HIGHLIGHTED TOPIC | Skeletal and Cardiac Muscle Blood Flow

Vasodilatory mechanisms in contracting skeletal muscle

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Clifford, Philip S., and Ylva Hellsten. Vasodilatory mechanisms in contracting skeletal muscle. J Appl Physiol 97: 393–403, 2004; 10.1152/japplphysiol.00179.2004.—Skeletal muscle blood flow is closely coupled to metabolic demand, and its regulation is believed to be mainly the result of the interplay of neural vasoconstrictor activity and locally derived vasoactive substances. Muscle blood flow is increased within the first second after a single contraction and stabilizes within ~30 s during dynamic exercise under normal conditions. Vasodilator substances may be released from contracting skeletal muscle, vascular endothelium, or red blood cells. The importance of specific vasodilators is likely to vary over the time course of flow, from the initial rapid rise to the sustained elevation during steady-state exercise. Exercise hyperemia is therefore thought to be the result of an integrated response of more than one vasodilator mechanism. To date, the identity of vasoactive substances involved in the regulation of exercise hyperemia remains uncertain. Numerous vasodilators such as adenosine, ATP, potassium, hypoxia, hydrogen ion, nitric oxide, prostanoids, and endothelium-derived hyperpolarizing factor have been proposed to be of importance; however, there is little support for any single vasodilator being essential for exercise hyperemia. Because elevated blood flow cannot be explained by the failure of any single vasodilator, a consensus is beginning to emerge for redundancy among vasodilators, where one vasoactive compound may take over when the formation of another is compromised. Conducted vasodilation or flow-mediated vasodilation may explain dilation in vessels (i.e., feed arteries) not directly exposed to vasodilator substances in the interstitium. Future investigations should focus on identifying novel vasodilators and the interaction between vasodilators by simultaneous inhibition of multiple vasodilator pathways.

blood flow; metabolic vasodilation; functional vasodilation

Precise matching of blood flow and metabolism is required for virtually all living tissues. This is especially important for skeletal muscle, where metabolism can vary considerably from periods of inactivity to periods of repeated contractions. Despite intense research efforts during the past century, our understanding of the mechanism for exercise hyperemia remains inadequate.

It has long been known that skeletal muscle blood flow increases in proportion to the metabolic demands of the tissue during steady-state dynamic exercise. There is a direct relationship between the increase in muscle blood flow and increased contraction frequency (100), increased running speed (1, 96), and increased muscle oxygen consumption (6, 107). Because of the direct relationship between skeletal muscle blood flow and metabolic activity, it is logical to assume that there must be some substance(s) released from the contracting muscle that causes vascular smooth muscle relaxation and consequent vasodilation. However, definitive support for involvement of any specific vasodilator substance is lacking.

Blood flow to any vascular bed is determined by the perfusion pressure and vascular tone. Under some conditions, muscle blood flow can be altered via changes in perfusion pressure without changes in smooth muscle tone. During exercise, increases in muscle perfusion pressure could be a consequence of elevated arterial pressure (the exercise pressor reflex) or reductions in venous pressure (mediated by the skeletal muscle pump). In the absence of major changes in perfusion pressure, the primary controller of muscle blood flow is vascular smooth muscle tone.

The autonomic nervous system does not appear to be responsible for vasodilation (12, 14, 15), although sympathetic vasoconstriction restrains blood flow to active muscle during exercise (11, 13, 60, 77, 114, 138, 163). Despite its appeal in explaining the rapidity of the blood flow response to contraction, acetylcholine spillover from the motor nerve (162) does not seem to be an adequate explanation for the initial vasodilation (35, 111). Thus the profound vasodilation in the vascular bed of exercising muscle is thought to be initiated by local factors, including substances released from skeletal muscle and
endothelial cells, as well as red blood cells (see Fig. 1). This initial response is magnified through dilation of upstream vessels by conducted dilation or flow-mediated dilation.

TIME COURSE OF VASODILATION

A common finding of in vivo experiments is that there is an immediate (within 1 s) increase in muscle blood flow after the release of muscle contraction (Refs. 111, 140, 153; see Fig. 2). The notion that this increase in blood flow represents vasodilation is supported by direct observations of terminal arterioles in rat spinotrapezius muscle (101), in which dilation began within 2 s of the onset of contractions. On the other hand, two other studies (55, 73) reported that the latency for vasodilation varies between ∼5 and 20 s, with the delay being directly proportional to vessel size. The latency for vasodilation to direct application of vasodilator agents was reported to be ≥4 s according to Wunsch et al. (165), who examined the response of cannulated first-order skeletal muscle arterioles to adenosine, acetylcholine, nitric oxide (NO), and K⁺. They concluded that “none of the vasodilators produced a response rapid enough to contribute to the initial hyperemia (1–2 s) at the onset of exercise.” There are two limitations to the interpretation of these findings: 1) first-order arterioles may not be the most responsive portion of the skeletal muscle vasculature.

Fig. 1. Cellular sources and interactions of vasodilating compounds in skeletal muscle tissue. A: skeletal muscle cells have the capacity to form vasodilator prostaglandins (PGs) from cyclooxygenase (COX), adenosine from 5′-nucleotidase (5′ NUC), and nitric oxide (NO) from NO synthase (NOS). All of these vasodilators have been found to increase in the muscle interstitium in response to contraction. Note that the formation of adenosine (ADO) occurs extracellularly via the action of membrane-bound 5′-nucleotidase on AMP. In addition, contraction results in a marked release of K⁺ from the skeletal muscle cells. B: endothelial cells can form NO, endothelium-derived hyperpolarizing factor (EDHF), eicosatrienoic acids (EETs), PGs, and ADO in response to stimulus by other compounds or by mechanical factors such as shear stress. Although the identity of EDHF is uncertain, candidates include 11,12-EET, formed by cytochrome P450 2C9 (CYP 2C9), and K⁺. C: erythrocytes may release NO and ATP in response to hemoglobin desaturation. The release of NO occurs independently of NOS and is derived either from nitrosohemoglobin or by reduction of nitrite via the deoxygenated hemoglobin molecule. The vasodilating effect of ATP is believed to be mediated by binding to P2Y purinergic receptors on the endothelium, possibly leading to release of NO, PGs, or EDHF. D: putative interactions between vasodilator enzyme systems. Vasodilator PGs have been described to exert a negative effect on the formation of NO by a cAMP-dependent mechanism. ADO promotes the release of NO as well as vasodilator PGs. NO has been shown to inhibit cytochrome P450 2C (CYP 2C), and thus the formation of EETs, and to promote the formation of vasodilator PGs. These interactions may occur between cell types as well as in the same cell. [Ca²⁺], Ca²⁺ concentration; eNOS, endothelial NOS; nNOS, neuronal NOS.
CONDUCTED AND FLOW-MEDIATED VASODILATION

Exercise hyperemia represents the coordinated response of a vascular network within skeletal muscle. The responses of a single vessel may not be representative of the entire vascular bed because there is substantial heterogeneity along the vascular tree (73). The highest resistance to flow is at the level of the terminal arterioles, but without vasodilation of feed arteries there is inadequate perfusion pressure to downstream vessels (93, 134). Feed arteries are not within the skeletal muscle and thus are not exposed to vasodilator substances in the interstitium. Two mechanisms postulated to explain dilation of feed arteries are conducted (also called propagated) vasodilation and flow-mediated vasodilation.

Conducted vasodilation was first demonstrated by topical application of acetylcholine to arterioles (135). In addition to a local response, upstream vasodilation was observed. The conducted response travels along the vessel wall by direct coupling between endothelial cells and/or smooth muscle cells (134). A crucial piece of evidence to support a role for this mechanism in exercise vasodilation is the demonstration that conducted vasodilation occurs in response to muscle contraction (Ref. 5; see Fig. 3), an effect that is dependent on NO and ATP-sensitive K⁺ channels (23).

Flow-mediated dilation reflects the response of endothelial cells to shear stress (118, 142) acting via release of NO, prostacyclin, and endothelial-derived hyperpolarizing factor (EDHF) (69, 106, 131). Theoretically, dilation of terminal arterioles should increase blood flow through the feed arteries, resulting in dilation through a shear stress mechanism. However, it has been suggested that flow-mediated dilation is already maximally activated at rest in rat soleus muscle (75). Thus the role for flow-mediated vasodilation in coordinating responses of the skeletal muscle vascular network is presently uncertain.

>Fig. 2. Muscle blood flow response to a 1-s tetanic contraction (top) and to mild-intensity dynamic exercise (bottom). Blood flow was measured in dogs with implanted ultrasonic flow probes. Note the immediate increase after a single contraction or initiation of dynamic exercise (denoted by arrows). A satisfactory explanation of the mechanisms underlying exercise hyperemia must account for this initial response as well as the ability to adapt to changes in metabolic rate during dynamic exercise. [Adapted from Hamann et al. (60, 61).]

(73), and 2) it is quite possible that vasodilator agents other than the ones used in this investigation are responsible for rapid vasodilation.

The delayed time course of vasodilation from the in situ and in vitro experiments cited above has been used to support the idea that, if the initial increase in blood flow at the onset of exercise is not attributable to vasodilatation, it must be due to a widening of the arteriovenous pressure gradient by the skeletal muscle pump (43, 95, 136). However, under in vivo conditions where the smooth muscle was rendered unable to dilate, muscle contractions did not increase blood flow (33, 59, 61). Preliminary data from Hamann et al. (59) show that the increase in blood flow after tetanic contraction can be prevented by clamping the smooth muscle membrane potential with intra-arterial infusion of K⁺. Thus it appears that immediate vasodilatation is obligatory for the elaboration of contraction-induced hyperemia. It should be noted that this review focuses on vasodilatory factors affecting muscle blood flow during exercise. The controversial role of the skeletal muscle pump in exercise hyperemia will be addressed in a separate mini-review dedicated to an in-depth exploration of this topic (152a).

A particularly thorny problem has been the identification of a vasodilator mechanism capable of responding rapidly enough to explain the prompt increase in blood flow at the onset of exercise. Because metabolic processes are too slow to account for the initial blood flow response, it seems likely that the mechanism or the vasoactive compound that initiates vasodilation is different from that which sustains it. A comprehensive understanding of exercise hyperemia requires explication of the immediate response after release of a single contraction as well as the adjustment to changes in metabolic rate in sustained dynamic exercise.

>Fig. 3. Dilation in upstream arterioles during electrical stimulation of hamster cremaster muscle fibers at graded intensities. Arteriolar diameter was measured by videomicroscopy. Arteriole under observation was not in proximity of contracting muscle fibers and therefore not exposed to any vasodilators released by muscle fibers. Blockade by local application of high osmolar sucrose indicates that the dilation is the result of a conducted signal. [Adapted from Berg et al. (5).]
PUTATIVE VASODILATORS

Lactate. Lactate accumulates in skeletal muscle when the rate of anaerobic glycolysis is increased to supply ATP to fuel contractions (51). On the basis of perfusion experiments showing that lactic acid could cause vasodilation, Gaskell (50) proposed over 100 years ago that vasodilation occurred in contracting muscle because of the release of metabolites by the muscle fibers. It is now known that lactate-induced vasodilation is dependent on activation of soluble guanylate cyclase (21). The major problem with the notion that lactate is an important metabolic vasodilator is the fact that there are exercise intensities that evoke measurable elevations in muscle blood flow with no change in interstitial lactate concentrations. In a recent study by Lott et al. (98), interstitial lactate concentration measured with microdialysis did not correlate with the changes in blood flow during one-legged knee extension. This finding shows that it is unlikely that lactate plays an essential role in exercise hyperemia.

Hydrogen ion. Changes in pH have been proposed to be involved in the regulation of vascular tone, and a lowering of both hypercapnic and normocapnic pH can reduce the intracellular Ca\(^{2+}\) concentration, thereby causing relaxation of smooth muscle cells (116). The mechanism behind this effect, however, is not certain. One possibility is that extracellular decreases in pH hyperpolarize the smooth muscle cells by increasing K\(^{+}\) permeability and modulating the ATP-sensitive K\(^{+}\) channels (27, 31, 121). An early study revealed no correlation between interstitial pH and blood flow in isolated canine gastrocnemius muscle (148). In humans, the muscle interstitial pH is ~7.4 at rest, and the level is lowered at a rate related to the exercise intensity, reaching levels of ~7 after one-legged knee-extensor exercise performed at 70 W (150). However, there does not appear to be a direct relationship between pH and exercise hyperemia as the interstitial pH shows a progressive decrease during steady-state exercise with the nadir observed 1 min after exercise has ceased (150). This pattern is clearly different from that exhibited by blood flow using the same exercise paradigm (124). The inescapable conclusion is that, although changes in pH may modify other vasodilatory mechanisms, they do not determine the muscle blood flow response to exercise.

Oxygen. Several studies have reported that leg blood flow is higher during hypoxic than during normoxic exercise (91, 129, 130). On the other hand, studies of hyperoxia have shown no effect on leg blood flow at submaximal or maximal exercise intensities (85, 128). In addition, increasing oxygen delivery by polycythemia did not affect blood flow to contracting muscle (48). These findings indicate that the skeletal muscle vasculature responds to oxygen lack but that oxygen delivery is not the primary controller of blood flow to active muscle. There is evidence that arteriolar oxygen saturation does not change during muscle contractions elicited by electrical stimulation, but there is a decrease in venular oxygen saturation (94). On this basis it has been postulated that decreases in venous PO\(_2\) may evoke release of relaxing factors from the venular endothelium, causing dilation of adjacent (paired) arterioles (132). This hypothesis remains to be tested in more intact preparations.

Potassium. During muscle contraction, K\(^{+}\) diffuses rapidly from the muscle fiber via voltage-dependent K\(^{+}\) channels. This results in an elevated concentration of K\(^{+}\) in the interstitial fluid surrounding the vasculature. Dawes (29) first proposed K\(^{+}\) as a mediator of exercise hyperemia based on the observation that intra-arterial injection caused vasodilation. Subsequent studies (67, 78, 84, 98) have shown that muscle contractions can raise interstitial K\(^{+}\) concentration ([K\(^{+}\)]) to ~9 mM. The magnitude of elevation in interstitial [K\(^{+}\)] is proportional to duration (67) and intensity (56, 78) of contractions. Recent investigations with microdialysis (78, 98) have substantiated the contraction-induced increase in interstitial [K\(^{+}\)], but these studies were unable to provide adequate temporal resolution to address the time course relative to immediate changes in blood flow at the onset of exercise. Using K\(^{+}\)-selective electrodes, Hnik et al. (67) showed that K\(^{+}\) accumulated very rapidly in the interstitium following contraction. The prompt increase in [K\(^{+}\)] makes this ion the only muscle-derived vasodilator studied to date that could potentially explain the blood flow response to a single contraction (111, 153). Studies in both humans and animals demonstrate that the timing of the increase in [K\(^{+}\)] in venous blood is well correlated with the observed time course of the initial changes in blood flow (81, 108).

Acquisition of compelling evidence for the involvement of K\(^{+}\) in exercise hyperemia has been hampered by the lack of understanding of the mechanism by which elevated [K\(^{+}\)] produces smooth muscle relaxation and vasodilation. The Nernst equation predicts progressive depolarization and vasoconstriction with increasing concentrations of K\(^{+}\). Above [K\(^{+}\)] of ~20 mM, the observations concur with the predictions of the Nernst equation; at [K\(^{+}\)] below ~20 mM, however, hyperpolarization and vasodilation are elicited. Increased activity of the Na\(^{+}\)-K\(^{+}\) pump (20, 30) and activation of inwardly rectifying K\(^{+}\) (Kir) channels have been suggested as mediators of the vasodilator response. Recent evidence demonstrates that the primary component of the vasodilation is activation of Kir channels (72, 143), since vasodilation to elevated [K\(^{+}\)] is absent when the Kir channel is blocked by barium administration (76, 86) or in Kir-deficient mice (167). Recognition of the involvement of Kir channels in K\(^{+}\)-mediated vasodilation suggests the need for future studies that examine the blood flow response to contractions in the presence of Kir blockade.

Adenosine and ATP. It has long been believed that adenosine stems from the contraction-induced degradation of adenosine nucleotides within the skeletal muscle cells; however, more recent evidence suggests that adenosine is formed mainly on the extracellular side of muscle cells via the action of membrane-bound ecto-5'-nucleotidase (22, 99). Extracellular formation of adenosine during contraction can explain how muscle interstitial adenosine in humans can increase substantially even at low submaximal intensities (62). In fact, exhaustive exercise, even at supramaximal intensities causing marked ATP degradation, does not result in an increase in adenosine content in normal human skeletal muscle cells (113, 154). Adenosine has been a leading candidate for metabolic vasodilation for many years because it is a potent vasodilator (123) known to be produced by skeletal muscle contraction (62). Indeed, Hellsten et al. (62) reported a strong correlation (r = 0.98) between interstitial adenosine concentrations measured by microdialysis and leg blood flow during exercise. Infusion of the adenosine receptor antagonist theophylline reduced exercise-induced blood flow by ~20% in humans (123). On the other hand, neither adenosine receptor blockade (89) nor ade-
osine receptor desensitization (64) altered exercising muscle blood flow in dogs. The latter result raises doubts about adenosine playing an essential role in exercise hyperemia, although potential redundancy with other control systems has not been examined.

ATP is recognized as a potent vasodilator, eliciting dilation by activation of P2Y purinergic receptors on vascular endothelial cells and thereby causing a release of vasodilators such as NO, prostaglandins, and EDHF (126). Additionally, ATP has been implicated as a mediator of conducted vasodilation (24). Circulating levels of ATP (53) as well as muscle interstitial ATP (62) have been shown to increase in an exercise-intensity-dependent manner, but the relationship between changes in ATP concentration and blood flow is not known. The source of ATP is unlikely to be skeletal muscle cells because extracellular ATP levels do not increase during electrical stimulation of cultured muscle cells (99). Other potential sources of extracellular ATP during exercise include 1) endothelial cells (112), 2) sympathetic nerve terminals (10, 80), and 3) release by red blood cells in response to mechanical deformation (144, 145) or hemoglobin deoxygenation (74). Unfortunately, the lack of specific antagonists for vasodilatory P2Y purinergic receptors has hampered firm conclusions regarding the involvement of ATP in exercise hyperemia.

**Nitric oxide.** Both the endothelium and the skeletal muscle cells are potential sources of NO in skeletal muscle tissue during contraction. The enzyme nitric oxide synthase (NOS), which catalyzes the formation of NO from L-arginine, exists in two constitutive isoforms: endothelial NOS (eNOS) and neuronal NOS (nNOS) (147). In human skeletal muscle eNOS and nNOS are localized in the vascular endothelium and in the skeletal muscle cells, respectively (46, 57), whereas in rodents eNOS has also been detected in type I muscle fibers (87, 88).

The generation of NO from the endothelium may be increased in response to receptor binding of compounds such as bradykinin, acetylcholine, and ATP (49) or by mechanical factors such as shear stress (118, 119). Activation of NOS in skeletal muscle cells may be related to the contraction-related increase in intracellular Ca^{2+} concentration or by factors such as phosphorylation of NOS (42) and binding of NOS to caveolin-3 (160).

NO may be derived not only from NOS but may also originate from the red blood cells. According to Stamler and coworkers (105, 146), NO can bind to hemoglobin as nitroso-hemoglobin and be released in response to off-loading of oxygen from the hemoglobin molecule. Moreover, nitrite that is present in large quantities in blood can be reduced to NO by deoxygenated hemoglobin (25). This provides an appealing mechanism whereby deoxygenation of hemoglobin could be the link that couples release of NO by the red blood cells to the metabolism of skeletal muscle.

NO has been described to be involved in the regulation of basal vascular tone in a large number of tissues, including skeletal muscle. The main mechanism underlying the vasodilatory effect of NO is via activation of guanylate cyclase in the smooth muscle cells (109). In addition, NO may indirectly participate in vasodilation by stimulating the release of vasodilator prostaglandins from endothelial cells (133, 159). Recent evidence also suggests that NO is involved in conducted vasodilation (16).

The role of NO in skeletal muscle hyperemia has been much debated due to contradictory results from different studies on both animals and humans. There is reasonable certainty that muscle contraction triggers formation of NO by skeletal muscle cells, and possibly by vascular endothelial cells, and that NO is subsequently released to the extracellular space (2, 141, 152). It is also known that arterial infusion of NO donors elicits marked increases in skeletal muscle blood flow, although infusion of the NO donor S-nitroso-N-acetyl-penicillamine at a high concentration has been observed to cause only about one-half of maximal exercise-induced skeletal muscle flow in humans (125). Most studies on laboratory animals and humans have shown NO to be involved in the regulation of basal muscle blood flow and flow in recovery from exercise. For example, infusion of NOS inhibitors in humans has been observed to lower basal flow by 30–50% and recovery blood flow by 30–40% (45, 83, 125, 137), and a similar effect of NOS inhibition on basal flow has been observed in laboratory animals (47, 120). Several studies on humans (34, 36, 52) have also reported that inhibition of NOS lowers contraction-induced increases in blood flow by up to 30%. However, the human studies reporting an effect of NOS inhibition on exercise hyperemia have been questioned due to the use of venous occlusion plethysmography for the determination of blood flow. This method requires exercise to be terminated before measurements, and thus flow is actually measured in early recovery from exercise. This methodological limitation could potentially explain why, in studies in which either the ultrasound-Doppler technique (125, 137) or the thermodilution technique (45, 83) has been used, a lack of effect of systemic NOS inhibition on exercise hyperemia has been observed. This is not a complete explanation; however, there is also a discrepancy on the effect of NOS inhibition on contraction-induced hyperemia among studies of laboratory animals in which other methods for blood flow assessment were used (63, 66, 70, 102). One factor that could contribute to the variation in results obtained in animal studies is the use of muscle groups of differing fiber type, since NO-dependent vasodilation is more prominent in high-oxidative than in low-oxidative muscle groups (66, 103, 165).

Another possible explanation for the divergent findings on the effect of NOS inhibition, apart from differences in methods for blood flow measurements and possible differences between muscle groups, is the efficacy of NOS inhibitors used. An infusion dose of 4 mg/kg body wt of N\textsuperscript{n} -nitro-L-arginine methyl ester in humans has, for example, been observed to lower the total NOS activity in skeletal muscle homogenates by close to 70% (45), and the possibility exists that the remaining NOS activity is sufficient to maintain blood flow. Nevertheless, a reduction in exercise hyperemia has indeed been demonstrated with combined inhibition of NOS and either cytochrome P-450 2C9 (65) or cyclooxygenase (COX) (9), suggesting that a lack of effect of NOS inhibition on exercise hyperemia can be explained, not solely by an insufficient inhibition of NOS but by redundancy in the regulation of muscle blood flow.

**Prostanoids.** The enzyme COX catalyzes the conversion of arachidonic acid to prostaglandin H\textsubscript{2} from which prostaglandin E\textsubscript{2}, prostacyclin, and thromboxane A\textsubscript{2} are derived. Of these, prostacyclin and prostaglandin are vasodilators, whereas thromboxane A\textsubscript{2} induces vasoconstriction. Vascular endothelial cells as well as skeletal muscle cells are potential sources of vasoactive prostaglandins (28, 156, 157).
The formation of vasodilator prostanoids is believed to be regulated by the availability of arachidonic acid, which is enhanced on activation of phospholipase by increased intracellular Ca\textsuperscript{2+} levels (28). Therefore, prostanoid synthesis is enhanced in a manner similar to that of NO, by agonists that increase the intracellular Ca\textsuperscript{2+} level in endothelial cells (e.g., bradykinin) and by mechanical factors such as shear stress (90). In addition, prostaglandin formation is enhanced by adenosine (127), NO, and peroxynitrite (54, 133, 155). Once produced, prostaglandin and prostacyclin evoke vasodilation by acting on receptors on the smooth muscle cells, leading to activation of adenylate cyclase and a consequent decrease in smooth muscle cell Ca\textsuperscript{2+} levels (158). Arterial infusion of prostaglandin E\textsubscript{1} leads to a marked increase in blood flow in the human forearm (149). Infusions of prostaglandin E\textsubscript{2} and prostacyclin in canine skeletal muscle arteries cause vasodilation, with prostacyclin being the more potent vasodilator (18).

The production of prostanoids is influenced by muscle contraction. Both prostaglandin E\textsubscript{2} and prostacyclin are formed intramurally (151), and their concentrations in the venous effluent are elevated during exercise (164, 166). More recent data obtained with microdialysis reveal increased muscle interstitial concentrations of prostaglandin E\textsubscript{2} (79) and prostacyclin (44) during exercise, with the concentration of prostaglandin E\textsubscript{2} being related to exercise intensity (79). Interestingly, thromboxane A\textsubscript{2} has been found to decrease during exercise and more so with increasing exercise intensity (79).

Studies on humans that used venous occlusion plethysmography to measure blood flow show that COX inhibition reduces the magnitude of exercise hyperemia by ~20% (34, 82, 164), whereas other studies show no effect of COX inhibition on exercise hyperemia in humans either with plethysmography (26) or with ultrasound-Doppler technique (139). Findings from studies on laboratory animals are equally divergent (4, 38, 106, 161, 166), making definitive conclusions difficult. From the negative findings above, it would appear that prostanoids are not essential for exercise hyperemia. However, the demonstration of a diminution in exercise hyperemia with combined NOS and COX inhibition (9) means that a backup role for prostanoids cannot be excluded.

**EDHF.** The concept of an EDHF originates from the observation that compounds such as bradykinin and acetylcholine induce hyperpolarization of smooth muscle cells and consequent arterial vasodilation in the presence of COX and NOS inhibitors (110). The mechanism underlying this effect has not been fully clarified, but it is believed that the agonist-induced increase in intracellular Ca\textsuperscript{2+} in the endothelial cells leads to hyperpolarization of the endothelial cells by activation of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels (17). This event is followed by hyperpolarization of the smooth muscle cells. Transmission of the hyperpolarization from endothelium to smooth muscle cells has been proposed to be achieved by one or several of the following mechanisms: 1) diffusion of a compound from the endothelium to the smooth muscle cells (19), 2) transmission via myoendothelial gap junctions (104), or 3) release of K\textsuperscript{+} from the endothelial cells causing activation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and/or activation of K\textsuperscript{+} channels (37).

There is a dearth of direct evidence regarding the involvement of EDHF in exercise hyperemia. However, Halcox and coworkers (58) observed that bradykinin evoked an increase in resting forearm blood flow after NOS and COX inhibition. Because the increase in forearm blood flow was attenuated by prevention of hyperpolarization with simultaneous infusion of high concentrations of KCl, they attributed this increase to EDHF. Studies have moreover shown that infusion of the K\textsuperscript{+} channel blocker tetraethylammonium chloride, after inhibition of NOS and COX, reduces the bradykinin-induced increase in forearm blood flow, suggesting an involvement of EDHF (68, 71). The fact that bradykinin is a potent vasodilator of skeletal muscle arteries (32, 68) and has been shown to increase in the skeletal muscle interstitium in response to exercise (92) raises the possibility that EDHF may be involved in exercise hyperemia via bradykinin.

The identity of EDHF varies between tissues, and there may be several EDHFs in one given tissue. In many vascular beds, metabolites of cytochrome P-450 epoxygenases appear to be involved (19, 40, 122). In skeletal muscle, the enzyme cytochrome P-450 2C (CYP 2C), which forms the vasodilator 11,12-eicosatrienoic acid (11,12-EET), has been proposed to be an EDHF synthase. 11,12-EET induces hyperpolarization of smooth muscle (41), and recombinant 11,12-EET elicits a marked vasodilation of microvessels (115). By use of antisense oligonucleotides to CYP 2C in isolated skeletal muscle resistance arteries, leading to a decreased presence of the enzyme in the endothelium, it has been shown that CYP 2C is involved in the acetylene-induced vasodilation in the presence of NOS and COX inhibitors (7). Cytochrome P-450 2C9 is present in human skeletal muscle tissue, primarily in the endothelial cells of microvessels; with no detectable presence within the skeletal muscle cells (65). Intra-arterial infusion of the specific CYP 2C inhibitor sulfaphenazole in humans does not alter the magnitude of muscle blood flow during exercise, but a combined inhibition of CYP 2C and NOS reduces the exercise-induced blood flow by ~15% (Ref. 65; Fig. 4). This observation suggests that CYP 2C is involved in the regulation of exercise hyperemia in humans and that it interacts with NOS. It should be emphasized, however, that in addition to CYP 2C, there

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**Fig. 4.** Reduction in blood flow during one-legged knee extensor exercise by simultaneous inhibition of NOS with L-N\textsuperscript{5}-monomethyl-L-arginine and CYP 2C9 with sulfaphenazole. Blood flow was measured in 7 human volunteers by the thermodilution technique under control conditions (○) and during inhibition (●). Because inhibition of NOS or CYP 2C9 alone has no effect on blood flow, these results suggest redundancy between the two vasodilator systems in regulating muscle blood flow during exercise. [From Hillig et al. (65), with permission from Blackwell Publishing.]
may well be other cytochrome P-450 isoforms as well as other compounds involved in an EDHF effect in skeletal muscle. As an example, K\(^+\) has been implied to be involved in several tissues (37), including rat skeletal muscle arteries where it has been observed to cause endothelium-independent and ouabainsensitive vasodilation (30).

**Interaction between vasodilator systems.** From the large number of studies that have been performed with pharmacological interventions either to inhibit the synthesis of a vasodilator or to block the effect of a vasodilator, it appears that none of the vasodilators tested to date is essential for exercise hyperemia. However, the fact that a compound is not required for the increase in blood flow does not exclude its involvement. Instead, the lack of effect of impaired synthesis or action of a vasodilator on the magnitude of exercise hyperemia may reflect a compensation by other vasodilators, i.e., that there is redundancy in the system. In vitro studies show numerous examples of interactions between vasodilator systems, and evidence that redundancy exists in vivo is beginning to emerge. Combined inhibition of COX and NOS reduces the exercise-induced blood flow in humans by \(\sim 15\%\) (65). Because neither CYP 2C nor NOS inhibition alone alters exercise hyperemia in this experimental model (45, 65, 125), these data suggest that there is an interaction between NOS and CYP 2C. In accordance with this observation, studies on isolated arteries have shown an interaction between CYP 2C and NOS. In coronary arteries, NOS is the primary vasodilator system and NO suppresses the activity of CYP 2C, so that, when NO formation is impaired, the activity of CYP 2C is enhanced (3).

Another interaction between vasodilator systems reported to occur in vivo is that between NOS and COX. Boushel and coworkers (9) used near-infrared spectroscopy to determine regional skeletal muscle blood flow in humans and showed that combined inhibition of COX and NOS reduced exercise hyperemia by \(\sim 30\%\). A synergistic effect of COX and NOS inhibition has also been shown for peak reactive hyperemia of legs (45). None of the vasodilators tested to date is essential for exercise hyperemia, as the effect of one vasodilator can generally be blocked without a change in blood flow. Nevertheless, these observations do not exclude the possibility that the lack of effect could be due to a compensatory effect of another vasodilator. Such redundancy between vasoactive compounds has been observed in vivo as well as in vitro. It is likely that the importance of specific vasodilator compounds varies over different phases of blood flow, i.e., onset of exercise to steady state. Conducted or flow-mediated vasodilation coordinate the response of the vascular network to allow dilation of vessel segments not directly exposed to vasodilator compounds.

It would be worthwhile in the future to focus on the interaction between different vasodilator compounds and the integration between the different cellular sources of vasodilator compounds. The importance of the red blood cell as a source of vasodilators, i.e., ATP and NO, deserves further investigation in the context of exercise hyperemia. Finally, it will be essential to continue the search for novel vasodilators, recognizing that research on EDHF, for example, is still in its infancy.

**REFERENCES**

Invited Review


EXERCISE HYPEREMIA


