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Changes in the expression of calcitonin gene-related peptide after exposure to injurious stretch-shortening contractions☆

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ABSTRACT

One of the factors that can result in musculoskeletal injuries, and time off work, is exposure to repetitive motion. The goal of this study was to determine if skeletal muscle injury induced by exposure to injurious stretch-shortening cycles (iSSCs), resulted in hyperalgesia in the hind limb and changes in calcitonin-gene related peptide (CGRP) immunolabeling in the dorsal root ganglia (DRG) in young and old male rats.

Methods: Young (3 months) and old (30 months) male Fisher 344 × BN F1 rats were anesthetized with isoflurane and the left hind limbs were exposed to 15 sets of 10 SSCs. Control animals were exposed to a single bout of SSCs of equal intensity. Sensitivity to mechanical stimulation was assessed using von Frey filaments prior to beginning the experiment, and on days 2 and 9 following exposure to iSSCs. Rats were euthanized one, 3 or 10 days after the exposure. The ipsilateral DRG were dissected from the L4–5 region of the spine, along with the left *tibialis anterior* (LTA) muscle.

Results: Rats exposed to iSSCs were more sensitive to mechanical stimulation than control rats 2 days after the exposure, and showed a reduction in peak force 3 days after exposure. Changes in sensitivity to pressure were not associated with increases in CGRP labeling in the DRG at 3 days. However, 9 days after exposure to iSSCs, old rats still displayed an increased sensitivity to mechanical stimulation, and this hyperalgesia was associated with an increase in CGRP immunolabeling in the DRG. Young rats exposed to iSSC did not display a change in CGRP immunolabeling and sensitivity to mechanical stimulation returned to control levels at 10 days.

Conclusions: These findings suggest that hyperalgesia seen shortly after exposure to iSSC is not influenced by CGRP levels. However, in cases where recovery from injury may be slower, as it is in older rats, CGRP may contribute to the maintenance of hyperalgesia.

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1. Introduction

Exposure to repetitive muscle contractions of high velocity, either in occupational settings or as a result of exercise, can result in a strain injury to the muscle. If not alleviated this injury can result in the development of chronic pain. According to the Bureau of Labor Statistics (BLS), approximately 33% of all non-fatal injuries that occur in the workplace are the result of injuries and strains to the musculoskeletal system (2014). Most of these injuries are due to overexertion and the repetitive use of the upper-limbs, or overexertion of the lower back. Although workers usually regain function following injury, time away from work is often extended in workers with musculoskeletal disorders (MSDs) because of persisting pain that may be present even after the injury has appeared to heal. This pain may be more prominent in older workers (i.e., >45 years of age), and keep them out of work for longer periods (BLS, 2014).

Animal models have been developed to determine how various work-related exposure factors (i.e., velocity, duty cycle, repetitive motion) contribute to the risk of developing an MSD (Baker et al., 2007; Cutlip et al., 2007a; Kehl et al., 2000). One of the models that has been used and characterized involves exposing the hind-limbs of rats and mice to repeated bouts of injurious eccentric contractions or stretch-shortening contractions, or more physiologically-relevant injurious stretch-shortening contractions (iSSCs; reciprocal eccentric/concentric contractions; (Baker et al., 2006; Brooks and Faulkner, 1990, 1996; Cutlip et al., 2004, 1997). When the repetition number is high (e.g., ≥70 repetitions) there is injury to the muscle that is characterized by an increase in edema, inflammation and myofiber degeneration 2–3 days after the exposure (Baker et al., 2006). As the muscle heals

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and performance recovers, inflammation is reduced and there is an increase in central nuclei within regenerating myofibers, and the presence of satellite cells (Krajnak et al., 2006). The studies describing the effects of iSSCs were performed in Sprague Dawley rats. A companion study (Rader et al., 2015) analyzed the effects of iSSCs in Fisher 344 × Brown Norway F1 rats, a model commonly used to study the effects of aging, to determine if exposure also resulted in anatomical, physiological and molecular changes associated with muscle injury and dysfunction. This study found that 3 days after exposure to iSSCs, there was a reduction in force production in both young and old rats. By 10 days after the exposure, muscle forces had returned to baseline levels in young rats, but there was still a force deficit in old rats. In addition, changes in gene transcription were different between young and old rats (Rader et al., 2015). These age-related changes may be due to a delayed response to healing in older animals, or they could be the result of changes due to pain or discomfort that may occur as a result of the exposure.

To determine if age-related differences in recovery from iSSC exposure were the result of changes in responsiveness to pain, the present study used iSSCs to assess whether this exposure resulted in muscle injury and hyperalgesia in Fisher 344 × Brown Norway F1 rats. This study also determined if aging affects either the expression of, or the recovery from iSSC-induced hyperalgesia. Studies in older humans suggest that depending on the exposure, recovery of muscle function and development of pain may vary, and be resolved at a slower rate in younger humans (Lilje et al., 2015; Reid et al., 2015). To begin to understand the mechanisms underlying prolonged hyperalgesia, or the development of neuropathic pain, we also examined the effects of this exposure on calcitonin-gene related peptide (CGRP) labeling in the muscle and dorsal root ganglia (DRG) of these animals. Previous studies have demonstrated an increase in CGRP in the DRG of cells innervating muscles after exposure to eccentric contractions (Dessem et al., 2010). There is also evidence to suggest that muscle injury and inflammation are associated with an increase in CGRP and the development of allodynia and/or hyperalgesia (Reinert et al., 1998). Tracing studies performed in rats have also demonstrated that the majority of sensory neurons innervating the gastrocnemius muscle contain CGRP (Barry et al., 2015). Hyperalgesia due to muscle inflammation via adjuvant injection or eccentric contractions has been associated with changes in CGRP concentrations in injured muscle and DRG (Bulling et al., 2001; Dessem et al., 2010). Because aged animals often show a delayed or attenuated response to muscle injury (Cutlip et al., 2009; Hollander et al., 2010; Rader et al., 2015), there may be changes in the development of hyperalgesia in response to iSSCs, and changes in CGRP may accompany or be a marker of this hyperalgesia. Therefore, in this study, we tested the hypothesis that iSSC-induced muscle injury would result in hyperalgesia in the exposed limb, and that aging may affect the development of hyperalgesia. We also examined the relationship between hyperalgesia and CGRP-labeling in both the exposed muscle and in the DRG.

2. Materials and methods

2.1. Animals

Young (n = 32; 3 months old; 309.1 ± 27.9 g) and old (n = 30; 30 months old; 587.7 ± 42.2 g) male Fischer 344 Brown Norway hybrid (F344 × BN F1) rats were obtained from the National Institutes on Aging colony. Rats were single housed in an AAALAC accredited animal facility where room temperature and humidity were held constant, with a reversed light/dark cycle (dark cycle was from 7:00 a.m. to 7:00 p.m.). Food and water was provided ad libitum. After one week of acclimatization, rats underwent exposure to an acute iSSC protocol (Baker et al., 2006). All procedures were approved by the National Institute for Occupational Safety and Health (NIOSH) Animal Care and Use Committee.

2.2. Exposure

Rats were anesthetized with isoflurane gas using a small animal anesthetic system (Surgivet Anesco, Waukesha, Wisconsin). The knee was secured in flexion (90°) with a knee holder. The left foot was secured in the load cell fixture using a custom-built foot holder with the ankle axis (assumed to be between the medial and lateral malleoli) aligned with the axis of rotation of the load cell fixture. Each animal was monitored during the protocol to ensure proper anesthetic depth and body temperature.

After being placed on the dynamometer, the joint position of each rat was defined by the angle between the tibia and the plantar surface of the foot. The angular position of the load cell fixture corresponded with the angular position of the ankle. A calibrated potentiometer measured the angular position of the load cell fixture in real-time during testing. Vertical forces applied to an aluminum sleeve fitted over the dorsum of the foot were translated to a load cell transducer (Sensotec, Columbus, Ohio) in the load cell fixture. The force produced by the dorsiflexor muscles was measured at the interface of the aluminum sleeve and the dorsum of the foot. Platinum stimulating electrodes (Grass Medical Instruments, Quincy, Massachusetts) were placed subcutaneously to span the peroneal nerve. The first electrode was placed lateral to the tibial notch and the second electrode was placed 5 mm distal and 3 mm posterior to the first electrode. Activation of the electrical stimulator resulted in muscle contraction of the dorsiflexor muscle group. We optimized muscle length for the dorsiflexor muscles via multi-positional isometric contraction and the stimulator settings (i.e., frequency and voltage) were titrated to the minimum value to elicit maximal dorsiflexor isometric force (unpublished data). Muscle stimulation for all protocols was a 120-Hz square-wave pulse at 0.2-ms pulse duration and 4 V.

The iSSC exposure protocol consisted of 15 sets of 10 continuous high-velocity (i.e., 500°/s) stretch-shortening contractions of the left limb (for a total of 150 SSCs). Each set was administered at 1-min intervals. This protocol previously was used to generate injury in the *tibialis anterior* (TA) muscle and reductions in force in young Sprague Dawley rats (Cutlip et al., 2009). Dynamic performance of the dorsiflexors was assessed both before and after iSSC exposure and on days 3 and 10, prior to euthanasia. To assess dynamic performance, dorsiflexor muscles were exposed to a single SSC which maximally activates the dorsiflexor muscle for 300 ms, then the ankle was rotated from 70° to 140° at 500° per second and returned to 70° at the same velocity. (Baker et al., 2007; Cutlip et al., 2004). After cessation of ankle rotation, activation continued for an additional 300 ms. Following this test the rats were exposed to the iSSC protocol. Control rats underwent the dynamic performance test (i.e., a single set of SSCs), but were not exposed to iSSCs (or repetitive SSCs). After the post-exposure dynamic performance test, rats were allowed to recover, placed in their cages, and put back into the colony room. Rats were euthanized 3 or 10 days after the exposure. These time points were chosen because at 3 days after the exposure there is an intense inflammatory response, and at 10 days muscle performance and fiber morphology return to control levels in young rats (Baker et al., 2007; Krajnak et al., 2006).

2.3. Mechanosensitivity testing with von Frey filaments

Sensitivity to mechanical stimulation of the left TA was measured prior to iSSC exposure, and on days 2 and 9 after the exposure, using von Frey filaments. Rats were placed into a mesh wire container that limited their ability to walk, but still allowed them to move their limbs. Filaments of different tensile strengths were used to detect sensitivity of the exposed limb. A von Frey fiber was pushed against the lateral side of the injured limb and the fiber that induced a withdrawal response prior to the fiber bending was recorded as the level of pressure inducing a response. Animals (4/age/treatment) were tested three times on each day, with a 1 min inter-test interval between tests. The test

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