Delayed onset muscle soreness (DOMS) is a common myogenic condition with principal symptoms of tenderness or soreness of affected muscles accompanied by a reduction in their range of movement. It usually occurs 24 to 48 h after unaccustomed exercise or eccentric contractions [1, 2]. However, its underlying mechanisms are not clearly understood. DOMS is considered to be related to a complex set of reactions involving micro-injury of the muscle fibers and connective tissues [3], and the participation of an inflammatory response is suggested [4]. A cellular infiltration of neutrophils, macrophages, and inflammatory mediators has also been demonstrated [5]. In fact, a repetitive administration of relatively high doses of indomethacin, an inhibitor of cyclooxygenase, could protect against the histological and biochemical changes of DOMS in mice [6]. Thus mechanical and biochemical injuries might be responsible for the development of DOMS [7].

On the other hand, most reports on the experimental DOMS described only the appearance of muscle soreness [8, 9]. A few studies reported the distribution of areas of tenderness [10, 11] and pointed out that the region of the muscle-tendon attachment was the main site of tenderness [10]. In our previous study in human subjects, eccentric exercise of the extensor muscle of the forearm was found to produce a localized tender region on the palpable band accompanying the development of DOMS [12]. The taut band was palpated in the extensor muscle of the forearm near the exercise (a total of 60 mg/kg in 12 doses). A clear rropy taut band was palpated at the GS muscle on the second day after the exercise and a localized sensitive region for evoked BF EMG was detected at the depth of the fascia of the band in the exercise and vehicle groups, whereas no such phenomena appeared in the control and indomethacin groups. The palpable band and sensitive region disappeared on the seventh day after the exercise. That indomethacin inhibits the development of DOMS and the localized sensitive region suggests that a sensitization of polymodal-type nociceptors in the fascia mediated by prostaglandins is a possible mechanism for the development of DOMS and the localized sensitive region. [Japanese Journal of Physiology, 52, 173–180, 2002]

Effect of Indomethacin on the Development of Eccentric Exercise–Induced Localized Sensitive Region in the Fascia of the Rabbit

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Abstract: The effect of indomethacin on the development of delayed onset muscle soreness (DOMS) and localized sensitive region produced by eccentric exercise was examined in lightly anesthetized rabbits (n=12, 2.0–3.3 kg). Repeated eccentric contractions of the gastrocnemius (GS) muscle were made by manual extensions during the tetanic contractions induced by electrical stimulation of the tibial nerve. The development of DOMS was confirmed by evoked reflex EMG in the biceps femoris (BF) muscle elicited by a quantitative manual extension of the GS muscle. The distribution of thresholds for the evoked BF EMG was measured by focal electrical stimulations of the GS muscle. Indomethacin (5 mg/kg in 2% sodium bicarbonate) or a vehicle was injected subcutaneously before, during, and after the exercise (a total of 60 mg/kg in 12 doses). A clear rropy taut band was palpated at the GS muscle on the second day after the exercise and a localized sensitive region for evoked BF EMG was detected at the depth of the fascia of the band in the exercise and vehicle groups, whereas no such phenomena appeared in the control and indomethacin groups. The palpable band and sensitive region disappeared on the seventh day after the exercise. That indomethacin inhibits the development of DOMS and the localized sensitive region suggests that a sensitization of polymodal-type nociceptors in the fascia mediated by prostaglandins is a possible mechanism for the development of DOMS and the localized sensitive region. [Japanese Journal of Physiology, 52, 173–180, 2002]

Key words: localized sensitive region, eccentric exercise, rabbit, palpable band, indomethacin.
the muscle-tendon attachment. The measurement of pain thresholds in deep tissues clearly demonstrated a decrease in pain threshold at the fascia [12]. These results suggested that the sensitization of muscular nociceptors by an inflammatory mediator might be important for the development of DOMS and the focal tender region on the palpable band.

The aim of this study was to examine the effect of indomethacin on the localized sensitive region, which appears after the DOMS procedures, and to clarify the participation of the inflammation in its pathogenic mechanisms.

**METHODS**

**Animal preparation.** Twelve rabbits (2.0–3.3 kg, 24 legs) were anesthetized with sodium pentobarbital (30 mg/kg, I.V.). A polyethylene catheter (PE50: outer diameter of 1.0 mm) was then chronically implanted into the auricularis caudalis vein, allowing supplemental doses or continuous infusion of anesthetics (20 mg·kg⁻¹·h⁻¹) during the experimental period.

Rectal temperature was monitored and maintained at 39.0±0.3°C with the use of a heating pad (MK-900, Muromachi Kikai Co., Tokyo, Japan). Heart rate was monitored continuously.

The protocol for this study was approved by the Ethics Committee of the Meiji University of Oriental Medicine.

**Eccentric exercise procedure.** A lightly anesthetized rabbit (20 mg/kg sodium pentobarbital, I.V.) was set on a frame where its hind leg was fixed on a board to regulate the direction of tetanic muscular contraction. Repetitive eccentric contractions of the gastrocnemius (GS) muscle were performed by manual extensions during tetanic contraction produced by electrical stimulation of the tibial nerve (50 Hz, 1 ms duration, 500 pulses at 0.067 Hz, bipolar insulated needle electrode).

A threshold current for the contraction of GS muscle of less than 0.3 mA was assumed to reflect an adequate electrode placement. Eccentric contractions were repetitively applied for 20 min (80 times) and repeated 3 times with 5 min resting periods (a total of 240 times).

**Measurements.** Rabbits were lightly anesthetized by sodium pentobarbital (20 mg/kg, I.V.) every time for measurement, then set on a frame.

**Measurement of DOMS.** The amplitude of the EMG of the biceps femoris (BF) muscle elicited by an extension of the GS muscle was used as an indicator of DOMS. A surface electrode was attached to the skin over the BF muscle. The plantar was fixed on a board and the knee joint firmly held; then plantar was pushed up about 15° in a dorsal direction by a constant force of about 1 kg to extend the GS muscle. The peak-to-peak amplitudes of the BF EMG were measured three times, and the median value was used for statistical analysis. Each reflex EMG was monitored on an oscilloscope (VC-11, Nihon Kohden, Tokyo, Japan), and recorded on a thermal array recorder (RTA-1200M, Nihon Kohden) and data recorder (RD-1235T, TEAC, Tokyo, Japan).

**Distribution of the thresholds and amplitude for reflex EMG activity.** The distribution of thresholds of BF EMG evoked by focal electrical stimulation of various tissues of the GS (RT: reflex thresholds) was examined. A stainless steel needle electrode insulated with acrylic resin (180 μm diameter, impedance 391±30 kΩ at 1 kHz; Nishin Medical Institute, Osaka, Japan) was used as a cathodal monopolar stimulating electrode.

A anodal metal surface electrode was attached to the skin 10 mm distant to the needle insertion site, and the insulated needle was inserted manually in 0.5–1.0 mm steps from the skin surface and held in a guide tube attached to the skin with adhesive tape. A train of 5 pulses (100 μs in duration) was applied once every 5 s. The threshold of evoked BF EMG was determined when the reflex responses appeared with a probability over 70%. The cutoff current intensity was 3.0 mA.

The amplitudes of the reflex EMG of the BF muscle (RA: reflex amplitude) evoked by focal electrical stimulation of the tissues on or around the fascia of the GS muscle at a constant intensity (0.5 mA) were also recorded. They were averaged 10 times at intervals of 5 s (Averager, DAT 1100, Nihon Kohden), and the averages were recorded on a thermal array recorder (RTA-1200M, Nihon Kohden) and data recorder (RD-1235T, TEAC). The peak-to-peak amplitudes of the BF EMG were measured and used for statistical analysis.

**Experimental schedules.**

*Experiment 1.* Six rabbits (12 legs) were allocated to the control group (n=6, no exercise) and exercise group (n=6, exercise only) in a crossover manner at intervals of about 1 month. The measurements of BF EMG evoked by the extension of the GS muscle were made before, immediately after, and 1, 2, 3, and 7 d after the exercise.

On the second day after exercise, the GS muscle was carefully palpated and the skin over the detected ropy band was marked. Our previous study in human subjects demonstrated that the minimum pain thresh-
old region (tender point) could be detected on a taut band in the eccentric exercised muscle. The distribution of the RT and RA over the taut band were then measured systematically at 2 mm intervals in the exercise group. Similar measurements were made on the seventh day after exercise.

Experiment 2. Six rabbits (12 legs) were allocated to the indomethacin group \((n=6, \text{exercise+indomethacin})\) and the vehicle group \((n=6, \text{exercise+vehicle})\) in a crossover manner at intervals of about 1 month. Indomethacin (Wako Pure Chem. Ind., Ltd., Osaka, Japan) was dissolved in 2% sodium bicarbonate (1 ml) and titrated to pH 7.4 with sodium monophosphate. Two percent sodium bicarbonate was used as the vehicle. Indomethacin (5 mg/kg) or the vehicle was injected subcutaneously before, during, immediately after, and 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 7 d after the exercise (a total of 60 mg/kg indomethacin).

Measurements of the development of DOMS were made before, immediately after, and daily on days 1 through 7 after the exercise. The distribution of RTs and RAs was examined on the second day after the exercise. The area corresponding to where the palpable band appeared in experiment 1 was also examined.

Statistics. The data are reported as mean±standard deviation (mean±SD). The nonparametric multiple test of Tukey and Dunnett’s multiple test (StatView ver. 5; SAS Institute Inc., NC, USA) was used for the statistical analysis. A \(p<0.05\) was defined as statistically significant.

RESULTS

Exercise-induced muscle soreness and localized sensitive region

In the exercise group, the exercised legs were kept in a slightly flexed position, and withdrawal response in several cases occurred when the GS muscle was palpated on the 2nd day after the exercise. Increased stiffness of the GS muscle was frequently observed, and these phenomena disappeared on the seventh day. Clear EMG activities were evoked by manual extension of the GS muscle immediately after the exercise and increased to a maximum on the second day (Fig. 1A). They decreased to baseline on the seventh day after the exercise (Fig. 1B). The baseline EMG amplitude of 7.50±4.37 \(\mu\text{V}\) increased to 166.1±66.0 \(\mu\text{V}\), and this increase was found to be statistically significant \((p<0.01, \text{Dunnett’s multiple test})\). The EMG activities of the BF muscle were usually brief bursting discharges. However, prolonged EMG activities were also observed in several cases. The spontaneous EMG activity was also increased to 23.33±15.68 \(\mu\text{V}\), from 7.50±4.37 \(\mu\text{V}\) (pre-exercise), on the second day after exercise in the exercise group (Dunnett’s multiple test, \(p<0.05\)), the change in spontaneous activity had disappeared on the seventh day after the exercise. On the other hand, in the control group (no exercise), extension of the GS muscle evoked no reflex activities during the 7-d experimental period.

A ropy taut band was palpated and a localized sensitive region detected on the band on the second day after exercise. The taut ropy band \((1.3±0.5 \text{ mm wide}, 17.7±4.7 \text{ mm long}, n=6, \text{mean±SD})\) was palpated in the GS muscle near the muscle-tendon attachment. The low RT region on the palpable band usually appeared at the restricted area to 4–8 mm distal to the external condyle of the tibia.

The spatial distributions of RT on and around the palpable band are summarized in Fig. 2. On the second day after the exercise, the RTs were remarkably decreased at the depth of the fascia on the palpable band (Fig. 2A). A significant difference between the minimum RT region and other measured points was detected on the second day (Tukey, \(p<0.05\)). No localized low RT region was detected on the seventh day (Fig. 2B). On the second day after the exercise, the latency of the evoked BF EMG was 47.7±4.4 ms.
(mean±SD, n=6), and this had not changed significantly on the seventh day (46.8±2.1 ms).

Figure 3 shows the spatial distributions of RA elicited by a constant current stimulus intensity (upper columns) and representative examples of evoked EMGs (lower traces). At the center of the columns is the largest EMG activity provoked by the stimulation of the fascia, and no EMG activity was elicited by stimulation 1 mm above or below the fascia in the exercised muscle on the second day after exercise. The evoked EMG amplitude at the most sensitive region on the palpable band was 0.95±1.01 mV (central column), and the other eight measured RAs were lower than in the central region (0–0.41±0.35 mV). On the
seventh day (Fig. 3B), the restricted high RA disappeared.

**Effect of indomethacin on exercise-induced muscle soreness and localized sensitive region**

The amplitude of the BF EMG elicited by the extension of the GS muscle is shown in Fig. 4. In the vehicle group, the evoked EMG was not recorded except for the spontaneous EMG before the exercise, and it appeared on the second day after exercise. It increased to $197.3 \pm 83.3 \mu V$, from $8.8 \pm 8.1 \mu V$. The evoked EMG was usually brief bursting discharges (Fig. 4A). On the other hand, in the indomethacin group, the amplitude of evoked EMG was slightly increased on the second day after exercise (Fig. 4B), though the difference was not significant (Dunnett’s multiple test, $p=0.281$).

On the second day after exercise, spontaneous EMG activity had also increased to $15.3 \pm 9.2 \mu V$, from $8.8 \pm 8.1 \mu V$, in the vehicle group, and to $14.8 \pm 8.8 \mu V$, from $11.2 \pm 7.9 \mu V$, in the indomethacin group. These differences, however, were insignificant.

In the exercise group, a ropy taut band was palpated over the GS muscle at the muscle-tendon attachment about 1.4–1.6 mm lateral to the tibia. The minimum threshold region was restricted to 4–8 mm distal to the external condyle of the tibia. The RT and RA were measured 10–22 mm lateral to the tibia and 0–14 mm distal to the external condyle of the tibia.

The contour line illustrates the distribution of RT and RA in the vehicle and indomethacin groups. In the vehicle group (Fig. 5A), at the depth of the fascia, needling stiffness increased on reaching the palpable band, and RTs were decreased. At the center of the measured area (16 mm lateral to the tibia and 6 mm...
distal to the external condyle of the tibia), the RT was lowest (Fig. 5A, upper) and its RA highest of all areas (Fig. 5A, lower). A palpable band could also be detected in the measurement areas of GS muscle, and a localized sensitive region existed on the palpable band. The latency of the reflex EMG was 44.3 ± 5.6 ms. On the other hand, in the indomethacin group at the depth of the fascia, the change of needling stiffness was not detected. The latency of the reflex EMG was 46.7 ± 7.0 ms. The distribution of the RT (Fig. 5B, upper) and RA (Fig. 5B, lower) were almost similar, and the location of the palpable band could not be determined. It is clear that the RTs in the indomethacin group were much higher than those in the vehicle group.

**DISCUSSION**

In the present study, a localized sensitive region at the depth of the fascia of the palpable band developed after eccentric exercise in rabbits. An administration of indomethacin inhibited the development of the sensitive region and palpable band as well as DOMS. These results suggest that the sensitization of muscular nociceptors by prostaglandins might be important for the development of DOMS and the localized sensitive region on the palpable band.

**Experimental DOMS in rabbit.** Many investigators have demonstrated that the most effective way of inducing DOMS is through exercise that incorporates eccentric muscular contractions [13]. Eccentric contraction induces greater tension and is considered to be the major cause of mechanical disruption of the muscle fibers and connective tissue [5, 14]. In this study we used the rabbit as an experimental animal. Under light anesthesia, tetanic contractions were provoked by electrical stimulation of the tibial nerve, and the GS muscle was then manually extended during contractions. These procedures could produce tenderness in the exercised muscle with a focal sensitive region on a palpable band in the rabbits. In our previous study in humans, we demonstrated that a localized tender region appears on the palpable band on the second day after eccentric exercise [12]. A similar localized sensitive region on a palpable band developed in the present study.

The amplitude of evoked EMG of the BF muscle was used as an index in this study. The evoked EMG could not be elicited by mechanical distension of the GS muscle in the control group, and it appeared when the affected muscle was extended several days after the exercise. The time course of the changes in the amplitude of evoked EMGs was quite similar to that measured in subjective soreness [12].

The evoked EMG of the BF muscle elicited by an electrical stimulation of the foot, called withdrawal reflex, has long been used as a useful index of pain in human subjects [15, 16] and of nociception in experimental animals [17–19]. In human psychophysical study, the amplitude of withdrawal reflex was shown to be correlated with the subjective pain magnitude ratings [15, 16]. The responsible afferents for the withdrawal reflex, however, were estimated from its latency in experimental animals [18, 19], and A-delta and C-afferent fibers in the withdrawal reflex has also been strongly suggested [17].

In this study the latency of EMGs evoked by focal electrical stimulation of the GS muscle exceeded 40 ms, and a rough estimation of the conduction velocity of the afferent fibers was less than 10 m/s, which suggested the participation of thin afferents in this reflex circuit. Thus the amplitude of the evoked EMG of the BF muscle might be a reasonable indicator for the measurement of DOMS.

**Effect of indomethacin on DOMS.** The present study clearly shows that a repetitive administration of indomethacin inhibits the development of a localized sensitive region as well as DOMS, and it also suppresses the formation of a palpable band. We used a relatively large dose of repetitively administered indomethacin, following a protocol in mice that succeeded in preventing the development of DOMS [6]. The adequacy of the dose and its form of administration are undoubtedly important. That the rabbits in the indomethacin group showed no abnormal behavior and had no apparent illness indicated that the dose used and the method of administration were suitable for the experiment. In contrast, several studies have demonstrated that the administration of nonsteroidal anti-inflammatory drugs (NSAIDs) could not protect against the development of DOMS in humans [20, 21], suggesting a role for noninflammatory mechanisms in DOMS. The importance of dose, administration times, and route of administration has been indicated [22], so the negative results of the NSAID studies should be carefully reconsidered.

Thus it is suggested that the development of DOMS and a localized sensitive region might be associated with various changes in the inflammatory process, such as minor tissue injury and sensitization of nociceptors by prostaglandins.

**Mechanisms of appearance of a localized sensitive region.** It is well known that the activity of group III and IV afferent receptors are related to muscle pain [23] and found mostly within the connective tissue of muscle [2]. In particular, the fascia
one of the most sensitive among the deep tissues [24, 25]. Most thin afferent fibers in muscle are thought to innervate polymodal-type receptors. They are easily sensitized by various chemical mediators such as prostaglandins [23, 26, 27], which are released in injured muscular tissue [7, 28]. In various inflamed tissues, silent or sleeping nociceptors have been found [23]. Although these receptors have not yet been found in skeletal muscle, they may be one cause of the development of DOMS and the localized sensitive region. Thus the inflammatory process following muscle injury may be important in the production of the muscle soreness [3, 7].

Usually the inflammatory process provoked by the tissue injury takes several minutes to hours, but not days. However, the time courses of serum prostaglandin E2 (PGE2) level changes and the degree of muscle soreness are closely related [4, 29]. It is hypothesized that elevated muscle calcium stimulates macrophages to synthesize PGE2 [29]. In muscle, high [Ca2+] stimulates the production of PGE2 [30]. Moreover, the macrophage is the predominant cell type at the site of injury 2–3 d after eccentric contraction and is capable of releasing large quantities of PGE2 [31].

Indomethacin is a kind of NSAID and a potent inhibitor of prostaglandin synthesis, which suppresses peripheral inflammation, and inflammation-induced pain [32]. Because indomethacin inhibits the development of a localized sensitive region, the sensitization of polymodal-type nociceptors in the fascia mediated by prostaglandins might be one of the possible mechanisms.

In the present study, the musculotendinous attachment area was the most sensitive region, and a sensitive region appeared on the palpable taut band. The mechanisms of the formation of a palpable band and the sensory region on it are not clear. However, the inflammatory process elicited by tissue injury may be closely related to their appearance. The musculotendinous attachment might be the area sensitive to the eccentric contraction, and an application of intense tension here might easily cause a minor tissue injury.

**Possibility of central sensitization in DOMS.** Recent ischemic conduction block experiments demonstrate the participation of thick afferent fibers in the development of a muscle soreness sensation and suggest central sensitization (allodynia) in DOMS [33]. A decrease in the thresholds of nociceptive dorsal horn neurons in the acute inflammation is well known [34]. An expansion of receptive fields and the appearance of new receptive fields were induced by the intramuscular injection of chemical algesic agents [35]. The data from these animal experiments also suggest that central changes occur when muscle tissue is injured by DOMS. The possibility of central sensitization in DOMS cannot therefore be excluded. However, the appearance in the present study of a very localized sensitive region in the fascia on the palpable band is difficult to explain in regard to central sensitizing mechanisms.

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