American Pain Society
RESEARCH
EDUCATION
TREATMENT
ADVOCACY
PUBLISHED BY ELSEVIER

The Journal of Pain, Vol 14, No 11 (November), 2013: pp 1425-1436
Available online at www.jpain.org and www.sciencedirect.com

Muscular Heat and Mechanical Pain Sensitivity After Lengthening Contractions in Humans and Animals

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Abstract: **Mechanical sensitivity of muscle nociceptors was previously shown to increase 2 days after lengthening contractions (LC), but heat sensitivity was not different despite nerve growth factor (NGF) being upregulated in the muscle during delayed-onset muscle soreness (DOMS). The discrepancy of these results and lack of other reports drove us to assess heat sensitivity during DOMS in humans and to evaluate the effect of NGF on the heat response of muscle C-fibers. Pressure pain thresholds and pain intensity scores to intramuscular injection of isotonic saline at 48°C and capsaicin were recorded in humans after inducing DOMS. The response of single unmyelinated afferents to mechanical and heat stimulations applied to their receptive field was recorded from muscle-nerve preparations in vitro. In humans, pressure pain thresholds were reduced but heat and capsaicin pain responses were not increased during DOMS. In rats, the mechanical but not the heat sensitivity of muscle C-fibers was increased in the LC group. NGF applied to the receptive field facilitated the heat sensitivity relative to the control. The absence of facilitated heat sensitivity after LC, despite the NGF sensitization, may be explained if the NGF concentration produced after LC is not sufficient to sensitize nociceptor response to heat.**

Perspective: ***This article presents new findings on the basic mechanisms underlying hyperalgesia during DOMS, which is a useful model to study myofascial pain syndrome, and the role of NGF on muscular nociception. This might be useful in the search for new pharmacologic targets and therapeutic approaches.***

Key words: *Muscle heat sensitivity, capsaicin, delayed-onset muscle soreness, lengthening contraction, nerve growth factor.*

Muscle pain is a common condition with multiple etiologies. As an example, delayed-onset muscle soreness (DOMS) is a frequently occurring condition that usually, but not necessarily, appears after intense exercise and lengthening contractions (LC) of the muscle. It is characterized by muscle soreness on movement, but not during rest, and tenderness that appear after some delay, peak 24 to 48 hours after the exercise, and usually disappear within a week.[7,8] DOMS in humans and its corresponding animal models have been used to study the mechanisms of muscle mechanical hyperalgesia.[17,23,41]

Nerve growth factor (NGF) has been proposed as an important substance involved in the muscle hyperalgesia following LC. NGF is an essential neurotrophic protein involved in maintenance and differentiation of sensory and sympathetic neurons and, later in adulthood, in nociception and pathologic pain condition.[9,28] NGF is released from various structures including skeletal muscle tissue.[2,32] Moreover, NGF sensitizes muscle nociceptors[29,32,40] and induces mechanical hyperalgesia, which lasts for up to 7 days in humans[39] and up to 2 days in rats.[32] NGF plays an important role in hyperalgesia during inflammation.[19,27,37] In addition to mechanical sensitization, heat sensitization has been

Received March 22, 2013; Revised June 25, 2013; Accepted July 6, 2013.
The authors report no conflict of interest.
This work was supported in part by a collaboration grant between the Japan Science and Technology Agency (JST, Strategic International Cooperative Program) and the Danish Agency for Science, Technology and Innovation.
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1526-5900/$36.00

http://dx.doi.org/10.1016/j.jpain.2013.07.010

observed in models where NGF has been injected intradermally,[14,36] and neutralization of NGF has been reported to block both heat and mechanical hyperalgesia during skin inflammation.[25] In a DOMS model, the NGF protein has been reported to be upregulated after LC, and upregulated NGF is considered to induce mechanical sensitization because administration of an anti-NGF antibody reversed the mechanical hyperalgesia after LC.[32] There are, however, no reports on the effect of NGF on muscle nociceptor heat sensitivity. Interestingly, the mechanically sensitized C-fiber afferents in the DOMS model are probably due to NGF,[32] but the nociceptor sensitivity to heat is reported to remain unchanged.[42] The discrepancy of these results and the lack of other reports drove us to assess the heat sensitivity during DOMS in humans, to reassess the heat sensitivity of primary muscle afferents after LC, and to evaluate the effect of NGF on their heat sensitivity.

The mechanical hyperalgesia after LC seems to be mediated, at least partially, by the transient receptor potential vanilloid type 1 (TRPV1) ion channel as TRPV1 antagonists reduce the increased mechanical sensitivity.[15] TRPV1 is known to respond to noxious heat, capsaicin, and low pH.[11] TRPV1 itself is not sensitive to mechanical stimulation, but a role for TRPV1 not only in heat but also in mechanical hyperalgesia induced by muscle inflammation has been reported.[43] In humans, thermally induced muscle pain has been demonstrated by injections of warm and cold isotonic saline,[18] but it is not known if this type of muscle pain is facilitated in the DOMS model.

In this study, it was hypothesized that heat and capsaicin nociception was facilitated during DOMS, possibly mediated by NGF, in humans. It was also examined if muscle heat and capsaicin-induced pain and nociceptor activity are facilitated after LC in animals and if NGF can sensitize rat muscle nociceptors to heat in vitro.

Methods

Human Study

Subjects

Eleven healthy adult subjects participated (6 male and 5 female); subjects were aged 20 to 37 years (mean, 25.8). We used a number similar to previous studies on the subject.[17] The subjects had no pain complaints or history of injuries to the lower leg and were not taking any medication. Subjects were given a detailed verbal explanation of the experimental procedure and signed an informed consent form prior to inclusion in the study. The study was conducted in Aalborg, Denmark, after approval by the ethics committees of Aalborg University (N-20070042) and Nagoya University (No. 298) and in accordance with the Declaration of Helsinki.

Human Experimental Protocol

The experimental protocol (Fig 1A) consisted of 4 sessions (day 0, day 3, day 4, and day 7) in which pressure pain thresholds (PPTs) were measured on the tibialis anterior (TA) muscle of both legs. DOMS was induced during the second session (day 3) and hyperalgesia development was assessed on the following sessions. On days 0 and 4 (baseline measurement and 24 hours after DOMS induction, respectively), PPTs were measured before and after intramuscular (i.m.) injections of isotonic saline at room temperature (control saline), isotonic saline heated to 48°C (heated saline), and capsaicin (Fig 1A). Injections were given to the 3 most mechanically sensitive sites of the TA muscle and the same injection procedure was repeated at the anatomically corresponding site on the control leg. Selection of injection sites was done on day 0 and again on day 3 before inducing DOMS. Selection of control/DOMS (right or left leg) was randomized.

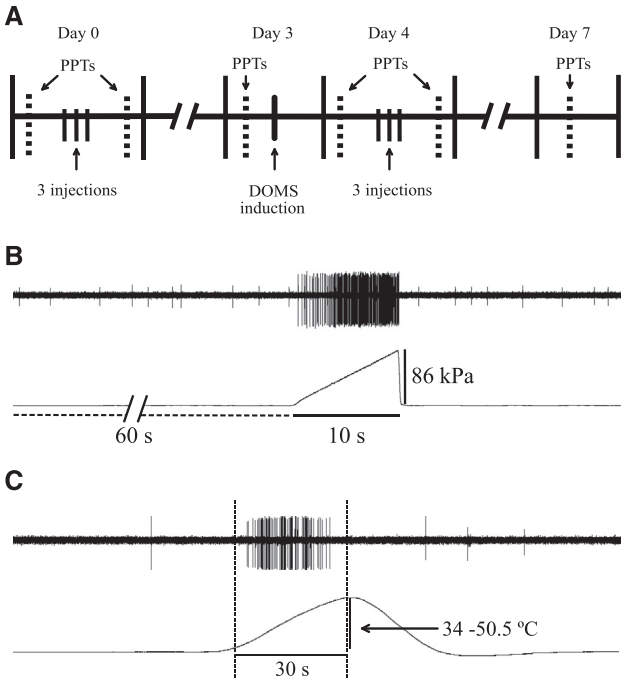

Figure 1. Experimental protocols and stimulation procedures. **(A)** Protocol timeline for the injections and assessments. **(B)** Sample of a single-fiber activity responding to mechanical stimulation and stimulus recording. **(C)** Sample of a single-fiber activity responding to thermal stimulation and stimulus recording.

DOMS Induction and Assessment

DOMS was induced by LC of the TA muscle as previously reported.[16] Briefly, the subject stood on a 13-cm-high metal platform, placed approximately 45 cm from a wall. Subjects were instructed to stand with the heel of the experimental leg on the edge of the platform with the mid- and forefoot extending over the edge. The palms of the subject's hands were placed on the wall for support only. After that, the subject raised the nonexperimental leg over the platform to the hip and knee level, and while standing on a single leg, the subject performed a slow plantar flexion of the foot and ankle allowing the forefoot to descend until the toes touched a cushion (approximately 2 cm thick) placed below the platform. At this point, the nonexperimental leg was extended until weight bearing and was used to assist in returning the subject to the initial starting position. This process was repeated 10 times per set for 3 sets