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Vascular K_{ATP} channels mitigate severe muscle O_2 delivery-utilization mismatch during contractions in chronic heart failure rats

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Abstract

The vascular ATP-sensitive K^+ (K_{ATP}) channel is a mediator of skeletal muscle microvascular oxygenation (PO_2mv) during contractions in health. We tested the hypothesis that K_{ATP} channel function is preserved in chronic heart failure (CHF) and therefore its inhibition would reduce PO_2mv and exacerbate the time taken to reach the PO_2mv steady-state during contractions of the spinotrapezius muscle. Moreover, we hypothesized that subsequent K_{ATP} channel activation would oppose the effects of this inhibition. Muscle PO_2mv (phosphorescence quenching) was measured during 180 s of 1-Hz twitch contractions (~6 V) under control, glibenclamide (GLI, K_{ATP} channel antagonist; 5 mg/kg) and pinacidil (PIN, K_{ATP} channel agonist; 5 mg/kg) conditions in 16 male Sprague-Dawley rats with CHF induced via myocardial infarction (coronary artery ligation, left ventricular end-diastolic pressure: 18 ± 1 mmHg). GLI reduced baseline PO_2mv (control: 28.3 ± 0.9 , GLI: 24.8 ± 1.0 mmHg, $p < 0.05$), lowered mean PO_2mv (average PO_2mv during the overall time taken to reach the steady-state; control: 20.6 ± 0.6 , GLI: 17.6 ± 0.3 mmHg, $p < 0.05$), and slowed the attainment of steady-state PO_2mv (overall mean response time; control: 66.1 ± 10.2 , GLI: 93.6 ± 7.8 s, $p < 0.05$). PIN opposed these effects on the baseline PO_2mv , mean PO_2mv and time to reach the steady-state PO_2mv ($p < 0.05$ for all vs. GLI). Inhibition of K_{ATP} channels exacerbates the transient mismatch between muscle O_2 delivery and utilization in heart failure rats and this effect is opposed by PIN. These data reveal that the K_{ATP} channel constitutes one of the select few well-preserved mechanisms of skeletal muscle microvascular oxygenation control in CHF.

Keywords

glibenclamide; kinetics; phosphorescence quenching; exercise hyperemia; pinacidil

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Conflict disclosures
None.

1. Introduction

Oxygen transfer to peripheral tissues via both convective and diffusive transport routes is routinely interrupted as part of the inexorable progression of chronic, congestive heart failure (Hirai et al., 2015; Poole et al., 2012). Decrements in skeletal muscle blood flow and vascular conductance combined with derangement of capillary structure, geometry and hemodynamics lower the muscle microvascular O_2 pressure (PO_{2mv}) and impair blood-myocyte O_2 flux thereby reducing work capacity commensurate with the severity of chronic heart failure (CHF) (Miyazaki et al., 2007; Piepoli et al., 2010a, 2010b). Muscle PO_{2mv} is a powerful measurement as it represents the final pressure head driving blood-myocyte O_2 flux. More importantly, because PO_{2mv} is set by the ratio of O_2 -delivery to O_2 -utilization, and occurs at the terminal cell-to-cell interface of the O_2 transport chain, it is impacted by the collective upstream cardiopulmonary perturbations. This makes the PO_{2mv} sensitive to both the spatial and temporal aspects of vascular function and therefore invaluable in revealing the O_2 transport depredations of diseases such as CHF (Diederich et al., 2002) as well as the role of vasoactive pathways including nitric oxide (NO) function (Ferreira et al., 2006).

Maintaining adequate muscle PO_{2mv} requires tight matching of O_2 -supply to O_2 -demand given that even transient mismatch can deplete muscle phosphocreatine stores and exacerbate the accumulation of muscle metabolites associated with fatigue such as ADP, H^+ , K^+ and P_i (Hogan et al., 1992; Richardson et al., 1995; Wilson et al., 1977). Potassium channels that are sensitive to ATP and ADP (K_{ATP}) constitute a potential coupling between tissue metabolism and vasomotor control (i.e. O_2 supply) via K^+ efflux-induced hyperpolarization. Accordingly, recent studies in isolated skeletal muscle arterioles demonstrate that the K_{ATP} channel opener levcromakalim evokes both local and conducted vasodilation (Dora, 2017). K_{ATP} channel inhibition *in vivo* exaggerates baroreflex-mediated vasoconstriction (Keller et al., 2004) whereas its activation attenuates α -adrenergic vasoconstriction (Nakai and Ichihara, 1994; Tateishi and Faber, 1995). However, it remains unclear under which circumstances this channel is obligatory for adequate skeletal muscle O_2 transport in health and disease.

Individual studies of K_{ATP} channel inhibition are equivocal; some demonstrating robust effects on the magnitude of the hyperemic response to exercise (Banitt et al., 1996; Bank et al., 2000; Bijlstra et al., 1996; Holdsworth et al., 2015) with others finding no change (Duncker et al., 2001; Farouque and Meredith, 2003; Schrage et al., 2006). However, conditions of the local microenvironment are crucial to the role of the channel *in vivo* and will differ among species, disease states and degree of metabolic stress (e.g. exercise). In particular, K_{ATP} channel function is likely to be an essential response to ischemia and the resultant tissue hypoxia (Bijlstra et al., 1996; Nakai and Ichihara, 1994; Suzuki et al., 2002; Tateishi and Faber, 1995; Wheaton and Chandel, 2011). Since these are common attributes of skeletal muscle in CHF patients blocking vascular K_{ATP} channel function may exaggerate muscle O_2 transport dysfunction in this condition. This is a relevant scenario in patients with diabetes-CHF comorbidities because sulphonylureas, such as glibenclamide (GLI), are widely prescribed for the management of hyperglycemia, and despite apparent high *in vitro* pancreatic K_{ATP} channel affinity, their clinical use is associated with cardiovascular

morbidity and mortality (Fadini et al., 2015; Morgan et al., 2014; Simpson et al., 2006). While the cardiovascular effects of sulphonylureas in diabetic patients are a consideration for prescription guidelines, the direct, peripheral vascular consequences of sulphonylurea use in CHF are not well described.

The purpose of this study was to test the hypothesis that vascular K_{ATP} channel function is preserved in CHF and, therefore, its inhibition via GLI would impair the matching of muscle O_2 delivery-to-utilization following the onset of electrically-induced contractions. This would be reflected by lowered PO_2mv and slowed kinetics (i.e. increased overall time to reach steady-state) following GLI superfusion of the spinotrapezius muscle of CHF rats. Moreover, we hypothesized that these effects of GLI would be opposed by the K_{ATP} channel activator pinacidil (PIN).

2. Methods

2.1. Ethical approval

All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University under the guidelines established by the National Institutes of Health. 16 adult male Sprague-Dawley rats (3-6 months old) were maintained in accredited animal facilities (Association for the Assessment and Accreditation of Laboratory Animal Care) at Kansas State University on a 12-h light/12-h dark cycle with food and water provided *ad libitum*.

2.2. Myocardial infarction procedures

All rats underwent induction of a myocardial infarction (MI) via ligation of the left main coronary artery which has been shown to result reliably in the development of moderate to severe CHF (Musch and Terrell, 1992). Briefly, each rat was anesthetized with a gas mixture of 5% isoflurane- O_2 and intubated for mechanical ventilation on a rodent respirator (model 680, Harvard Instruments, Holliston, USA) with subsequent maintenance on a 3% isoflurane- O_2 gas mixture for the duration of the procedure. A left thoracotomy was performed to expose the heart through the fifth intercostal space. Exteriorization of the heart provided access to the left main coronary artery which was ligated with a 6-0 silk suture ~1-2 mm distal to the edge of the left atrium. The muscles of the thorax were then closed with 4-0 gut, and the skin incision was closed with 3-0 silk followed by an administration of the analgesic agents bupivacaine (1.5 mg/kg subcutaneously) and buprenorphine (0.01–0.05 mg/kg i.m.) as well as ampicillin (50 mg/kg i.m.) to reduce the risk of infection. Upon removal from mechanical ventilation and withdrawal of anesthesia the rats were monitored ~8-12 h post-operatively for the development of arrhythmias and undue stress with care administered as needed. The recovery duration prior to the final, PO_2mv protocol was 21 days which is consistent with the time course for complete remodeling of necrotic myocardial tissue (Fishbein et al., 1978). During this time the rats were monitored daily (appetite, weight loss, gait/posture, etc.) in conjunction with the university veterinary staff.

2.3. Surgical instrumentation

On the day of the final protocol the rats were anesthetized initially with a 5% isoflurane-O₂ mixture and maintained on a 3% isoflurane-O₂ mixture for the duration of the surgical instrumentation. The carotid artery was cannulated and a two-French-catheter-tipped pressure transducer (Millar Instruments, Houston, USA) was advanced into the left ventricle (LV) for the measurement of LV end diastolic pressure (LVEDP). Subsequently, cannulation of both the carotid and caudal arteries was performed with PE-10 connected to PE-50 (Intra-Medic polyethylene tubing, Clay Adams, Spark, USA) for the measurement of mean arterial pressure (MAP) and heart rate (HR) as well as infusion of the phosphorescent probe Oxyphor G2 (Oxygen Enterprises, Philadelphia, PA, USA; carotid) and for blood sampling and administration of anesthetics (caudal). Isoflurane-O₂ inhalation was progressively removed as anesthesia was maintained with pentobarbital sodium administered via the caudal artery catheter to effect. Depth of anesthesia was monitored at frequent and regular intervals via the blink and toe-pinch reflexes as well as magnitude and frequency of ventilation. Access to the spinotrapezius was achieved by surgically reflecting the overlying skin and fascia. The spinotrapezius muscle was selected because the fiber-type composition and oxidative capacity closely resemble that of the human quadriceps muscle group which makes it a useful analog of human locomotor muscle (Delp and Duan, 1996). During both the surgical preparation and experimental protocol the muscle was superfused frequently with Krebs-Henseleit bicarbonate-buffered solution consisting of (in mM) 4.7 KCl, 2.0 CaCl₂, 2.4 MgSO₄, 131 NaCl, and 22 NaHCO₃ equilibrated with 5% CO₂ and 95% N₂ (pH 7.4, 37-38°C). Exposed tissue surrounding the spinotrapezius muscle was covered with Saran wrap (Dow Brands, Indianapolis, IN) to reduce dehydration and confine the exposure of GLI and PIN to the spinotrapezius muscle. Platinum iridium electrodes were sutured to the rostral (cathode) and caudal (anode) regions of the spinotrapezius muscle for electrically-induced twitch contractions. As previously reported by our laboratory these surgical procedures do not impact the microvascular integrity and responsiveness of the rat spinotrapezius muscle (Bailey et al., 2000).

2.4. Drugs

The pharmacological sulphonylurea derivative GLI (494 g/mol; 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-methoxybenzamide; Sigma-Aldrich, St Louis, MO, USA) was used to inhibit vascular K_{ATP} channels. Given that GLI is difficult to maintain in solution, 25 mg of GLI was dissolved in 99:1 ratio of distilled water/NaOH (1 M) during continuous sonication to produce a 2.5 mg/ml stock solution. A 5 mg/kg dose was drawn from the stock solution and diluted to ~3 ml with Krebs-Henseleit solution. PIN (245 g/mol; *N*-cyano-*N'*-pyridin-4-yl-*N''*-(1,2,2-trimethylpropyl)guanidine; Sigma-Aldrich, St Louis, MO, USA) is readily dissolved in distilled water and was diluted to ~3 ml with Krebs-Henseleit solution before being administered to oppose vascular K_{ATP} channel inhibition at the same dose as GLI.

2.5. Experimental protocol

Three separate contraction bouts were performed under control (vehicle), GLI (5 mg/kg) and PIN (5 mg/kg) conditions. Drug doses and experimental protocol were selected based on

previous investigations examining the functional role of K_{ATP} channels on skeletal muscle blood flow and PO_2mv (Holdsworth et al., 2016, 2015) and preliminary studies in our laboratory. The vehicle was administered for control via superfusion (3 ml) of the spinotrapezius during 180 s of continuous PO_2mv recording. The recording was extended for an additional 90-180 s to ensure that baseline PO_2mv had stabilized at which time muscle contraction was evoked via electrical stimulation (1 Hz, ~6 V, 2-ms pulse duration, model s48; Grass Technologies, Quincy, MA) for 180 s. This protocol has been shown to increase spinotrapezius muscle blood flow four-to five-fold and metabolic rate six- to seven-fold without altering blood pH and is consistent with moderate intensity exercise (Behnke et al., 2002; Hirai et al., 2013). Immediately upon cessation of electrical stimulation an arterial blood sample was drawn (~0.8 ml) for the determination of blood gases, hematocrit, pH, [lactate] and [glucose]. This was followed by a brief washout with Krebs-Henseleit solution and a 20-30 min recovery period, after which the second contraction bout was performed in an identical fashion to the first after the superfusion of GLI (5 mg/kg). This was repeated for the third contraction bout with the administration of PIN. As previously reported by our laboratory, >20 min recovery elicits reproducible PO_2mv responses (Copp et al., 2009; Herspring et al., 2008). Given the long-lasting effects of GLI and the possibility that this drug may not washout completely with Krebs-Henseleit superfusion and the recovery period employed herein (Thomas et al., 1997; cf. Brayden, 2002), PIN was compared to GLI only as described in the *Statistical analysis* section below. At the end of the protocol, rats were euthanized with intra-arterial pentobarbital sodium overdose (>50 mg/kg) and subsequent removal of the heart. The lungs, right ventricle (RV), LV and atria were removed and weighed. Measurement of infarct size in the LV was made via planimetry as described previously (Ferreira et al., 2006).

2.6. Spinotrapezius PO_2mv measurement

PO_2mv was measured via phosphorescence quenching using a frequency domain phosphorometer (PMOD 5000; Oxygen Enterprises, Philadelphia, PA). As described previously (Behnke et al., 2001), this technique applies the Stern-Volmer relationship (Rumsey et al., 1988), which describes the quantitative O_2 dependence of the phosphorescent probe G2 via the equation:

$$PO_{2mv} = [(\tau^\circ/\tau) - 1] / (k_Q \cdot \tau^\circ)$$

where k_Q is the quenching constant and τ and τ° are the phosphorescence lifetimes at the ambient O_2 concentration and in the absence of O_2 , respectively. For the phosphorescent probe G2, k_Q is $273 \text{ mmHg}^{-1} \text{ s}^{-1}$ and τ° is 251 μs (Bodmer et al., 2012; Dunphy et al., 2002). These characteristics do not change over the physiological range of pH (~7.4) and temperature (~38°C) *in vivo* and, therefore, the phosphorescence lifetime is affected solely by the O_2 partial pressure. The Pd-porphyrin cores of phosphor probes bind to albumin; the primary macromolecule in plasma. This quality as well as the probe's negative charge ensure restriction to the vascular compartment, of which, the capillary bed volumetrically constitutes the major intramuscular space (Poole et al., 2004). After infusion of G2 the common end of the bifurcated light guide with a >500 μm penetration depth was positioned

~2-4 mm superficial to the dorsal surface of the exposed spinotrapezius muscle in an area devoid of macro-vessels. The phosphorometer modulates sinusoidal excitation frequencies between 100 Hz and 20 kHz and allows phosphorescence lifetime measurements from 10 μ s to ~2.5 ms. PO₂mv was measured continuously and recorded at 2-s intervals throughout the duration of the experimental protocol.

2.7. Analysis of spinotrapezius PO₂mv kinetics

PO₂mv time course was determined via exponential regression analysis by applying the Levenberg-Marquardt algorithm (SigmaPlot 11.0; Systat software, San Jose, CA) to the contraction-induced PO₂mv transient. PO₂mv responses were fit with either a one- or two-component model as follows.

One component:

$$PO_{2mv}(t) = PO_{2mv(BL)} - \Delta_1 PO_{2mv} \left[1 - e^{-(t-TD_1/\tau_1)} \right]$$

Two component:

$$PO_{2mv}(t) = PO_{2mv(BL)} - \Delta_1 PO_{2mv} \left[1 - e^{-(t-TD_1/\tau_1)} \right] + \Delta_2 PO_{2mv} \left[1 - e^{-(t-TD_2/\tau_2)} \right]$$

PO₂mv_(t) is the PO₂mv at a given time *t*; PO₂mv_(BL) corresponds to the pre-contracting resting PO₂mv; Δ_1 and Δ_2 are the amplitudes for the first and second components, respectively; TD₁ and TD₂ are the independent time delays for each component; and τ_1 and τ_2 are the time constants (i.e. time to 63% of the response) for each component. Goodness of fit was determined using three criteria: the coefficient of determination, sum of squared residuals, and visual inspection (Behnke et al., 2002).

The mean response time (MRT₁) was used to describe the overall dynamics of the PO₂mv fall following the onset of muscle contractions via the equation:

$$MRT_1 = TD_1 + \tau_1$$

The MRT₁ analysis was limited to the first component of the PO₂mv response given that inclusion of an emergent second component underestimates the speed of the PO₂mv fall at the onset of contractions. In the event of a second component the MRT₂ was calculated using the following equation:

$$MRT_2 = TD_2 + \tau_2$$

To determine the overall time taken to reach the steady-state PO₂mv the MRT_{Total} was calculated via the equation:

$$MRT_{Total} = MRT_1 + MRT_2$$

The MRT_{Total} does not equal the sum of the average MRT_1 and MRT_2 because the calculation of MRT_{Total} is independent of the average of MRT_2 . The purpose of the second component kinetics parameters is to represent the speed with which the $PO_{2mv(SS)}$ is reached after the initial fall in PO_{2mv} . For this reason, the MRT_2 averages are calculated from rats with a two-component fit only. However, the calculation of MRT_{Total} necessarily reflects the absence of MRT_2 (i.e. entries of zero) for rats fit with a one-component model.

Mean PO_{2mv} was calculated as the average PO_{2mv} from the contractions onset through the duration of MRT_{Total} .

2.8. Statistical analysis

MAP and HR were compared between conditions and across time points via 2-way repeated measures ANOVA with Student Newman Keuls *post-hoc* tests as necessary. Kinetics parameters were compared between conditions with 1-way, repeated measures ANOVA on the basis of the *a priori* hypotheses. Student Newman Keuls *post-hoc* tests were used to compare control/GLI and GLI/PIN when necessary. Results are presented as means $\pm$ SE. The level of significance was set at $p < 0.05$.

3. Results

The final body weight for all CHF rats was 463 ± 12 g. Table 1 summarizes the descriptive statistics for the central indices of CHF. Arterial blood [lactate], [glucose], PO_2 , PCO_2 , O_2 saturation, hematocrit and pH were not different with GLI relative to control and were also not different after PIN superfusion compared to GLI ($p > 0.05$ for all; data not shown). The MAP (control: 110 ± 4 , GLI: 114 ± 5 mmHg) and HR (control: 326 ± 10 , GLI: 337 ± 14 beats/min) at the start of contractions with GLI were not different from control ($p > 0.05$ for both). PIN reduced MAP at the start of contractions compared to GLI (PIN: 100 ± 6 , GLI: 114 ± 5 mmHg, $p < 0.05$), but did not change HR (PIN: 367 ± 14 , GLI: 337 ± 14 beats/min, $p > 0.05$).

Muscle PO_{2mv} responses from rest to contractions are shown in Fig. 1 and the kinetics parameters are provided in Table 2. The goodness of fit for the models was demonstrated by an r^2 of 0.97 ± 0.01 and the sum of squared residuals of 18.9 ± 3.0 . Overall, the mean PO_{2mv} over the early contractions transient was significantly reduced with GLI relative to control (control: 20.6 ± 0.6 , GLI: 17.6 ± 0.3 mmHg, $p < 0.05$). During the control condition 11 of 16 rats exhibited PO_{2mv} profiles that transiently fell below the contracting steady-state (termed an “undershoot”, ${}_2PO_{2mv}$) necessitating a two-component model fit. Conversely, during GLI the two-component model was required for 15 of 16 rats and during PIN for 5 of 12 rats. Compared to control, GLI reduced the PO_{2mv} baseline ($PO_{2mv(BL)}$) prior to contractions. In addition, GLI increased the amplitude of ${}_2PO_{2mv}$ and slowed the attainment of the steady-state as indicated by an increase in the total mean response time (MRT_{Total}).

PIN increased the mean PO_{2mv} across MRT_{Total} compared to GLI (GLI: 17.6 ± 0.3 , PIN: 25.7 ± 0.5 mmHg, $p < 0.05$). This is in direct opposition to the effect of GLI during the early contraction transient relative to control. PIN also increased both the baseline and steady-

state PO_{2mv} with no difference in $_{1}PO_{2mv}$ relative to the GLI condition. The effects of PIN were such that a faster attainment of the steady-state (i.e. lower MRT_{Total}) was observed when compared to GLI.

4. Discussion

K_{ATP} channel inhibition via GLI fundamentally altered the spinotrapezius PO_{2mv} profile such that mean PO_{2mv} was lowered at rest and following the onset of contractions in CHF rats. The major effects of GLI were 1) a reduction in the baseline PO_{2mv} ; 2) a near doubling of the PO_{2mv} undershoot amplitude with a 42% increase in the overall time to reach the contracting steady-state and MRT_{Total} ; and 3) a reduced mean PO_{2mv} during the transition from contraction onset to the steady-state PO_{2mv} . These data indicate that vascular smooth muscle and/or endothelial K_{ATP} channel function are essential to the control of muscle PO_{2mv} kinetics at the onset of contractions in CHF, and the K_{ATP} channel may represent one of the select few well-preserved mechanisms of skeletal muscle vascular control in CHF. This K_{ATP} channel-conferred protection against severe O_2 -delivery to O_2 -utilization mismatch during contractions should raise serious concerns when sulphonylureas such as GLI are prescribed for patients with coincident diabetes and CHF.

The central indices of dysfunction (Table 1) support that moderate CHF was induced in these animals (Ferreira et al., 2006) which is consistent with New York Heart Association (NYHA) class II and III CHF in humans (Musch et al., 1988). The spectrum included animals with elevated LVEDP's (up to 27 mmHg) and MI size's greater than 40% (severe CHF) however the sample size was not sufficient for adequately powered statistical comparisons between moderate and severe sub-groups. Thus, the moderate CHF herein may underestimate the effect of GLI on the PO_{2mv} profiles during contractions. Nonetheless, moderate CHF induced via the rat model of coronary artery ligation does result in decrements to skeletal muscle blood flow, vascular conductance, PO_{2mv} kinetics, $\dot{V} O_{2max}$, and time-to-exhaustion during exercise akin to NYHA Class II and III CHF in humans (Hirai et al., 2015; Musch and Terrell, 1992; Poole et al., 2012; Richardson et al., 2003).

K_{ATP} channel manipulation of the steady-state O_2 delivery has been characterized previously for *in vivo* electrical stimulation (Thomas et al., 1997), reactive hyperemia (Banitt et al., 1996; Bank et al., 2000; Bijlstra et al., 1996) and exercise-induced hyperemia (Holdsworth et al., 2015). Our laboratory has also demonstrated that GLI increased the prevalence of the undershoot and prolonged the time to reach the contracting steady-state PO_{2mv} in the spinotrapezius muscle of healthy rats (Holdsworth et al., 2016). The available evidence is equivocal in regards to $\dot{V} O_2$ and mitochondrial respiration with modulation of K_{ATP} channel function. Some investigations indicate no appreciable change in $\dot{V} O_2$ during whole body exercise with GLI in diabetic patients (Larsen et al., 1999) and that mitochondrial K_{ATP} channel inhibition does not impact the O_2 cost of myocardial contractions (Chen et al., 2001). Other groups have found that GLI reduces diaphragmatic $\dot{V} O_2$ in canines (largely driven by reductions in O_2 delivery) (Vanelli et al., 1994) and that skeletal muscle mitochondrial respiration is impaired by small doses of GLI (Montoya-Pérez et al., 2010). While GLI-induced changes in O_2 delivery may constitute the primary driver for the

perturbed PO_2mv profile, it is not possible to identify whether the superfusion method utilized herein affected K_{ATP} channels present specifically within the arteriole wall and/or myocyte (Flagg et al., 2010; Jackson, 2000). Further studies in CHF are required to establish whether manipulation of K_{ATP} channel function could be influenced by the mode of drug delivery and/or specific tissue of interest.

The small-muscle mass preparation used herein poses an advantage for investigations of peripheral vascular control because it does not tax the limits of cardiac output. This is a strength for investigations of vascular K_{ATP} channels in CHF because the channel's role in cardiac function may obfuscate detection and/or interpretation of the impact on peripheral vascular control (Nichols et al., 1991; Noma, 1983). Although no direct assessment of K_{ATP} channel activity and/or expression was performed in the current study, in CHF rats it appears that the vascular K_{ATP} channel pathway remains functional given the marked sensitivity of the PO_2mv profile to both GLI and PIN. The PO_2mv profiles of the CHF rats herein exhibit the characteristic undershoot demonstrated previously by our laboratory (Behnke et al., 2001; Hirai et al., 2014) and permitted the statistical comparison of the second kinetics component (i.e. undershoot) between control and GLI which was not possible in our previous investigation of K_{ATP} function in healthy rats (Holdsworth et al., 2016). Specifically, the present investigation revealed that GLI elevated the magnitude of the PO_2mv undershoot and slowed the overall time taken to reach steady-state. In contrast to previous results in healthy rats (Holdsworth et al., 2016), baseline PO_2mv was reduced with GLI indicating a role for K_{ATP} -mediated function in resting O_2 delivery-utilization matching in CHF. In addition, our findings indicate that PIN opposed the major effects promoted by GLI on muscle PO_2mv thus indicating modulation of K_{ATP} channel function rather than some non-specific effect with the present experimental protocol.

Intrinsic impairments in specific vasodilatory mechanisms in CHF skeletal muscle such as reduced NO bioavailability (Ferreira et al., 2006) will place greater reliance on K_{ATP} channel function during contractions. Figure 2 demonstrates different responses to GLI on the spinotrapezius muscle PO_2mv in healthy (data from Holdsworth et al., 2016) versus the present investigation with CHF rats. That GLI induced a greater reduction in muscle PO_2mv during the early contraction transient in CHF compared to healthy animals is consistent with augmented reliance of K_{ATP} channel function on O_2 delivery-utilization matching in this disease. Crucially, greater O_2 delivery-utilization mismatch with GLI in CHF occurs concomitantly with the slowing of second component (phase II) $\dot{V}O_2$ kinetics following the onset of contractions (Behnke et al., 2002). Exacerbating the fall in skeletal muscle PO_2mv due to a sluggish increase in O_2 delivery is also likely to impact cellular metabolism and contribute to the slowing of $\dot{V}O_2$ kinetics and premature fatigue that is prominent in CHF patients (Ferreira et al., 2005; Hepple et al., 1999; Wheaton and Chandel, 2011; Wilson et al., 1977).

GLI clearly exaggerates many of the CHF-induced perturbations of skeletal muscle PO_2mv kinetics including elevating the undershoot amplitude and delaying attainment of the contracting steady-state PO_2mv (Ferreira et al., 2006). This effect is also well-characterized in other O_2 supply-limited conditions such as aging (Behnke et al., 2005) and diabetes (Padilla et al., 2007) which are often coincident conditions for CHF patients. The

exacerbation of this dysregulation with GLI is clinically relevant given that these populations present with severe exercise intolerance (Piepoli et al., 2010a, 2010b; Sacre et al., 2015) due, in part, to a lowering of the skeletal muscle PO_2mv pressure head intrinsic to those diseases (Hirai et al., 2015; Poole et al., 2012). Both the magnitude and time course (i.e. concomitant with the rise in $\dot{V} \text{O}_2$ following contractions onset) of the decrements in PO_2mv suggest that degradation of the intracellular metabolic environment is likely to impair muscle work capacity and/or $\dot{V} \text{O}_2$ kinetics with GLI.

Impairments in contracting muscle PO_2mv in CHF have been attributed, in part, to reductions in NO bioavailability (Ferreira et al., 2006) and the results herein suggest that preserved K_{ATP} function protects against a more severe mismatch of O_2 -delivery to O_2 -utilization in this disease. Taken together, the shared contribution of NO and K_{ATP} channel signaling to muscle PO_2mv suggests that these pathways are not merely independent, parallel mechanisms, but rather synergistic mediators of skeletal muscle vascular and metabolic control. NO has been shown to cause arterial hyperpolarization, in part, via K_{ATP} channel activation (Murphy and Brayden, 1995) which may occur through the cGMP intermediary as demonstrated previously in cardiac myocytes (Chai et al., 2011) and cultured smooth muscle cells (Kubo et al., 1994). Furthermore, as a reactive nitrogen species, NO can cause direct S-nitrosylation of cysteine residues found on the SURx subunit (Kawano et al., 2009; Yoshida et al., 2006). Thus, K_{ATP} channel activation via NO is likely mediated through a combination of these two mechanisms. The notion that K_{ATP} channels can amplify vasodilatory signaling (Cole et al., 2000; Silva et al., 2008) via modulation of membrane potential deserves further investigation and may be valuable clinically (Akrouh et al., 2009).

Both the results herein and large retrospective studies of the association of sulphonylurea monotherapy with cardiovascular mortality and morbidity are cause for concern in diabetic and CHF patients (Fadini et al., 2015; Morgan et al., 2014; Simpson et al., 2006). Our findings demonstrate the additive effect of GLI on PO_2mv dysregulation in CHF which has been minimally addressed in the risk assessments of sulphonylurea therapy. That the primary muscle $\dot{V} \text{O}_2$ kinetics are significantly slowed in diabetic patients raises an intriguing question as to whether it is a *de facto* diabetes- or alternatively sulphonylurea-induced dysfunction of the K_{ATP} channel that drives the $\dot{V} \text{O}_2$ kinetics limitation (Bauer et al., 2007; O'Connor et al., 2015; Regensteiner et al., 1998). Clearly, when CHF attenuates other pathways vascular K_{ATP} channels remain one of the few potential defenses protecting against a more severe mismatch of O_2 -delivery to O_2 -utilization during muscle contractions. The administration of sulphonylureas may pose a previously unrecognized danger in patients afflicted with both CHF and diabetes where an additional drag on O_2 delivery could severely compromise exercise tolerance. The findings herein extend previous investigations of GLI in healthy rats (Holdsworth et al., 2016) by demonstrating a robust perturbation of the PO_2mv profile at the onset of electrically-induced contractions of the spinotrapezius muscle in CHF rats. The apparent sensitivity of the vascular K_{ATP} channels to pharmacologic intervention suggests that, in contrast to the well-established disruption of other mechanisms of vascular control in CHF such as NO synthase uncoupling (Maier et al., 2000; Sindler et al., 2009) and autonomic imbalance (Coats et al., 1994; Wang et al., 2010), preserved vascular K_{ATP}

channel function protects against exaggerated transient skeletal muscle hypoxia during contractions.

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Highlights

- Chronic heart failure impairs skeletal muscle microvascular oxygenation (PO_2mv) and exercise tolerance.
- Vascular ATP-sensitive K^+ (K_{ATP}) channels are important modulators of muscle PO_2mv during contractions in health.
- Herein we demonstrate that preserved K_{ATP} channel function helps preserve PO_2mv during muscle contractions in chronic heart failure.

Figure 1.

Mean absolute spinotrapezius muscle PO_{2mv} profiles for (A) control and GLI and (B) GLI and PIN in CHF rats. (C) Mean relative spinotrapezius PO_{2mv} profiles for control, GLI and PIN in CHF rats. Contractions began at time zero. Notice the divergence of the GLI profile resulting in a marked undershoot of the steady-state PO_{2mv} .

Figure 2.

Magnitude of change in spinotrapezius muscle PO_2mv with GLI during the early transient from rest to contractions in healthy (data from Holdsworth et al., 2016) versus the present investigation with CHF rats. Contractions began at time zero. GLI superfusion of the spinotrapezius muscle was utilized herein to minimize potential alterations in MAP and HR (primarily due to reduced conductance in the renal and splanchnic vascular beds) (Duncker et al., 2001; Holdsworth et al., 2015) observed previously with intra-arterial drug administration in healthy rats (Holdsworth et al., 2016). Note the different effects of GLI on muscle PO_2mv in CHF compared to healthy rats, indicating a greater reliance of K_{ATP} channel function on O_2 delivery-utilization matching in this disease. See text for further details. * $p < 0.05$ vs. healthy.

Table 1

Morphological and hemodynamic characteristics of CHF rats

	Mean $\pm$ SEM	Range
LVEDP, mmHg	18 $\pm$ 1	10 – 27
LV dp/dt, mmHg/s	6270 $\pm$ 239	4900 – 8800
LV/body mass, mg/g	1.91 $\pm$ 0.03	1.73 – 2.07
RV/body mass, mg/g	0.59 $\pm$ 0.02	0.51 – 0.78
Lung/body mass, mg/g	3.56 $\pm$ 0.16	2.78 – 5.29
Infarct size, %	30 $\pm$ 1	18 – 42

Data are mean $\pm$ SEM. LVEDP, left ventricular end diastolic pressure; LV dp/dt, left ventricular dp/dt; RV, right ventricle; LV, left ventricle. $n = 16$.

Table 2

Spinotrapezius PO₂mv parameters at rest and following the onset of contractions under control, GLI and PIN conditions

	Control	GLI	PIN
PO ₂ mv(BL), mmHg	28.3 ± 0.9	24.8 ± 1.0 [*]	32.5 ± 2.0 [†]
₁ PO ₂ mv, mmHg	11.0 ± 0.7	9.7 ± 0.6	9.7 ± 1.2
₂ PO ₂ mv, mmHg	3.3 ± 0.2	4.3 ± 0.4 [*]	2.4 ± 0.4 [†]
PO ₂ mv(SS), mmHg	19.6 ± 0.6	19.1 ± 0.9	23.8 ± 1.4 [†]
TD ₁ , s	5.5 ± 0.9	4.7 ± 0.6	3.0 ± 0.7
TD ₂ , s	39.0 ± 6.9	36.8 ± 5.2	57.0 ± 12.7
τ ₁ , s	10.6 ± 0.7	10.3 ± 1.0	16.8 ± 3.6
τ ₂ , s	33.6 ± 4.6	47.1 ± 5.4 [*]	32.5 ± 7.2
MRT ₁ , s	16.2 ± 1.1	15.0 ± 1.0	19.8 ± 3.3
MRT ₂ , s	72.6 ± 7.9	83.9 ± 6.3	89.5 ± 12.2
MRT _{Total} , s	66.1 ± 10.2	93.6 ± 7.8 [*]	57.1 ± 14.1 [†]

Values are mean ± SE. PO₂mv(BL), resting PO₂mv; ₁PO₂mv, amplitude of the first component; ₂PO₂mv, amplitude of the second component; PO₂mv(SS), contracting steady-state PO₂mv; TD₁, time delay of the first component; TD₂, time delay of the second component; τ₁, time constant of the first component; τ₂, time constant of the second component; MRT₁, mean response time of the first component; MRT₂, mean response time of the second component; MRT_{Total}, sum of the first and second component mean response times. The two-component model was used to describe the PO₂mv kinetics in the following conditions: CON (11/16); GLI (15/16); PIN (5/12).

^{*}
p < 0.05 vs. control.

[†]
p < 0.05 vs. GLI.