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Plasticity of microvascular oxygenation control in rat fast-twitch muscle: Effects of experimental creatine depletion

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ABSTRACT

Aging, heart failure and diabetes each compromise the matching of O_2 delivery ($\dot{Q}O_2$)-to-metabolic requirements (O_2 uptake, $\dot{V}O_2$) in skeletal muscle such that the O_2 pressure driving blood–myocyte O_2 flux (microvascular PO_2, $PmvO_2$) is reduced and contractile function impaired. In contrast, β-guanidinopropionic acid (β-GPA) treatment improves muscle contractile function, primarily in fast-twitch muscle (Moerland and Kushmerick, 1994). We tested the hypothesis that β-GPA (2% wt/BW in rat chow, 8 weeks; $n = 14$) would improve $\dot{Q}O_2$-to-$\dot{V}O_2$ matching (elevated $PmvO_2$) during contractions (4.5 V @ 1 Hz) in mixed (MG) and white (WG) portions of the gastrocnemius, both predominantly fast-twitch). Compared with control (CON), during contractions $PmvO_2$ fell less following β-GPA (MG −54%, WG −26%, $P < 0.05$), elevating steady-state $PmvO_2$ (CON, MG: 10 ± 2, WG: 9 ± 1; β-GPA, MG 16 ± 2, WG 18 ± 2 mmHg, $P < 0.05$). This reflected an increased $\dot{Q}O_2/\dot{V}O_2$ ratio due primarily to a reduced $\dot{V}O_2$ in β-GPA muscles. It is likely that this adaptation helps facilitate the β-GPA-induced enhancement of contractile function in fast-twitch muscles.

1. Introduction

Skeletal muscle fiber types are highly stratified with respect to contraction speed (Armstrong and Phelps, 1984; Baldwin et al., 1972; Barclay et al., 1993; Bottinelli et al., 1996; Delp and Duan, 1996), oxidative capacity (Delp and Duan, 1996; Gollnick et al., 1972), capillarity (Folkow and Halicka, 1968), and O_2 uptake ($\dot{V}O_2$) kinetics (Barstow et al., 1996), as well as blood flow ($\dot{Q}$) and O_2 delivery ($\dot{Q}O_2$) (Ferreira et al., 2006b). Investigation of the mechanistic bases for the heterogeneity of $\dot{Q}$ and hence $\dot{Q}O_2$ among muscles of disparate fiber types supports that inter fiber-type differences are attributable, in part, to a differential arteriolar vasomotor control (Behnke et al., 2002a, 2011; Laughlin et al., 1997; McDonough et al., 2005).

We have previously demonstrated that, compared with its fast-twitch counterparts (medial and white gastrocnemius, peroneal), the contracting, slow-twitch soleus muscle achieved a higher $\dot{Q}O_2$ per unit $\dot{V}O_2$ (Behnke et al., 2002a, 2004). The $\dot{Q}O_2 : \dot{V}O_2$ ratio is of crucial importance because it determines the microvascular O_2 partial pressure ($PmvO_2$) which drives blood–myocyte O_2 flux and also helps "set" intracellular PO_2 and thus muscle energetics (Behnke et al., 2002a; Haseler et al., 2004; Hogan and Welch, 1986; McDonough et al., 2005). The regulation of $PmvO_2$ demonstrates considerable plasticity, for example, in aging (Behnke et al., 2005) and chronic diseases such as heart failure (Diederich et al., 2002) and diabetes (Behnke et al., 2002c; Padilla et al., 2007). Specifically, $PmvO_2$ in the spinotrapezius muscle falls more rapidly and to far lower levels during contractions in aging and these disease states than observed in young healthy controls. These conditions are associated with slowed pulmonary $\dot{V}O_2$ kinetics and compromised muscle oxidative function (Behnke et al., 2002c, 2004; Belardinelli et al., 1997; Brandenburg et al., 1999; Chilibeck et al., 1997; McDonough et al., 2004a; Pfeifer et al., 2001; Regensteiner et al., 1998; Sietsema et al., 1994; though see Wilkerson et al., 2011 for an exception in long-term diabetic patients) and the lowered $PmvO_2$ indicates a reduced $\dot{Q}O_2$-to-$\dot{V}O_2$ ratio in skeletal muscle.

In addition to depleting intramuscular creatine and phosphocreatine stores (Moerland and Kushmerick, 1994; Moerland et al., 1989), chronic dietary β-guanidinopropionic acid (β-GPA) treatment up-regulates adenosine monophosphate-activated protein kinase (AMPK) activity (Bergeron et al., 2001; Williams et al., 2009). AMPK is a serine/threonine kinase that is expressed in several

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tissues including endothelial and smooth muscle cells and contributes to the regulation of endothelial nitric oxide synthase (eNOS) activation and NO synthesis (Morrow et al., 2003). Further, Bradley et al. (2010) have recently demonstrated that activation of AMPK has a direct vasodilatory action on skeletal muscle resistance arteries through increased NO activity. In muscle-specific AMPK dominant negative transgenic mice there is a faster $Pmv\dot{O}_2$ decline (*i.e.*, reduced time-constant) during the rest-to-contractions transition *versus* that observed from mice demonstrating a normal AMPK phenotype (Kano et al., 2011). Thus, the absence of AMPK induces a disproportionate slowing of $\dot{Q}O_2$ *versus* $\dot{V}O_2$ kinetics across the rest-to-contractions transition (Kano et al., 2011). Therefore, long-term β-GPA treatment would likely have an indirect influence on vasomotor regulation (*e.g.*, faster vasodilatory dynamics) through enhanced nitric oxide signaling elicited by increased AMPK activity. Based upon this reasoning, we investigated whether β-GPA treatment would elevate the $\dot{Q}O_2$-to-$\dot{V}O_2$ ratio during contractions in fast-twitch muscle (which is affected more than slow twitch muscle with β-GPA treatment at least with respect to mitochondrial adaptations; Bruton et al., 2003). Specifically, we tested the hypotheses that chronic β-GPA supplementation would reduce the magnitude and slow the rate of the $Pmv\dot{O}_2$ fall (presumably due to faster blood flow kinetics) during muscle contractions resulting in an elevated steady-state contracting $Pmv\dot{O}_2$. As β-GPA treatment improves muscle contractile function primarily in fast-twitch muscle (Moerland and Kushmerick, 1994), we reasoned that these contractile improvements in fast-twitch muscle would result, in part, from an enhanced $Pmv\dot{O}_2$, which would then help facilitate transcapillary O_2 flux and act to increase intramyocyte PO_2.

2. Methods

All procedures were approved by the Institutional Animal Care and Use Committee at Kansas State University. Rats were housed individually at 23 °C and were maintained on a 12:12-h light–dark cycle. All rats were fed rat chow (control or containing 2% β-GPA for 8 weeks, see below) and water *ad libitum*.

2.1. Surgical preparation

All rats were anesthetized prior to experimentation with pentobarbital sodium (40 mg/kg ip to effect) and supplemented (5–10 mg/kg) as needed. The carotid and tail (caudal) arteries were catheterized with polyethylene tubing (PE-10 connected to PE-50). This allowed for the infusion of the phosphorescent probe [palladium *meso*-tetra (4-carboxyphenyl) porphine dendrimer (R2)], measurement of arterial blood pressure (Digi-Med BPA model 200, Louisville, KY) and withdrawal of arterial blood for blood gas measurement (Nova Stat Profile M, Waltham, MA).

The muscles used in the current study (*i.e.*, mixed gastrocnemius, (MG) and white gastrocnemius, (WG); McDonough et al., 2005) were chosen as previous research demonstrates that the effects of β-GPA are manifest primarily in fast-twitch musculature (Freyssenet et al., 1995; Moerland, 1995). Both the MG (3% type I, 6% type IIa, 34% type IId/x and 57% type IIb) and the WG (8% type IId/x, 92% type IIb; Delp and Duan, 1996) are comprised primarily of fast-twitch fiber phenotypes. Whereas the soleus (predominantly slow-twitch) may have provided an interesting 'control' comparison this would have required more animals for what was expected to be a negative result.

Each muscle was exposed for $Pmv\dot{O}_2$ measurements as previously detailed (McDonough et al., 2005). The tibial nerve was isolated and a stimulating electrode was attached. The ground electrode was attached distally, near the Achilles tendon. Care was taken to minimize the extent of the surgery in all cases. The exposed tissue was superfused with a Krebs–Henseleit bicarbonate-buffered solution (38 °C, equilibrated with 5% CO_2–N_2 balance) and body temperature was maintained at ~38 °C via a heating pad.

2.2. β-GPA supplementation

Experimental animals were fed β-GPA for 8 weeks which has been demonstrated to reduce phosphocreatine (PCr) (Moerland and Kushmerick, 1994) and up-regulate AMPK activity (Bergeron et al., 2001; Chaturvedi et al., 2009). The β-GPA was incorporated into the rat chow at 2% wt/wt.

2.3. Contractions protocol

The rat was positioned on a custom-built ergometer and secured as detailed previously (McDonough et al., 2005). Fifteen minutes later the MG or WG was stimulated at 1-Hz for 3 min (twitch 4.5 V, 2 ms pulse duration) using a Grass S88 stimulator. This contraction intensity was chosen as it corresponds to approximately 65% of the voltage which produces a minimal $Pmv\dot{O}_2$ for these two muscles (McDonough, Behnke, Musch, Poole, unpublished observations). All animals were euthanized with an overdose of pentobarbital sodium (>80 mg/kg i.a.) following the conclusion of the experimental protocol.

2.4. Phosphorescence quenching

Fifteen minutes prior to the beginning of the contraction protocol the R2 probe was infused (15 mg/kg via the arterial catheter) and the probe of a PMOD 1000 Frequency Domain Phosphorimeter (Oxygen Enterprises Ltd., Philadelphia, PA) was positioned ~2 mm above the exposed muscle. A light guide contained within the probe focuses excitation light (524 nm) on the medial region of the exposed muscle (~2.0 mm diameter, to ~500 μm deep). The PMOD 1000 uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and repeated every 2 s (for review see Rumsey et al., 1988; Vinogradov and Wilson, 1994). The phosphorescence lifetime was obtained computationally based on the decomposition of data vectors to a linearly independent set of exponentials (Vinogradov et al., 2001).

The Stern–Volmer relationship allows the calculation of $Pmv\dot{O}_2$ from a measured phosphorescence lifetime using the following equation:

$$Pmv\dot{O}_2 = \frac{[(t^o/t) - 1]}{(k_Q^* t^o)}$$

where k_Q is the quenching constant (mmHg/s) and t^o and t are the phosphorescence lifetimes in the absence of O_2 and at the ambient O_2 concentration, respectively. For R2, in *in vitro* conditions similar to those found in the blood, k_Q is 409 $mmHg^{-1}\ s^{-1}$ and t^o is 601 μs (Lo et al., 1997). Since the R2 is tightly bound to albumin in the plasma and is negatively charged, in combination with the extremely high albumin reflection coefficients in skeletal muscle (Renkin and Tucker, 1998), the PO_2 measurements are ensured to result from signals within the microvasculature, rather than the surrounding muscle tissue (Poole et al., 2004). The phosphorescence lifetime is insensitive to probe concentration, excitation light intensity, and absorbance by other chromophores in the tissue (Pawlowski and Wilson, 1992). The effects of pH and temperature are negligible within the normal physiological range which was maintained herein.