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

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Running Title: Mechanisms of Anemic Coronary Vasodilation

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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 $32 \pm 1\%$ to $10 \pm 1\%$ ($P < 0.001$). Overall, coronary flow corresponded with the level of myocardial oxygen consumption, was dependent on arterial pressures $\geq$ ~60 mmHg, and occurred with little/no change in coronary venous PO_2. 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^+ (K_V) channels (4-aminopyridine: 0.3 mg/kg iv; $P = 0.52$). However, administration of the K_{ATP} 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 (K_{ATP}) 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 K_V channels.

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

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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 of the coronary blood flow in response to acute, euvolemic 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 O_2-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) K_V channel inhibition with 4-aminopyridine (4-AP, 0.3 mg/kg iv with Hespan replacement); 5) K_{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

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, PCO_2, PO_2, 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]. MVO_2 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 PO_2) plotted vs. hematocrit or MVO_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 hematocrit or MVO_2. 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 MVO_2 (from 61 ± 4 to 27 ± 4 ml O_2/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 O_2/min/g; **Figure 1D**; $P < 0.001$) as hematocrit was reduced from ~35% to ~15%. In contrast, aortic blood pressure (**Figure 1A**) and MVO_2 (**Figure 1B**) were not significantly altered by hemodilution in swine that received Hespan (**Table 1**). With the relative maintenance of blood pressure and MVO_2, 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 O_2/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 MVO_2 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 MVO_2 (**Figure 3D**). Interestingly, coronary venous PO_2 remained unchanged with reductions in

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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 $32 \pm 2\%$ to $9 \pm 1\%$. 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 $33 \pm 1\%$ to $8 \pm 1\%$ (**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 $\leq 10\%$ [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%)

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hematocrits (**Figure 1A**; **Figure 3A**). These findings support that adequate coronary perfusion pressure ($\geq$ 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 PO_2, which are indexed by changes in coronary venous PO_2, are proposed to invoke the production of vasodilator factors that act to increase coronary blood flow and restore tissue PO_2 to normal levels via negative feedback loop [25]. However, the consistency of coronary venous PO_2 (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 PO_2 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 (≥ 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_{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_{ATP} channels has not been previously investigated. Data from this study are the first to demonstrate that the inhibition of K_{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_2 by ~45-50% at all levels of hematocrit (**Table 2**) and that such reductions in MVO_2 could result in decreases in coronary blood flow, possibly via effects on mitochondrial K_{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_2 (**Table 2**). Furthermore, the Bache laboratory previously documented that glibenclamide-mediated reductions in MVO_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_{ATP} channel antagonist 5-hydroxydecanoate (5-HD) has no effect on MVO_2 supports that glibenclamide-mediated decreases in MVO_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^+ channel subtypes (e.g. IK_1 and K_{V1}) [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_{ATP}) channels.

The mechanisms responsible for the activation of K_{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_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_2 (increase

in myocardial oxygen extraction). These findings indicate that reductions in myocardial tissue PO_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_{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_V channels.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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

Control								
Hematocrit (%)	32 ± 1	26 ± 1	21 ± 1	16 ± 1	10 ± 1	**Drug**	**Hematocrit**	**Interaction**
Sample size	n = 6	n = 6	n = 5	n = 6	n = 6			
Arterial oxygen content (ml O$_2$/dl)	14.7 ± 0.4	11.6 ± 0.2*	9.1 ± 0.2*	7.0 ± 0.1*	4.7 ± 0.1*	N/A	*P < 0.001*	N/A
Coronary blood flow (ml/min/g)	0.39 ± 0.05	0.54 ± 0.06	0.79 ± 0.10*	1.02 ± 0.12*	1.63 ± 0.16*	N/A	*P < 0.001*	N/A
Mean blood pressure (mmHg)	92 ± 3	89 ± 4	87 ± 4	84 ± 4	81 ± 5	N/A	P = 0.400	N/A
Heart rate (bpm)	64 ± 6	68 ± 7	82 ± 11	81 ± 7	93 ± 7	N/A	P = 0.070	N/A
Arterial pH	7.47 ± 0.03	7.51 ± 0.02	7.50 ± 0.02	7.51 ± 0.02	7.49 ± 0.01	N/A	P = 0.563	N/A
Coronary venous pH	7.40 ± 0.03	7.44 ± 0.02	7.44 ± 0.02	7.42 ± 0.04	7.44 ± 0.02	N/A	P = 0.825	N/A
Arterial pO$_2$ (mmHg)	173 ± 23	177 ± 22	175 ± 25	173 ± 23	173 ± 24	N/A	P = 1.000	N/A
Coronary venous pO$_2$ (mmHg)	19.4 ± 1.8	20.5 ± 1.4	19.5 ± 1.3	18.7 ± 1.7	19.2 ± 1.7	N/A	P = 0.952	N/A
MVO$_2$ (µl O$_2$/min/g)	41 ± 4	42 ± 5	50 ± 6	50 ± 5	55 ± 6	N/A	P = 0.293	N/A
L-NAME								
Hematocrit (%)	32 ± 2	24 ± 1	19 ± 1	14 ± 1	9 ± 1	**Drug**	**Hematocrit**	**Interaction**
Sample size	n = 5	n = 5	n = 5	n = 4	n = 5			
Arterial oxygen content (ml O$_2$/dl)	15.3 ± 0.3	11.2 ± 0.3*	8.9 ± 0.4*	6.7 ± 0.2*	4.9 ± 0.1*	P = 0.851	*P < 0.001*	P = 0.326
Coronary blood flow (ml/min/g)	0.42 ± 0.03	0.47 ± 0.02	0.72 ± 0.04*	1.10 ± 0.12*	1.63 ± 0.24*	P = 0.921	*P < 0.001*	P = 0.958
Mean blood pressure (mmHg)	124 ± 6†	130 ± 3†	122 ± 7†	117 ± 8†	116 ± 9†	*P < 0.001*	P = 0.206	P = 0.925
Heart rate (bpm)	65 ± 5	63 ± 7	75 ± 7	85 ± 6	84 ± 7	P = 0.521	P = 0.005	P = 0.900
Arterial pH	7.52 ± 0.01	7.54 ± 0.03	7.49 ± 0.02	7.46 ± 0.02	7.44 ± 0.03	P = 0.702	P = 0.157	P = 0.079
Coronary venous pH	7.45 ± 0.02	7.43 ± 0.01	7.42 ± 0.02	7.37 ± 0.03	7.37 ± 0.04	P = 0.230	P = 0.643	P = 0.250
Arterial pO$_2$ (mmHg)	195 ± 13	194 ± 15	190 ± 14	186 ± 21	185 ± 16	P = 0.241	P = 0.998	P = 0.999
Coronary venous pO$_2$ (mmHg)	16.8 ± 2.4	14.5 ± 2.7†	14.5 ± 1.7	14.7 ± 0.9	18.0 ± 0.7	*P = 0.002*	P = 0.831	P = 0.681
MVO$_2$ (µl O$_2$/min/g)	50 ± 5	48 ± 4	56 ± 7	68 ± 8	66 ± 10	*P = 0.013*	*P = 0.036*	P = 0.868
4-AP								
Hematocrit (%)	33 ± 1	25 ± 1	20 ± 1	14 ± 2	8 ± 1	**Drug**	**Hematocrit**	**Interaction**
Sample size	n = 5	n = 5	n = 4	n = 4	n = 5			
Arterial oxygen content (ml O$_2$/dl)	15.1 ± 0.3	11.7 ± 0.4*	9.1 ± 0.3*	7.0 ± 0.3*	4.8 ± 0.2*	P = 0.541	*P < 0.001*	P = 0.900
Coronary blood flow (ml/min/g)	0.37 ± 0.05	0.52 ± 0.06	0.76 ± 0.08	0.98 ± 0.14*	1.52 ± 0.19*	P = 0.524	*P < 0.001*	P = 0.993
Mean blood pressure (mmHg)	90 ± 7	93 ± 7	89 ± 5	98 ± 6	91 ± 5	P = 0.097	P = 0.753	P = 0.546
Heart rate (bpm)	68 ± 8	67 ± 5	71 ± 6	73 ± 6	77 ± 5	P = 0.195	P = 0.055	P = 0.625
Arterial pH	7.55 ± 0.02†	7.53 ± 0.03	7.55 ± 0.01	7.51 ± 0.03	7.51 ± 0.03	*P = 0.018*	P = 0.847	P = 0.363
Coronary venous pH	7.48 ± 0.03†	7.48 ± 0.02	7.47 ± 0.01	7.47 ± 0.01	7.46 ± 0.01	*P = 0.022*	P = 0.966	P = 0.785
Arterial pO$_2$ (mmHg)	176 ± 17	178 ± 15	183 ± 12	206 ± 14	205 ± 9	P = 0.267	P = 0.934	P = 0.899
Coronary venous pO$_2$ (mmHg)	15.7 ± 1.2	14.3 ± 1.5†	13.3 ± 2.0†	15.5 ± 1.8	16.0 ± 1.5	*P < 0.001*	P = 0.962	P = 0.815
MVO$_2$ (µl O$_2$/min/g)	39 ± 3	47 ± 4	53 ± 3	56 ± 8	60 ± 13	P = 0.380	*P = 0.049*	P = 0.947

* = $P < 0.05$ 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.

Glibenclamide Vehicle								
Hematocrit (%)	29 ± 1	23 ± 1	18 ± 1	12 ± 1	8 ± 1	**Drug**	**Hematocrit**	**Interaction**
Sample size	n = 4	n = 3	n = 4	n = 3	n = 4			
Arterial oxygen content (ml O_2/dl)	14.8 ± 0.5	11.4 ± 0.2*	8.7 ± 0.2*	6.4 ± 0.2*	4.6 ± 0.2*	P = 0.176	*P < 0.001*	P = 0.755
Coronary blood flow (ml/min/g)	0.53 ± 0.07	0.66 ± 0.07	1.01 ± 0.09*	1.45 ± 0.13*†	2.00 ± 0.17*	*P < 0.001*	*P < 0.001*	P = 0.522
Mean blood pressure (mmHg)	100 ± 4	98 ± 5	96 ± 5	100 ± 6†	98 ± 5†	P < 0.001	P = 0.977	P = 0.799
Heart rate (bpm)	69 ± 11	64 ± 2	75 ± 10	75 ± 6	83 ± 2	P = 0.419	*P = 0.040*	P = 0.911
Arterial pH	7.47 ± 0.04	7.44 ± 0.04	7.44 ± 0.03	7.42 ± 0.02†	7.41 ± 0.01†	*P < 0.001*	P = 0.673	P = 0.345
Coronary venous pH	7.40 ± 0.04	7.38 ± 0.03	7.38 ± 0.02	7.37 ± 0.02	7.37 ± 0.02	*P = 0.012*	P = 0.931	P = 0.741
Arterial pO_2 (mmHg)	165 ± 29	159 ± 26	181 ± 20	127 ± 28	167 ± 14	P = 0.366	P = 0.623	P = 0.881
Coronary venous pO_2 (mmHg)	15.3 ± 1.7	14.8 ± 0.9†	15.8 ± 0.8	16.3 ± 0.9	19.9 ± 0.5*	*P = 0.003*	*P = 0.028*	P = 0.293
MVO_2 (µl O_2/min/g)	66 ± 6†	66 ± 5†	76 ± 8†	80 ± 5†	73 ± 3†	*P < 0.001*	P = 0.433	P = 0.847
Glibenclamide								
Hematocrit (%)	32 ± 1	25 ± 1	19 ± 1	14 ± 1	9 ± 1	**Drug**	**Hematocrit**	**Interaction**
Sample size	n = 5	n = 5	n = 5	n = 5	n = 5			
Arterial oxygen content (ml O_2/dl)	14.9 ± 0.4	11.7 ± 0.3*	9.0 ± 0.3*	7.0 ± 0.2*	4.6 ± 0.1*	P = 0.217	*P < 0.001*	P = 0.932
Coronary blood flow (ml/min/g)	0.30 ± 0.03‡	0.40 ± 0.04‡	0.57 ± 0.04*‡	0.79 ± 0.05*‡	1.12 ± 0.08*‡	*P < 0.001*	*P < 0.001*	*P < 0.001*
Mean blood pressure (mmHg)	107 ± 7	102 ± 12	104 ± 10	102 ± 10	94 ± 10	P = 0.541	P = 0.933	P = 0.951
Heart rate (bpm)	56 ± 7	61 ± 9	64 ± 6	70 ± 7	78 ± 7	P = 0.139	P = 0.101	P = 0.960
Arterial pH	7.46 ± 0.04	7.40 ± 0.04	7.39 ± 0.03	7.39 ± 0.03	7.39 ± 0.03	P = 0.213	P = 0.419	P = 0.975
Coronary venous pH	7.37 ± 0.04	7.33 ± 0.04	7.33 ± 0.04	7.33 ± 0.03	7.33 ± 0.03	*P = 0.044*	P = 0.782	P = 0.993
Arterial pO_2 (mmHg)	200 ± 31	209 ± 31	211 ± 30	218 ± 25‡	224 ± 21	*P = 0.005*	P = 0.908	P = 0.832
Coronary venous pO_2 (mmHg)	10.8 ± 1.7‡	12.2 ± 1.4	14.0 ± 1.5	15.0 ± 1.0	17.0 ± 1.1*	*P = 0.003*	*P = 0.002*	P = 0.838
MVO_2 (µl O_2/min/g)	42 ± 4‡	43 ± 4‡	45 ± 2‡	50 ± 4‡	44 ± 4‡	*P < 0.001*	P = 0.237	P = 0.913

* = $P < 0.05$ vs. baseline hematocrit, same treatment. † = $P < 0.05$ vs. control, same level of hematocrit. ‡ = $P < 0.05$ vs. vehicle, same level of hematocrit.
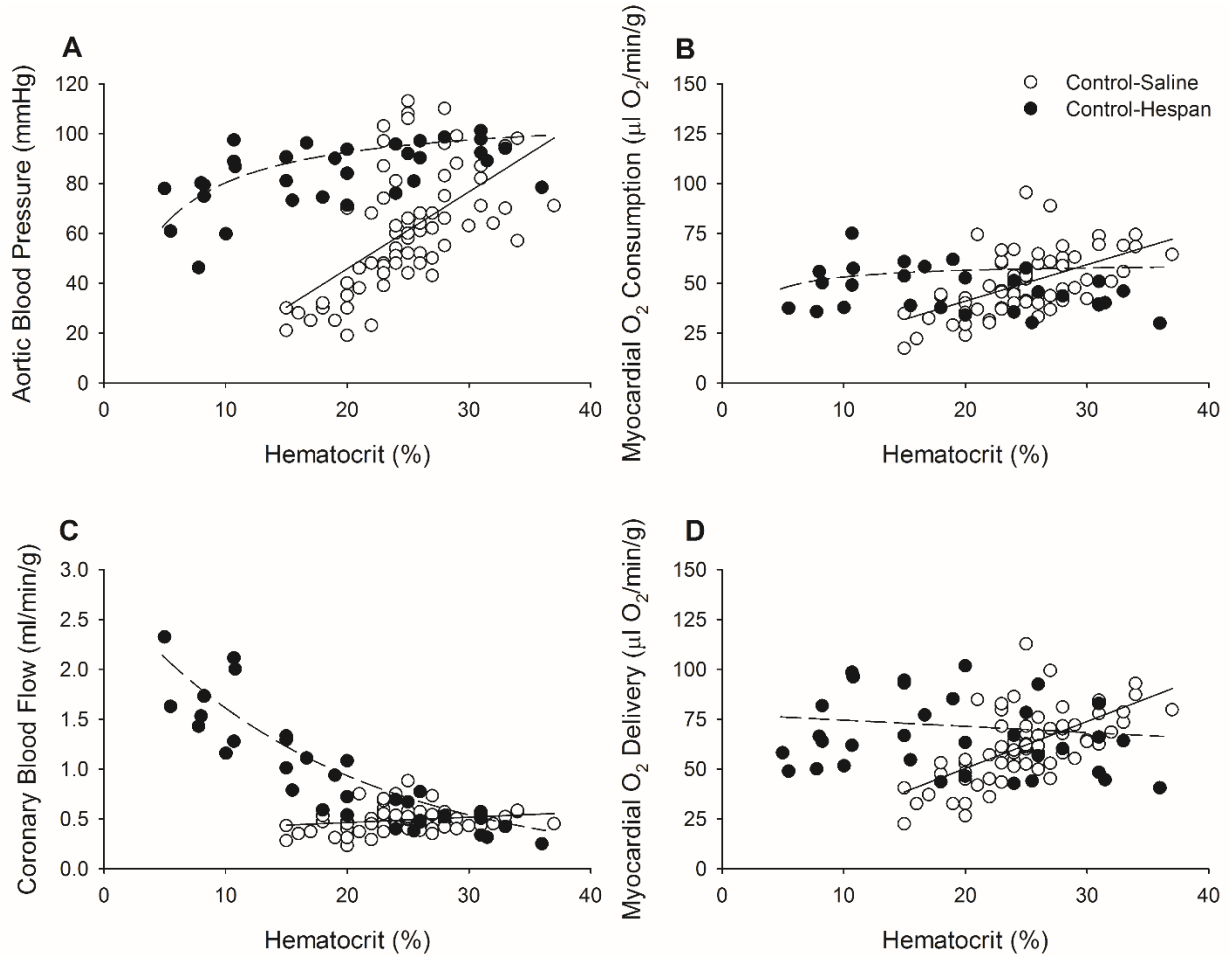
Figure 1. Relationship between aortic blood pressure (A), myocardial oxygen consumption (B), coronary blood flow (C) and myocardial oxygen delivery (D) vs. hematocrit for control swine that received volume replacement with saline and Hespan.
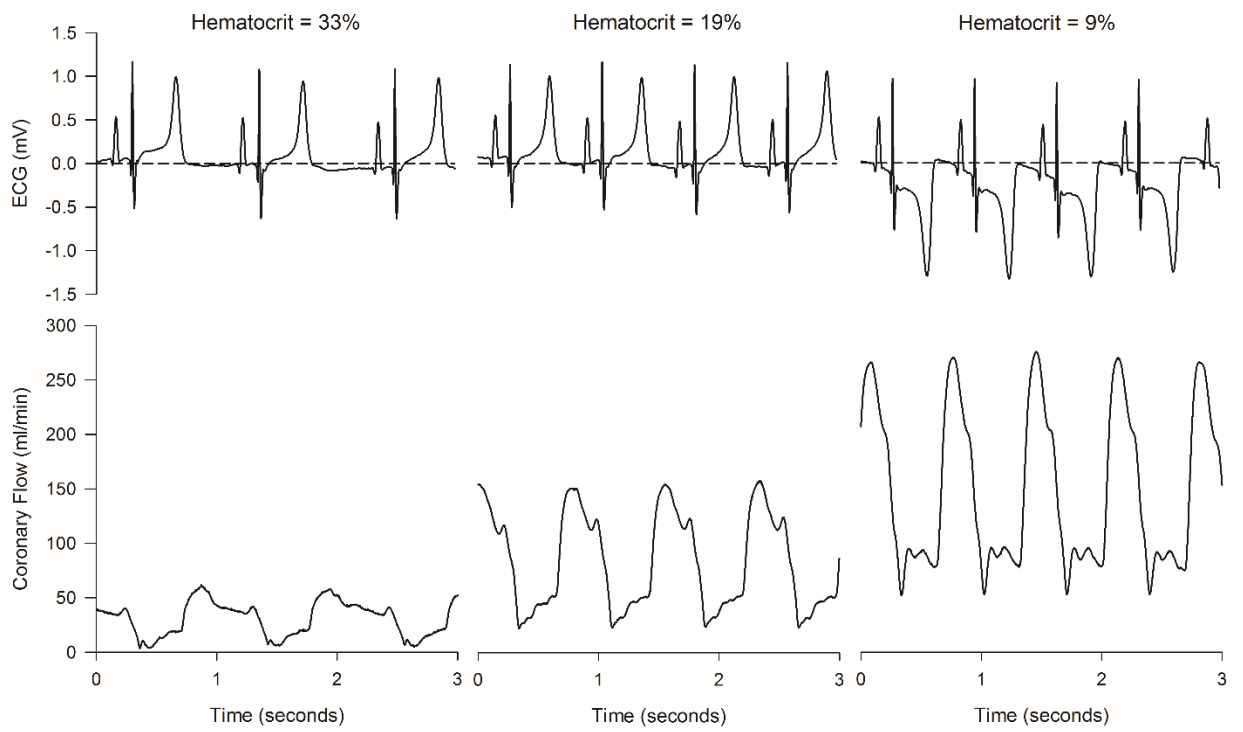
Figure 2. Representative tracing of the effects of decreasing hematocrit on ECG and coronary blood flow over time in untreated control swine that received volume replacement with Hespan.
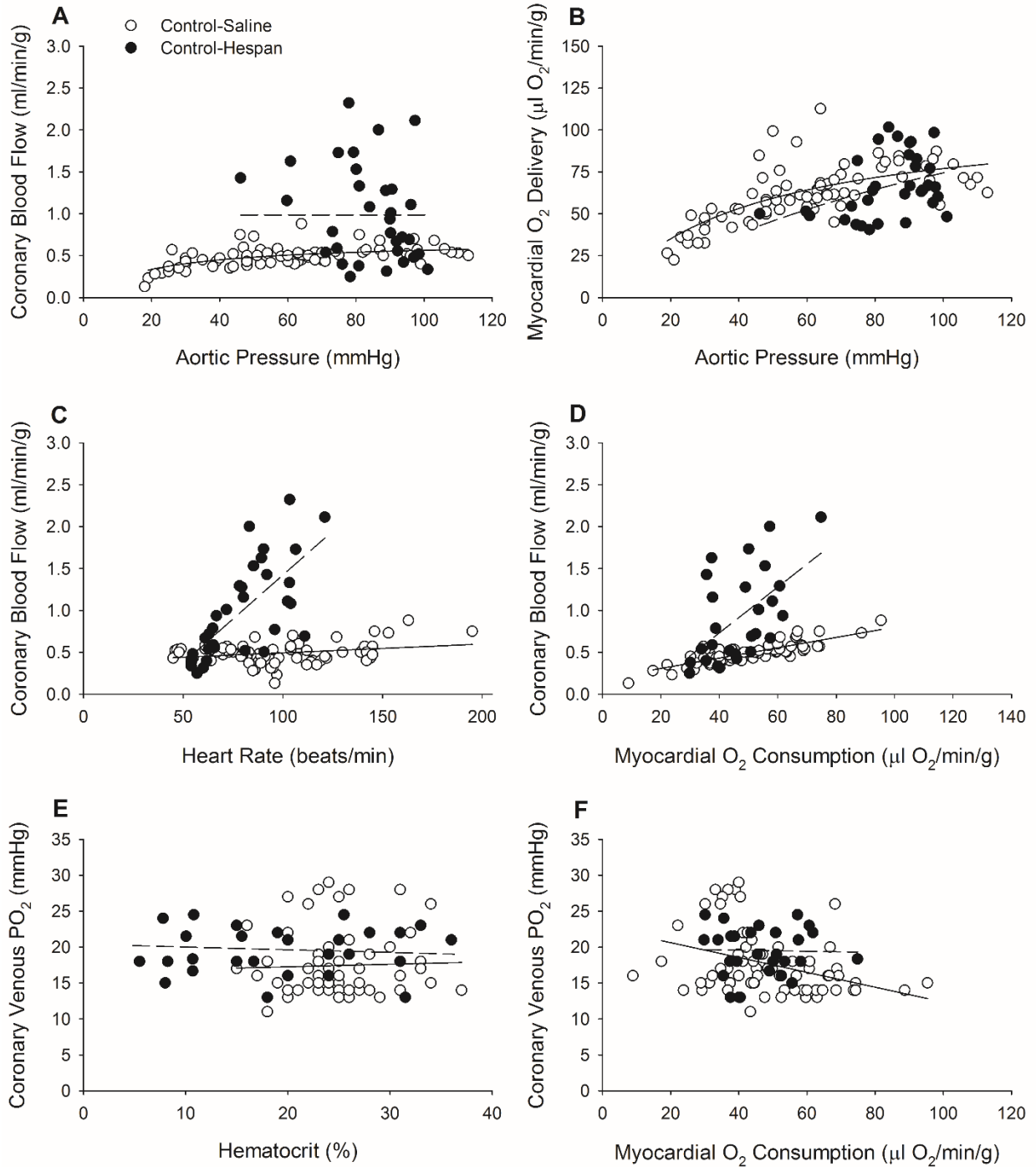
Figure 3. Relationship between coronary blood flow (A) and myocardial oxygen delivery (B) vs. aortic pressure, coronary blood flow vs heart rate (C) and myocardial oxygen consumption (D), and coronary venous oxygen partial pressure vs. hematocrit (E) and myocardial oxygen consumption (F) for control swine that received volume replacement with saline and Hespan.
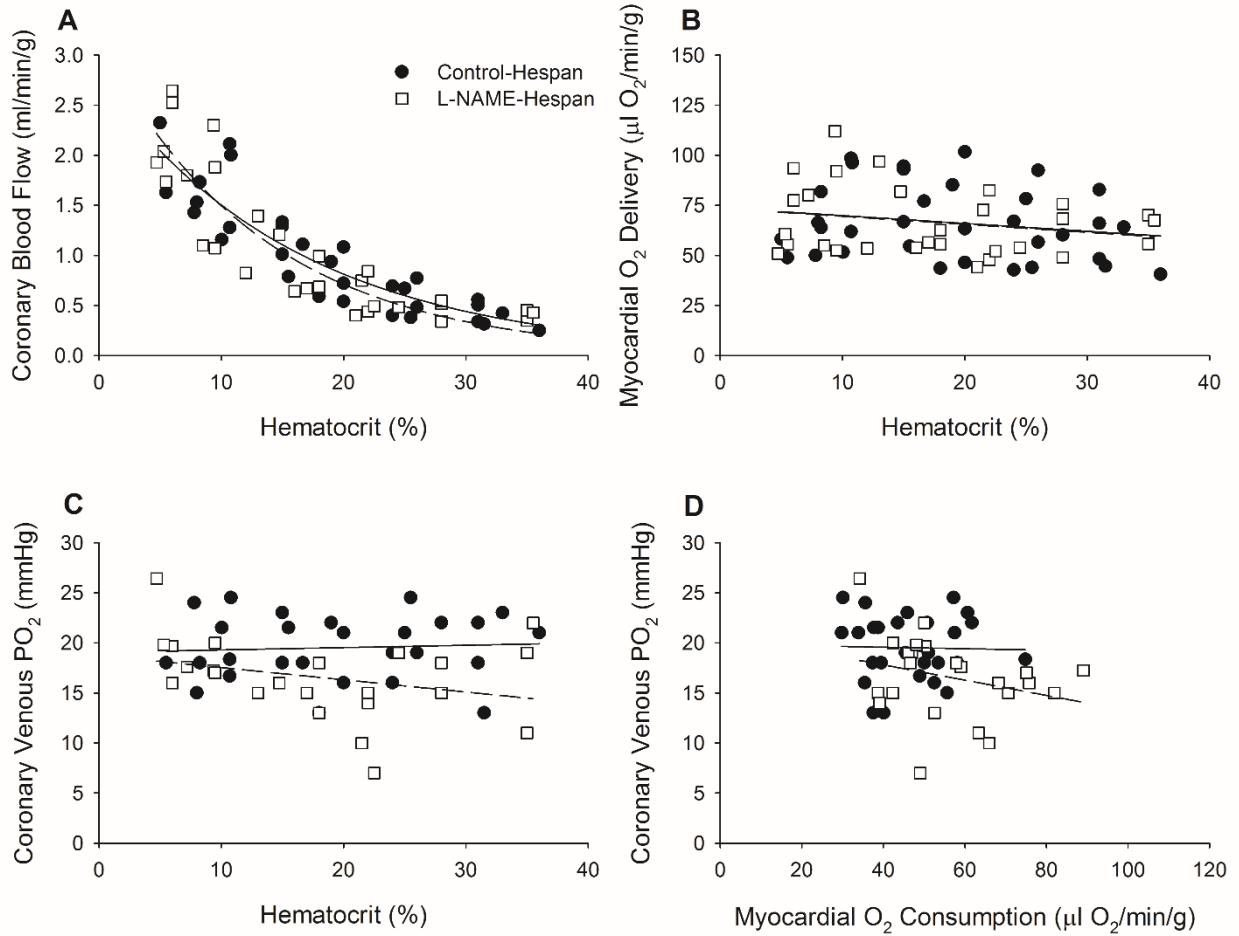
Figure 4. Relationship between coronary blood flow (A), myocardial oxygen delivery (B), and coronary venous oxygen tension (C) vs. hematocrit and coronary venous oxygen tension vs. myocardial oxygen consumption (D) for control and L-NAME treated swine that received volume replacement with Hespan.
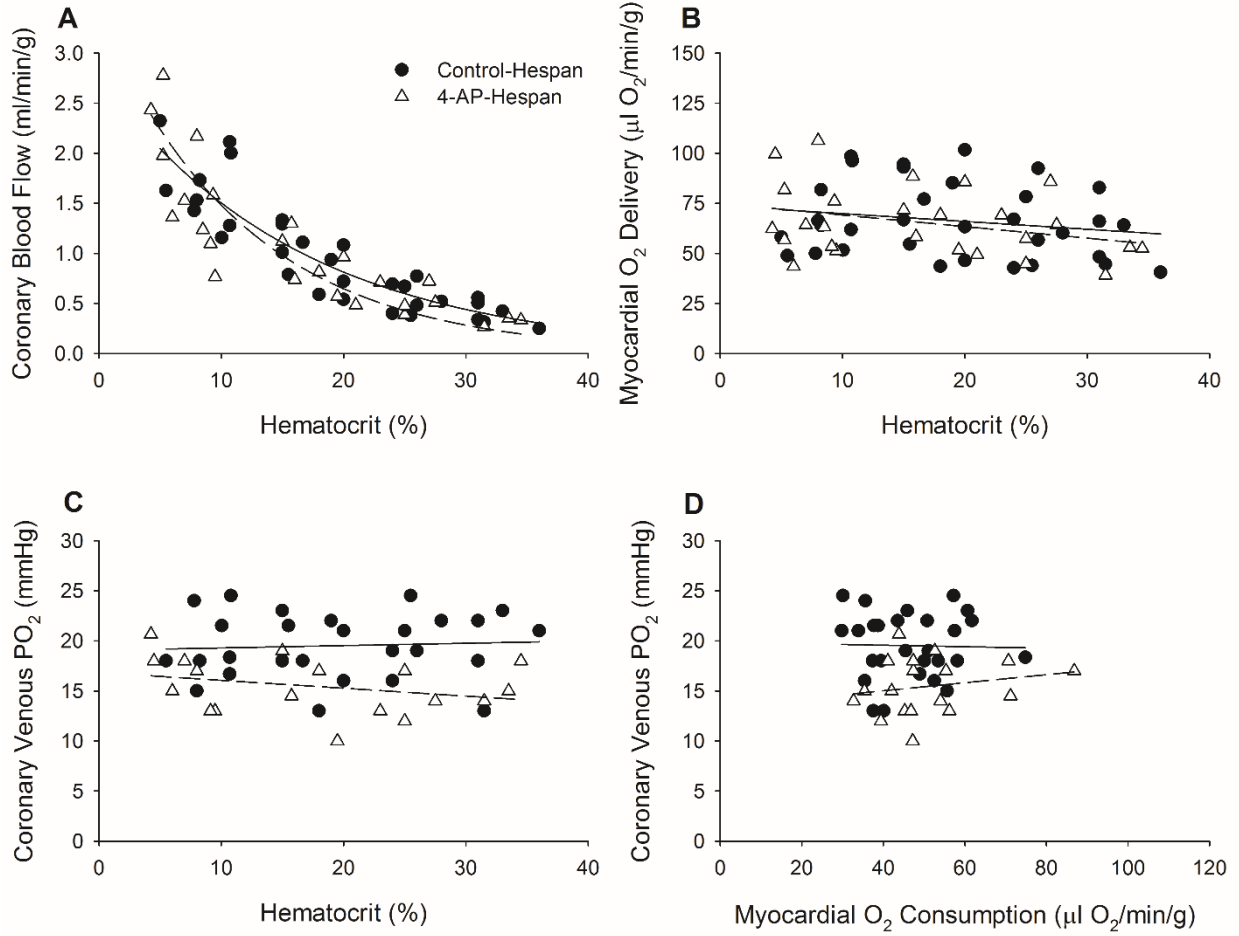
Figure 5. Relationship between coronary blood flow (A), myocardial oxygen delivery (B), and coronary venous oxygen tension (C) vs. hematocrit and coronary venous oxygen tension vs. myocardial oxygen consumption (D) for control and 4-AP treated swine that received volume replacement with Hespan.
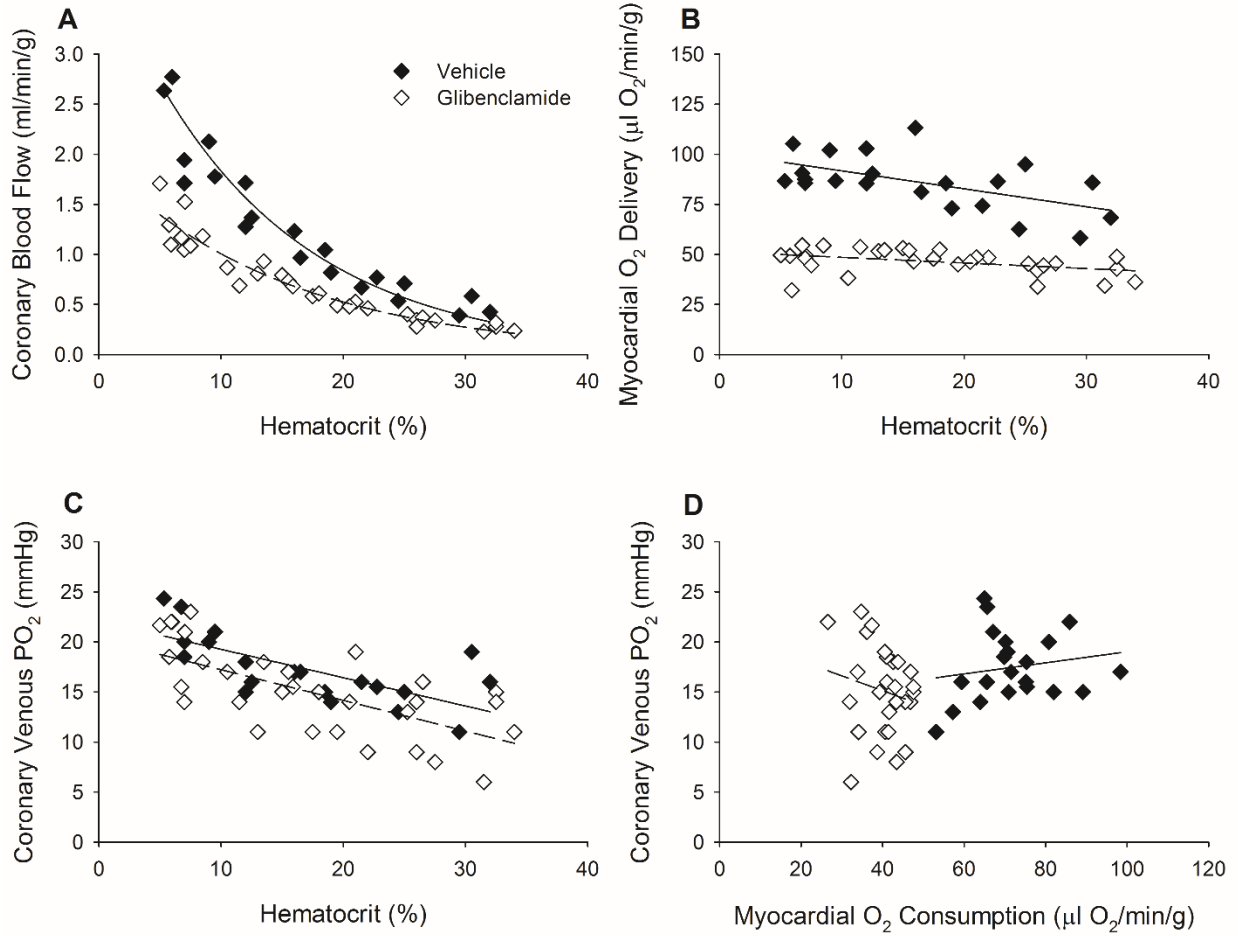
Figure 6. Relationship between coronary blood flow (A), myocardial oxygen delivery (B), and coronary venous oxygen tension (C) vs. hematocrit and coronary venous oxygen tension vs. myocardial oxygen consumption (D) for vehicle and glibenclamide treated swine that received volume replacement with Hespan.