Regulation of myocardial oxygen delivery in response to graded reductions in hematocrit: Role of K⁺ channels

Alexander M. Kiel¹,², Adam G. Goodwill¹, Jillian N. Noblet¹, April L. Barnard¹, Daniel J. Sassoon¹, Johnathan D. Tune¹

¹Department of Cellular & Integrative Physiology, Indiana University School of Medicine, ²Weldon School of Biomedical Engineering, Purdue University

Running Title: Mechanisms of Anemic Coronary Vasodilation

Correspondence:
Johnathan D. Tune, PhD
Department of Cellular & Integrative Physiology
Indiana University School of Medicine
635 Barnhill Drive
Indianapolis, IN 46202
Phone: 317-274-3433
Email: jtune@iu.edu

This is the author's manuscript of the article published in final edited form as:
ABSTRACT

This study was designed to identify mechanisms responsible for coronary vasodilation in response to progressive decreases in hematocrit. Isovolemic hemodilution was produced in open-chest, anesthetized swine via concurrent removal of 500 ml of arterial blood and the addition of 500 ml of 37°C saline or synthetic plasma expander (Hespan, 6% hetastarch in 0.9% sodium chloride). Progressive hemodilution with Hespan resulted in an increase in coronary flow from 0.39 ± 0.05 to 1.63 ± 0.16 ml/min/g (P < 0.001) as hematocrit was reduced from 32 ± 1% to 10 ± 1% (P < 0.001). Overall, coronary flow corresponded with the level of myocardial oxygen consumption, was dependent on arterial pressures ≥ ~60 mmHg, and occurred with little/no change in coronary venous PO₂. Anemic coronary vasodilation was unaffected by the inhibition of nitric oxide synthase (L-NAME: 25 mg/kg iv; P = 0.92) or voltage-dependent K⁺ (Kᵥ) channels (4-aminopyridine: 0.3 mg/kg iv; P = 0.52). However, administration of the Kₐtp channel antagonist (glibenclamide: 3.6 mg/kg iv) resulted in an ~40% decrease in coronary blood flow (P < 0.001) as hematocrit was reduced to ~10%. These reductions in coronary blood flow corresponded with significant reductions in myocardial oxygen delivery at baseline and throughout isovolemic anemia (P < 0.001). These data indicate that vasodilator factors produced in response to isovolemic hemodilution converge on vascular smooth muscle glibenclamide-sensitive (Kₐtp) channels to maintain myocardial oxygen delivery and that this response is not dependent on endothelial-derived nitric oxide production or pathways that mediate dilation via Kᵥ channels.

**Keywords:** coronary; anemia; nitric oxide; Kᵥ channels; Kₐtp channels; swine
INTRODUCTION

The coronary circulation is tightly regulated in order to ensure adequate matching between myocardial oxygen delivery and metabolism. This control of coronary blood flow is essential as the myocardium extracts ~70-80 percent of the oxygen delivered while at rest [18, 19, 40, 50]. Thus, any physiologic perturbation that alters the overall balance between oxygen delivery and myocardial oxygen consumption (MVO$_2$) requires the subsequent modulation of coronary microvascular resistance to ensure oxygen supply/demand balance. As such, the coronary circulation has a remarkable ability to increase blood flow upwards of 10-fold (from ~0.5 ml/min/g at rest to ≥ 5.0 ml/min/g with maximal dilation) [63]. Although this intricate coupling has been recognized for many years, our understanding of the underlying mechanisms remains rather limited.

Prior studies to examine the balance between coronary blood flow and MVO$_2$ have established that coronary blood flow increases exponentially (>4-fold) with ~70% reductions in arterial oxygen content in response to hemodilution (anemia), hypoxemia, and carbon monoxide poisoning [9, 29, 36, 37, 39, 43, 54, 64, 66, 71]. This progressive augmentation of coronary flow maintains overall myocardial oxygen delivery and occurs with a ~2-fold increase in MVO$_2$ and little/no change in myocardial oxygen extraction [10, 19, 34, 35, 47, 64, 67, 68]. However, evidence of enhanced myocardial lactate release and impairments to both sub-endocardial blood flow and cardiac contractile function have been reported with more severe reductions in hematocrit (≤ 10%) [1, 3, 37, 45, 64]. Earlier studies have suggested a role for reduced blood viscosity in the coronary response to anemia [5, 31, 33, 46, 62]. However, data demonstrating diminished vasodilator reserve to hemodilution in the presence of a critical coronary stenosis or in response to a brief coronary occlusion (i.e. reactive hyperemia) directly implicate that progressive reductions in hematocrit lead to the activation of vasodilator pathways [7, 9, 22, 31, 37, 67]. Nonetheless, elucidation of the mechanisms responsible for anemic coronary vasodilation has proven challenging. In particular, circulating catecholamine concentrations are not
Mechanisms of Anemic Coronary Vasodilation

significantly altered by reductions in hematocrit to ~9% [64] and consequently, increases in coronary blood flow observed during β-adrenoceptor blockade are sufficient to sustain myocardial oxygen delivery thereby reducing the likelihood that these effects are sympathetically mediated [11]. Inhibition of nitric oxide production with N^G^-nitro-L-arginine methyl ester (L-NAME) was also found to have little/no effect on the coronary blood flow in response to acute, euvoletic reductions in hematocrit from 40% to 20% [8]. However, whether nitric oxide contributes to the coronary dilator response as hematocrit is reduced below 20% has not been determined. Alternatively, end-effector K^+ channels in vascular smooth muscle are regulated by a variety of influences including endogenous endothelial and metabolic factors [25], cellular energy status (ATP/ADP ratio) [13, 25, 48], redox-dependent signaling [53], and the overall degree of oxygenation [21, 28]. Yet, whether these channels contribute to the balance between myocardial oxygen delivery and metabolism in response to progressive hemodilution has not been determined.

The purpose of this study was to identify mechanisms responsible for coronary vasodilation and the maintenance of myocardial oxygen delivery in response to moderate and severe reductions in hematocrit. Experiments were designed to test the hypothesis that the contributions of nitric oxide, voltage-dependent (K_v), and/or ATP-sensitive (K_ATP) K^+ channels progressively increase in response to acute isovolemic hemodilution in open-chest, anesthetized domestic swine. Our findings provide novel insight into the end-effector channels required for anemic coronary vasodilation.
METHODS

All experiments involving animals 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 Publication No. 85-23, Revised 2011). Lean adult male domestic swine (n = 29) were sedated with telazol, xylazine, and ketamine (5, 2.5, and 2.5 mg/kg) prior to anesthesia with morphine (0.5 mg/kg) and intravenous α-chloralose (60 mg/kg).

Experimental preparation

Anesthetized swine were intubated and ventilated with O₂-supplemented room air. Femoral cut downs were performed and catheters placed in the femoral artery and vein for continuous measurement of systemic blood pressure and heart rate and for administration of anesthetic and antagonists, respectively. Succinylcholine (0.5 mg/kg) was administered prior to a thoracotomy in the left 5th intercostal space. The left anterior descending (LAD) coronary artery was isolated and a perivascular flow probe (Transonic Systems Inc.) placed around the artery. A catheter was placed in the interventricular vein to sample coronary venous blood. Systemic heparin (500 units/kg) was administered to prevent clotting in the coronary venous catheter. Following a ~15 min stabilization period, data were continuously recorded on IOX data acquisition software from EMKA Technologies (Falls Church, VA).

Acute isovolemic anemia protocol

Pigs were randomly assigned to one of the following six groups: 1) Control with saline replacement; 2) Control with Hespan (hydroxyethyl starch) replacement; 3) nitric oxide synthesis inhibition with nitro-L-arginine methyl ester (L-NAME, 25 mg/kg iv with Hespan replacement); 4) Kᵥ channel inhibition with 4-aminopyridine (4-AP, 0.3 mg/kg iv with Hespan replacement); 5) Kᵥ ATP channel inhibition glibenclamide (3.6 mg/kg with Hespan replacement); 6) glibenclamide vehicle (equal parts 95% ethanol, 1N NaOH, propylene glycol). Following administration of drugs (for
Mechanisms of Anemic Coronary Vasodilation

groups 3-6) and the ~15 min stabilization period of the animal, arterial and coronary venous blood samples were obtained at baseline and at stepwise serial reductions in hematocrit. Progressive isovolemic anemia was produced by withdrawing 500 ml of arterial blood that was simultaneously replaced by intravenous administration of 500 ml of saline or Hespan, which were warmed to 37°C prior to infusion. Following completion of experimental protocols, hearts were fibrillated and excised as recommended by the American Veterinary Medical Association Guide on Euthanasia.

Blood gas analyses

Arterial and coronary venous blood samples were collected, immediately sealed and placed on ice. The samples were analyzed for pH, PCO₂, PO₂, glucose, lactate, and oxygen content with an Instrumentation Laboratories automatic blood gas analyzer (GEM Premier 3000) and CO-oximeter (682) system. Hematocrit was determined by centrifugation of capillary tubes containing blood collected at each replacement on a StatSpin micro-hematocrit centrifuge (CritSpin M961-22). LAD perfusion territory was estimated to be 30% of total heart weight, as previously described by Feigl [20]. MVO₂ was calculated by multiplying coronary blood flow by the arterial coronary venous difference in oxygen content.

Statistical analysis

Data are presented as mean ± SE. Statistical comparisons for data presented in Tables 1 and 2 were made by a two-way analysis of variance (ANOVA; Factor A: drug treatment; Factor B: level of hematocrit). Experimental variables were averaged within and between animals relative to the following hematocrit levels: (≥ 28.0) → (27.9 – 22.0) → (21.9 – 17.0) → (16.9 – 11.0) → (≤ 10.9). Differences were considered statistically significant when $P < 0.05$. If significance with ANOVA was detected, a Student–Newman–Keuls multiple comparison test was performed. The relationship between coronary blood flow and hematocrit was fit to an inverse, second-order equation for each animal and a statistical comparison (t-test) of the predicted coronary flow based
on the fit equation was performed at hematocrits of 10%, 20% and 30% to establish differences in relationships between treatments. Multiple linear regression analysis was used to compare slopes of response variables (oxygen delivery, coronary venous PO₂) plotted vs. hematocrit or MVO₂. 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 hematocrit or MVO₂. Statistical analyses were performed with Sigma Plot 11.0 software (Systat Software Inc., San Jose, CA, USA), ANCOVA analyses were performed with VassarStats (Arlington, New York, USA).
RESULTS

Control responses to isovolemic hemodilution

Hemodynamic and coronary responses to graded reductions in hematocrit in untreated control swine that received volume replacement with saline or the synthetic colloid Hespan (hydroxyethyl starch) are shown in Figure 1. Saline based isovolemic hemodilution produced significant decreases in blood pressure (from 74 ± 6 mmHg to 26 ± 2 mmHg; Figure 1A; P < 0.01) and MVO₂ (from 61 ± 4 to 27 ± 4 ml O₂/min/g; Figure 1B; P < 0.01). These reductions were associated with minimal change in coronary blood flow (0.49 ± 0.02 to 0.40 ± 0.06 ml/min/g; Figure 1C; P = 0.63) and marked decreases in myocardial oxygen delivery (78 ± 4 to 36 ± 6 ml O₂/min/g; Figure 1D; P < 0.001) as hematocrit was reduced from ~35% to ~15%. In contrast, aortic blood pressure (Figure 1A) and MVO₂ (Figure 1B) were not significantly altered by hemodilution in swine that received Hespan (Table 1). With the relative maintenance of blood pressure and MVO₂, coronary blood flow increased ~4-fold as hematocrit was reduced to ≤10% (Figure 1C and Table 1). This increase in coronary blood flow was sufficient to maintain myocardial oxygen delivery at ~53 ± 5 µl O₂/min/g (Figure 1D). However, examination of electrocardiograms revealed evidence of sub-endocardial ischemia (ST segment depression and T wave inversion) under these conditions (Figure 2).

Examination of coronary responses relative to changes in aortic pressure (i.e. coronary perfusion pressure), heart rate, and MVO₂ are provided in Figure 3. These relationships demonstrate that coronary blood flow (Figure 3A) remained relatively constant over a wide range of blood pressures, down to ~40 mmHg, in swine that received saline replacement. However, myocardial oxygen delivery progressively decreased as aortic pressure fell with hemodilution in these animals (Figure 3B). In contrast, coronary vasodilation in response to isovolemic hemodilution in Hespan infused animals was not influenced by underlying changes in aortic pressure (Figure 3A) but was directly related to increases in heart rate (Figure 3C) and MVO₂ (Figure 3D). Interestingly, coronary venous PO₂ remained unchanged with reductions in
hematocrit (Figure 3E; \(P = 0.97\)) and was unaffected by underlying differences in MVO\(_2\) in swine that received Hespan (Figure 3F; \(P = 0.90\)). In contrast, coronary venous PO\(_2\) decreased as MVO\(_2\) increased in swine that received saline replacement (Figure 3F; \(P < 0.01\)).

**Role of nitric oxide in anemic coronary vasodilation**

Inhibition of nitric oxide synthase with L-NAME resulted in a significant increase in mean arterial pressure from 92 ± 4 to 124 ± 6 mmHg (Table 1; \(P < 0.001\)) at baseline. This was associated with a significant increase in MVO\(_2\), from 41 ± 4 to 50 ± 5 µl O\(_2\)/min/g (Table 1; \(P = 0.013\)) at baseline. These effects of nitric oxide inhibition were evident throughout the isovolemic anemia protocol. Despite these hemodynamic effects, inhibition of nitric oxide did not affect coronary blood flow (Figure 4A; \(P = 0.36\)), myocardial oxygen delivery (Figure 4B; \(P = 0.92\)), or heart rate (Table 1; \(P = 0.521\)) as hematocrit was decreased from 32 ± 2% to 9 ± 1%. However, administration of L-NAME diminished coronary venous PO\(_2\) (Table 1; \(P = 0.002\)), primarily at higher hematocrits (Figure 4C; \(P < 0.01\)) and levels of MVO\(_2\) (Figure 4D; \(P = 0.03\)).

**Role of \(K_V\) and \(K_{ATP}\) channels in anemic coronary vasodilation**

Blockade of \(K_V\) channels with 4-AP did not significantly affect blood pressure (\(P = 0.097\)), heart rate (\(P = 0.195\)), or MVO\(_2\) (\(P = 0.38\)) as hematocrit was reduced from 33 ± 1% to 8 ± 1% (Table 1). Inhibition of \(K_V\) channels also did not significantly alter coronary blood flow (Figure 5A; \(P = 0.21\)) or myocardial oxygen delivery (Figure 5B; \(P = 0.63\)) at baseline or in response to isovolemic anemia. In contrast, 4-AP significantly decreased coronary venous PO\(_2\) (Table 1; \(P < 0.001\)) irrespective of underlying hematocrit (Figure 5C; \(P < 0.001\)) or MVO\(_2\) (Figure 5D; \(P < 0.001\)).

Inhibition of \(K_{ATP}\) channels with glibenclamide had no effect on blood pressure (\(P = 0.541\)) or heart rate (\(P = 0.139\)), however, MVO\(_2\) decreased ~35% relative to vehicle-control swine,
irrespective of underlying hematocrit (Table 2; $P < 0.001$). Administration of glibenclamide induced significant decreases in coronary blood flow (Table 2; $P < 0.001$) as hematocrit was reduced from $32 \pm 1\%$ to $9 \pm 1\%$ (Figure 6A; $P < 0.001$). These reductions in coronary blood flow corresponded with significant reductions in myocardial oxygen delivery at baseline and throughout isovolemic anemia (Figure 6B; $P < 0.001$). Glibenclamide also significantly decreased coronary venous PO$_2$, (Table 2; Figure 6C; $P = 0.003$) but did not significantly alter the relationship between coronary venous PO$_2$ and MVO$_2$ (Figure 6D; $P = 0.36$).
DISCUSSION

This investigation was designed to identify mechanisms responsible for coronary vasodilation and the maintenance of myocardial oxygen delivery in response to moderate and severe reductions in hematocrit. Experiments tested the hypothesis that the contribution of nitric oxide, $K_V$, and/or $K_{ATP}$ channels increase in response to acute isovolemic hemodilution in open-chest, anesthetized domestic swine. Our findings are consistent with prior studies which have demonstrated that progressive augmentation of coronary blood flow in response to anemia is sufficient to maintain overall myocardial oxygen delivery, although subendocardial ischemia is apparent as hematocrit falls to ≤ 10% [1, 3, 37, 45, 64]. Overall, coronary blood flow corresponds with MVO$_2$, is dependent on arterial driving pressures ≥ 60 mmHg, and occurs with little/no change in myocardial oxygen extraction (coronary venous PO$_2$) [10, 19, 34, 35, 47, 64, 67, 68]. The major novel findings of this study are that inhibition of $K_{ATP}$ channels with glibenclamide significantly attenuates anemic coronary vasodilation and that this response occurs independent of alterations in endothelial nitric oxide production or the activation of $K_V$ channels. These data are the first to implicate that vasodilator factors produced in response to graded reductions in hematocrit (arterial oxygen content) converge on vascular smooth muscle glibenclamide-sensitive $K_{ATP}$ channels to mediate increases in coronary blood flow and maintain myocardial oxygen delivery.

Myocardial Oxygen Supply/Demand Balance During Acute Isovolemic Anemia

It is well established that reductions in hematocrit lead to marked hemodynamic responses including increases in cardiac output, heart rate, contractility, and MVO$_2$ [8, 30, 37, 64], all of which are important determinants of coronary blood flow [25]. In the present study, we noted significant differences in the coronary response to hemodilution in swine that received volume replacement with saline vs Hespan. We propose that the lack of a change in coronary blood flow to progressive anemia in the saline group (Figure 1C) is most likely due to marked reductions in arterial pressure which fell beyond the normal autoregulatory range at relatively high (~30%)
Mechanisms of Anemic Coronary Vasodilation

hematocrits (Figure 1A; Figure 3A). These findings support that adequate coronary perfusion pressure (≥ 60 mmHg) is required to ensure the maintenance of myocardial oxygen delivery in response to progressive reductions in hematocrit. Importantly, when arterial pressure is maintained by volume replacement with Hespan, increases in coronary blood flow are directly related to the degree of hemodilution (Figure 1) and reductions in coronary vascular resistance. The central question surrounding this phenomenon is “how” changes in hematocrit induce increases in coronary blood flow precisely to the degree necessary to preserve myocardial oxygen delivery.

The simplest explanation for reductions in coronary vascular resistance in response to anemia is a reduction of blood viscosity. While analysis of vascular hindrance (resistance/viscosity) supports a role for viscosity at hematocrits ranging from ~60% to 20% [33], studies which have documented diminished vasodilator reserve to hemodilution in the presence of a critical coronary stenosis or in response to a brief coronary occlusion (i.e. reactive hyperemia) directly demonstrate that progressive reductions in hematocrit lead to the activation of vasodilator pathways [7, 9, 22, 31, 37, 67]. We propose that the discrepant coronary responses to volume replacement with saline are not related to differences in the viscosity as the lower dynamic viscosity of saline vs. Hespan would be predicted to augment the overall degree of anemic coronary vasodilation. However, reductions in arterial pressure in swine that received saline confound interpretation of the role of viscosity.

Although it is apparent that coronary vasodilation occurs in response to anemia, the mechanisms responsible for anemic coronary dilation have remained elusive. More specifically, how changes in hematocrit are sensed is simply not understood. Classically, changes in myocardial tissue PO2, which are indexed by changes in coronary venous PO2, are proposed to invoke the production of vasodilator factors that act to increase coronary blood flow and restore tissue PO2 to normal levels via negative feedback loop [25]. However, the consistency of coronary venous PO2 (myocardial oxygen extraction) as hematocrit is lowered to < 10% (Figure 3) has
been found in rats [69], dogs [7, 12, 37, 61], pigs [64], baboons [68], and humans [24] and directly argues against this traditional paradigm. How and why coronary venous PO2 remains unchanged, even during severe hemodilution when ischemia is evident, is still yet another mystery. Proposed mechanisms for this paradoxical response include the flow-limited diffusion of oxygen, alterations in oxygen binding properties of blood due to altered plasma protein and buffer content, and the diminished release of oxygen by erythrocytes due to reduced intracellular convection [37, 67].

Role for Nitric Oxide in Anemic Coronary Vasodilation

Nitric oxide is an endothelial-derived vasodilating factor whose release is stimulated by pharmacological agonists (e.g. acetylcholine and bradykinin) and mechanical stimulation of the endothelium via shear stress, pulsatile flow, and/or axial strain [4, 38, 58, 59]. Prior studies also indicate that nitric oxide is scavenged and transported by hemoglobin in the form of S-nitrosohemoglobin [41, 57]. To examine the role of nitric oxide in the regulation of coronary blood flow during progressive reductions in hematocrit, we performed isovolemic hemodilution experiments in the absence and presence of the nitric oxide synthase blocker L-NAME. While the inhibition of nitric oxide production resulted in significant (> 25 mmHg) increases in blood pressure (Table 1) and increased myocardial oxygen extraction at higher hematocrits (>20%) (Figure 4C), L-NAME had essentially no effect on coronary blood flow (Figure 4A) or myocardial oxygen delivery (Figure 4B) at hematocrits ranging from ~30% to ~10%. These data are consistent with the prior studies by Crystal et al. in dogs which documented no effect of intracoronary L-NAME on anemic coronary vasodilation down to hematocrits of ~20% [8]. Importantly, the use of intracoronary L-NAME prevented changes in systemic blood pressure and thus argue against hypertension as a confounding influence in the present study. The lack of effect of either systemic or intracoronary L-NAME demonstrates that alterations in nitric oxide bioavailability or endothelial shear stress do not play a significant role in modulating coronary blood flow in response to progressive isovolemic hemodilution.
Role for $K^+$ Channels in Anemic Coronary Vasodilation

$K^+$ channels dominate membrane conductance of coronary vascular smooth muscle and serve as important end-effector mechanisms of endogenous and exogenous vasodilator compounds [17, 25]. In particular, $K_V$ channels have been shown to contribute to the control of coronary blood flow at rest, during increases in $MVO_2$, and following a brief coronary artery occlusion [2, 16, 26, 27, 49, 53]. To test the hypothesis that anemic coronary vasodilation is mediated by endogenous factors that converge on $K_V$ channels, we performed isovolemic hemodilution experiments in the absence and presence of the non-selective $K_V$ channel inhibitor 4-AP [51-53]. Similar to our results with L-NAME, we found that 4-AP diminished coronary venous $PO_2$ primarily at higher hematocrits ($\geq 20\%$) (*Table 1; Figure 5C*). However, inhibition of $K_V$ channels did not significantly influence anemic coronary vasodilation as 4-AP had no effect on coronary blood flow (*Figure 5A*), myocardial oxygen delivery (*Figure 5B*) in response to progressive hemodilution. Prior studies from our laboratory have documented that the 0.3 mg/kg dose used in this investigation is sufficient to significantly impair coronary vasodilation and the balance between myocardial oxygen delivery and metabolism in response to increases in $MVO_2$ and to a brief coronary artery occlusion [2, 16, 53]. These findings importantly demonstrate that 4-AP significantly reduces coronary blood flow, under specific physiological conditions, and indicate that the vasodilator “metabolites” produced in response to progressive anemia are different than those produced in response to exercise or acute myocardial ischemia. While we cannot rule out potential effects of 4-AP on cardiomyocytes or other $K^+$ channel subtypes, it is noteworthy that 4-AP did not produce any changes in the ECG or $MVO_2$ (*Table 1*) and that similar doses of 4-AP do not significantly influence coronary vasodilation in response to the $K_{ATP}$ channel agonist pinacidil [16]. Taken together, our findings do not support a requisite role for $K_V$ channels, or the pathways that converge on these channels, in mediating coronary vasodilation in response to hemodilution.
K\textsubscript{ATP} channels are highly expressed in coronary vascular smooth muscle and are known to be activated by cellular energetic state (ATP/ADP ratio), intracellular pH, and by pathophysiologic conditions such as hypoxia and ischemia [14, 17]. However, whether isovolemic hemodilution mediates coronary vasodilation via activation of K\textsubscript{ATP} channels has not been previously investigated. Data from this study are the first to demonstrate that the inhibition of K\textsubscript{ATP} channels with glibenclamide markedly diminishes increases in coronary blood flow (Figure 6A) and myocardial oxygen delivery (Figure 6B) in response to progressive reductions in hematocrit. It is important to point out that glibenclamide significantly decreased MVO\textsubscript{2} by \(-45-50\%\) at all levels of hematocrit (Table 2) and that such reductions in MVO\textsubscript{2} could result in decreases in coronary blood flow, possibly via effects on mitochondrial K\textsubscript{ATP} channels [25]. However, we propose that this is likely not the case as the progressive reduction of the coronary blood flow response to decreases in hematocrit in glibenclamide treated swine (Figure 6A) does not correspond with augmented decreases in MVO\textsubscript{2} (Table 2). Furthermore, the Bache laboratory previously documented that glibenclamide-mediated reductions in MVO\textsubscript{2} are restored to normal levels by increasing coronary blood flow with intracoronary sodium nitroprusside [32]. This finding combined with additional evidence that the mitochondrial K\textsubscript{ATP} channel antagonist 5-hydroxydecanoate (5-HD) has no effect on MVO\textsubscript{2} supports that glibenclamide-mediated decreases in MVO\textsubscript{2} are the result of coronary vasoconstriction (limitation of myocardial oxygen delivery) rather than primary reductions in mitochondrial respiration per se [6]. However, we acknowledge that we cannot definitively rule out effects of glibenclamide on sarcolemmal or mitochondrial channels in cardiomyocytes and/or the potential for glibenclamide to antagonize other K\textsuperscript{+} channel subtypes (e.g. IK\textsubscript{1} and KV\textsubscript{1}) [56, 70]. Despite the potential for these confounding influences, findings from this investigation directly support that anemia results in the production of factors that mediate coronary dilation via pathways that converge on glibenclamide-sensitive (K\textsubscript{ATP}) channels.
The mechanisms responsible for the activation of K\textsubscript{ATP} channels during hemodilution are yet to be determined. While prior studies indicate that hypoxia-induced hyperpolarization of vascular smooth muscle and vasodilation is diminished by glibenclamide [21], data from the Gutterman laboratory indicate that the direct vasodilator effect of hypoxia on isolated coronary arterioles occurs over a time period of 10-15 min [44]; i.e. development of smooth muscle hypoxia is unlikely to contribute to anemic coronary vasodilation. Evidence of overt myocardial ischemia (ST segment depression, T wave inversion, myocardial lactate release) and contractile dysfunction following more severe reductions in hematocrit (≤ 10%) implicates the activation of ischemic vasodilator pathways such as adenosine could be involved. This hypothesis is supported by earlier studies which have established that myocardial adenosine release increases exponentially with the severity of hypoxia [15, 29, 60, 65] and that the inhibition of adenosine receptors reduces hypoxic coronary vasodilation by ~20-25% [23, 42, 43, 55]. We propose that studies to examine the role of adenosine in response to progressive anemia should include inhibition of not only specific adenosine receptor subtypes (A1, A2A, A2B, and A3 receptors), but also involve experiments to interrogate the potential effects of other purine nucleotides (AMP, ADP), purinergic receptors (various P2Y subtypes), and potentially cyclooxygenase products. Furthermore, based on previous studies we hypothesize that unidentified factors (other than purinergic metabolites) are responsible for the majority (~75-80%) of anemic coronary vasodilation.

**IMPLICATIONS AND CONCLUSIONS**

Data from this study highlight many unanswered questions that remain central to the field of coronary physiology. Namely, how alterations in myocardial oxygen supply and/or MVO\textsubscript{2} are ultimately sensed and regulated. With regard to the coronary response to anemia, it is particularly intriguing that coronary blood flow increases precisely to the degree necessary to maintain oxygen delivery, and that this preservation occurs without a decrease in coronary venous PO\textsubscript{2} (increase
in myocardial oxygen extraction). These findings indicate that reductions in myocardial tissue PO\textsubscript{2} are not required for maintaining myocardial oxygen supply/demand balance and suggest that other “oxygen sensing” mechanisms could be at play. While the present data do not provide evidence to support how progressive reductions in hematocrit are sensed, they do directly implicate a significant role for glibenclamide-sensitive (K\textsubscript{ATP}) channels in the response. Whether the involvement of these channels occurs directly via changes in the energy status of vascular smooth muscle and/or through the production of vasoactive factors that converge on these channels remains to be determined. Importantly, our findings also demonstrate that the anemic coronary vasodilation is not dependent on endothelial-derived nitric oxide production or involve pathways that converge on smooth muscle K\textsubscript{V} channels.

**ACKNOWLEDGEMENTS**

The authors wish to thank Joshua Sturek for expert technical assistance. This study was supported by U01HL118738.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.
REFERENCES


Table 1. Hemodynamic and coronary responses to graded reductions in hematocrit (Hespan replacement) in untreated control, L-NAME, and 4-AP treated swine.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hematocrit</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32 ± 1</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 6</td>
<td>n = 6</td>
</tr>
<tr>
<td>Arterial oxygen content (ml O₂/dl)</td>
<td>14.7 ± 0.4</td>
<td>11.6 ± 0.2*</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min/g)</td>
<td>0.39 ± 0.05</td>
<td>0.54 ± 0.06†</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>92 ± 3</td>
<td>89 ± 4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>64 ± 6</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.47 ± 0.03</td>
<td>7.51 ± 0.02</td>
</tr>
<tr>
<td>Coronary venous pH</td>
<td>7.40 ± 0.03</td>
<td>7.44 ± 0.02</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min/g)</td>
<td>173 ± 23</td>
<td>177 ± 22</td>
</tr>
<tr>
<td>Coronary venous pH</td>
<td>19.4 ± 1.8</td>
<td>20.5 ± 1.4</td>
</tr>
<tr>
<td>MVO₂ (µl O₂/min/g)</td>
<td>41 ± 4</td>
<td>42 ± 5</td>
</tr>
</tbody>
</table>

L-NAME

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hematocrit</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Arterial oxygen content (ml O₂/dl)</td>
<td>15.3 ± 0.3</td>
<td>11.2 ± 0.3°</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min/g)</td>
<td>0.42 ± 0.03</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>124 ± 6¢</td>
<td>130 ± 3¢</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>65 ± 5</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.52 ± 0.01</td>
<td>7.54 ± 0.03</td>
</tr>
<tr>
<td>Coronary venous pH</td>
<td>7.45 ± 0.02</td>
<td>7.43 ± 0.01</td>
</tr>
<tr>
<td>Coronary pO₂ (mmHg)</td>
<td>195 ± 15</td>
<td>194 ± 15</td>
</tr>
<tr>
<td>Coronary venous pO₂ (mmHg)</td>
<td>16.8 ± 2.4</td>
<td>14.5 ± 2.7¢</td>
</tr>
<tr>
<td>MVO₂ (µl O₂/min/g)</td>
<td>50 ± 5</td>
<td>48 ± 4</td>
</tr>
</tbody>
</table>

4-AP

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hematocrit</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Arterial oxygen content (ml O₂/dl)</td>
<td>15.1 ± 0.3</td>
<td>11.7 ± 0.4*</td>
</tr>
<tr>
<td>Coronary blood flow (ml/min/g)</td>
<td>0.37 ± 0.05</td>
<td>0.52 ± 0.06</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>90 ± 7</td>
<td>93 ± 7</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>68 ± 8</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.55 ± 0.02¢</td>
<td>7.53 ± 0.03</td>
</tr>
<tr>
<td>Coronary venous pH</td>
<td>7.48 ± 0.03¢</td>
<td>7.48 ± 0.02</td>
</tr>
<tr>
<td>Coronary pO₂ (mmHg)</td>
<td>176 ± 17</td>
<td>178 ± 15</td>
</tr>
<tr>
<td>Coronary venous pO₂ (mmHg)</td>
<td>15.7 ± 1.2</td>
<td>14.3 ± 1.5¢</td>
</tr>
<tr>
<td>MVO₂ (µl O₂/min/g)</td>
<td>39 ± 3</td>
<td>47 ± 4</td>
</tr>
</tbody>
</table>

* = P < 0.05 vs. baseline hematocrit, same treatment. † = P < 0.05 vs. control, same level of hematocrit.
Table 2. Hemodynamic and coronary responses to graded reductions in hematocrit (Hespan replacement) in vehicle and glibenclamide treated swine.

<table>
<thead>
<tr>
<th>Hematocrit (%)</th>
<th>Drug</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide</td>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 4</td>
<td>n = 3</td>
</tr>
<tr>
<td>Arterial oxygen content (ml O₂/dl)</td>
<td>14.8 ± 0.5</td>
<td>11.4 ± 0.2*</td>
</tr>
<tr>
<td></td>
<td>0.53 ± 0.07</td>
<td>0.66 ± 0.07</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>100 ± 4</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69 ± 11</td>
<td>64 ± 2</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.47 ± 0.04</td>
<td>7.44 ± 0.03</td>
</tr>
<tr>
<td>Coronary venous pH</td>
<td>7.40 ± 0.04</td>
<td>7.38 ± 0.03</td>
</tr>
<tr>
<td>Arterial pO₂ (mmHg)</td>
<td>165 ± 29</td>
<td>159 ± 26</td>
</tr>
<tr>
<td>Coronary venous pO₂ (mmHg)</td>
<td>15.3 ± 1.7</td>
<td>14.8 ± 0.9†</td>
</tr>
<tr>
<td>MVO₂ (μl O₂/min/g)</td>
<td>66 ± 6†</td>
<td>66 ± 5†</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>Arterial oxygen content (ml O₂/dl)</td>
<td>14.9 ± 0.4</td>
<td>11.7 ± 0.3*</td>
</tr>
<tr>
<td>Coronaory blood flow (ml/min/g)</td>
<td>0.30 ± 0.03‡</td>
<td>0.40 ± 0.04‡</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>107 ± 7</td>
<td>102 ± 12</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>56 ± 7</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.46 ± 0.04</td>
<td>7.40 ± 0.04</td>
</tr>
<tr>
<td>Coronary venous pH</td>
<td>7.37 ± 0.04</td>
<td>7.33 ± 0.04</td>
</tr>
<tr>
<td>Arterial pO₂ (mmHg)</td>
<td>200 ± 31</td>
<td>209 ± 31</td>
</tr>
<tr>
<td>Coronary venous pO₂ (mmHg)</td>
<td>10.8 ± 1.7‡</td>
<td>12.2 ± 1.4</td>
</tr>
<tr>
<td>MVO₂ (μl O₂/min/g)</td>
<td>42 ± 4‡</td>
<td>43 ± 4‡</td>
</tr>
</tbody>
</table>

* = P < 0.05 vs. baseline hematocrit, same treatment. † = P < 0.05 vs. control, same level of hematocrit. ‡ = P < 0.05 vs. vehicle, same level of hematocrit.
Figure 1. Relationship between aortic blood pressure (A), myocardial oxygen consumption (B), coronary blood flow (C) and myocardial oxygen delivery (D) vs. hematocrit for control swine that received volume replacement with saline and Hespan.
Figure 2. Representative tracing of the effects of decreasing hematocrit on ECG and coronary blood flow over time in untreated control swine that received volume replacement with Hespan.
Figure 3. Relationship between coronary blood flow (A) and myocardial oxygen delivery (B) vs. aortic pressure, coronary blood flow vs heart rate (C) and myocardial oxygen consumption (D), and coronary venous oxygen partial pressure vs. hematocrit (E) and myocardial oxygen consumption (F) for control swine that received volume replacement with saline and Hespan.
Mechanisms of Anemic Coronary Vasodilation

Figure 4. Relationship between coronary blood flow (A), myocardial oxygen delivery (B), and coronary venous oxygen tension (C) vs. hematocrit and coronary venous oxygen tension vs. myocardial oxygen consumption (D) for control and L-NAME treated swine that received volume replacement with Hespan.
Figure 5. Relationship between coronary blood flow (A), myocardial oxygen delivery (B), and coronary venous oxygen tension (C) vs. hematocrit and coronary venous oxygen tension vs. myocardial oxygen consumption (D) for control and 4-AP treated swine that received volume replacement with Hespan.
Figure 6. Relationship between coronary blood flow (A), myocardial oxygen delivery (B), and coronary venous oxygen tension (C) vs. hematocrit and coronary venous oxygen tension vs. myocardial oxygen consumption (D) for vehicle and glibenclamide treated swine that received volume replacement with Hespan.