

Skeletal muscle contraction-induced vasodilation in the microcirculation

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Maximal whole body exercise leads skeletal muscle blood flow to markedly increase to match metabolic demands, a phenomenon termed exercise hyperaemia that is accomplished by increasing vasodilation. However, local vasodilatory mechanisms in response to skeletal muscle contraction remain uncertain. This review highlights metabolic vasodilators released from contracting skeletal muscle, endothelium, or blood cells. As a considerable skeletal muscle vasodilation potentially results in hypotension, sympathetic nerve activity needs to be augmented to elevate cardiac output and blood pressure during dynamic exercise. However, since the enhanced sympathetic vasoconstriction restrains skeletal muscle blood flow, intramuscular arteries have an indispensable ability to blunt sympathetic activity for exercise hyperaemia. In addition, we discuss that mechanical compression of

the intramuscular vasculature contributes to causing the initial phase of increasing vasodilation following a single muscle contraction. We have also chosen to focus on conducted (or ascending) electrical signals that evoke vasodilation of proximal feed arteries to elevate blood flow in the microcirculation of skeletal muscle. Endothelial hyperpolarization originating within distal arterioles ascends into the proximal feed arteries, thereby increasing total blood flow in contracting skeletal muscle. This brief review summarizes molecular mechanisms underlying the regulation of skeletal muscle blood flow to a single or sustained muscle contraction.

Keywords: Blood flow control, Endothelium, Exercise hyperaemia, Intramuscular vasculature, Microcirculation, Vascular smooth muscle cell


INTRODUCTION

During steady-state dynamic exercise, blood flow to active skeletal muscle is dramatically increased by nearly 100 times (i.e., exercise hyperaemia) (Richardson et al., 1993). Skeletal muscle blood flow is regulated by metabolic vasodilatory substances (Clifford and Hellsten, 2004). Furthermore, increased sympathetic nerve activity that causes vasoconstriction in 'noncontracting' skeletal muscle to prevent hypotension is interestingly reduced in 'contracting' skeletal muscle to increase vasodilation and provide adequate blood flow during exercise (Hearon and Dineno, 2016). A number of leading investigators have also attempted to demonstrate how skeletal muscle vasodilation is initiated from a single muscular contraction. Potential mechanisms such as acetylcholine (ACh) spillover, potassium release following skeletal muscle con-

traction, mechanosensitive component-induced vasodilation, and conducted vasodilation would be discussed in this review.

METABOLIC VASODILATORY FACTORS

Nitric oxide (NO), an endogenous vasoactive candidate, is generated by endothelial nitric oxide synthase or neuronal nitric oxide synthase (nNOS) in the endothelium or skeletal muscle cells, respectively (Frandsen et al., 1996; Grozdanovic et al., 1996). Since exercise-induced increases in endogenous agonists (e.g., ACh, bradykinin, and adenosine triphosphate [ATP]) elicit NO generation (Nosarev et al., 2015), NO seems to play a crucial role in vasodilation in contracting skeletal muscle. However, roles of NO in exercise hyperaemia are still far from clear. Prior studies have been shown that pharmacological inhibition of NOS with L-NAME

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does not diminish vasodilation of skeletal muscle arteries in contracting leg muscle (Frandsen et al., 2001; Rådegran and Saltin, 1999), indicating that NO may be a nonobligatory molecule for exercise hyperaemia. Contrarily, it has been reported that skeletal muscle contraction leads matricryptic type II repeat of fibronectin (FNIII-1) receptors on vascular smooth muscle cells (VSMCs) to be exposed, inducing NO-mediated vasodilation by stimulating nNOS (Hocking et al., 2008). Collectively, further studies are required for a better understanding of NO in exercise hyperaemia.

Prostaglandin E₂ and prostacyclin (PGI₂) are derived from prostaglandin H₂ that is converted from arachidonic acid through cyclooxygenase (COX). These prostaglandins are commonly generated from endothelium or skeletal muscle cells (Davidge, 2001). Exercise is shown to increase levels of prostaglandin E₂ and PGI₂ in the interstitial space or venous efflux of contracting skeletal muscle (Frandsen et al., 2000). However, similar to NO, some debates exist as to whether prostaglandins are involved in exercise hyperaemia. Once COX inhibitors were infused to reduce prostaglandin production, blood flow of human forearm and leg was not considerably altered at rest or during exercise (Schrage et al., 2004; Schrage et al., 2010). Interestingly, simultaneous suppression of COX and NOS reduced blood flow in the exercise leg by 30% (Schrage et al., 2004), suggesting that NO-dependent vasodilation may compensate for COX inhibition-mediated decrease in vasomotor reactivity to provide adequate blood flow during exercise.

The fact that each skeletal muscle is innervated by a motor nerve at the neuromuscular junction suggests the intriguing hypothesis that ACh spillover results in skeletal muscle arteriolar vasodilation (Welsh and Segal, 1997). ACh released from the end plate of motor nerves stimulates nicotinic receptors on the sarcolemma to elicit muscle contraction. ACh spillover is sufficient to functionally reach small-sized arteries and capillaries in skeletal muscle for vasodilation (Welsh and Segal, 1997). ACh directly binds to muscarinic receptors in endothelial cells (ECs) and in turn alters intracellular Ca²⁺ dynamics including Ca²⁺ release from the endoplasmic reticulum (ER). These Ca²⁺ signaling networks provoke NO production and endothelium-derived hyperpolarization (EDH) that are responsible for a prompt vasodilation following a brief muscle contraction or during exercise. However, the 'ACh spillover' has been debated and questioned in subsequent studies. Blood flow in response to muscle contraction was assessed in the absence or presence of atropine (nicotinic receptor antagonist) that would not suppress ACh release but would inhibit muscle contraction (Brock et al., 1998). As a consequence, the atropine application did not abolish increased vasodilation and blood

flow after forearm muscle contraction. Further, pharmacological neuromuscular blockade with vecuronium (for inhibition of muscle contraction) profoundly attenuated increases in vasodilation and blood flow following a single or repeated muscle contraction (Naik et al., 1999). These results suggest that ACh spillover from the motor nerves may not be necessary for muscle contraction-induced vasodilation. Preferably, vasoactive substances released/produced by muscle contraction may play an important role in increasing vasomotor reactivity and blood flow.

Several criteria have been established to determine whether endogenous molecules act as metabolic vasodilatory factors during physiological stresses (Feigl, 1983): (a) the endogenous candidates that regulate vasomotor reactivity at rest or during exercise have to be measurable; (b) the vasoactive responses similarly or identically occur when the candidate agents are exogenously applied; and (c) stimulating or inhibiting vascular responses to the vasodilatory molecules has to be consistent. On the basis of the criteria, substantial attention has been paid to intravascular ATP as a potent vasodilatory molecule in contracting skeletal muscle. Indeed, ATP concentration in venous plasma draining from skeletal muscle was progressively and considerably elevated along with increasing intensity of rhythmic handgrip contraction or single-leg knee extensor exercise (Mortensen et al., 2011; Kirby et al., 2012). ATP-mediated activation of purinergic (P₂) receptors in ECs evokes a robust vasodilation during exercise through protein kinase C (PKC)-Akt-NO signaling pathways (Mortensen et al., 2009a). The increased ATP has been identified to be rapidly degraded by ectonucleotidases (< 1 sec of half-life), implicating that ATP should be locally released in the microcirculation to promptly cause vasodilation during exercise (Creelius et al., 2015). Regarding to the cellular sources of ATP, it was conventionally predicted that ATP is likely released from sympathetic nerves or contracting skeletal muscle itself (Mortensen et al., 2009b). However, exogenous ATP administration does not alter interstitial ATP levels (Mortensen et al., 2009b). It is implied that sympathetic nerve and/or skeletal muscle-released ATP may not be able to pass across the plasma membrane of VSMCs and exert endothelial P₂ receptor-mediated vasodilation (Creelius et al., 2015). On the other hand, previous studies have elucidated that perfusion occlusion markedly diminishes exercise-induced increase in venous plasma ATP concentration (Kirby et al., 2013), suggesting that the increased ATP from steady-state exercise is closely related to elevated intravascular perfusion. As the increased perfusion augments blood supply and shear stress, a general belief is that the sources of ATP release during exercise may be erythrocytes and/or endothelial cells.

In general, potassium (K^+) is the most abundant ion in cells and K^+ channels largely regulate the membrane potential (Haddy et al., 2006). Since intracellular K^+ concentration is much higher than one in extracellular space, this chemical gradient result in K^+ efflux and more negative membrane potential (i.e., hyperpolarization or repolarization). It is important to note that K^+ ions are released from contracting skeletal muscle fibers for the repolarization phase of the contraction/relaxation cycle (Clifford, 2007). Previous studies support the notion that interstitial K^+ levels are profoundly increased following a single muscle contraction (Hnik et al., 1976) or during exercise (Green et al., 2000; Juel et al., 2000; Lott et al., 2001). Interestingly, alteration in interstitial K^+ concentration is dependent of exercise duration (Hnik et al., 1976) or intensity (Green et al., 2000) of skeletal muscle contraction. K^+ released from skeletal muscle fibers is considered a key player for vasodilation or exercise hyperaemia by inducing K^+ channel activation-dependent hyperpolarization of VSMCs (Joyner and Wilkins, 2007). Specifically, inwardly rectifying K^+ (K_{ir}) channels are activated by increased extracellular K^+ to cause K^+ efflux-mediated hyperpolarization (Knot et al., 1996). While the relatively high K^+ provokes vasoconstriction through K^+ entry through K_{ir} channels expressed in VSMCs (Quayle et al., 1993) based on the Nernst equation, K_{ir} channels stimulated by the low K^+ level result in K^+ efflux and vasodilation (Knot et al., 1996). It has been defined in animal and human studies that vasodilation following a single skeletal muscle contraction or during steady-state exercise is significantly abrogated in the presence of K_{ir} channel inhibitor barium chloride (Armstrong et al., 2007; Creelius et al., 2014). Thus, extracellular K^+ and K_{ir} channel-mediated VSMC hyperpolarization and vasodilation is a novel and innovative mechanism underlying onset and steady-state exercise hyperaemia.

FUNCTIONAL SYMPATHOLYSIS

Skeletal muscle blood flow increases significantly during whole-body maximal exercise, which exceeds the pumping capacity of the heart and causes excessive vasodilation-mediated hypotension (Hearon and Dinno, 2016). Thus, during high intensity exercise, sympathetic nerve activity has to be markedly enhanced to elevate cardiac output and peripheral vasoconstriction through the binding of norepinephrine to α_1 - and α_2 -adrenergic receptors in VSMCs (Thomas, 2015). The increased sympathetic nerve activity contributes to regulation of systemic blood pressure and redistribution of cardiac output towards contracting skeletal muscle (Hearon and Dinno, 2016). However, in contrast to inactive tissues, peripheral

vasoconstriction signaling in response to sympathetic nerve activity should be attenuated in vascular beds of contracting skeletal muscle to optimize blood flow and oxygen delivery during exercise, which is termed 'functional sympatholysis' (Remensnyder et al., 1962).

Although the exact mechanisms underlying functional sympatholysis remain unclear, mounting evidence has shown that it is regulated by a number of local vasoactive substances including NO, prostaglandins, ATP, and K^+ ions (Clifford and Hellsten, 2004; Saltin and Mortensen, 2012). In addition, functional sympatholysis is directly proportional to exercise intensity (Thomas and Segal, 2004) and primarily occurs in the small distal arterioles compared to the proximal arteries or feed arteries in the skeletal muscle microcirculation (VanTeeffelen and Segal, 2003). This heterogeneity in functional sympatholysis leads the distal arterioles to ensure adequate blood flow to the most metabolically active skeletal muscle (Thomas, 2015). Meanwhile, impaired functional sympatholysis is associated with vascular diseases such as hypertension (Thomas, 2015) and sympathetic overactivity considerably abolishes skeletal muscle vasodilation during exercise (Calbet and Lundby, 2012). Taken together, appropriate functional sympatholysis contributes to regulating blood flow distribution in contracting skeletal muscle.

MECHANICAL COMPRESSION

A large number of studies have investigated vasodilatory mechanisms during steady-state muscle contraction. However, these mechanisms do not account for a fundamental question as to how the vasodilation of skeletal muscle feed arteries or arterioles are initiated at the onset of muscle contraction. With respect to this interesting issue, emphasis has been placed on external compression (elicited by muscle contraction) on intramuscular arterioles. It has been reported that intramuscular pressures of 270 or 600 mmHg are observed during moderate-intensity running or maximal muscle contraction, respectively (Ballard et al., 1998; Sejersted et al., 1984). As ECs and VSMCs are mechanosensitive to shear stress, stretch, tension, or pressure, it is conceivable that muscle contraction-evoked mechanical compression may activate mechanosensors in ECs or VSMCs to initiate vasodilation following a brief muscle contraction.

Clifford et al. (2006) attempted to directly explore this intriguing hypothesis by manipulating extravascular pressure in a custom-designed chamber that mimics mechanical compression caused by skeletal muscle activity. Pressure pulses of 600 mmHg were applied on isolated rat soleus feed arteries for 1 sec, 5 sec, or a

series of 5 repetitions with 1-sec duration. While the skeletal muscle feed arteries were entirely collapsed during the extravascular pressure application, release of the pressure immediately led to a significant vasodilation (Clifford et al., 2006). The degree of vasodilation was independent of the duration of compression, whereas increasing the number of compressions markedly augmented vasodilation in soleus feed arteries (Clifford et al., 2006). Consistently, it has been shown in human studies that elevation in extravascular pressure significantly increases blood flow and vascular conductance in forearm brachial arteries (Kirby et al., 2007). Recent work has mechanistically identified that blockade of integrins (mechanosensors in ECs and VSMCs) with antibodies against $\alpha_5\beta_1$ $\alpha_v\beta_3$ integrins significantly diminishes mechanical compression-evoked vasodilation of intramuscular arteries (Lu and Kassab, 2015).

ENDOTHELIUM-DERIVED HYPERPOLARIZATION AND CONDUCTED VASODILATION

Endothelial Ca^{2+} signaling plays an essential role in regulating vascular diameter and blood flow by changing membrane potential in ECs and VSMCs (Garland et al., 2011). ACh-dependent activation of endothelial muscarinic receptors stimulates phospholipase C to induce cleavage of phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). The binding of the second messenger to IP_3 receptors (IP_3Rs) in ER results in Ca^{2+} release from the ER. The released Ca^{2+} activates intermediate/small conductance Ca^{2+} -sensitive K^+ channels (IK_{Ca}/SK_{Ca}) and leads to K^+ efflux. The K^+ loss elicits EDH that is transferred to VSMCs through myoendothelial gap junctions (MEGJs). MEGJs are indispensable for EC-SMC heterocellular communication that allows the movement of ionic charges between those cells (Figuroa and Duling, 2009). The hyperpolarization to VSMCs causes vasodilation by suppressing Ca^{2+} influx through voltage dependent calcium channels (VDCCs) that are required for vasoconstriction. ACh-induced VSMC hyperpolarization and vasodilation in small-sized arteries or arterioles were not significantly abolished in the presence of inhibitors of NO, guanylyl cyclase, or prostaglandins (Garland and McPherson, 1992). In contrast, ACh-dependent vasodilation was completely abrogated when IK_{Ca} and SK_{Ca} were simultaneously inhibited (Holzmann et al., 1994). These previous results indicate that vasodilatory mechanisms are primarily dependent of EDH in small-sized resistance arteries.

Arteriolar networks in skeletal muscle are typically comprised of feed arteries and branch arterioles including first-, second-,

third-order, and terminal arterioles. While distal branch arterioles are embedded in skeletal muscle, proximal feed arteries are not directly involved in blood flow delivery necessary for muscle contraction. However, vasodilation along and among feed arteries and branches of vascular resistance network has to be coordinated to supply substantial blood flow into active skeletal muscle during exercise (Segal and Duling, 1986; Segal and Jacobs, 2001). It should be recognized that vasomotor reactivity of intramuscular distal arterioles are regulated by metabolic vasoactive factors, blunted sympathetic nerve activity, and dilatory mechanisms elicited by mechanical compression. From this perspective, a compelling question has been suggested: how are proximal feed arteries dilated to provide appropriate blood flow during skeletal muscle contraction? Regarding to this, the concept of 'ascending or conducted vasodilation' has shed novel insight into the coordinated vasodilation of skeletal muscle vasculature. In brief, vasodilation originating from the microcirculation embedded in skeletal muscle ascends into proximal feed arteries to increase total blood flow entering the skeletal muscle (Bagher and Segal, 2011). Accumulating evidence has shown that ascending vasodilation is attributed to the conduction of electrical signaling including EDH between EC-EC and EC-SMC through gap junctions (Segal and Duling, 1986; Segal and Jacobs, 2001). EDH initiated from the intramuscular vasculature is bi-directionally (i.e., up- and downstream) conducted from EC to EC through homocellular gap junctions (Domeier and Segal, 2007; Milkau et al., 2010). EDH is also transmitted to adjacent VSMCs through MEGJs, which in turn induces ascending vasodilation by inhibiting Ca^{2+} influx through VDCCs (Figuroa and Duling, 2008; Segal and Duling, 1986). Taken together, it is suggested that EDH-mediated conducted (or ascending) vasodilation from terminal arterioles to feed arteries is a major determinant for achieving appropriate muscle blood flow during exercise.

CONCLUSIONS

An ability to elevate blood flow within the skeletal muscle microcirculation plays a vital role in matching metabolic demands and eliminating waste products of muscle metabolism. As blood flow is related to the fourth power of arterial diameter (the Poiseuille's Law), we have sought to summarize multiple vasodilatory mechanisms including metabolic, neuronal, mechanical, and conducted responses of intramuscular vasculature to muscle contraction. However, while the individual mechanism has been well defined, the mechanisms by which those contributors to vasodilation are coordinated each other to achieve optimal blood flow during

dynamic exercise have not been fully delineated. Therefore, further studies have to be done to address integrative dilatory mechanisms in the microcirculation of skeletal muscle tissue.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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