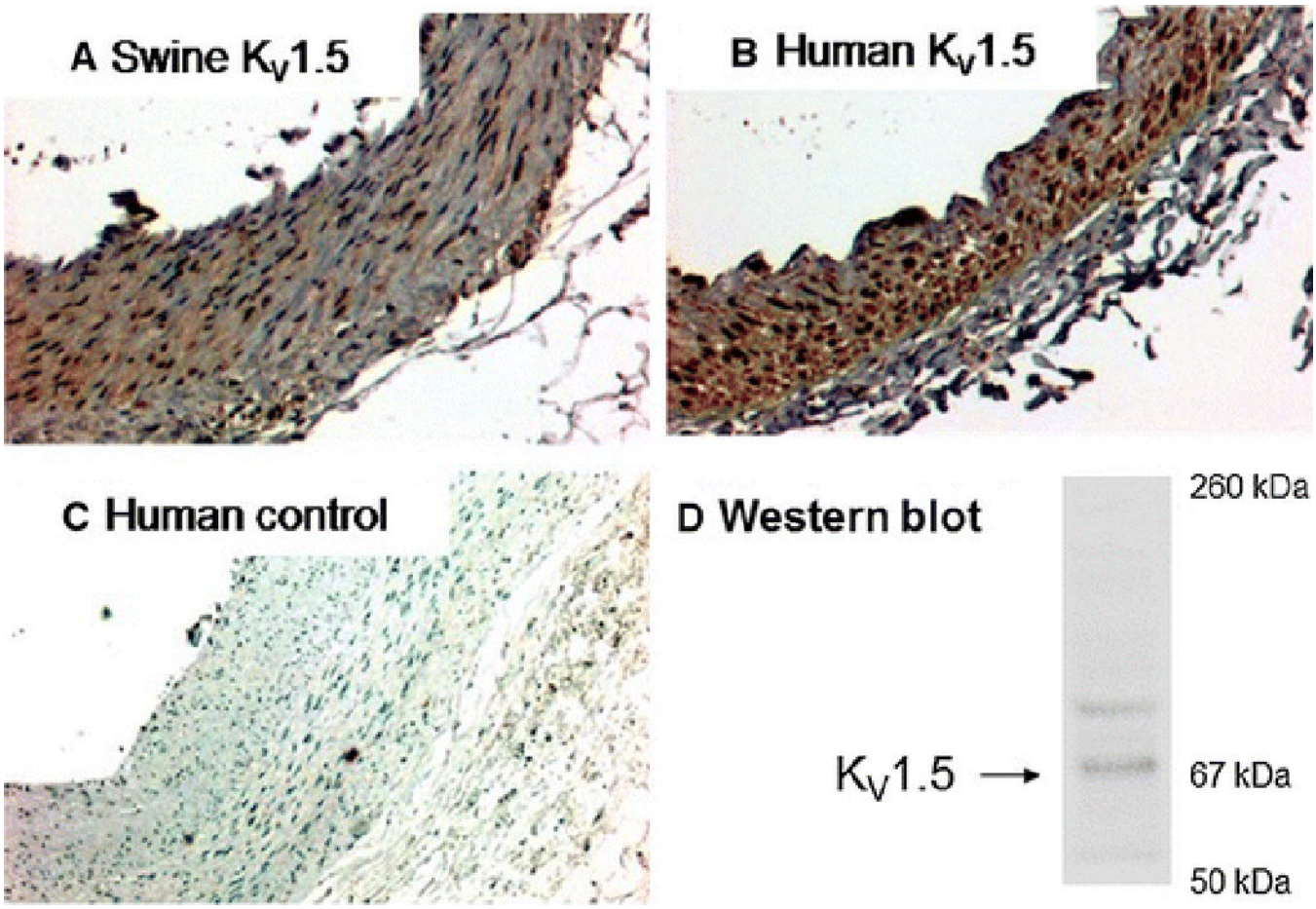

Fig. 2. $K_V1.5$ channel protein is expressed in swine and human coronary arteries

Coronary artery sections from swine and humans are shown. In panels A (swine) and B (human), coronary arteries were stained for $K_V1.5$ (brown indicates positive immunoreactivity). A negative control in human tissue is shown in panel C. Panel D contains a Western blot performed on proteins extracted from the swine coronary artery. $K_V1.5$ immunoreactivity appears at approximately 67 kDa.

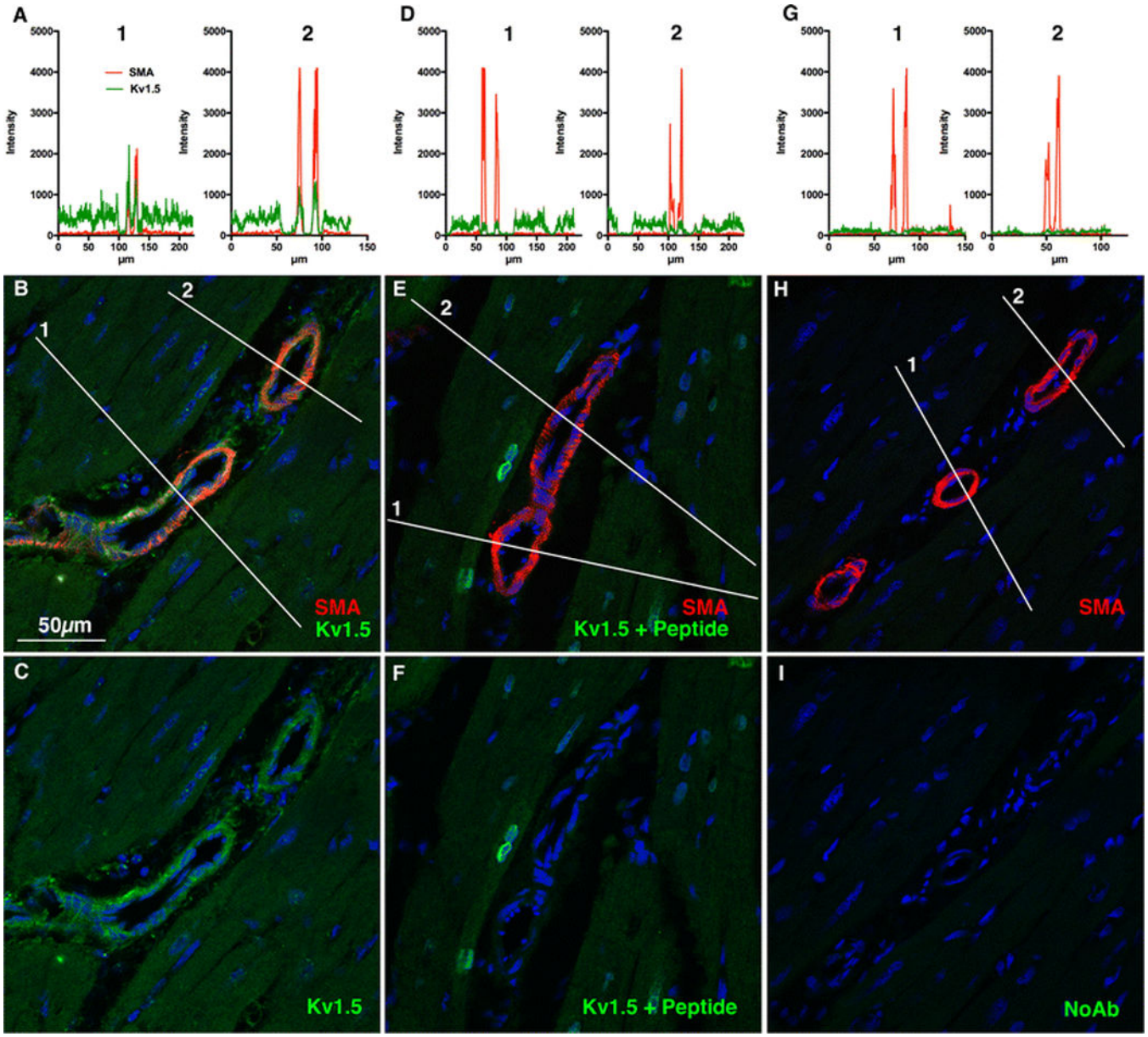

Fig. 3. K_V1.5 channel protein is expressed in the coronary microcirculation

Representative immunofluorescence experiments from swine myocardium are shown. Panel A contains line scans of the intensity of green (K_V1.5) and red (smooth muscle actin, SMA) fluorescence across a section of heart muscle containing resistance-sized vessels (Panel B). Note the spikes in K_V1.5 immunofluorescence intensity that overlap with the peaks of SMA. Panel C shows the green and blue (nuclear) channels from Panel B. Panel D contains line scans of the intensity of green (K_V1.5) and red (SMA) fluorescence across a section of myocardium stained with K_V1.5 antibody pre-adsorbed to its antigenic peptide (Panel E; the green and blue channels are shown in Panel F). Note the lack of green immunofluorescence in the red peaks. Panel G contains line scans of the intensity of green (K_V1.5) and red (SMA) fluorescence across a section of heart incubated without the primary K_V1.5 antibody

(Panel H; the green and blue channels are shown in Panel I). Note the lack of green immunofluorescence.

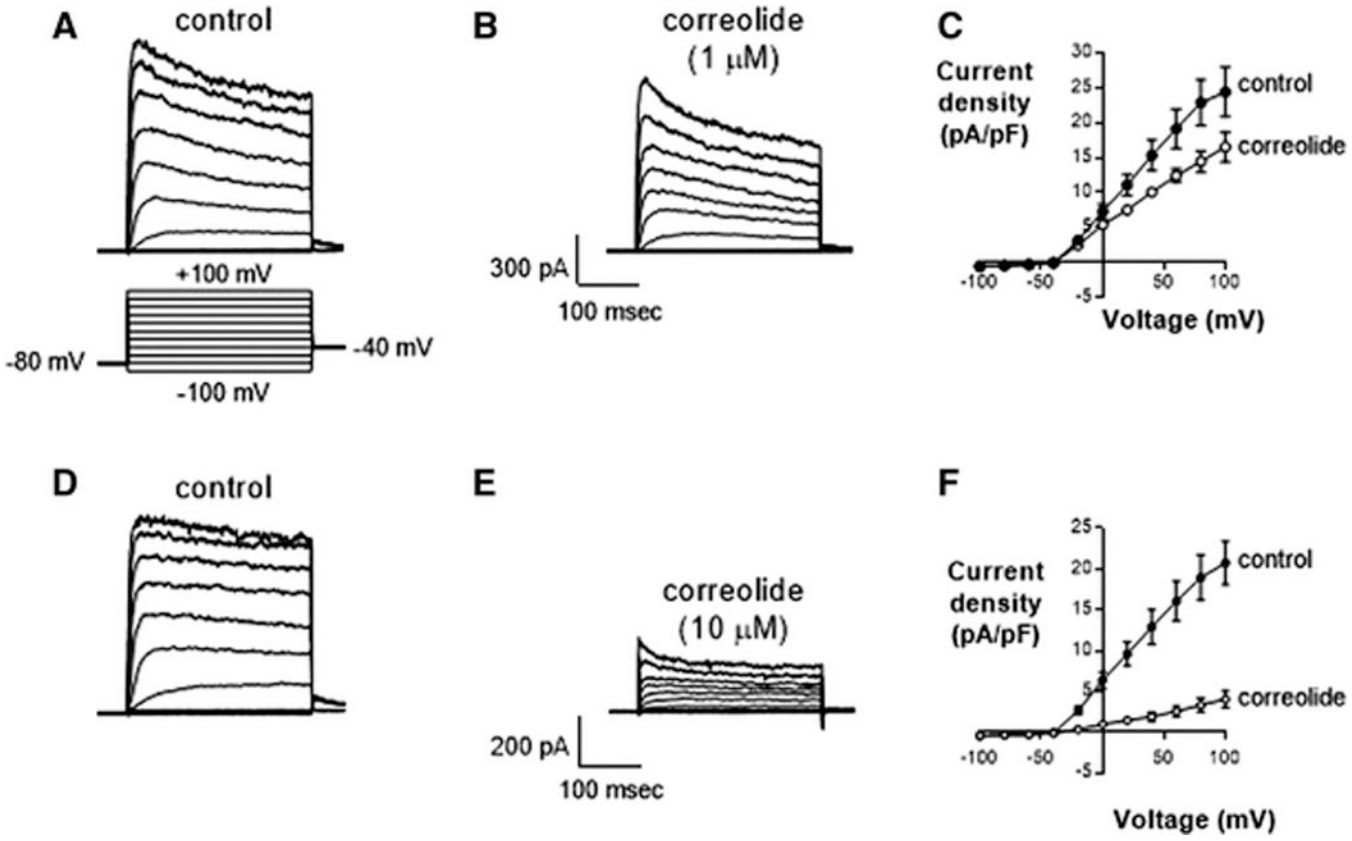

Fig. 4. Correolide inhibits K_V1 current in swine coronary smooth muscle cells

Patch clamp recordings from representative cells are shown for experiments with 1 and 10 μM correolide. Depolarization elicited time- and voltage-dependent currents (panel A; the voltage template is shown below the control trace). Correolide (1 μM) inhibited current approximately 30% (panel B). Panel C contains group data (n = 4 cells) for the current-voltage relationship before and after 1 μM correolide. Current is normalized to membrane capacitance, an index of cell size ($P < 0.05$ for effect of correolide on currents at membrane potentials between +20 and +100 mV). Panels D and E show the effect of 10 μM correolide. This higher concentration of correolide inhibited the majority of whole-cell current in coronary vascular smooth muscle cells. Panel F contains group data (n = 5 cells) for the current-voltage relationship ($P < 0.05$ for currents at membrane potentials between −20 and +100 mV).

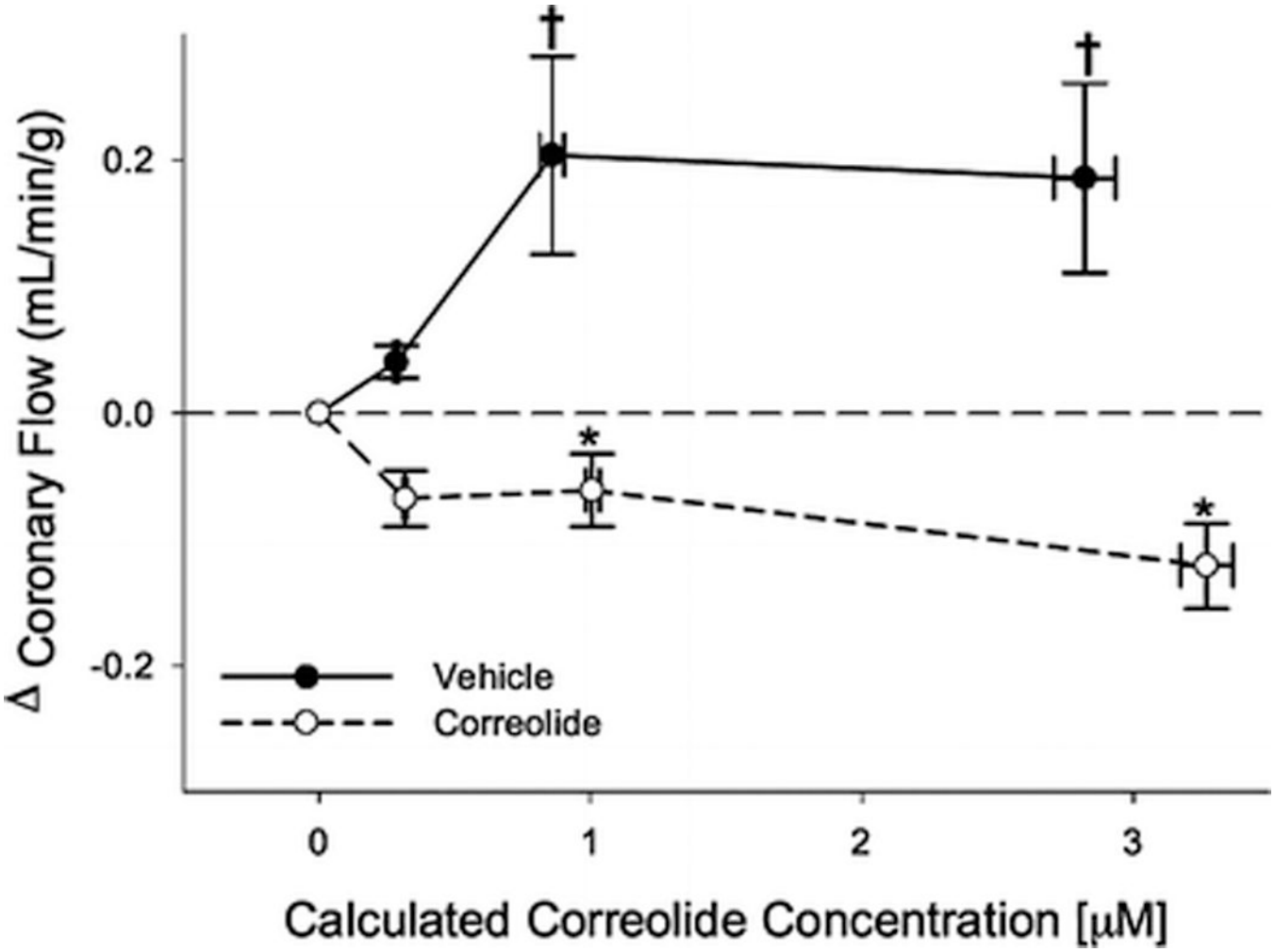

Fig. 5. K_V1 channels regulate coronary vascular resistance at the resting heart rate

Coronary blood flow vs. the calculated intracoronary plasma concentration of correolide is shown. Error bars in the x direction represent variability in the calculated plasma concentrations due to changes in coronary blood flow, as the infusion rate of drug was held constant. Results are from n = 5 pigs in the vehicle group and n = 5 pigs in the correolide-treated group. Daggers indicate the significant effect of vehicle (diluted DMSO) to cause coronary vasodilation (i.e., on average, vehicle alone increased coronary blood flow 0.18 ml/min/g). Asterisks indicate significant correolide-induced coronary vasoconstriction at a constant perfusion pressure of 100 mm Hg.

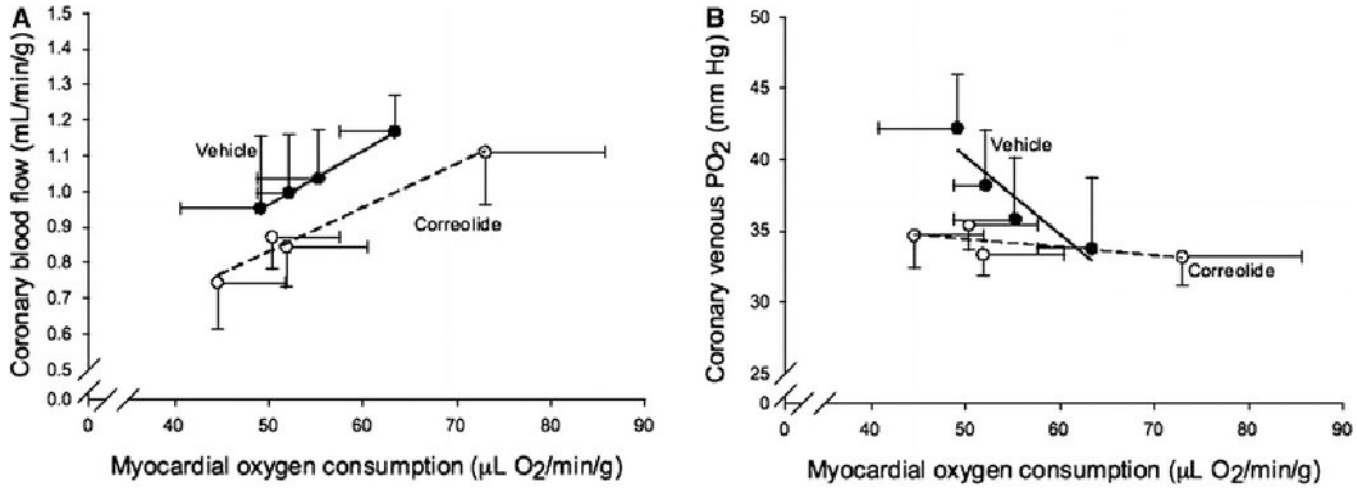

Fig. 6. K_V1 channels function in coronary metabolic vasodilation

Panel A illustrates the relationship between coronary blood flow and myocardial oxygen consumption. Results are from n = 4 pigs in the vehicle group (5 pigs were tested, but all coronary venous blood samples from 1 pig were not collected due to a technical problem) and n = 5 pigs in the correolide-treated group. The rate of myocardial oxygen consumption was increased by infusing dobutamine at 4 increasing dose rates (Table 2). In vehicle-treated pigs, coronary blood flow increased linearly with the rate of myocardial oxygen consumption (filled symbols, solid line). In correolide-treated pigs (3 μM; open symbols, dashed line), the relationship between coronary blood flow and myocardial oxygen consumption was shifted down in a parallel manner ($P < 0.001$). Panel B shows the relationship between coronary venous PO_2 and myocardial oxygen consumption. In the vehicle group, coronary venous PO_2 fell as myocardial oxygen consumption increased. In the correolide-treated group, coronary venous PO_2 was lower at rest and did not fall further as myocardial oxygen consumption increased. The regression lines are not parallel and different ($P < 0.01$).

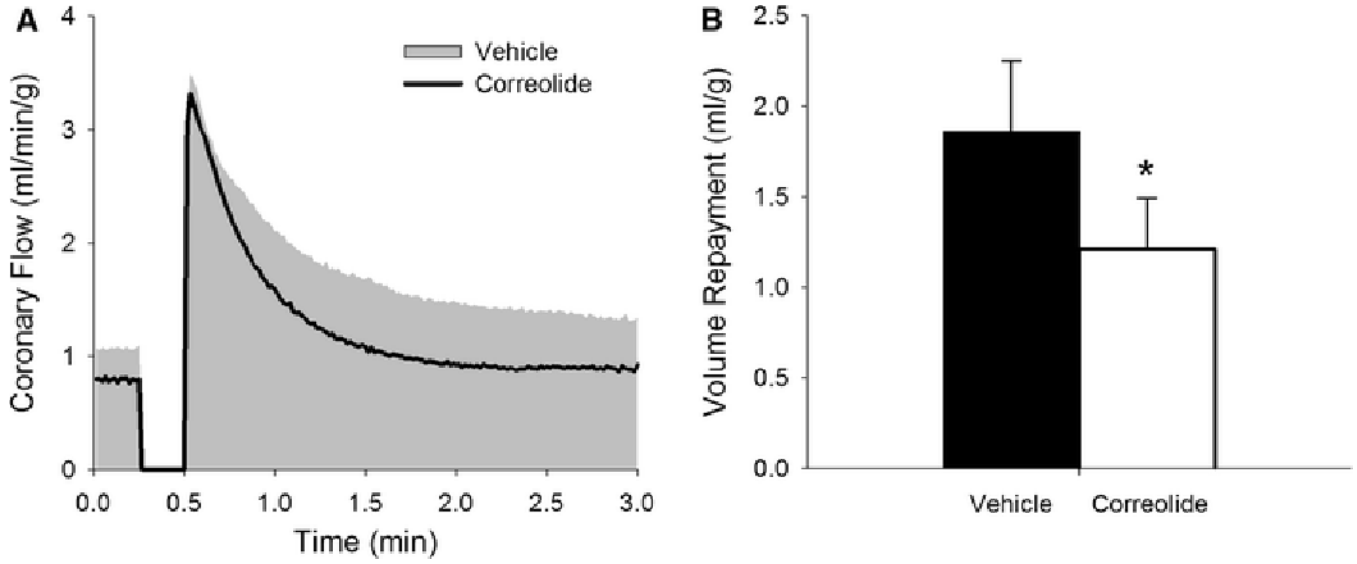

Fig. 7. K_V1 channels participate in ischemic coronary vasodilation: reactive hyperemia

Coronary blood flow over time is shown: before, during, and after a 15 s occlusion. Results are from n = 5 pigs in the vehicle group (grey filled) and n = 5 pigs in the correolide-treated group (3 μM; solid line). Correolide reduced coronary blood flow at rest and accelerated the decay of ischemic hyperemia. Peak hyperemia (i.e., maximal vasodilation) was unaffected by inhibition of K_V1 channels.

Table 1

Primers used for qPCR

Gene	Primers (5' to 3')	Length	Amplicon	Accession number
KCNA1	*F* TTACGAACTGGGTGAGGAGGC	21	165	AB033207.1
	R ACCGAGACGATGGCGATGAC	20		
KCNA2	*F* GGCAGCTAGAAGGCGTAGGG	20	124	NM_001110418.1
	R CCTCTGGGTCATAGGTGTCCTG	22		
KCNA3	*F* CAGCTTCGACGCCATCCT	18	137	XM_001924029.3
	R CCTCGCGGAACTTCTCCAT	19		
KCNA4	*F* GGAGGATGAGGGTTTTGTGAGG	22	132	XM_005658133.1
	R ACGGAGACGATGGCTATGCC	20		
KCNA5	*F* CGGAGGAAGAGGAGGGAGAT	20	105	NM_001006593.2
	R CCGAGATATTGATGAGGACGC	21		
KCNA6	*F* GCGCTCTGGAGTTCGTGTTGT	21	145	XM_003481699.2
	R GGAAGTCTCCCGCATCCTGTT	21		
KCNA7	*F* GATGACCCGTTCTTTGTGGTG	21	148	XM_005664712.1
	R CGAAGTAGGGCAGAATAGCCAC	22		
KCNA10	*F* ATCTGGCGGGAAAATCCG	18	155	XM_003125855.1
	R TGTCATTGGAGGGGAGCAGT	20		
GAPDH	*F* TGGGAAACTGTGGCGTGAT	19	123	AF017079.1
	R AAGGCCATGCCAGTGAGC	18		

Table 2

Hemodynamic parameters during metabolic vasodilation: dobutamine challenge in vehicle- (n = 4) and correolide-treated (3 μM; n = 5) swine.

Dobutamine Dose (μg/kg/min)	Group	Heart Rate (beats/min)	Systolic Pressure (mm Hg)	Diastolic Pressure (mm Hg)	Mean Pressure (mm Hg)
Baseline	Vehicle	82 ± 6	84 ± 4	55 ± 2	69 ± 3
	Correolide	87 ± 11	77 ± 9	49 ± 6	62 ± 8
0.3	Vehicle	86 ± 9	83 ± 12	55 ± 6	68 ± 9
	Correolide	82 ± 5	78 ± 10	49 ± 8	62 ± 10
1	Vehicle	92 ± 10	89 ± 11	59 ± 6	73 ± 8
	Correolide	86 ± 5	78 ± 11	51 ± 7	64 ± 10
3	Vehicle	98 ± 12	113 ± 8	75 ± 4^{a}	91 ± 6^{a}
	Correolide	96 ± 11	86 ± 11	55 ± 8^{b}	70 ± 10
10	Vehicle	117 ± 20^{a}	126 ± 5^{a}	81 ± 4^{a}	98 ± 4^{a}
	Correolide	113 ± 13^{a}	97 ± 9^{ab}	58 ± 8^{b}	74 ± 9^{ab}

The study contained 10 swine (5 each in the correolide- and vehicle-treated groups); however, in this experiment (Table 2 and Fig. 6), a technical problem in measuring MVO_2 reduced the number of observations in the vehicle only group to n = 4.

"a" denotes a difference from baseline within a group (i.e., a significant effect of dobutamine),

"b" indicates a difference between groups at a given dose of dobutamine (i.e., a significant effect of correolide).

Basic Res Cardiol. Author manuscript; available in PMC 2017 September 01.