Effects of low pH on the mechanical response of thin-fiber muscle afferents that may be associated with exercise pressor reflex

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Abstract Cardiovascular and respiratory responses are reflexly augmented during exercise, and the former is known as the exercise pressor reflex. The exercise pressor reflex is thought to be caused by both mechanical and metabolic stimuli to thin-fiber muscle afferents innervating active skeletal muscles. Ischemia induced-acidosis has been reported to augment the exercise pressor reflex. However, protons alone do not excite many thin-fiber afferents. In this short review, we show that protons lower the response threshold and increase the response magnitude to mechanical stimulation of thin-fiber muscle afferents, thus possibly contributing to the exercise pressor reflex. Furthermore, this short review introduces a new sensitizing mechanism by protons (low pH) of thin-fiber muscle afferents via a type of extracellular matrix proteoglycan, versican. The possibility of controlling the augmented reflex during ischemic exercise in people with cardiovascular disease is also discussed.

Keywords : ischemia, acid, mechanical sensitization, extracellular matrix proteoglycan, versican

Introduction

Cardiovascular and respiratory responses are reflexly augmented during exercise to meet the oxygen demand of active skeletal muscles. Two neural mechanisms contribute to this potentiation. One is “central command,” defined as descending signals from higher brain centers, which send motor commands to skeletal muscles and at the same time to cardiovascular and respiratory regulatory centers within the medulla^{1,2)}. The other is the “exercise pressor reflex” via thin-fiber muscle afferents. These afferents are excited by mechanical stimuli occurring during muscle contractions and by accumulated metabolites produced by contracting muscles, which then activate respiratory and circulatory circuits in the brain stem^{3,4)}.

Ischemia often occurs in active muscles, and is known to facilitate the exercise pressor reflex⁵⁾. The exercise pressor reflex is also exaggerated in cardiovascular diseases such as hypertension, heart failure, and peripheral arterial disease^{6,7)}. Individuals with these conditions are potentially at increased risk for life-threatening cardiovascular events when they perform exercise⁵⁾. Ischemia-induced decline in tissue pH or acidosis has been assumed to heighten the responsiveness of thin-fiber muscle afferents, resulting in an augmented cardiovascular response. However, not much is known about the modulation of thin-fiber muscle afferent sensitivity by acidity. Therefore, in this paper we review the acute effects of acid on thin-fiber muscle afferents.

Thin-fiber muscle afferents not excited by acid only

Ischemia induces a decrease in pH in skeletal muscles. Although acid is known to be related to the exercise pressor reflex⁸⁻¹⁰⁾, acid alone probably does not excite thin-fiber muscle afferents, for the following reasons. First, only 20 of 137 (14.6%, our data, unpublished) or 7 of 30 (23%)¹¹⁾ thin-fiber muscle afferents recorded by a single fiber recording technique (see the method section in Hotta et al.¹²⁾) were excited by acid (pH 6.2 or pH 5.5). Second, Lewis¹³⁾ found that although ischemia alone did not cause muscle pain to develop, pain occurred when muscles were rhythmically contracted under ischemia. Third, Light et al.¹⁴⁾ demonstrated that an acidic condition within the physiological range alone failed to activate cultured dorsal root ganglion (DRG) neurons (primary sensory neurons) innervating skeletal muscles, but when combined with lactate and ATP these neurons were activated. Lactic acid is generated via glycolysis facilitated by ischemia. ATP is released as a result of muscle contractions^{15,16)}. Fourth, some researchers reported the existence of thin-fiber muscle afferents that were not stimulated by non-ischemic muscle contraction, but were stimulated by ischemic contraction^{17,18)}.

Acid augments mechanical response in thin-fiber muscle afferents

As mentioned above, thin-fiber muscle afferents seldom respond to acid. However, a previous study demonstrated that low pH sensitized rat thin-fiber skin afferents to me-

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chanical stimuli¹⁹). Thus, we hypothesized that acid lowers the mechanical threshold and augments responsiveness of thin-fiber muscle afferents regardless of whether it excites the afferents. We recorded single-fiber activities from rat muscle nerve preparations, and demonstrated that their response threshold was significantly lowered and the magnitude of the response to mechanical stimulation (0–198 mN compression) was significantly increased by a pH 6.2 solution (Fig. 1)¹²). This level of acidosis has been observed in exercising muscles⁹).

Fig. 1 Changes in mechanical threshold and magnitude of the mechanical response of thin-fiber muscle afferents. The application of pH 7.4 buffer solution had no significant influence on the mechanical response of thin-fiber muscle afferents. However, the mechanical threshold significantly decreased after application of pH 6.2. The total number of evoked discharges during a ramp mechanical stimulus (0–196 mN in 10 s) significantly increased after the application of pH 6.2 acidic buffers. Values are means $\pm$ SE. Asterisks show $P < 0.05$ (paired t test). Redrawn from Hotta et al.¹².

Possible mechanism for acid-induced augmentation of the mechanical response in thin-fiber muscle afferents

In previous patch-clamp experiments using rat small DRG neurons²⁰, mechanically activated (MA) currents were sensitized by acid that was mainly observed in cells labelled with isolectin B4 (IB4). IB4 is a plant lectin that binds to a versatile extracellular matrix proteoglycan, versican, side chains of which are chondroitin sulfates²¹. Acid-sensing ion channels (ASICs) and transient receptor potential vanilloid 1 (TRPV1) have been found to respond to low pH^{22,23}). This low pH-induced sensitization of MA currents was not suppressed by inhibitors of these acid-sensitive channels (amiloride and capsazepine)²⁰. A combination of inhibitors of intracellular signaling pathways

also failed to block this sensitization²⁰. On the other hand, low pH-sensitized MA currents in IB4-positive neurons were attenuated by chondroitin sulfate or chondroitinase ABC, an enzyme that destroys the integrity of versican²⁰. We also found, although still a preliminary finding, that at the tissue level, injection of chondroitin sulfate into the receptive field in skeletal muscle attenuated acid-induced augmentation of the mechanical response²⁴. Although it was not possible to examine whether sensitized afferents contained versican, the majority of them probably did given that a previous report showed that over 70% of thin-fiber muscle afferents were IB4-positive²⁵.

We therefore propose a novel mechanism in which, at least in IB4-positive neurons, alteration in interactions between mechanosensitive channels and versican side chains (chondroitin sulfate) is involved in acid-induced sensitization in thin-fiber muscle afferents.

Limitations of acid-induced sensitizing mechanism of thin-fiber muscle afferents via versican

This hypothesis has three limitations. First, we have not shown which neuronal channels are influenced by acid-induced alteration in electric charges of chondroitin sulfate in versican. Many channels, such as TRPV4, TRP ankyrin 1 (TRPA1), and ASICs, have been proposed as candidate mechano-gated channels²⁶. In 2010, two mechanically activated cation channels, i.e., Piezo 1 and Piezo 2, were identified in mice²⁷. However, mechanically activated channels responsible for thin-fiber afferents have not yet been identified; thus, it is not possible to examine which channels are influenced by versican in acidosis. At minimum, Piezo 1 is not involved in this mechanism because low pH significantly reduced Piezo 1 activation²⁸. The second limitation is that thin-fiber muscle afferents are able to respond to both noxious and innocuous stimuli. The former afferents are classified as nociceptors and the latter as ergoreceptors. Although many thin-fiber muscle afferents are polymodal receptors²⁹, we cannot determine whether neurons tested by the patch-clamp method²⁰ and single-fiber recordings¹² contribute not only to perception of pain but also to the exercise pressor reflex. Third, the extent to which the compressive mechanical stimulation used in patch-clamp experiments²⁰ and single-fiber recordings¹² simulates the mechanical force exerted on the muscle thin-fiber afferents during muscle contractions is not known.

Physiological and practical implications

During high intensity exercise, exercising muscles are exposed to acidosis, and cardiovascular and respiratory responses need to be augmented to supply more oxygen to the active skeletal muscles. Low pH-induced augmentation of the mechanical response of thin-fiber muscle afferents may contribute to this potentiation of the exercise

pressor reflex.

Chondroitin sulfate has been utilized for treatment of joint inflammation, especially in elderly people, because it is well tolerated with no side effects from over dosage and is without any multi-drug interactions³⁰. If administration of chondroitin sulfate suppresses exaggerated cardiovascular responses to exercise, it could be useful in developing exercise prescriptions for elderly people and people with cardiovascular risk. We have started experimental research on the effects of oral administration of chondroitin sulfate on cardiovascular response to exercise in humans.

Conclusion

Low pH augments the mechanical response of thin-fiber muscle afferents, which are considered to be associated with the exercise pressor reflex. At minimum, versican, a versatile extracellular matrix proteoglycan, participates in this mechanism.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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