A perspective on the muscle reflex: implications for congestive heart failure

Lawrence I. Sinoway1,2 and Jianhua Li1

1Division of Cardiology, Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center, Hershey; and 2Lebanon Veterans Affairs Medical Center, Lebanon, Pennsylvania

Sinoway, Lawrence I., and Jianhua Li. A perspective on the muscle reflex: implications for congestive heart failure. J Appl Physiol 99: 5–22, 2005; doi:10.1152/japplphysiol.01405.2004.—In this review we examine the exercise pressor reflex in health and disease. The role of metabolic and mechanical stimulation of thin fiber muscle afferents is discussed. The role ATP and lactic acid play in stimulating and sensitizing these afferents is examined. The role played by purinergic receptors subdivision 2, subtype X, vanilloid receptor subtype 1, and acid-sensing ion channels in mediating the effects of ATP and H+ are discussed. Muscle reflex activation in heart failure is then examined. Data supporting the concept that the metaboreflex is attenuated and that the mechanoreflex is accentuated are presented. The role the muscle mechanoreflex plays in evoking renal vasoconstriction is also described.

autonomic nervous system; sympathetic nervous system; metaboreceptors; mechanoreceptors

EXERCISE AND THE SYMPATHETIC NERVOUS SYSTEM: GENERAL CONCEPTS

DURING EXERCISE the sympathetic nervous system is activated. This contributes to an increase in blood pressure, heart rate, and peripheral vasoconstriction (101, 117). The neural mechanisms that activate the sympathetic nervous system are not completely understood. Two basic theories of neural control have evolved. The first, termed “central command,” suggests that neural motor and sympathetic activation occur in parallel, i.e., there is a volitional signal emanating from central motor areas that leads to increased sympathetic activation during exercise. It has been suggested that this system is linked to skeletal muscle metabolic needs via parallel rostral brain activation of motor and autonomic centers. In this way, as muscle fatigues, more motor units are recruited and a commensurate increase in sympathetic outflow occurs (28, 31, 33, 49, 117, 118, 122).

The second theory of sympathoexcitation states that the discharge of mechanically and chemically sensitive afferents in the exercising muscle increases during contraction evoking the “exercise pressor reflex” (63, 88). Alam and Smirk (3) were the first to offer evidence suggesting that chemical byproducts of muscle contraction could evoke a pressor reflex. These authors demonstrated that blood pressure remained above baseline values if the circulation to the exercising limb was arrested at the end of exercise. When this metabolite sensitive system is activated, sympathetic tone increases and is directed toward blood vessels in a variety of organs including the skeletal muscle (58, 67, 94, 101, 117), evoking a limb vasoconstrictor response (94, 101). This system is activated as the muscle fatigues (89, 95) and is capable of evoking dramatic increases in sympathetic discharge (103).

Coote et al. (22) provided evidence in support of the exercise pressor reflex in an anesthetized animal preparation. Electrical stimulation of the ventral roots evoked pressor and heart rate responses that were blocked by cutting the dorsal roots. The magnitude of the response was proportional to the generated muscle tension. These authors also demonstrated that ischemic muscle contractions evoked a greater pressor reflex than nischemic contractions, a finding confirmed by others (110). McCloskey and Mitchell (63) demonstrated that the afferent arm of this reflex involved the stimulation of group III and IV muscle afferents. Anodal blockade of the L7–S1 dorsal roots of the cat blocked thickly myelinated group I and II afferents but did not block the cardiovascular responses. Topical application of a local anesthetic to the dorsal roots did not block group I and II afferents but did block the cardiovascular responses to contraction. The role of group III and IV afferents has been confirmed by others (68, 90, 113, 126). The free nerve endings of both group III and IV afferent fibers have been identified in the interstitial spaces and appear to be in close proximity to lymphatics and blood vessels of muscle and tendon tissue. These loci would seem ideal for chemotransduction. Separate populations of group III and IV fibers have been identified within the interstitium in close proximity to collagen bundles. These receptors presumably are appropriately situated to act as mechanically sensitive receptors (5).

Important studies by Kaufman and colleagues (45, 46) using a static contraction paradigm demonstrated that group III fibers in the triceps surae muscle of the cat are predominantly mechanically sensitive, whereas unmyelinated group IV muscle afferents are chemically sensitive. Moreover, in this model, ischemia increased the discharge of group IV but not group III fibers.

Experiments by Kaufman and colleagues (1) using a “walking cat preparation” with recordings from single afferent fibers have recharacterized the discharge properties of group III and IV afferents. In these studies, both fiber groups were stimulated by dynamic exercise induced by electrical stimulation of the

Address for reprint requests and other correspondence: L. I. Sinoway, Cardiology, H047, Pennsylvania State Univ. College of Medicine, P.O. Box 850, Hershey, PA 17033 (E-mail: lsinoway@psu.edu).

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mesencephalic locomotor region. At relatively low levels of tension generation and muscle oxygen consumption, all group III and 8 of 10 group IV fibers increased their discharge frequency (1) with 12 of 18 group III and 2 of 8 group IV discharging synchronously with the contraction phase of the gait cycle. Interestingly, Adreani and Kaufman (2) again using the walking cat preparation demonstrated that ischemia increased the discharge of 44% group III and 47% of group IV afferent fibers tested.

MUSCLE REFLEX ENGAGEMENT AND SYMPATHETIC ACTIVATION

The muscle reflex is engaged when muscle contraction occurs (Fig. 1). As the muscle contracts, concentrations of a variety of chemicals rise in the interstitial space where the free nerve endings in group III and IV muscle afferents reside. Contraction also deforms the receptor field of the muscle afferent and increases the discharge of mechanically sensitive muscle afferents. Once these fibers are stimulated, a powerful central neural signal is generated that contributes to the increase in sympathetic outflow seen with exercise. This increase in sympathetic outflow is important because if peripheral blood flow capacity is greater than maximal cardiac output (4, 6, 73), then some controlling influence must be interposed between the heart and the muscle vasculature to maintain blood pressure, thereby ensuring that cerebral and cardiac flow are preserved. The sympathetic nervous system plays a crucial role in this regulatory process.

BLOOD FLOW DELIVERY TO EXERCISING MUSCLE

The interrelationship between metabolic vasodilatation and neurally mediated vasoconstriction during exercise has been an area of considerable investigation. It is known that muscle blood flow capacity can greatly exceed maximal cardiac output (4, 6, 73). Given the fact that blood pressure does not fall during exercise, it must be concluded that these peak levels of muscle flow are not achieved. Rowell (87) has hypothesized that myogenic and sympathetic vasoconstrictor influences prevent muscle flow from outstripping cardiac output during maximal exercise. This hypothesis is based on work demonstrating that marked increases in sympathetic drive can reduce the flow response to a variety of vasodilatory stimuli. For example, in humans, when sympathetic tone is markedly and acutely increased, maximal flow responses to ischemia and/or exercise are substantially reduced (98, 100). Similar observations have been made in animals (9, 25, 78, 116). Recent work by VanTeeffelen and Segal (115) using a hamster retractor muscle preparation has demonstrated that sympathetic nerve stimulation restricts blood flow to contracting muscle by constricting feed arteries and first order arterioles. This effect was contrasted with the effect on second and third-order arterioles, where sympathetic activation did not evoke vasoconstriction. The authors concluded that this combination of effects served to vasoconstrict the active muscle and to also redistribute flow within exercising muscle (Fig. 1).

THE MUSCLE REFLEX AND THE RENAL CIRCULATION

In resting humans the kidney receives approximately one-quarter of the total body blood supply (48). In turn, renal blood flow is directly linked to the glomerular filtration rate. Despite the kidneys impressive ability to autoregulate blood flow, exercise causes a prominent reduction in renal blood flow, thus allowing a greater percentage of total body flow to be directed to the exercising muscle (32). One of the major determinants of this prominent renal vasoconstriction is an increase in sympathetic discharge directed to the kidney (24). Thus understanding the neural mechanisms that regulate renal blood flow has important ramifications for the overall understanding of exercise capacity during exercise in human subjects. Animal reports suggest that neural regulation of the kidney circulation is different than it is for skin and muscle (38–40, 119). Specifically, prior reports in animals suggest that mechanoreceptor engagement increases renal sympathetic nerve activity (SNA), in turn evoking renal vasoconstriction. Important studies by Victor et al. (119) demonstrated that hindlimb contractions in cats evoked an increase in renal SNA that was synchronized
with tetanic contractions. Peak increases in renal SNA were seen within 3 s of contraction initiation. In paralyzed cats, electrical stimulation of the tibial nerve at five times motor threshold evoked renal sympathetic discharge (119). This level of stimulation is sufficient to stimulate group III but not group IV afferents. Mueller et al. (72) have shown that muscle contraction increases renal vascular resistance within 10 s of the onset of treadmill exercise. These authors further demonstrated that this early increase in vasoconstriction could be blocked with phentolamine. Matsukawa et al. (60) demonstrated that there is a relationship between tension developed and the magnitude of the increase in renal SNA. This group also demonstrated that metaboreceptors as well as mechanoreceptors can contribute to increases in renal sympathetic tone (Fig. 1) (61).

Middlekauff et al. (67) used positron emission tomography (PET) scanning techniques to examine cortical blood flow during a variety of handgrip exercise interventions in humans. This group demonstrated that handgrip evokes renal vasoconstriction. The limited time resolution of the PET method made it difficult for these authors to examine the relative contributions of metaboreceptor and mechanoreceptor stimulation to the observed renal vasoconstrictor response. In a separate report, this group attempted to overcome this limitation of PET by having subjects perform a 3-min intermittent involuntary biceps contraction paradigm. The authors observed a greater increase in renal resistance in heart failure than in controls. A period of posthandgrip circulatory arrest (PHG-CA) was performed in the control subjects. The data suggested that this paradigm did not evoke a metaboreceptor response in the normal subjects (65).

In the following sections we will focus on three areas germane to understanding the muscle reflex and its impact on circulatory control in health and disease. Specifically, we will address three questions: 1) does adenosine triphosphate (ATP) evoke the muscle reflex and 2) does lactic acid evoke this reflex. Finally, we will examine whether the muscle reflex is abnormal in disease states. Specifically, we will ask: 3) is the reflex abnormal in heart failure? We acknowledge that by discussing two specific metabolites and one specific disease entity we are greatly limiting the scope of discussion. We have done this in an effort to present a timely and specific view of issues we believe are important.

THE ROLE OF ATP IN STIMULATING SKELETAL MUSCLE AFFERENTS

There are a number of reasons to suspect that ATP may play a role in evoking the exercise pressor reflex. First, it is known that touch-induced sensory nerve discharge increases when ATP is injected subcutaneously in frogs. This effect can be blocked by P2 purinoceptor antagonists (75). Second, arterial or intra-articular injections of the selective purinergic receptor subdivision 2, subtype X (P2X) receptor agonist, α,β-methylene ATP, causes rapid short-lasting excitation of subpopulations of group III and IV knee joint afferent nerves (26). Third, ATP stimulates P2X and P2Y receptors, which are found on sensory neurons (11, 12, 21, 37, 41, 47, 75). Such afferent stimulation can evoke both nerve impulse generation, as well as sensory neurotransmitter release (11, 12, 47).

On the basis of these data, this laboratory began experiments examining the role ATP may play in evoking the muscle reflex. In these studies the ATP analog α,β-methylene ATP was infused into the arterial supply of the decerebrate cat. The doses of α,β-methylene ATP were 0.1, 0.2, and 0.5 mM (0.5 ml of each). Injections of the three concentrations of the P2X agonist evoked 6.2 ± 2.5, 22.5 ± 4.4, and 35.2 ± 3.9 mmHg increases in mean arterial pressure, respectively (Fig. 2). After occlusion of the femoral vein, the 0.5 mM dose still increased mean arterial pressure by ~35 mmHg, suggesting that the response was localized to the cat hindlimb. Cutting the sciatic nerve reduced the pressor response to 8.4 ± 2.7 mmHg (Fig. 2). The effect of α,β-methylene ATP was blocked by pyridoxalphosphate-6-azophenyl-2',4'-disulfonate (PPADS; a P2 receptor blocker), but not by reactive blue Z (P2Y receptor blocker). This suggests that the majority of the increase in blood pressure was due ATP stimulating afferents fibers subserved by the sciatic nerve. Because care was taken to eliminate all sensory input from the hindlimb skin, it was concluded that the afferents stimulated by the arterial injections were predominantly located within skeletal muscle.

Hanna et al. (34) performed studies showing that intraarterial infusions of α,β-methylene ATP but not 2-chloroadenosine evoke a pressor response in unanesthetized decerebrate cats. In a second report from this group, it was demonstrated that when PPADS was infused into the arterial supply of the cat...
hindlimb before muscle contraction, the exercise pressor reflex was attenuated (36). They also found that the pressor response seen during a period of postcontraction circulatory occlusion was attenuated. The authors concluded that P2 receptors play a role in stimulating mechano- and metaboreceptors. The response was blocked by cutting the sciatic nerve or by prior infusions of PPADS. Finally, Hanna and Kaufman (35) have examined the discharge of group III and IV afferents in response to arterial infusions of α,β-methylene ATP. In this important study, they observed that 3 of 18 group III and 7 of 9 group IV fibers increased their discharge in response to α,β-methylene ATP. Thus α,β-methylene ATP preferentially stimulates slowly conducting sensory afferent fibers.

**ATP AND MUSCLE MECHANORECEPTOR SENSITIZATION**

In a prior report from this laboratory, muscle sympathetic nerve responses to static quadriceps contractions were examined in human subjects (Fig. 3). Subjects performed 20-s bouts of contraction at 25% maximum voluntary contraction (MVC) separated by 5 s of rest. This was done for 5 min. Of note, the muscle sympathetic nerve response to this intervention did not reach a steady state until three or four 20-s bouts of contraction were performed. This phenomenon was examined in greater detail by combining the contractions into four groups (i.e., contractions 1–3, 4–6, 7–9, and 10–12). The nerve activity was then signal averaged and integrated. The increase in the integrated signal averaged muscle sympathetic nerve activity (MSNA) was less for contractions 1–3 than for the other groupings of contractions. The amount of work performed did not change from contractions 1–3 to 4–12 (Fig. 4) (38a).

Why is the integrated signal averaged MSNA for contractions 4–12 greater than for 1–3? It is unlikely that this is a metaboreflex response because metaboreflex responses are generally seen close to the time of fatigue. It is also unlikely that this response is due to a classic mechanoreflex response because the tension generated during contractions 1–3 was identical to that seen during contractions 4–12. At the time this report was published, we postulated that muscle metabolites released during contractions sensitized mechanically sensitive afferents. Thus the difference between contractions 1–3 and contractions 4–12 was due to sensitization of the reflex. Studies using rhythmic handgrip contractions were also consistent with the concept of chemical sensitization of the muscle mechanoreflex (7).

It is with these data in mind that studies were performed to determine whether ATP could sensitize muscle mechanoreceptors. A series of studies was performed in which the muscle mechanoreflex was engaged by performing muscle hindlimb stretch in the decerebrate cat model. Muscle stretch causes deformation of the mechanoreceptors’ receptive field without leading to dramatic changes in the metabolic profile of the skeletal muscle (109). Thus muscle stretch evokes preferential stimulation of mechanoreceptors without the confounding influences of metaboreceptor stimulation. In these studies, hindlimb muscle stretch was performed in the decerebrate cat.

![Fig. 3](image-url) Muscle sympathetic nerve activity (MSNA) during bouts of quadriceps contraction of 20-s duration with 5 s of rest in between contractions. Reprinted with permission from Ref. 38a.

![Fig. 4](image-url) Top: integrated MSNA neurogram is signal averaged and then integrated. Bottom: integrated signal averaged MSNA during quadriceps contractions 1–3, 4–6, 7–9, and 10–12. Reprinted with permission from Ref. 38a.
In this group of experiments, hindlimb muscle stretch to a tension of 2 kg raised blood pressure by $27 \text{ mmHg}$. Preadministration of 0.1 mM $\alpha_\beta$-methylene ATP or 2 mM ATP increased the pressor response to stretch. These effects of ATP and $\alpha_\beta$-methylene ATP were reversible because a third bout of muscle stretch in the absence of these metabolites (recovery) evoked a pressor response similar to that seen during the control stretch (Fig. 5) (55).

Summary data for these muscle stretch experiments are shown in Fig. 6A. It is interesting to note that the effect of the $\alpha_\beta$-methylene ATP was more sustained, i.e., it was present during 20 s of recovery, whereas the effects of ATP returned more rapidly toward baseline. It was speculated that the difference between $\alpha_\beta$-methylene ATP and ATP during recovery may have been due to the fact that the $\alpha_\beta$-methylene ATP is not readily metabolized by ectonucleotidases (83). In a separate set of studies, bouts of muscle stretch were performed before and after blocking purinergic receptors with the drug PPADS (Fig. 6B). When ATP was given in the presence of PPADS, a pressor response was attenuated, suggesting that a large component of the increase in blood pressure seen with muscle stretch was due to sensitization of purinergic receptors. This group of experiments suggests that ATP does sensitize mechanically sensitive afferents and that this sensitizing effect requires stimulation of purinergic P2X receptors (Fig. 6) (55).

In the next series of studies, it was determined whether skeletal muscle interstitial ATP rises as muscle tension increases during muscle contraction. To address this issue, a series of studies was performed in which the microdialysis method (57) was used to measure interstitial concentrations of ATP as muscle contraction was initiated. Muscle contraction was induced by electrical stimulation of the L7 and S1 ventral roots of decerebrate cats. Contractions were performed at 3 and 5 Hz at $3 \times$ motor threshold (pulse duration of 0.1 ms). The bouts of twitch contraction were sustained for 10 min. Of note, interstitial ATP rose in the contracting muscle by $150\%$ during...
3-Hz stimulation and by 200% at 5 Hz. An increase in ATP was not found in the opposite control hindlimb. Importantly, interstitial ATP rose as a function of the peak muscle tension generated (Fig. 7). The fact that ATP did not rise in the opposite control leg suggests that the ATP was not likely released from sympathetic nerves as part of the muscle reflex (52). ATP AND SYMPATHETIC NERVES

In addition to its sensory effects (34, 55), ATP can affect adrenergic transmission by stimulating purinergic receptors on sympathetic nerve endings (10). For example, in cultured cervical ganglion neurons and cardiac synaptosomes, ATP-sensitive P2X purinoceptors have been shown to enhance norepinephrine (NE) exocytosis (96, 107). Accordingly, this group recently speculated that a rise in interstitial ATP would lead to a rise in interstitial NE concentrations ([NE]i). In these experiments, [NE]i was measured in the hindlimb of the rat using the microdialysis technique as attempts were made to raise ATP by two methods: 1) by muscle stretch; and 2) by infusions of ATP into the hindlimb arterial supply. Muscle stretch (0.5 kg of tension) increased interstitial ATP concentration (P < 0.05), and [NE]i (P < 0.05) in active muscle (Fig. 8A). The rise in [NE]i was linearly linked to the elevated interstitial ATP (r = 0.878, P < 0.001) (Fig. 8B). This effect of muscle stretch was attenuated by infusions of PPADS and was augmented when the nucleotidase inhibitor 6-N,N-diethyl-β-γ-dibromo-methylene-δ-adenosine-5’-triphosphate (ARL67156) were infused into the rat hindlimb (Fig. 9A). [NE]i was also elevated by 76% (P < 0.05) after ATP (3 μM) was injected into the arterial blood supply of the hindlimb muscles (Fig. 9B). This dose of ATP was insufficient to raise [NE]i in the opposite control leg or to raise blood pressure (Fig. 9B). Thus this series of studies documents two important physiological observations: 1) ATP rises with muscle stretch; and 2) ATP in concentrations insufficient to stimulate sensory afferents does stimulate sympathetic nerves, leading to the release of NE. This regional mechanism may play an important role in regulating blood flow to exercising muscle (51). Studies are now underway to examine whether muscle stretch in humans raises [NE]i.

WHAT ROLE DOES LACTIC ACID PLAY IN STIMULATING MUSCLE AFFERENTS?

Considerable controversy surrounds the role lactic acid plays in stimulating muscle afferents (30, 82, 117, 120). For example, some reports suggest that in McArdle’s disease, muscle contraction evokes reduced sympathetic responses to handgrip, whereas others have not observed a reduced sympathetic response to muscle contraction (30, 82, 120, 121). McArdle’s disease is an inborn error of metabolism in which glycogen degradation is blocked because of myophosphorylase deficiency. The inability to break down glycogen leads to reduced lactate levels with muscle contraction.

It is clear that when lactic acid is infused into the arterial supply of the triceps surae muscle of the cat (85, 102), there is an increase in the discharge frequency of not only group IV fibers (85) but also group III muscle afferents (85, 102) (Fig. 10). Thus even though group III fibers are classically termed “mechanoreceptors,” infusions of lactic acid increase their discharge. For example, in a prior report from Kaufman’s laboratory, 13 of 20 group III fibers tested increased their discharge frequency with lactic acid. Twelve of 20 of these increased their discharge in response to muscle contraction (102). In this prior report, the effects of repeated boluses of lactic acid on group III discharge was also tested. The first of three injections (4 ml) of lactic acid led to a large increase in group III fiber discharge within 2 s of infusion. With the second injection of lactic acid, the response was less and the onset latency was greater. Interestingly, when lactic acid was given during a bout of muscle contraction, a large increase in

![Fig. 7. Relationship between peak muscle tension and interstitial concentrations of ATP in the decerebrate cat. Of note, as muscle tension increases, interstitial ATP rises. Reprinted with permission from Ref. 52.](image)

![Fig. 8. A: effects of muscle stretch on ATP and norepinephrine (NE) concentrations in the decerebrate rat model. Of note, as tension is increased, both ATP and NE increased. B: correlation between the increase in ATP and the increase in NE. *P < 0.05 vs. control before stretch. Values are means ± SE.](image)
the discharge frequency of the group III afferents was noted. Thus group III afferent fibers respond to chemical stimulation with lactic acid, their discharge decreases with a second dose, and discharge can be restored when contraction is coupled with lactic acid boluses (Fig. 11).

Another interesting feature regarding group III afferents is that the resting interstitial lactic acid concentration helps determine the discharge seen during a bout of muscle contraction. This characterization was based on studies giving dichloroacetate (DCA) to anesthetized cats before a 1-min bout of static hindlimb muscle contraction in the anesthetized cat. DCA increases the active form of pyruvate dehydrogenase so that, for a given amount of pyruvate generated during glycolysis, less lactate is generated (108, 129). DCA lowered resting and exercise lactic acid levels (venous resting lactate before DCA: 2.5 ± 0.1 mM; after DCA: 1.2 ± 0.3 mM, P < 0.05; contraction lactate before DCA: 3.0 ± 0.2; after DCA: 2 ± 0.3 mM, P < 0.05) and this was associated with a decrease in the discharge of group III afferents (Fig. 12A). This effect was most prominent during the first 10 s of contraction. In control studies, two bouts of contraction were performed without administration of DCA before the second trial (Fig. 12B). Under these circumstances there was no difference in the discharge frequency during the two trials.

DCA studies were also performed in human subjects. In these experiments, seven subjects performed two bouts of static exercise at 20% MVC until fatigue as MSNA was measured. These bouts of contraction were performed before and after infusing DCA. DCA lowered lactic acid at rest and in response to muscle contraction (rest: trial one 0.8 ± 0.1 mM; trial two 0.4 ± 0.1 mM, P < 0.01; contraction: trial one 1.7 ± 0.2; trial two 1.3 ± 0.3 mM). We found that, in six of the seven subjects tested, the DCA led to a reduction in the level of MSNA that was seen at the end of the bout of exercise and during PHG-CA (Fig. 13). The MSNA response during the DCA bout of contraction was approximately one-half of that seen without DCA. Control studies were also performed in which no DCA was given between the two bouts of contraction. Under these circumstances, there was no attenuation in the muscle sympathetic nerve response in the second bout of exercise. Moreover, the effects of DCA were not due to a generalized effect of this agent on the autonomic nervous system because MSNA responses to the cold pressor test (CPT) were not attenuated after DCA (CPT before DCA: 307 ±
On the basis of this series of animal and human studies, we would suggest that the lactic acid can 1) directly stimulate afferents; 2) sensitize muscle afferents; and 3) the response to lactic acid can become desensitized and then resensitized by muscle contraction. However, before leaving this section, we must point out that not all data support these three conclusions. Studies performed

100% increase in MSNA; CPT after DCA 292 ± 83%, NS). On the basis of this series of animal and human studies, we would suggest that the lactic acid can 1) directly stimulate afferents and 2) sensitize muscle afferents; and 3) the response to lactic acid can become desensitized and then resensitized by muscle contraction.

Fig. 11. Effects of lactic acid on the discharge of group III afferent fiber. Of note, the first injection of lactic acid led to a large increase in impulses per second. The second injection lead to a smaller increase in discharge frequency. When lactic acid was coupled with a bout of contraction, the group III afferent was resensitized. Reprinted with permission from Ref. 102.

Fig. 12. A: discharge frequency of group III afferents to a bout of static contraction. Of note, discharge frequency was attenuated when dichloroacetate (DCA) was first infused. *P < 0.5 at 10 s. B: 2 trials of static contraction without DCA infused between trial 1 and trial 2. Reprinted with permission from Ref. 102.
Fig. 13. Percent change in MSNA (%Δ MSNA) in 7 separate subjects to a bout of static handgrip to fatigue. Of note, in 6 of 7 subjects, the Δ% MSNA during end grip (G₆) was attenuated. During circulatory arrest (CA), Δ% MSNA was again attenuated in 6 of 7 subjects. B, baseline; G₁–G₃, minutes 1 through 3 of handgrip. Reprinted with permission from Ref. 29.
in this laboratory suggested that lactic acid infusions (into the arterial supply of the triceps surae muscle of the cats) only evoke a pressor response when the pH of the infusate was below pH 6 (104). Second, a number of studies have shown that lactate and H\(^+\) remain elevated after muscle contraction at a time that the specific index of autonomic engagement returns to baseline (86, 104). These studies would argue against a direct stimulatory effect of lactic acid on muscle afferents. Finally, Middlekauff et al. (64) have recently shown that DCA infusions in human subjects do not attenuate MSNA responses during low-level rhythmic exercise. This would argue against a role for lactic acid in sensitizing muscle afferents.

**RECEPTOR STIMULATION AND LACTIC ACID**

A key question that arises from these data is, if lactate stimulates muscle afferents, what type of receptor does lactic acid stimulate? If lactic acid plays a role it likely does so via altering the pH or the sensitivity of H\(^+\)-sensing channels on sensory afferents (43). This is perhaps why lactic acid is more potent in evoking autonomic muscle reflexes than is equimolar H\(^+\) (86). Hydrogen ion can activate sensory nerves by potentially opening two types of ion channels. These include the type 1 vanilloid receptor and the acid-sensing ion channel (ASIC) (13, 114, 124, 125). Vanilloid receptor subtype 1 (VR1) appear on C fibers in a variety of tissues. Vanilloid substance capsaicin activates pulmonary C fibers (20, 79), cardiac afferents (131), and the hindlimb musculature of the dog (23, 127), cat (44), and rat (105). Specifically, capsaicin preferentially stimulates group IV skeletal muscle afferents (44). The ASIC belongs to the amiloride-sensitive degenerin/epithelial sodium channel (ENaC) channel family. These channels are frequently observed on sensory neurons (14, 123). In an effort to determine how lactic acid might be mediating its effect on skeletal sensory afferents, a series of studies was performed in which bolus infusions of lactic acid were given before and after VR1 and ASIC were blocked. A decerebrate rat model was employed for these studies. When lactic acid was infused into the hindlimb of the rat, a large increase in blood pressure was noted. When capsaizapine, a blocker of the VR1 receptor, was given the effect of lactic acid was un-attenuated by blockade of VR1 stimulant of capsaicin-sensitive sensory afferents (131), and when it is given a few days before the terminal experiments it destroys VR1-containing sensory nerves. When capsaizapine was given into the hindlimb arterial supply of the decerebrate rat, a biphasic response was noted. This response was characterized by an initial fall in blood pressure followed a few seconds later by a rise in blood pressure. In animals treated with RTX 4–5 days earlier, no pressor response was seen when capsaizapine was given (Fig. 15). Studies were then performed in which lactic acid was given to both control animals and to RTX-treated animals. In the RTX-treated animals, there was a substantial attenuation in the ability of infused lactic acid to raise blood pressure (Fig. 15). On the basis of these data above, it was concluded that lactic acid mediates its response by stimulating ASIC and that these ASIC are likely to be present on sensory fibers that also have VR1 receptors (and would thus have been destroyed by RTX) (53). It is clear that work needs to be done in humans to examine the role that these receptor subtypes play in evoking the exercise pressor reflex.

**THE MUSCLE REFLEX IN CONGESTIVE HEART FAILURE**

Congestive heart failure is a chronic condition that is characterized by impaired cardiac function that leads to reduced blood supply to metabolizing tissues. It is a common disease with ~500,000 patients being diagnosed each year. It is a lethal disease with ~300,000 deaths each year (42). The common causes of this disease include coronary artery disease, hypertension, and idiopathic cardiomyopathy. It is known that sympathetic excitation plays a prominent role in disease progression (8). Moreover, it is known that sympathoexcitation is inversely related to disease prognosis. This laboratory has focused on how the muscle reflex is engaged during heart failure.

In 1991 we performed studies in which we compared muscle reflex responses in patients with congestive heart failure to control subjects. Nine subjects were studied: four were class II, four class III, and one New York Heart Association (NYHA)
class IV congestive heart failure. In these studies, subjects with and without congestive heart failure performed static handgrip exercise at 30% of MVC as MSNA was measured using microneurography. At the end of the 2-min bout of contraction, the circulation to the forearm was arrested, evoking posthandgrip muscle ischemia. Sympathetic nerve responses during this intervention are thought to represent specific stimulation of the forearm muscle metaboreflex (Fig. 16).

The increase in MSNA during handgrip was not statistically different between the heart failure and control subjects. However, during PHG-CA, SNA continued to rise in the control subjects whereas in heart failure subjects it fell toward baseline. Of note, forearm muscle pH responses to the handgrip paradigm were similar in the two groups ($^{31}$P-NMR). These findings were interpreted as suggesting that the predominant mechanism leading to sympathetic activity in the control subjects was due to stimulation of the muscle metaboreflex. This finding was consistent with earlier studies in human subjects (58). However, in congestive heart failure the metaboreceptor reflex response was attenuated and this led to the decreased sympathetic response during handgrip itself. It was suggested that this normalized response in heart failure was due to enhanced stimulation of muscle mechanoreceptors. Subsequent studies from this laboratory demonstrated that limb congestion, a common feature of congestive heart failure, increases sympathetic nerve response to handgrip exercise (62). Moreover, further studies suggested that limb congestion could sensitize muscle mechanoreceptors and in the process increase synchrony between contraction and sympathetic discharge (71). Negrão et al. (76) found that metaboreflex control of MSNA was attenuated in severe heart failure (NYHA class III and IV) but were maintained in those with mild heart failure (NYHA class I and II). This paper for the first time suggested that metaboreceptor desensitization may be a function of the severity of heart failure. This finding has recently been confirmed in a rat model by Li et al. (54). Floras and colleagues (77) had heart failure and control subjects perform static and rhythmic handgrip paradigms as MSNA was measured. They observed that, compared with age-matched controls, MSNA responses in heart failure were exaggerated under all conditions tested. They concluded that the muscle metaboreflex is accentuated in heart failure. The heart failure subjects in this report had a

**Fig. 15.** Pressor responses to capsaicin (CAP) and lactic acid in control animals and in previously treated resiniferatoxin (RTX) animals. Of note, RTX treatment abolished the response to capsaicin and RTX treatment markedly attenuated the pressor response to lactic acid. *P < 0.05 vs. control (solid bars). Reprinted with permission from Ref. 53.

**Fig. 16.** Schematic representations of MSNA responses to a bout of static handgrip for 2 min. Of note, the increase in MSNA at end grip was relatively similar in the 2 groups; however, during posthandgrip circulatory arrest (PHG-CA), MSNA remained elevated in control subjects and fell toward baseline in the heart failure subjects.
\( \dot{V}O_2 \text{max} \) of 18.6 ml/kg, which places them in the upper half of the mild to moderate heart failure range (128). The reasons why these findings differ from those reported by Sterns et al. (111) and Negra˜o et al. (76) (in severe heart failure) are unclear.

The autonomic responses to rhythmic exercise are distinctly different from those seen with static exercise (16, 80, 81, 92, 93, 97). In an effort to further examine the relationship between muscle metabolism and sympathoexcitation, studies were performed in heart failure subjects and age-matched controls in which rhythmic handgrip was performed at 25% MVC with a two-contraction–3-s rest cycle for 20 min or until the subjects fatigued. The heart failure subjects ranged from NYHA class II to IV. Measurements included skin SNA, MSNA, and \(^{31}\)P-NMR spectroscopy using a coil placed over the exercising flexor digitorum superficialis muscle in the forearm.

Sixteen of 20 control subjects were able to complete 20 min of this paradigm, whereas only 4 of 24 heart failure subjects completed the 20-min rhythmic handgrip paradigm. Thus 20 of 24 heart failure subjects (83%) fatigued “prematurely,” whereas only 4 of 20 controls (20%) fatigued prematurely. Importantly, the greater propensity for fatigue in the heart failure subjects was associated with marked muscle acidosis, a prominent accumulation of \( H_2PO_4^- \) and an increase of \( P_i/P_{i+PCr} \) (NMR spectroscopy). With regards to sympathetic outflow it was observed that MSNA rose earlier in handgrip and both MSNA and skin SNA remained elevated during PHG-CA in heart failure but not in the controls. These findings suggest that the muscle metaboreflex was engaged in the heart failure subjects and not in the controls because the metabolic changes in skeletal muscle were so much more pronounced in the heart failure than in the control subjects (99) (Fig. 17).

These findings are consistent with a number of papers (16, 80, 81, 92, 93, 97) that suggest that ventilatory and sympathetic responses to rhythmic exercise are exaggerated in heart failure. It has been proposed that these exaggerated responses were due to intrinsic abnormalities in skeletal muscle. Hence the term the “muscle hypothesis” was coined (17, 18).

It is clear that the accumulation of by-products of muscle metabolism are greatly increased in heart failure (59, 130). There are many potential reasons for this predilection to altered muscle metabolism in heart failure. These include muscle atrophy in heart failure (112), differences in blood flow distribution (74), reductions in muscle blood flow in heart failure

\[ \text{Changes in Renal Blood Flow Velocity} \]

![Fig. 17. Data demonstrating that increased MSNA seen during rhythmic handgrip was sustained during PHG-CA in heart failure (A). \( *P < 0.05 \) between congestive heart failure (CHF) and controls at PHG-CA. Of note, nuclear magnetic resonance (NMR) data showed that greater changes in hydrogen ion (H\(^+\)), diprotonated form of inorganic phosphate (H\(_2\)PO\(_4\)), and interstitial phosphate (Pi) were seen in the heart failure patients (B). \( *P < 0.05 \) between controls and CHF. Rec, recovery. Reprinted with permission from Ref. 99.](image)

![Fig. 18. Renal blood flow velocity in control subjects and heart failure (HF) subjects. Of note, group effects were noted for all 3 time periods examined, suggesting that renal flow reductions during handgrip in heart failure are much more prominent then they are in a control group. \( *P < 0.05 \) between HF and controls at the indicated % maximal voluntary contraction (MVC). Reprinted with permission from Ref. 69.](image)
(50, 56, 74), reduced diffusive and conducive \( \text{O}_2 \) delivery in heart failure (84), differences in muscle fiber type in heart failure and controls (27, 112), and differences in mitochondrial enzyme concentrations in heart failure and control subjects (59, 112). All of these potential changes seen in heart failure would tend to increase muscle by-product release and lead to more rapid fatigue.

In summary, the autonomic adjustments to exercise depend on the type of muscle work being performed. The metabolic derangements of heart failure are much more likely to be manifested during rhythmic than during static exercise (99, 111) because isotonic compared with isometric exercise is more metabolically “costly” (91). Moreover, flow abnormalities that may be present in heart failure will be exaggerated under the freely perfused conditions seen with rhythmic exercise because static contractions \( \geq 20\% \text{ MVC} \) can impede limb flow (29). Thus metabolite release differences between heart failure and control subjects will be minimized with static and accentuated with rhythmic exercise. The importance of altered muscle metabolism to the exaggerated ventilatory and autonomic responses to exercise is likely to be an important reason why conditioning paradigms are particularly useful in correcting many autonomic abnormalities seen with rhythmic exercise patients with heart failure (80).

**MUSCLE REFLEX CONTROL OF THE RENAL CIRCULATION IN HEART FAILURE**

The hypothesis that the muscle mechanoreflex is accentuated in heart failure may be particularly important to understanding renal circulatory control in this disease. Specifically, one would hypothesize that an accentuated mechanoreflex would lead to enhanced renal vasoconstriction in heart failure.

Studies by Victor et al. (119) have suggested that renal SNA is augmented at the initiation of bouts of static contraction of the cat hindlimb. Work by Middlekauff and colleagues (67) using PET scan have suggested that reductions in renal blood flow occurred during bouts of handgrip exercise, and this group has suggested that this renal constriction was in part due to engagement of the muscle mechanoreflex.

Middlekauff et al. (67) examined renal vasoconstrictor responses to muscle contraction in humans by using PET scanning techniques to measure renal cortical blood flow. These authors observed that renal vascular resistance increased during the first 2 min of a 2.5-min static handgrip period at 10% MVC.

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**Fig. 19.** Pressor responses to intra-arterial infusions of capsaicin in control animals, animals with small myocardial infarctions (MI), and those with large infarcts. Top: mean arterial pressure. Bottom: heart rate. Of note, the pressor response to capsaicin was markedly attenuated in animals with large infarcts. *\( P < 0.05 \) vs. respective baselines. †\( P < 0.05 \) vs. small-MI and control animals. Values are means ± SE. Reprinted with permission from Ref. 54.

**Fig. 20.** Response to muscle stretch in the 3 animal groups. Of note, decerebrate rats with large myocardial infarctions had a much greater increase in blood pressure with muscle stretch than was seen in the control animals or those with small infarcts. Top: *\( P < 0.05 \) vs. respective baselines; †\( P < 0.05 \) vs. small-MI and control animals. Values are means ± SE. Bottom: *\( P < 0.05 \) vs. baseline. Reprinted with permission from Ref. 54.
MVC. The magnitude of the increase in renal vascular resistance during the first 2 min of the 10% MVC was similar to the values seen during the last 2 min of a 3.5-min bout of 30% MVC as well as the value seen during PHG-CA.

Our laboratory has recently employed duplex scanning of the renal artery to obtain beat-by-beat measurements of renal blood flow velocity. The excellent time resolution of this method affords the opportunity to examine the flow velocity and time course and thus gain more specific information into the mechanisms responsible for renal vasoconstriction. Using this approach has demonstrated that in humans, renal vasoconstriction occurs within the first few seconds of forearm muscle contraction. This response was linked to the amount of tension generated (i.e., the greater the muscle tension generated the greater the renal vasoconstrictor response seen), and renal vasoconstrictor responses to involuntary contractions are not attenuated compared with responses seen during voluntary contractions (70). Thus, in humans, muscle contraction evokes renal vasoconstriction. The rapid nature suggests the response is not due to a muscle metaboreflex. The findings of this group of studies suggests that, in normal humans, the muscle mechanoreflex is the major determinant of renal vascular resistance (70).

Middlekauff and colleagues (66) used dynamic PET to measure renal blood flow in heart failure patients during static muscle contraction. They found that 30% MVC led to a greater increase in vascular resistance in heart failure than in controls, whereas the posthandgrip response was less in heart failure than in controls. The authors concluded that the greater renal constriction in heart failure was due to mechanoreflex engagement.

The renal vascular responses to contraction in congestive heart failure have also been examined using renal duplex scanning. In a recent report by Momen et al. (69), three paradigms were performed. In the first protocol, fatiguing handgrip at 40% reduced renal flow velocity with the reductions in flow velocity being larger in the heart failure group than in the control subjects. During PHG-CA, renal blood flow was similar in the two groups. In a second protocol, short bouts (15 s) of static handgrip at graded intensities led to greater reduction in renal blood velocity in heart failure than in a control group (Fig. 18). In a third protocol, involuntary and voluntary biceps contractions at 15–30% evoked similar renal constrictor responses in the two groups. These data suggest that

Fig. 21. Sensitizing effects of α,β-me ATP on the blood pressure response to muscle stretch. Of note, ATP increases the magnitude of the pressor response to muscle stretch, and this augmentation of α,β-me ATP is greatest in rats with large myocardial infarctions. *P < 0.05 vs. control (open bars), †P < 0.05 for large vs. small and control (i.e., comparison of solid bars). Values are means ± SE. Reprinted with permission from Ref. 54.

Fig. 22. Schematic representation of autonomic control in normal subjects and those with heart failure. Left: with exercise, muscle reflex is initiated, which leads to an increase in MSNA and renal vasoconstriction. These effects seem to be mediated by stimulation of VR1 and/or acid-sensing ion channels and purinergic receptor subdivision 2, subtype X (P2X) receptors. In heart failure (right), stimulation of VR1 acid-sensing ion channels is attenuated, and this likely contributes to the attenuated muscle metaboreceptor-mediated response. On the other hand, P2X-mediated stimulation of muscle mechanoreceptors is augmented, and this leads to accentuated renal vasoconstriction.
muscle mechanoreflex activity is enhanced in heart failure and serves to vasoconstrict the kidney (69). The role this accentuated engagement plays in redistributing cardiac output toward exercising muscle and in elevating salt and water retention remains to be explored.

POTENTIAL CAUSES OF THE MUSCLE REFLEX ABNORMALITIES IN CONGESTIVE HEART FAILURE

Recently, experiments were undertaken to further characterize the afferent mechanisms that regulate sympathetic outflow in heart failure. To accomplish this goal, a myocardial infarction (MI) model of congestive heart failure was employed in the decerebrate rat (106). This model affords the opportunity 1) to examine muscle metaboreceptor-mediated pressor responses as substances are infused into the arterial supply of the rat hindlimb and 2) to examine muscle mechanoreceptor-mediated pressor responses as the hindlimb musculature is stretched.

Decerebrate rats were studied 8 to 14 wk after ligation of the left anterior descending coronary artery. Animals with MIs were subdivided into those with “small” MIs involving less than 35% of the left ventricle, and into those with “large” MIs that involved more than 35% of the left ventricle. Infarct size was estimated after the rats were euthanized and the hearts were excised (15).

When capsaicin was injected into the arterial supply of the rat hindlimb, a large pressor response was observed in control animals and in those with small MIs. However, in the animals with large infarcts the pressor response to capsaicin was markedly blunted (Fig. 19). These data suggest that VR1 receptor-mediated processes are in fact attenuated in congestive heart failure. Because the VR1 receptor colocalizes with the ASIC (53), we would speculate that the attenuated VR1 responses may be a marker for impaired activity of chemically sensitive muscle afferents in congestive heart failure. The etiology of these attenuated responses requires further study.

In an effort to examine the sensitization of the mechanoreflex, hindlimb muscle stretch studies were performed in the decerebrate rat model. Compared with animals without infarcts and those with small infarcts, the magnitude of the pressor response in animals with large MIs and congestive heart failure was augmented, suggesting that the muscle mechanoreflex was accentuated (Fig. 20).

Muscle stretch was then performed after infusing α,β-methylene ATP, a stimulant of purinergic receptors. This was done in an effort to determine whether the ability of ATP to sensitize the muscle mechanoreflex was enhanced in heart failure. The pressor response to stretch after α,β-methylene ATP was greater in rats with large MIs than in control animals or those with small MIs (Fig. 21). The findings of this report suggest that alterations in afferent VR1 and P2X receptors alter the processing of sensory information in heart failure. These changes may alter the magnitude and sympathetic nervous system activity in heart failure.

Smith et al. (106) have shown that muscle stretch and muscle contraction performed in the dilated cardiomyopathy rat model led to an accentuated pressor response. These authors believed that the muscle metaboreflex was attenuated and the muscle mechanoreflex was accentuated.

Recently, Middlekauff and colleagues have performed an important group of experiments in humans with heart failure. Subjects performed two paradigms: rhythmic exercise at 20% MVC for 3 min followed by PHG-CA and passive exercise. In the first experiment, MSNA rose in both groups and fell during PHG-CA, suggesting a non metaboreceptor-mediated process. Of note, DCA did not alter MSNA despite lowering lactate and pH. Finally, MSNA rose earlier in heart failure than in controls. Passive exercise led to a greater rise in MSNA in heart failure than in controls. These studies suggest that muscle mechanoreceptor responses are accentuated in heart failure, and because the responses to passive muscle stretch were greater in heart failure they raise the possibility that the sensory afferents themselves have been modified by the disease (64). These findings are thus consistent with the muscle stretch studies of Li et al. (54) and Smith et al. (106) in the rat myocardial infarct model in which pressor response to a given level of stretch were increased in heart failure. The findings of Middlekauff et al. (64) finally suggest that lactic acid does not contribute to the heightened mechanoreceptor sensitization.

SUMMARY

The muscle reflex is mediated by stimulation of mechanically and metabolite sensitive muscle afferents. ATP and lactic acid can both stimulate and sensitize muscle afferents. The VR1, ASIC, and P2X receptors are involved in these responses. When the reflex is engaged, SNA increases and this leads to renal vasoconstriction (Fig. 22A). In congestive heart failure, the muscle mechanoreflex is accentuated. Whether the muscle metaboreflex is attenuated or accentuated may depend on the relative degree of muscle metabolic abnormalities, the degree of metaboreceptor desensitization, and the mode of exercise being performed (rhythmic vs. static). These findings are also related to attenuated VR1 and/or ASIC responses and to enhanced P2X receptor responses. The greater mechanoreflex engagement leads to a preferential vasoconstriction in the renal bed (Fig. 22B). Future studies will be necessary to better understand this exciting area of integrative pathophysiology.

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