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Muscle receptors close to the myotendinous junction play a role in eliciting exercise pressor reflex during contraction

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Abstract

Although a muscle mechanosensitive reflex contributes to regulation of the cardiovascular responses during exercise, the precise location of muscle mechanoreceptors responding to contraction has not been identified yet. We have recently reported that mechanosensitive receptors located at or close to the myotendinous junction play a role in eliciting the cardiovascular responses to passive stretch of skeletal muscle. The mechanoreceptors located at or near the myotendinous junction are hypothesized to respond to static contraction as well. To test this hypothesis, we had two interventions for the reflex cardiovascular responses to static contraction of the triceps surae muscle with the same tension development in decerebrate or pentobarbital-anesthetized rats; cutting the Achilles tendon and local injection of lidocaine into the myotendinous junction. The cardiovascular responses were evoked by static contraction regardless of the achillotomy, suggesting that mechanoreceptors terminating in the more distal part of the cut Achilles tendon did not contribute to the reflex cardiovascular responses. Lidocaine (volume, 0.04–0.1 ml) injected into the myotendinous junction blunted the reflex cardiovascular responses, indicating that muscle afferent fibers terminating at or passing through the myotendinous junction contribute to the exercise pressor reflex. The achillotomy did not affect the cardiovascular responses to passive stretch with the same tension as static contraction, but the localized injection of lidocaine similarly blunted the responses to passive stretch as contraction. We conclude that the mechanosensitive receptors eliciting the reflex cardiovascular responses may at least partly locate close to the myotendinous junction, to monitor tension development during muscular activity.

Keywords: Muscle thin fiber afferents; Mechanosensitive receptors; Decerebrate rats; Tension development; Static contraction; Passive mechanical stretch; Achilles tendon

1. Introduction

Feedback control elicited by activation of group III and IV thin afferent fibers contributes to the cardiovascular responses during exercise as well as feedforward control due to central command (Mitchell, 1990; Kaufman and Forster, 1996; Matsukawa et al., 1998). Group III muscle afferent fibers are mainly mechanosensitive, whereas group IV muscle afferent fibers are mainly metabosensitive (Kniffki et al., 1981; Kaufman et al., 1983). A role of the muscle mechanosensitive reflex in regulation of the cardiovascular responses during exercise has been studied using anesthetized or decerebrate animals (Kniffki et al., 1981; Kaufman et al., 1983; Stebbins et al., 1988; Matsukawa et al., 1992, 1994; Hayes and Kaufman, 2001; Smith et al., 2001; Hayes et al., 2005; Koba et al., 2006). Mechanical stimuli arising from contracting muscle are able to partly elicit the reflex cardiovascular responses, because gadolinium, a blocker of cation-selective mechanosensitive channels, blunted the cardiovascular responses to static contraction of the triceps surae muscle in anesthetized or decerebrate cats and rats (Hayes and Kaufman, 2001; Smith et al., 2005; Matsukawa et al., 2007; Nakamoto and Matsukawa, 2007).

Muscle group III afferent fibers have primarily free nerve endings, which are found in connective tissues, between extra- and intrafusal muscle fibers, in the tendon at the myotendinous

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junction, in the adventitia of arterioles and venules, in the capsule of tendon organs, in the wall of lymphatic vessels, and also associated with fat cells (Stacey, 1969; Barker, 1974; Andres et al., 1985; Abrahams, 1986; Von Düring and Andres, 1990). Thus free nerve endings of thin fiber afferents are present in every tissue of skeletal muscle except for capillaries. However, it is known that all of the mechanosensitive afferents do not always elicit the reflex cardiovascular responses (Mense and Meyer, 1985). We have recently reported that mechanosensitive receptors located near or at the myotendinous junction play a role in eliciting the cardiovascular responses to passive stretch of skeletal muscle (Nakamoto and Matsukawa, 2007). However, passive stretch and contraction behave somewhat differently as mechanical stimuli. Indeed, tendon stretch stimulated a different population of group III mechanosensitive afferents than did static contraction (Mense and Meyer, 1985; Hayes et al., 2005). Therefore it remained unknown whether the muscle receptors located near or at the myotendinous junction might respond to static contraction as well as to passive stretch and could elicit exercise pressor reflex.

To examine the precise location of the muscle receptors responding to static contraction, we evoked static contraction of the triceps surae muscle with two kinds of the experimental interventions in decerebrate or anesthetized rats; (1) cutting the Achilles tendon and (2) local injection of lidocaine into the myotendinous junction. First, to examine whether muscle receptors in the tendon might have a role in evoking the exercise pressor reflex, we evoked static contraction with the cut and reconnected Achilles tendon. Afferent signals from the more distal part of the cut Achilles tendon would be eliminated by the intervention. Second, to investigate whether muscle afferent fibers terminating at or passing through the myotendinous junction contributed to the exercise pressor reflex, static contraction was conducted before and after injecting lidocaine into the myotendinous junction. Although muscle afferent fibers passing through the myotendinous junction could not be excluded from the lidocaine intervention alone, the contribution of those afferents would be revealed by the former intervention with cutting Achilles tendon.

2. Methods

The experiments were performed in 17 Wistar male rats weighing 328±22 g, in accordance with the "Guiding Principles for the Care and Use of Animals in the Fields of Physiology Sciences" approved by the Physiological Society of Japan. The present experimental protocols were also approved by the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University.

2.1. Preparations

2.1.1. General procedure

A mixture of 4% halothane, N_2O, and O_2 was used to anesthetize the rats in a plastic box and then a face mask was attached. After the concentration of halothane was lowered to a level of 1.0–1.5% enough to keep anesthesia through the mask, surgery was started. Electrocardiogram (ECG), heart rate (HR), and rectal temperature were continuously monitored. Respiratory thoracic movement was visually observed. Rectal temperature was maintained at 37–38 °C with a heating pad. To maintain an appropriate level of surgical anesthesia, the concentration of halothane was usually preset in a range of 1.0–1.5% but was increased to 2.0–2.5% if an increase in HR and/or respiration and/or withdrawal of the limb in response to noxious pinch of the paw and/or a surgical procedure was observed. Catheters were inserted into the left external jugular vein for administering drugs and into the left carotid artery for measuring arterial blood pressure (AP). AP was continuously monitored with a pressure transducer (DPT-6100, Kawasumi Laboratories, Tokyo, Japan). The trachea was exposed and an endotracheal tube was inserted into the airway. Then the lungs were artificially ventilated by a respirator (SN-48087, Shinano, Tokyo, Japan), maintaining halothane anesthesia via the endotracheal tube. The animals were subsequently decerebrated ($n=14$) or anesthetized with pentobarbital ($n=3$).

2.1.2. Decerebrate preparation

The rats were placed in a stereotaxic apparatus. The upper skull and dura mater were removed. Decerebration at the precollicular and premammillary level (Bregma-4.5 mm) was performed by electrocoagulation as previously reported (Sadamoto and Matsukawa, 1997; Matsukawa et al., 1998). A stainless steel electrode, whose insulation was removed along a length of 4 mm from the tip, was inserted near the basilar bone. A negative direct current (1 mA) was passed for 30 s through the electrode. Then, the electrode was withdrawn by 4 mm and the current was passed again. This procedure was repeated over a total of 20 tracks at 0.5 mm intervals on the frontal plane. Dexamethasone (0.2 mg i.v.) was administered to minimize cerebral edema. After finishing decerebration, the pelvis and the knee and ankle joints of both hindlimbs were clamped to prevent movement of the body trunk and hindlimbs. The triceps surae muscle, the Achilles tendon, and the calcaneus bone of each hindlimb were exposed. The calcaneus bone was sectioned and the Achilles tendon was connected to a force transducer (TU-CR 50 N, TEAC, Tokyo, Japan) for measuring muscle tension. A pair of silver wire electrodes (bare diameter, 0.1 mm) insulated with silicone tubing was carefully wound on the tibial nerve to evoke muscle contraction and the electro-nerve complex was covered with silicone gel. A recovery period of more than 60 min was allowed to eliminate the effect of halothane anesthesia. To minimize spontaneous movement in decerebrate rats, ketamine hydrochloride (2–5 mg/kg iv) was administered as necessary. The animals were killed with an overdose of pentobarbital sodium at the end of experiments and the transected area of the brain was examined histologically. From the histological analysis, the cerebrum and the rostral part of the hypothalamus were disconnected from the brain stem and the caudal part of the hypothalamus left intact.