Environmental Medicine, Vol. 46 No.1, 2, 2002 (page 74 ~ 76)

A Combination of Eccentric Muscle Exercise and Repeated Cold Stress (RCS) Induced Prolonged Hyperalgesia—An Attempt to Develop an Animal Model of Chronic Muscle Pain

Toru TAGUCHI, Jun SATO and Kazue MIZUMURA
Department of Neural Regulation
Division of Regulation of Organ Function
Research Institute of Environmental Medicine
Nagoya University, Nagoya 464-8601, Japan

Abstract: Few studies have addressed the mechanism of chronic pain originating from musculoskeletal structure, in large part due to the lack of a reliable animal model of chronic muscle pain. The purpose of this study is to try to make an animal model of long-lasting muscle hyperalgesia, using a combination of eccentric muscle work and repeated cold stress (RCS). The mechanical withdrawal threshold in muscle was significantly decreased by RCS with or without eccentric muscle contraction (for at least 7 or 3 days after eccentric exercise or sham exercise, respectively). This result suggests that a combination of eccentric muscle work and RCS could prolong muscle hyperalgesia. However, the duration of the hyperalgesic state in this animal model was not considered long enough to be used in studies on the mechanism of chronic muscle pain. Further trials to elongate the period of hyperalgesia are needed.

Key words: repeated cold stress, extensor digitorum longus muscle, delayed onset muscle soreness, eccentric contraction, mechanical pain threshold

Fourteen percent of the population of the United States suffers from chronic musculoskeletal pain,[1] and the percentage is expected to be similar in Japan. Moreover, these percentages are growing. Yet in spite of the growing population of sufferers, there are few studies on chronic pain originating from musculoskeletal structure. Musculoskeletal pain in current animal models lasts only for 24 h or less, e.g. Berberich et al.[2] reported that the background activities of group III and IV single afferent units increased 2–8 h after injection of 2% carrageenan into the gastrocnemius-soleus muscle. Short-term pain and hyperalgesia may not reflect the actual changes that occur in chronic painful conditions. It is therefore important to develop an animal model of chronic muscle pain.

Repetitive eccentric muscle work can cause delayed onset muscle soreness in the exercised muscles, which usually reaches a peak some 24 to 48 hours after exercise in humans and disappears within 3 to 7 days.[3-6] Moreover, repeated cold stress (RCS) induces a decrease in pain threshold (hyperalgesia) measured at the rat paw for at least several days after cessation of the stress[7] and also induces several changes in autonomic functions.[8]

In the present study we tried to develop an animal model of long-lasting muscle hyperalgesia using a combination of eccentric muscle work and RCS.

Materials and Methods

1. Animals:

Twelve untrained Sprague-Dawley male rats (SLC Inc., Japan) weighing 200 g (7 wks) at the beginning of the experiments were used in this study. The animals were kept two per cage under a 12 h light/dark cycle (light between 07.00 h and 19.00 h) in an air-conditioned room (22°C). They had free access to food and water throughout the experiment. All experimental procedures were approved by the Animal Care Committee, Research Institute of Environmental Medicine, Nagoya University.

2. Experimental group:

The animals were divided into two groups, i.e. a sham exercised group (SHAM, n = 6) and an eccentric exercised group (ECC, n = 6). The rats in ECC group were loaded with repetitive eccentric contractions (contraction while the muscle is being stretched) of the right ankle dorsi-flexor muscle (extensor digitorum longus, EDL). The rats in the SHAM group were given repetitive stretch stimulation of the EDL similar to the ECC group but without muscle contraction, and served as a control.

3. Exercise protocol:

The animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Rectal temperature was kept in the physi-

Reprint requests to: Dr. Kazue MIZUMURA at the above address.

This work was supported in part by Grants-in Aid for Comprehensive Research on Aging and Health from the Ministry of Health, Labour and Welfare.

ological range (37–38°C) with a heating pad. A pair of needle electrodes, which were insulated except for the tips, was transcutaneously inserted near the common peroneal nerve that innervates the EDL (Fig. 1). Repetitive contraction of the EDL was induced by electrical stimuli applied to the common peroneal nerve through an isolator (SS-202J, Nihon Kohden Corp., Japan) connected to an electrical stimulator (SEN-7203, Nihon Kohden Corp., Japan). Before starting exercise, twitch threshold currents were determined. If the threshold current exceeded 100 microamperes, the electrodes were repositioned so that a lower threshold was obtained. The twitch threshold was 93.3±8.2 (mean±SD) microamperes in the SHAM group and 90.0±6.3 microamperes in the ECC group, respectively (not statistically significant, $P < 0.4475$). The current applied during exercise in the ECC group was set at three times the threshold. Rats in the SHAM group received no current to their nerves; that is, the EDL was not made to contract at all during the exercise period. The stimulus parameter to induce tetanic contraction was a frequency of 50 Hz with pulse duration of 1 ms. The ankle joint was stretched from the starting position (30 degree plantar-flexion) to 90 degrees plantar-flexion over a 1 s period (stretch) with use of a servomotor (CPL28T08B-06C2T, Oriental Motor Co. Ltd., Japan) and then returned to the starting position over a 3 s period (shorten). The movement of the servomotor was synchronized with the electrical stimulator. This pattern was repeated every 4 s for a total of 500 repetitions.

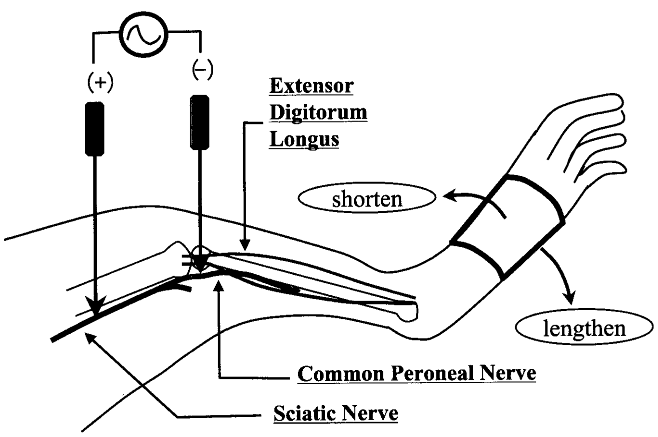

Fig. 1 Schematic drawing of the eccentric exercise applied in the experiment.
A pair of electrodes, which were insulated except for the tips, was inserted transcutaneously and placed near the common peroneal nerve. Repetitive contractions of the extensor digitorum longus (EDL) muscle were induced by cyclic electrical stimuli of the nerve innervating the muscle. The EDL muscle was stretched synchronously with contraction as indicated in the figure.

4. Repeated cold stress (RCS):

Repeated cold stress was applied according to the method described by Sato et al.[7] Briefly, rats were kept at 4°C from 19.00 h on the day of exercise to 09.30 h the following morning and then alternately exposed to 22 and 4°C at 30-minute intervals from 09.30 h to 19.00 h. These procedures were repeated for 2 days after exercise (Fig. 2).

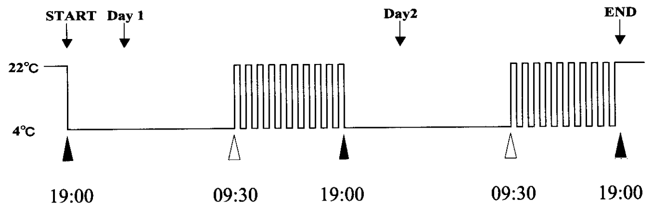

Fig. 2 Schedule of repeated cold stress (RCS).
RCS was given for 2 days by changing the environmental temperature from 22 to 4°C at 30-minute intervals from 09.30 h to 19.00 h. Day 0 corresponds to the day of exercise.

5. Measurement of the withdrawal threshold:

A Randall-Selitto apparatus (Ugo Basile, Italy) was used to measure the withdrawal threshold. The animal was held at the trunk with a towel. A cone-shaped pusher with a round tip was applied to the belly of the EDL through shaved skin. The speed of the applying force was set at 16 g/s, and the cut-off level was set at 250 g to avoid damaging the tissue. The intensity of the pressure causing a withdrawal reaction of the hind limb was defined as the withdrawal threshold. Measurements were performed 10 times at a few minute intervals, and the mean value of the latter 5 trials was taken as the threshold. The test was always done from 12.00 h to 15.00 h to avoid circadian fluctuations. Training sessions were carried out for four consecutive days to increase the sensitivity of the test[9] and the measurements were made 1, 2, 3, 7 and 10 days after exercise. The experimenter was blinded to the group to which an animal belonged.

6. Statistical analysis:

Results are expressed as mean±SD. Repeated measures ANOVA and Bonferroni's multiple comparison test as a post hoc test were done to compare the effects of days in each group. $P < 0.05$ was considered significant.

Results

In the training session for four consecutive days the mechanical withdrawal threshold in both groups was almost the same and constant (Fig. 3). The exposure to RCS with or without eccentric contraction in rats significantly decreased the threshold in both groups compared with the values on the fourth training day. In the SHAM group the threshold was decreased

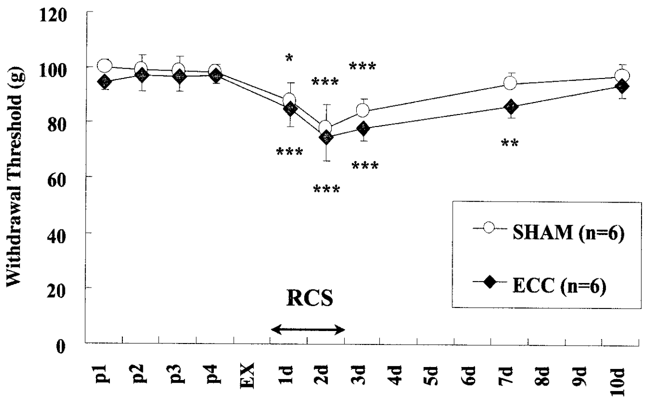

Fig. 3 Effects of a combination of eccentric muscle work and RCS on the withdrawal threshold responding to pressure applied to the EDL. "EX" in the transverse axis represents the day of exercise. Values are shown as mean±SD. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the values on the fourth day, Bonferroni's multiple comparison test).

during the exposure to RCS, and reached the lowest value on the second day after sham exercise (from 98.3±2.7 g on the fourth training day to a significantly lower 78.1±8.5 g, $P < 0.001$). The decreased withdrawal threshold gradually returned to the baseline level by the seventh day after exercise. In the ECC group the threshold was also decreased during the exposure to RCS, and was lowest on the second day after exercise (from 96.8±2.6 g on the fourth training day to a significantly lower 74.8±3.9 g, $P < 0.001$). The withdrawal threshold on the seventh day was still significantly lower than that on the fourth training day and returned to the baseline level by the tenth day after eccentric exercise.

Discussion

The present study showed that the exposure to RCS with or without eccentric muscle contraction caused significant decreases in the mechanical withdrawal threshold in both groups. The significant decrease in the threshold lasted for at least 3 days in the SHAM group and for at least 7 days after exercise in the ECC group.

In a pilot study, we showed that the eccentric exercise load used in the present study without RCS led to a significant decrease in the mechanical withdrawal threshold in the ECC group for 3 days after exercise (the lowest value was reached on the second day). In the control group, on the other hand, the threshold was kept constant (not decreased) throughout the observation period (data not shown). Considering these results together, it appears that a combination of eccentric muscle work and RCS prolongs the hyperalgesic state originating from the muscle longer than either of these two alone.

The mechanical withdrawal threshold was measured by applying mechanical stimulation to the EDL through shaved skin in the present study. It is therefore difficult to distinguish the pain threshold in the skin from that in the muscle. Our everyday experience suggests that hyperalgesia induced by muscle exercise occurs in the exercised muscle; therefore, the presently observed change in the withdrawal threshold might also indicate a change in the muscle pain threshold. However, evaluation with other means will be required to determine whether the change in withdrawal threshold observed in the present experiment really indicated a change in muscle pain threshold.

In conclusion, muscle hyperalgesia could be prolonged with a combination of eccentric muscle work and RCS compared with either of these modalities alone. The duration of the hyperalgesic state in the muscle, however, was still not long enough if compared with that seen in clinical practice. Further trials to develop an animal model of chronic muscle pain in order to elucidate the underlying mechanisms of this type of pain are urgently needed.

References

1) Magni G, Caldieron C, Rigatti-Luchini S, et al. Chronic musculoskeletal pain and depressive symptoms in the general population. An analysis of the 1st National Health and Nutrition Examination Survey data. Pain 1990; 43: 299–307.
2) Berberich P, Hoheisel U, Mense S. Effects of a carrageenan-induced myositis on the discharge properties of group III and IV muscle receptors in the cat. J Neurophysiol 1988; 59: 1395–1409.
3) Pyne DB. Exercise-induced muscle damage and inflammation: A review. Aust J Sci Med Sport 1994; 26: 49–58.
4) Armstrong RB. Mechanisms of exercise-induced delayed onset muscular soreness: A brief review. Med Sci Sports Exerc 1984; 16: 529–538.
5) Smith LL. Acute inflammation: The underlying mechanism in delayed onset muscle soreness? Med Sci Sports Exerc 1991; 23: 542–551.
6) Tiidus PM. Manual massage and recovery of muscle function following exercise: A literature review. J Orthop Sports Phys Ther 1997; 25: 107–112.
7) Sato M, Kuraishi Y, Kawamura M. Effects of intrathecal antibodies to substance P, calcitonin gene-related peptide and galanin on repeated cold stress-induced hyperalgesia: Comparison with carrageenan-induced hyperalgesia. Pain 1992; 49: 273–278.
8) Hata T, Kita T, Itoh E, et al. The relationship of hyperalgesia in SART (repeated cold)-stressed animals to the autonomic nervous system. J Auton Pharmac 1988; 8: 45–52.
9) Taiwo YO, Coderre TJ, Levine JD. The contribution of training sensitivity in the nociceptive paw-withdrawal test. Brain Res 1989; 487: 148–151.

Received June 24, 2002; accepted July 3, 2002