Cardiovascular Regulation by Skeletal Muscle Reflexes in Health and Disease

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Running Head: Exercise Pressor Reflex-Mediated Control of the Circulation

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Abstract

Heart rate and blood pressure are elevated at the onset and throughout the duration of dynamic or static exercise. These neurally mediated cardiovascular adjustments to physical activity are regulated, in part, by a peripheral reflex originating in contracting skeletal muscle termed the exercise pressor reflex. Mechanically sensitive and metabolically sensitive receptors activating the exercise pressor reflex are located on the unencapsulated nerve terminals of group III and group IV afferent sensory neurons, respectively. Mechanoreceptors are stimulated by the physical distortion of their receptive fields during muscle contraction and can be sensitized by the production of metabolites generated by working skeletal myocytes. The chemical by-products of muscle contraction also stimulate metaboreceptors. Once activated, group III and IV sensory impulses are transmitted to cardiovascular control centers within the brainstem where they are integrated and processed. Activation of the reflex results in an increase in efferent sympathetic nerve activity and a withdrawal of parasympathetic nerve activity. These actions result in the precise alterations in cardiovascular hemodynamics requisite to meet the metabolic demands of working skeletal muscle. Coordinated activity by this reflex is altered after the development of cardiovascular disease generating exaggerated increases in sympathetic nerve activity, blood pressure, heart rate and vascular resistance. The basic components and operational characteristics of the reflex, the techniques used in human and animals to study the reflex, and the emerging evidence describing the dysfunction of the reflex with the advent of cardiovascular disease are highlighted in this review.

Keywords: mechanoreflex, metaboreflex, sensory neurons, hypertension, heart failure
**Introduction**

Adjustments in autonomic nerve activity to the cardiovascular system during exercise are requisite to meet the metabolic demands of working skeletal muscle. Dynamic exercise is characterized by intensity dependent increases in heart rate (HR), stroke volume (SV) and cardiac output (CO) while total peripheral resistance (TPR) is reduced. These changes in circulatory hemodynamics produce moderate increases in arterial blood pressure (BP). As a result, skeletal muscle blood flow is rapidly augmented to provide adequate oxygen and nutrient delivery to the working muscle (116, 139). In contrast, muscle blood flow is reduced during static exercise due to the relatively large increases in intramuscular pressure produced by isometric contractions. HR mediated increases in CO are generated along with little to no change in TPR resulting in a markedly potentiated BP response. These changes occur in an attempt to maintain adequate perfusion of muscle during static exercise (116).

The autonomic nervous system mediates these cardiovascular responses to both dynamic and static exercise by increasing sympathetic nerve activity (SNA) and withdrawing parasympathetic nerve activity via three distinct neural mechanisms: central command, the exercise pressor reflex and the arterial baroreflex (152).

Central command is a feed forward neural mechanism that transmits excitatory impulses to descending motor neurons for locomotion and in a parallel fashion activates cardiovascular control circuits within the medulla oblongata (34). As early as 1895, Johansson (61) postulated that a neural mechanism of central origin was, in part, responsible for the modulation of the cardiovascular response to exercise. In 1913, Krogh and Linhard (83) provided evidence in support of this theory quantifying a rapid increase in ventilation and pulse rate within one second of the onset of voluntary exercise. In agreement with Johansson, they concluded that an irradiation of impulses from the motor cortex was the most likely explanation for such a rapid response. Since that time, numerous investigations in both animals and humans have firmly established an important role for central command in the regulation of the cardiovascular response to exercise. However, this input is only one facet of the integrated system that finely adjusts the autonomic response to physical activity.

In 1917, Krogh and Linhard (82) further demonstrated in humans that pulse rate and ventilation were rapidly increased at the onset of involuntary leg contraction induced by electrical stimulation of skeletal muscle. As this technique did not involve voluntary movement and hence eliminated ‘cortical irradiation’, it was concluded that reflex input from the skeletal muscle itself was likewise involved in mediating the cardiorespiratory response to exercise. In support of this concept, Alam and Smirk (4, 5) later demonstrated in 1937-1938 that dynamic calf exercise evoked increases in BP and HR that were maintained by circulatory arrest upon the cessation of exercise. However, upon the restoration of blood flow, both MAP and HR fell precipitously (4, 5). In a similar protocol, a spinal cord lesion patient with preserved motor control and absent sensation below one knee, performed calf exercise in the numb leg
which evoked typical increases in HR and BP (6). In this patient the sympathoexcitatory responses were not maintained during post-exercise circulatory occlusion. This elegant series of experiments demonstrated that a reflex originating in skeletal muscle, the exercise pressor reflex (EPR) as it is known today (115), was intimately involved in mediating the precise cardiovascular adjustments to exercise.

The contribution of the arterial baroreflex to the modulation of the circulatory response to exercise induced by central command and the EPR has been more recently recognized. Arterial baroreceptors located within the carotid sinuses and the aortic arch function as sensors in a negative feedback system that modulates beat-to-beat variations in BP by continually adjusting HR, SV and peripheral resistance (49, 67, 98). During steady-state exercise, the baroreflex is reset to function around the higher blood pressures induced by physical activity in conscious animals and humans (28, 80, 130). In doing so, the baroreflex maintains the ability to ‘finely tune’ the cardiovascular response to exercise.

Clearly, each of the described inputs (i.e. central command, the EPR and the arterial baroreflex) contributes importantly to the regulation of the autonomic nervous system during exercise. Not surprisingly, each is known to transmit information directly to cardiovascular regulatory centers within the medulla and, therefore, maintain the potential to modify one another functionally. Adequate discussion of these interactions and the individual contributions of each of these inputs is a large endeavor and beyond the scope of the current review. As such, this review will focus primarily on the EPR as much attention has been recently given to this reflex in both health and disease.

The Exercise Pressor Reflex

Skeletal muscle is innervated by several types of sensory neurons of which only Group III (thinnly myelinated, Aδ fibers) and Group IV (unmyelinated, C fibers) afferent fibers participate in the reflex regulation of cardiovascular function during exercise (69, 102). The majority of group III afferents are rapidly (2-5 sec) excited by mechanical distortion of their receptive field, located on unencapsulated nerve endings and pacinian corpuscles (2, 47, 69, 71, 76, 109). As such, receptors associated with these afferent fibers are termed ‘mechanoreceptors’; although a few are responsive to chemical stimuli (67). Group III nerve endings terminate in the collagenous connective tissue, and the endoneurium of the triceps surae and calcaneal tendon of the cat (1, 8, 67, 157). Activation of this group of afferent neurons primarily stimulates the mechanically-sensitive component of the EPR termed the muscle mechanoreflex. Group IV afferents are predominately excited by changes within the chemical milieu of skeletal muscle interstitium resulting from the accumulation of metabolites produced by contracting muscle (67, 76). As such, excitatory impulses are delayed (5-20sec) following muscle contraction and ischemia potentiates the activity of these neurons (64, 69, 71, 109). Group IV afferent fibers exhibit
heterogeneity with some sensitive to mechanical distortion (69). Anatomically, sensory receptors associated with group IV afferent neurons are located on unencapsulated nerve endings that terminate within the walls of capillaries, venules and lymphatic vessels of skeletal muscle (8). These locations are advantageous for direct sampling of the chemical environment within the vasculature of the skeletal muscle. Stimulation of this group of afferent neurons primarily activates the chemically-sensitive component of the EPR known as the muscle metaboreflex.

The first site of synapse for most skeletal muscle group III and IV afferents is the dorsal horn of the spinal cord, specifically Rexed’s laminae I, II, V and X (65, 93, 108, 186). Although the specific pathway remains unknown, muscle afferents project from the dorsal horn to the brainstem along a path that includes the dorsolateral sulcus and the ventral spinal cord (24, 56, 81). Spinal cord transection at the first cervical vertebrae eliminates the pressor response to static muscle contraction, whereas transection of the rostral medulla only partially attenuates the response (60). Therefore, an intact brainstem is required to process impulses from group III and IV afferents. Neuronal cells responsive to EPR stimulation have been described in the nucleus tractus solitarius (NTS), rostral ventral medulla (RVLM), caudal ventrolateral medulla, lateral tegmental field, nucleus ambiguus and the ventromedial region of the rostral periaqueductal grey (15, 57, 58, 88, 92). Of these, the NTS, is likely the primary site of central processing, with secondary projections to the aforementioned nuclei (65, 127).

Group III and IV afferent impulses evoke graded, intensity dependent increases in efferent SNA, and parasympathetic withdrawal (119, 120). Skeletal muscle contraction reduces the tonic excitation of vagal motor neurons serving as one mechanism by which HR is increased (107). In contrast, sympathetic outflow to the heart and systemic vasculature including the vasculature of skeletal muscle, kidneys, the splanchnic region and skin is increased with activation of skeletal muscle reflexes (42, 100, 101, 119, 170, 174). Preganglionic sympathetic neurons originating in the RVLM project to the intermediolateral cell columns of the spinal cord, synapse at the paravertebral sympathetic chain ganglia, and innervate the heart and vasculature. The EPR mediated adjustments in parasympathetic and sympathetic nerve activity results in increases in cardiac contractility, stroke volume, HR, and BP (144). It is through these pathways, outlined in Figure 1, that skeletal muscle reflexes contribute to cardiovascular regulation during physical activity.

**Experimental Models to Assess Exercise Pressor Reflex Function**

**Humans**

The cardiovascular response to exercise is tightly regulated by input from central command, the EPR and the baroreflex making it difficult to isolate the distinct contributions of each component. However, innovative models have been developed to examine the role of the EPR in humans. For example, the induction of epidural anesthesia using lidocaine reduces motor strength and blocks
sensory feedback from contracting skeletal muscle. The pressor response to both static and dynamic exercise is reduced when performed after epidural anesthesia compared to control exercise at the same relative intensity and perceived effort (7, 26, 30, 117, 161). Such investigations emphasize the important role skeletal muscle reflexes play in regulating the cardiovascular response to exercise in humans. Likewise, involuntary exercise induced by electrically stimulating skeletal muscle via electrodes placed on the limb significantly increases BP and HR while avoiding excitation of central command (10, 21, 29, 53, 54, 59, 82). However, electrical stimulation may alter the order of muscle fiber recruitment from that produced physiologically by direct activation of motor neurons (52). Passive cycling also removes the influence of central command while evoking EPR-induced increases in HR and BP (23, 84, 124, 125). As an added advantage, when performed at a low resistance, passive cycling increases BP and SV without altering lactic acid production, making it a useful model for examining the contribution of the muscle mechanoreflex to EPR function (84). Medical anti-shock (MAS) trousers, inflated to 60 to 100 mmHg, combined with suprasystolic inflation of thigh cuffs, to prevent the cephalad translocation of fluids, evokes a pressor response that is abolished with epidural anesthesia suggesting it is another technique that can be used to assess EPR activity (184).

Additionally, patients with Brown-Séquard syndrome are ideal candidates in which to examine the role of the EPR. These patients exhibit spinal hemiplegia to the lateral half of the spinal cord with unilateral damage to group III and group IV muscle afferents that project to the dorsal horn, rendering sensory function absent in one leg, while motor function is retained (95). Experiments in these patients have demonstrated that the pressor response to post-exercise circulatory occlusion in the leg with reduced sensory function is abolished establishing a critical role for the EPR in mediating the cardiovascular response to exercise (187).

In humans, the metabolically sensitive component of the EPR (i.e. the metaboreflex) has been the subject of many investigations, most likely due to the relative simplicity with which it can be examined. As stated previously, Alam and Smirk (4-6) demonstrated that BP and HR remained elevated after the cessation of calf exercise if the leg circulation was arrested by the suprasystolic inflation of thigh cuffs. This attenuates the removal of metabolites produced during muscle contraction and enhances the stimulation of metaboreceptors located on afferent sensory neurons. An added advantage of this technique is that it is performed after exercise is discontinued at a time point when both the muscle mechanoreflex and central command have been disengaged. This technique has also been used to demonstrate that the metaboreflex modulates the sympathetic response to exercise. The sympathetic response to handgrip exercise is sustained during post-exercise circulatory occlusion while increasing forearm blood flow (i.e. during the application of suction to the forearm) during circulatory arrest attenuates this response (63). Lower body positive pressure (LBPP) can also be used in humans to stimulate the metaboreflex. Reducing blood flow to exercising muscle via LBPP produces a greater
elevation in BP and plasma lactate concentration suggesting this technique reduces the removal of the
metabolic by-products of skeletal muscle work serving to supra-stimulate the metaboreflex (123).
Microdialysis studies within exercising skeletal muscle have demonstrated that interstitial potassium,
lactate, and phosphate concentrations increase concomitantly with elevations in SNA, BP and HR, and
are therefore potential chemical substances that stimulate metaboreflex activity (97).

Patient populations have also been useful in assessing metaboreflex function in humans.
Patients with McArdle’s disease, have a genetic deficiency in myophosphorylase expression which
prevents exercise-induced muscle acidosis. Several studies have demonstrated that the sympathetic
response to static exercise is attenuated in this patient population although this finding is not universal
(25, 131, 172, 173). In addition, animal studies have also demonstrated that when acid-sensing ion
channels located on skeletal muscle innervating sensory afferent neurons are inhibited there is a
significant reduction in the pressor response to hindlimb static contraction (46). Therefore, it is plausible
that exercise-induced skeletal muscle acidosis plays a partial role in the sympathetic response to
exercise. Collectively, use of these techniques has established an integral role for the muscle
metaboreflex in the reflex regulation of the cardiovascular system during exercise.

The mechanoreflex has been more difficult to isolate in humans, largely because the stimulus,
muscle contraction, is accompanied by metabolite production and central command activation. Low-
resistance passive cycling and alternative techniques (e.g. low intensity involuntary muscle contraction,
sustained passive muscle stretch, and leg compression) have been used in an attempt to preferentially
stimulate mechanoreceptors in the absence of input from the metaboreflex and central command (27,
33, 113). Batman et al (9) measured muscle SNA and metabolite production during prolonged low-
intensity rhythmic isotonic hand-grip exercise. Despite no alterations in metabolite production, there
was a progressive increase in muscle SNA that was not observed during post-exercise circulatory
occlusion confirming metaboreceptors were not stimulated (9). Although central command was intact in
this model, it has little or no affect on the sympathetic response to forearm hand-grip exercise (171).
Such experiments demonstrate that the mechanoreflex facilitates the regulation of the autonomic
response to exercise in humans.

Animals

Animal models are commonly used to assess the activity of the EPR, mechanoreflex and
metaboreflex while experimentally eliminating the influence of central command and the arterial
baroreflex. Involuntary hindlimb muscle contraction induced via electrical stimulation of spinal nerve
ventral roots is regularly utilized to examine the respiratory and cardiovascular response to stimulation
of the EPR (16, 66, 70, 102, 151). In many of these investigations, but not all, a decerebration
procedure is performed prior to EPR stimulation effectively removing the influence of central command
and the deleterious effects of anesthesia (151). McCloskey et al. (102) demonstrated that electrical
stimulation of the L7-S1 ventral roots in cats evoked a hindlimb tetanic muscle contraction that
directly increased BP, HR and respiration. Application of lidocaine to the dorsal roots attenuated the
cardiovascular and respiratory response to hindlimb contraction suggesting it was mediated by small
erve afferent sensory neurons (102). The dorsal roots remain intact in this preparation allowing skeletal
muscle afferent impulses to be transmitted to the brainstem. This model has also been reduced into a
viable preparation in the rat, allowing for the evaluation of EPR function in a variety of disease states
(106, 150, 151, 155).

Ischemic contraction and post-exercise circulatory occlusion are commonly used to examine the
role of the metaboreflex in animals (3). Additionally, intra-arterial infusion of exogenous metabolic
derivatives or metaboreceptor agonists into the hindlimb are used to stimulate metaboreflex activity
(68). For example, capsaicin (an exogenous substance demonstrated to primarily activate group IV
afferent fibers) infused and trapped in the hindlimb circulation significantly increases BP and HR in cats
and rats (68, 149). As shown in figure 2, metaboreceptor antagonists are also frequently used to
assess the contribution of the metaboreflex to activation of the EPR during muscle contraction (149).
With regard to the muscle mechanoreflex, passive stretch of the gastrocnemius and soleus muscles of
the hindlimb is commonly used to selectively stimulate mechanoreceptors evoking elevations in BP, HR
and SNA (119, 151). It should be noted, however, that Hayes et al (43) recently determined that stretch
excites a separate population of group III afferent neurons than those stimulated during muscle
contraction although significant overlap exists.

Animal models are valuable to researchers delineating the mechanisms by which skeletal
muscle reflexes modulate cardiovascular function in health and disease. However, there are limitations
to these models. For example, in rats, activation of the EPR under inhaled anesthesia evokes a
depressor response (151, 165, 174). Therefore, rats must be decerebrated in order to generate a
cardiovascular response similar to that observed during exercise in conscious animals. As another
example, in anesthetized or decerebrate animals, intra-arterial injection of gadolinium, a
mechanoreceptor antagonist, significantly attenuates the pressor, chronotropic and sympathetic
response to mechanoreflex stimulation (41, 45, 119). In contrast, in conscious cats, gadolinium has no
effect on the pressor response to exercise suggesting the mechanoreflex may be suppressed, and/or
its loss of input compensated for, in the conscious state (99). Despite such limitations, the assessment
of EPR function in animal models allows for a more direct examination of the reflex in isolation and has
played a key role in advancing our understanding of cardiovascular regulation during exercise in both
health and disease.
**The Skeletal Muscle Metaboreflex**

Metabolite accumulation in skeletal muscle tissue partially drives the autonomic response to exercise. Therefore, metabolites that excite metaboreceptors have been the subject of intense investigation. A candidate metabolite must evoke afferent impulses from metabolically sensitive afferent fibers, be present in exercising muscle, produce a pressor response when administered exogenously, and, when its production is inhibited, must attenuate the pressor response to exercise. Metabolites that fit these criteria are potassium, lactic acid, bradykinin, by-products of arachidonic acid metabolism, analogues of adenosine tri-phosphate (ATP), and di-protonated phosphate (87, 117, 135, 136, 138, 142, 148, 158, 163, 182) to name a few. Exogenous administration of these substances individually only partially recapitulates the cardiovascular response to static contraction suggesting a combination of substances may excite metaboreceptors during exercise. Intra-arterial administration of a cocktail of metabolites produced during muscle contraction (e.g. protons, ATP and lactate) evoked rapid elevations in calcium in dorsal root ganglion (DRG) neurons innervating skeletal muscle to a greater extent than individual administration of each substance (94). Inhibition of one or all of the receptors specific to changes in pH, ATP and lactate significantly reduced the number of DRG cells excited by the combination of substances (94). Although the exact mechanism by which the metaboreflex is excited remains undetermined, the aforementioned metabolites likely excite a variety of receptors that are directly involved. For example, cannabinoid receptors have been demonstrated to excite skeletal muscle reflexes while mu-opioid receptors may attenuate activation of the EPR (166, 183).

Similar to the endogenous chemical substances that stimulate the metaboreflex, several receptors located on sensory fibers are plausible candidates for mediating EPR function. Acid sensing ion channels (ASICs), proton-gated channels localized to group IV fibers, open when exposed to an extracellular pH of 7.0 or less such as occurs when lactic acid accumulates during static muscle contraction (14, 162, 188). In cats, intra-arterial injection of the ASIC antagonist A-317567 markedly attenuates the pressor response to electrically-induced static contraction (46). Infusion of amiloride, a non-specific ASIC and epithelial sodium channel antagonist, into the hindlimb circulation has no effect on the immediate (2-5 sec) autonomic response to static contraction or passive stretch (103). However, amiloride blunts the latent pressor response (> 5 seconds) to static contraction (103). Subsequent experiments have demonstrated that amiloride and A-317567 injection into the popliteal artery attenuates the change in BP and renal SNA after supra-stimulation of the metaboreflex by circulatory occlusion during and immediately after muscle contraction (105). Collectively, these data suggest that metabolites that stimulate ASICs channel opening partially mediate the excitation of metabolically sensitive sensory neurons during exercise.

Transient receptor potential vanilloid 1 (TRPV1) receptors are also predominantly localized to group IV fibers (110). Intra-arterial injection of capsaicin, a TRPV1 receptor agonist, markedly
increases BP, HR and SNA suggesting this receptor excites group IV afferent neurons (68, 156). TRPV1 receptors are sensitive to changes in muscle temperature, increases in extracellular hydrogen ion concentration (pH < 5.7), and inflammatory products such as bradykinin and prostaglandins (35, 62, 134, 164, 181). These potential “activators” of the TRPV1 receptor are present during exercise, and decreases in extracellular pH, characteristic of ischemia during static contraction, may sensitize the receptors making them more receptive to additional stimuli (35, 164). However, it has yet to be definitively determined whether these receptors play a significant role in stimulating afferent sensory neurons as the data from functional studies has been conflicting (13). Kindig et al (75) pharmacologically blocked TRPV1 receptors in cats with iodoresinaferatoxin (IRTX) during static contraction of the gastrocnemius and soleus muscles while the hindlimb was freely perfused or the circulation was occluded. In this study IRTX did not alter the cardiovascular response to contraction during either condition (75). On the contrary, in rats, Smith et al (149) separately infused three TRPV1 receptor antagonists, IRTX, capsazepine and ruthenium red prior to static contraction, passive stretch of the hindlimb or intra-arterial injection of capsaicin. Each antagonist significantly attenuated the pressor response to EPR and metaboreflex stimulation but had no affect the pressor or cardioaccelerator response to mechanoreflex activation (149). Furthermore, capsazepine has been shown to attenuate the pressor response to hindlimb intra-arterial administration of di-protonated phosphate in rats (31). The discrepancy among studies is not readily apparent. However, the majority of studies do raise the possibility that activation of TRPV1 receptors by skeletal muscle metabolites (e.g. protons) may contribute to excitation of the skeletal muscle metaboreflex during exercise. Clearly, further research is needed in this area.

Purinergic (P) ligand-gated ion channels have been localized to both group III and IV muscle afferent neurons (175-177). Skeletal muscle contraction triggers the release of purines such as adenosine and interstitial ATP which act as ligands for P1 and P2X receptors, respectively (11, 48, 89, 90). Both adenosine and ATP have been investigated as potential metabolic stimulators of the EPR. In humans, infusion of exogenous adenosine into the forearm arterial circulation has been shown to increase muscle SNA during post-exercise circulatory occlusion although this finding has been difficult to reproduce consistently (17, 38, 111). One reason for this difficulty may be that exogenously administering adenosine leads to its appearance in the systemic circulation resulting in direct stimulation of the aortic and carotid chemoreceptors producing a chemoreflex mediated change in muscle SNA (140, 141). As such, P1 receptors most likely do not play a role in the metaboreflex induced cardiovascular response to exercise. In contrast, intra-arterial administration of α,β-methylene ATP (a P2X receptor agonist) into the hindlimb of decerebrated cats elevates BP and enhances afferent impulses from group IV fibers by 67% (39, 90, 132). Further, arterial administration of the P2X receptor antagonist, pyridoxal phosphate-6-azophenyl-2’,4’-disulfonic acid (PPADS), attenuates the
pressor response to static muscle contraction by 38% in cats, and reduces the pressor response to post-contraction circulatory occlusion (40). Administration of PPADS also abolishes the afferent impulses transmitted by group IV fibers during post-contraction circulatory occlusion (74). In in vitro studies in kidney cells, PPADS was shown to also block the release of arachidonic acid suggesting the reduced pressor response observed in the previous experiments may not be exclusively due to the inhibition of P2X receptors (146). However, McCord et al (104) addressed this concern demonstrating that in vivo administration of PPADS to the hindlimb circulation during contraction in cats did not alter PGE2 production. Therefore, it is likely that arachadonic acid production is not significantly affected in this model. A recent investigation in cats has shown that the pressor response to post-contraction circulatory occlusion is attenuated after infusion of either a P2X2/3 or P2X3 selective antagonist into the arterial circulation of the hindlimb (104). Further, Cui et al (18) demonstrated in humans that the pressor and muscle SNA response to fatiguing hand-grip and post-exercise ischemia are attenuated by infusion of vitamin B6, a P2X receptor antagonist. Collectively, the emergent data suggest ATP produced during exercise binds P2X receptors thereby exciting skeletal muscle sensory afferent fibers (91).

The Skeletal Muscle Mechanoreflex

Group III sensory afferent neurons are predominately excited by mechanical distortion of their receptive fields, however, the area of the muscle where mechanoreceptors are optimally exposed to this stimulus has yet to be identified. In rats, injection of lidocaine into the myotendinous junction has been shown to abolish the cardiovascular response to mechanoreflex stimulation while severing and reattaching the Achilles tendon has no effect (121, 122). These studies suggest that mechanoreceptors are located proximally to the Achilles tendon within the myotendinous junction with the receptive fields of some group III afferent neurons located near the head of the muscle. Additionally, the specific receptor-type that excites mechanically sensitive neurons remains unknown. Gadolinium, a trivalent lanthanide, inhibits mechanoreceptor excitation to mechanical stimuli such as tendon stretch and static contraction (41, 45, 119). Gadolinium inhibits the opening of mechanogated potassium channels, L- and T-type calcium channels and mechanogated cation channels, but these channels have yet to be localized to group III afferent nerve endings (36).

A small population of group III afferent fibers are polymodal in nature, exhibiting a secondary excitatory response to muscle fatigue during static contraction. These neurons appear to be excited by the accumulation of metabolites such as bradykinin, potassium, by-products of arachidonic acid metabolism, and lactic acid (55, 69, 85, 136, 143, 147). Evidence suggests mechanoreceptors are sensitized by metabolites, specifically during conditions of limited perfusion, thereby enhancing the sympathetic response to mechanoreflex activation (3, 71). For example, extension of the wrist (a technique thought to selectively activate mechanoreceptors) significantly increases BP and muscle
SNA during post-exercise ischemia. This finding suggests metabolites produced during ischemia sensitize mechanoreceptors (19).

A number of investigations have sought to determine which metabolites are involved in the sensitization of mechanoreceptors. Cyclooxygenases (COX) and lipoxygenases enzymatically aid in the metabolism of arachidonic acid producing prostaglandins (PG), thromboxanes, and leukotrienes. In cats, intra-arterial infusion of arachidonic acid into the hindlimb circulation has been demonstrated to stimulate afferent impulses in 50% of Group III sensory fibers (136). Administration of arachidonic acid has also been shown to augment the number of group III impulses fired per second during static contraction while infusion of the COX inhibitor, indomethacin, markedly attenuates contraction-induced group III afferent impulses (44, 137). Furthermore, infusion of exogenous PG partially restores the pressor response to static contraction after treating the hindlimb with indomethacin (159). Collectively, these studies suggest that the by-products of COX mediated arachidonic acid metabolism sensitize mechanoreceptors during static exercise. In humans, dynamic and static exercise increases circulating PG (185). Consistent with animal data, intrabrachial infusion of indomethacin has been shown to abolish the muscle SNA response to low intensity rhythmic exercise in humans (9, 111). However, indomethacin was not localized to the brachial circulation and the results of these studies may have been influenced by a systemic response. In a separate experiment, ketorolac, a nonselective COX inhibitor was intravenously infused into the arm after Bier block, a technique used to reduce the systemic circulation of exogenously administered substrates (20). The sympathetic response to wrist extension and post-exercise ischemia was abrogated after inhibition of COX, possibly due to a reduction in thromboxane production (20).

The purine ATP is produced during exercise, acts as a ligand for P2X receptors localized on group III and IV fibers and as such may function as a metabolite that sensitizes mechanoreceptors. Hanna et al (40) demonstrated that inhibition of P2X receptors in the hindlimb of the cat attenuates the pressor response to passive stretch by 47%. More recently, this group measured group III afferent activity during electrically-induced static contraction and passive stretch in decerebrated cats before and after infusion of the P2X receptor antagonist, PPADS (72). PPADS reduced group III afferent impulses to static contraction and tendon stretch by 47% and 50%, respectively (72). Additionally, PPADS attenuated the rapid renal SNA response to static contraction and tendon stretch (73). Therefore, the evidence supports a role for purinergic receptors in the sensitization of mechanoreceptors during physical activity.

**Exercise Pressor Reflex Dysfunction in Disease**

Cardiovascular diseases, such as hypertension and heart failure, are characterized by chronic elevations in SNA. In such diseases the autonomic response to exercise is altered generating
exaggerated increases in BP, HR and vascular resistance during acute physical activity. Therefore, the risk of experiencing myocardial ischemia, myocardial infarction, cardiac arrest and/or stroke during or immediately after exercise is heightened. Recent research has demonstrated that skeletal muscle reflexes play a significant role in developing this potentially deleterious cardiovascular response to exercise in both hypertension and heart failure.

**Hypertension**

In hypertension, exercise evokes excessive increases in BP from a chronically elevated baseline (128). Smith et al (155) demonstrated that the pressor and tachycardic responses to activation of the EPR are exaggerated in spontaneously hypertensive rats (SHR) compared to their normotensive counterparts (Figure 4). This heightened cardiovascular responsiveness has been shown to manifest at both maximal and submaximal exercise intensities. Additionally, ganglionic and α-adrenergic blockade with hexamethonium and phentolamine, respectively, abolishes the exaggerated pressor response to EPR activation in SHR suggesting the heightened cardiovascular response to exercise is likely mediated by potentiated increases in efferent SNA (155). Direct measurement of renal SNA in SHR demonstrated that renal SNA is indeed abnormally elevated in response to activation of the EPR in these animals (119). Collectively, these studies support the contention that the exaggerated cardiovascular response to exercise in hypertension is mediated, in part, by abnormally large EPR induced elevations in SNA.

In an animal model of essential hypertension, investigations have demonstrated that both the mechanically and metabolically sensitive components of the EPR contribute significantly to its overactivity as shown in Figure 5 (86, 119). Selective activation of mechanically-sensitive afferent fibers via passive muscle stretch produces enhanced elevations in BP, HR, and renal SNA in hypertensive rats (86, 119). In addition, gadolinium infusion into the hindlimb while stimulating the EPR abrogates the abnormally large pressor, tachycardic and sympathetic responses produced in hypertensive animals (119). Moreover, preliminary studies performed in our laboratory have demonstrated that static contraction in an ischemic hindlimb (an experimental strategy designed to supra-stimulate the metaboreflex) likewise evokes excessive increases in MAP, HR and renal SNA in hypertensive rats (118). With respect to the latter, the findings reported in animals have recently been corroborated in a series of investigations in patients with hypertension of various etiologies (22, 145). In hypertensive humans, post-handgrip exercise circulatory occlusion (a predominantly metabolic stimulus) has been shown to produce significantly greater increases in BP compared to age-matched normotensive subjects (145). Extending these findings, Delaney et al. (22) recently demonstrated that the muscle SNA response to activation of the metaboreflex is abnormally large in older hypertensive patients although conflicting results have been reported in younger patients (133).
Collectively, the animal and human data support the hypothesis that skeletal muscle reflexes are exaggerated in hypertension. The etiology of this dysfunction is currently unclear but may result from a shift in the expression and/or sensitivity of metaboreceptors and mechanoreceptors. For example, TRPV1 receptor protein expression is augmented in the dorsal root ganglion of SHR compared to normotensive rats (118). Whether such changes in receptor protein expression lead to abnormal group III and/or group IV afferent activity remains unknown. As another possibility, central processing of sensory input within the brainstem may be altered in hypertension. Within the cardiovascular control circuits, nitric oxide synthase (NOS) catalyzes the oxidation of L-arginine producing nitric oxide (NO) and L-citrulline. In cats, increasing endogenous NO production within the NTS has been shown to buffer EPR activity such that the sympathetic response to contraction is not as large as it otherwise would be (153). Therefore, NO may either directly or indirectly function as a neuromodulator of the cardiovascular response to EPR activation by attenuating increases in sympathetic outflow. Several mechanisms could facilitate the dysfunction of this potential NO buffering system in hypertension. For example, in hypertensive rats, circulating Angiotensin II (Ang II) is elevated as are the substrates and enzymes necessary for Ang-II production and activity within brain stem nuclei important to cardiovascular control. In addition, Ang II binding receptor protein expression is greater within brain stem cardiovascular regulatory centers (169). Binding of Ang II to its receptor activates redox sensitive pathways resulting in a greater production of reactive oxygen species (ROS), such as superoxide, which have been shown to be increased in the brainstem of hypertensive rats (51, 126, 169). Moreover, superoxide is a well known scavenger of NO (51, 126). As such, increased superoxide production within the brainstem in hypertension could potentially reduce NO bioavailability. As another example, a reduction in NOS expression or an uncoupling of this enzymatic protein could likewise lead to a decrease in NO production reducing the sympathetic buffering capacity of NO. Although highly speculative at this point, such alterations in the neurochemistry of the brain could significantly contribute to EPR induced exaggerations in SNA during exercise in hypertension.

Heart Failure

Chronic hypertension may lead to deleterious structural changes in the myocardium, as a result of pumping blood against a heightened arterial resistance (i.e. afterload). Patients with heart failure exhibit a dilated left ventricle, decreased cardiac output, chronically elevated SNA, and peripheral endothelial dysfunction (50, 168). Additionally, in heart failure patients, exercise evokes excessive increases in BP, HR, and SNA similar to that observed in hypertension (37, 114). Research suggests that skeletal muscle reflexes are likewise dysfunctional in heart failure; however the etiology appears to be different than that observed in hypertension (150). A review of mechanoreflex and metaboreflex dysfunction in heart failure has been published previously (152) but will be briefly discussed. As seen
in figure 6, activation of the EPR via static contraction in rats with dilated cardiomyopathy (induced by ligation of the left anterior descending coronary artery) elicits a greater pressor, cardioaccelerator and renal sympathetic response compared to control and sham animals with similar baseline BP (79, 150). Animal and human data has suggested that the exaggerated EPR observed in heart failure is mediated by mechanoreflex overactivity, whereas metaboreflex function appears to be blunted (12, 154, 156, 160, 178).

Speculatively, the blunting of the metaboreflex in heart failure patients may result from a chronic reduction in skeletal muscle perfusion preventing sufficient removal of metabolites produced during exercise. As such, the potential chronic exposure to excess metabolites could result in a downregulation of metaboreceptors or a decrease in their sensitivity. In support of this concept, two separate groups have demonstrated that TRPV1 mRNA and protein expression is significantly reduced in the dorsal root ganglion subserving hindlimb skeletal muscle of rats with dilated cardiomyopathy compared to controls (156, 178). Furthermore, TRPV1 mRNA expression has also been shown to be reduced in the soleus muscle of the hindlimb (156). Collectively, these data suggest that metabolically sensitive group IV afferent impulses may be significantly reduced in an animal model of ischemia induced heart failure possibly due to a down regulation of metaboreceptors.

In heart failure, evidence suggests that an overactive mechanoreflex mediates the exaggerated EPR evoked cardiovascular response to physical activity. Isolated stimulation of mechanoreceptors produces a significantly greater increase in muscle and renal SNA in rats with dilated cardiomyopathy compared to normal rats (112, 113, 178). The exaggerated cardiovascular response may be due to a heightened sensitization of mechanoreceptors to metabolites due to a reduced ability to remove metabolites produced during contraction. For example, intra-arterial injection of a bradykinin receptor blocker reduced the renal SNA response to hindlimb static contraction in rats with dilated cardiomyopathy while having no affect on the renal SNA response in normal rats (78). Middlekauff et al (111) sought to determine whether the by-products of arachidonic acid metabolism sensitize mechanoreceptors in humans with heart failure. Low intensity rhythmic handgrip exercise evoked heightened increases in BP, HR and muscle SNA which were markedly reduced after infusion of indomethacin into the forearm arterial circulation (111). This is consistent with the tenent that excessive accumulation of arachidonic acid by-products sensitizes muscle mechanoreceptors during physical activity in heart failure patients. However, direct measurements of these metabolites in heart failure patients during exercise have yet to be obtained. As another example, intra-arterial administration of an antagonist of the P2X receptor, also thought to sensitize mechanoreceptors, significantly attenuated the discharge of Group III afferents during passive stretch and static contraction (178). Additionally, administration of a P2X receptor agonist increased the pressor response to muscle stretch by 72% in heart failure rats, compared to only 42% in control rats (32). Furthermore, P2X receptor protein
expression has been demonstrated to be significantly increased in the dorsal root ganglion subserving skeletal muscle afferent fibers in heart failure rats compared to healthy controls (32, 178).

Other mechanisms may have a role in generating abnormal EPR function in heart failure as well. For example, chronic peripheral ischemia due to an insufficient cardiac output in heart failure potentiates the generation of ROS (e.g. superoxide) within skeletal muscle (167). Augmented ROS production is strongly associated with endothelial dysfunction, and may contribute to the exaggerated sympathoexcitatory response to exercise in heart failure (96). In normal rats, hindlimb infusion of a superoxide dismutase inhibitor increased ROS production within the skeletal muscle and augmented the pressor response to static muscle contraction (180). This sympathoexcitatory response was significantly attenuated by intra-arterial infusion a superoxide dismutase mimetic, tempol (180). However it should be noted that in this investigation tempol was not trapped in the hindlimb circulation, therefore it is possible that the attenuated pressor response to EPR stimulation was mediated by a systemic effect of tempol, not an attenuation of muscle afferent activity. In support of this concern, a separate experiment in which tempol was injected and trapped in the hindlimb circulation of normal rats was unable to verify that tempol attenuated the pressor response to static contraction in normal rats (77). However, in rats with heart failure induced by myocardial infarction, entrapment of tempol within the hindlimb circulation did produce a marked reduction in BP, HR and renal SNA in response to activation of the EPR (77). Collectively, these data suggest that increases in ROS generation in the hindlimb skeletal muscle contributes to the exaggerated cardiovascular response to stimulation of the EPR in heart failure.

**Future Directions/Perspectives**

Skeletal muscle reflexes are essential to the initiation and regulation of the cardiovascular response to exercise. It is apparent that this reflex mechanism is altered in disease states such as hypertension and heart failure. In these disease states, an overactive EPR elicits a potentially dangerous exaggerated circulatory response to exercise. This acute response puts the patient at greater risk for the occurrence of a deleterious cardiovascular and/or cerebrovascular event. Seemingly contradictory, recent research has demonstrated that exercise training may attenuate the overactive EPR that develops in cardiovascular disease. For example, in heart failure patients, six weeks of forearm exercise training has been shown to improve the cardiovascular response to physical activity (129). In a similar regard, Wang et al (179) demonstrated that training heart failure rats on a treadmill for 7 weeks significantly reduced the peak change in BP, HR, and renal SNA to levels comparable to healthy untrained animals. The favorable adaption in heart failure animals resulted from both an attenuation of mechanoreflex overactivity and a partial restoration metaboreflex function. These findings are encouraging and suggest that exercise training may abrogate the abnormally exaggerated
cardiovascular response to exercise in heart failure. Unfortunately, it has yet to be determined whether exercise training can improve skeletal muscle reflex function in hypertension. Further, it remains unknown whether exercise training can be effective as a preventative measure against the development of muscle reflex dysfunction in the early stages of cardiovascular disease. Future research focused on determining the mechanisms of muscle reflex dysfunction as well as identifying potentially effective therapies for this dysfunction will continue to be important both clinically and for our understanding of cardiovascular regulation during exercise in health and disease.

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References


23


Figure Legends

Figure 1. The exercise pressor reflex arc. Skeletal muscle contraction excites group III and group IV sensory afferent neurons through several putative stimuli. Stretch or the application of pressure to skeletal muscle distorts the receptive field of mechanically sensitive receptors located predominately on group III afferent neurons. These mechanoreceptors have yet to be identified, but are likely mechanogated cation, potassium, or calcium channels. Metabolites released during muscle contraction such as potassium, lactic acid, bradykinin, analogues of ATP, by-products of arachidonic acid metabolism, and diprotonated phosphate excite metabolically sensitive receptors located predominately on group IV afferent neurons. Pharmacological agonists for acid-sensing ion channels (ASICs), bradykinin receptors, transient potential vanilloid receptor 1 (TRPV1), purinergic receptors, and cannabinoïd receptors have been demonstrated to evoke group IV afferent activity. However, there are likely other potential metaboreceptors that have yet to be identified. Group III and group IV afferent sensory neurons transmit impulses, via the spinal cord, to the cardiovascular control centers located within the medullary region of the brainstem, specifically the nucleus tractus solitarius (NTS), rostral ventrolateral medulla (rVLM), and caudal ventrolateral medulla (cVLM), as well as other secondary nuclei. Central processing of this input promotes increases in sympathetic nerve activity and withdrawal of parasympathetic nerve activity. Thus, heart rate, arterial blood pressure, and vascular resistance are reflexively increased upon the onset of muscle contraction.

Figure 2. Cumulative Histogram of group IV afferent impulses before, during, and after electrically-induced static contraction. A) In 8 group IV sensory neurons, afferent activity was elevated during static contraction (denoted by black bar) compared to the impulses recorded 1min prior to and 2min after contraction. B) The administration of indomethacin (5 mg/kg i.v.), markedly reduced the discharge of the same group IV afferent neurons during static contraction. Indomethacin did not affect the discharge of afferents prior to or following contraction. Data adapted from Rotto et al. (135).

Figure 3. Cumulative Histogram of group III afferent impulses before, during, and after electrically-induced static contraction. A) In 11 group III sensory neurons, afferent activity was elevated during static contraction (denoted by black bar, Cx) B) the discharge of group III afferents was markedly reduced during contraction 60min after the injection of gadolinium trichloride (10mM in 1mL) into the femoral artery. C) Group III afferent impulses during contraction were partially restored after 120min. Data adapted from Hayes et al. (41).
Figure 4. A representative tracing of the cardiovascular response to electrically-induced static contraction in normotensive and hypertensive rats. The pressor and sympathetic response to a given amount of tension generated during electrically induced static contraction is greater in spontaneously hypertensive rats (SHR) compared to normotensive Wistar Kyoto (WKY) rats. ABP, arterial blood pressure; RSNA, renal sympathetic nerve activity. Tracing adapted from Mizuno et al. (119).

Figure 5. Stimulation of the metaboreflex and the mechanoreflex evokes an exaggerated pressor response in hypertension. A) Injection of graded doses of capsaicin into the femoral artery of hypertensive rats produced an exaggerated increase in mean arterial pressure (MAP) compared to normotensive rats. B) Passive stretch of the hindlimb evoked a significantly greater pressor response in SHR compared to WKY specifically from 34% to 100% maximal tension development. * Denotes SHR significantly different from WKY. † Denotes significantly different from the preceding stimulus. Significance was set at P<0.05. Data adapted from Leal et al. (86).

Figure 6. Electrically-induced static contraction evokes an exaggerated sympathetic response in rats with dilated cardiomyopathy. A) Static contraction of the hindlimb for 30sec evoked a significantly (*, P<0.05) greater change in renal sympathetic nerve activity (RSNA) and B) lumbar SNA (LSNA) in rats with ischemia-induced dilated cardiomyopathy (DCM, black bars) compared to normal control rats (white bars). C) the change in RSNA and D) LSNA was significantly greater for a given amount of tension generated over the 30sec contraction (tension time index, TTI). Data adapted from Koba et al. (79).
Putative Stimuli
- Mechanical distortion of the receptive field via stretch or pressure

Possible Mechanoreceptors
- Mechanogated cation channels
- Mechanogated potassium channels
- Mechanogated calcium channels

Possible Metaboreceptors
- ASICS
- Bradykinin
- TRPV1

Group III (A-δ fibers)
- Group IV (C fibers)

Cardiovascular Control Centers

Sympathetic Nerve Activity:
- ↑ Heart Rate
- ↑ Blood Pressure
- ↑ Vascular Resistance

Parasympathetic Nerve Activity:
- ↓ Venous capacitance
- ↑ Norepinephrine secretion

Possible Stimuli
- Potassium
- Lactic acid
- Bradykinin
- ATP analogues
- Arachidonic acid by-products
- Diprotonated phosphate
- Purinergic
- Cannabinoid
WKY

ABP (mmHg)

Raw RSNA (μV)

Integrated RSNA (μV)

RSNA (%)

Tension (Kg)

SHR

10s