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Effect of local cooling on excitation-contraction coupling in myasthenic muscle: Another mechanism of ice-pack test in myasthenia gravis

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HIGHLIGHTS

- We evaluated the effects of local cooling on muscle twitch in myasthenia gravis.
- Local cooling induced amelioration of impaired excitation-contraction coupling in ice-pack-positive myasthenia gravis.
- The ice-pack test induces effects on synaptic transmission and excitation-contraction coupling.

ABSTRACT

Objective: The ice-pack test is a convenient diagnostic testing procedure for myasthenia gravis (MG). We investigated the underlying mechanism of the ice-pack test performed on bilateral masseters.
Methods: We performed trigeminal repetitive nerve stimulation (RNS), excitation-contraction (E-C) coupling assessment (Imai's method) and bite force measurement before and after cooling of the masseters in MG patients and normal controls. After placing the ice-pack on the masseters for 3 min, serial recordings of the three tests were performed at various time intervals during 10 min after cooling.
Results: The bite force increased significantly after cooling in ice-pack-positive MG patients. The acceleration and acceleration ratio (acceleration at a given time to baseline acceleration) of jaw movement increased significantly after cooling of the masseters in ice-pack-positive MG patients compared to ice-pack-negative patients and normal controls. The prolonged effect of cooling continued until the end of recording even though decremental response to RNS had returned to baseline value.
Conclusions: Cooling of myasthenic muscle may induce two effects. One is relatively short effect on electrical synaptic transmission at the endplate, and another is prolonged effect on E-C coupling in the muscle.
Significance: The ice-pack test induces a prolonged effect of ameliorating impaired E-C coupling in MG.

1. Introduction

Ptosis, the most common symptom in myasthenia gravis (MG) (Murai et al., 2011), can be improved by local cooling of the eyelid in some patients with MG. This phenomenon has been used in the "ice-pack test" to diagnose MG (Saavedra et al., 1979; Sethi et al., 1987; Ertaş et al., 1994). Possible physiological mechanisms of how cooling improves neuromuscular transmission include: (1) increased postsynaptic receptor sensitivity to acetylcholine (ACh) (Harris and Leach, 1968); (2) facilitated transmitter replacement in the presynaptic terminal (Hubbard et al., 1971); (3) efficient utilization of ACh (Borenstein and Desmedt, 1974; Ricker et al., 1977); (4) decreased hydrolysis of ACh by acetylcholine esterase (AChE), allowing sustained action of the transmitter already released from the axon terminal (Foldes et al., 1978); and (5) reduced rate of removal of calcium ions from the nerve terminal following stimulation (Maddison et al., 1998).

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Although previous studies focused on the amelioration of neuromuscular transmission at the endplate by local cooling as described above, cooling of the skeletal muscle is known also to induce contractile responses, probably caused by Ca^{2+} release from the sarcoplasmic reticulum (Talon et al., 2000; Protasi et al., 2004). The release of Ca^{2+} plays a significant role in the events that link the electrical stimulus to the mechanical response, which is excitation-contraction (E-C) coupling. Therefore, the ice-pack test may involve another mechanism that increases contractile response by enhancing E-C coupling in the myasthenic muscle.

Our group has developed a method for *in vivo* assessment of E-C coupling, so-called Imai's method. Using this method, we have found that bite force correlates significantly with masseteric E-C coupling time (ECCT) [difference in onset latencies between masseteric compound muscle action potentials (CMAPs) and mandibular movement-related potentials (MRPs)] (Tsuda et al., 2010). We have also revealed that anti-ryanodine receptor (RyR) antibody contributes to E-C coupling impairment in MG (Imai et al., 2011, 2012a), and that tacrolimus is a RyR enhancer in MG (Imai et al., 2012b).

In the present study, we attempted to elucidate another mechanism of the ice-pack test in MG by performing serial recordings of trigeminal repetitive nerve stimulation (RNS), mandibular MRP, and bite force before and after local cooling of bilateral masseters. We demonstrated the enhancement of E-C coupling by cooling.

2. Materials and methods

2.1. Subjects and protocol

Twenty-five patients (9 males and 16 females) aged 29–72 years (mean, 53.7 years) diagnosed with MG at Sapporo Medical University Hospital were studied. MG was diagnosed when a patient manifested typical clinical findings and defect in neuromuscular transmission revealed by electrophysiological tests. Such defect was indicated by either an abnormal decrement in repetitive nerve stimulation tests conducted on the nasalis, trapezius, abductor pollicis brevis and abductor digiti minimi; or increased jitter observed on concentric needle single fiber electromyography conducted on the extensor digitorum communis and orbicularis oculi (Kouyoumdjian and Stålberg, 2008; Kokubun et al., 2012). Disease severity was graded according to the Myasthenia Gravis Foundation of America (MGFA) clinical classification (Jaretzki et al., 2000). Acetylcholine receptor (AChR) binding antibodies, anti-muscle specific receptor tyrosine kinase (MuSK) antibodies (Shiraishi et al., 2005) and anti-RyR antibodies (Mygland et al., 1992; Takamori et al., 2004) were measured. Thymic lesions were diagnosed histopathologically after extended thymectomy.

At the time of this study, 11 of 25 patients were naive to specific MG therapy, while the remaining 14 patients had MG exacerbation during treatment with corticosteroids alone or corticosteroids with tacrolimus (4 and 10 patients, respectively). For patients who were taking pyridostigmine bromide, they stopped taking the drug more than 12 h before the study. Eight patients had undergone extended thymectomy.

We classified MG patients into two groups according to the ice-pack test for chewing. Ice-packs were placed on bilateral masseters (see: Section 2.2) for 3 min, and whether chewing ability improved after cooling was subjectively assessed by the MG activities of daily living profile (MG-ADL) (Wolfe et al., 1999; Tsuda et al., 2010). Twenty-five MG patients were divided into ice-pack-positive group (13 patients; 4 males and 9 females; aged 30–72 years, mean 52.2 years) or ice-pack-negative group (12 patients; 5 males and 7 females; aged 29–72 years, mean 55.3 years).

Each assessment was performed in the supine position. After cooling of bilateral masseter muscles by ice packs for 3 min, serial recordings of CMAP and MRP were performed at 15, 60, 120, 180, 240, 300, 360, 420, 480, 540, 600 s, and RNS at 30, 210, 390, 570 s after cooling. At the same time of the electrophysiological assessments, bite force was also measured before and within 2 min after the 3-min cooling, allowing adequate rest between recordings. Bite force was measured only once after cooling to avoid chewing fatigue. Normal ranges for the methods used in this study were established using the data of 9 healthy control subjects (5 males and 4 females) aged 35–66 years (mean, 49.3 years).

The study was approved by the ethics committee of Sapporo Medical University School of Medicine, Sapporo, Japan (number: 16–81, 23–86). All healthy control subjects and patients gave informed consent for participation in this study.

2.2. Cooling of masseter and temperature monitoring

We used commercially available cold packs (3 M Reusable Cold/Hot Pack; 3M Consumer Health Care, St Paul, MN, USA) to cool bilateral masseters. After the cold packs were stored in freezer for at least 2 h, two packs were wrapped in cloth and applied to both cheeks simultaneously for 3 min. The skin temperature above the masseters was measured by non-contact thermometer (Visiofocus 06400; Tecnimed Sri, Italy) just before each electrophysiological recording.

2.3. Bite force measurement

Details of the procedures to measure bite force have been described previously (Tsuda et al., 2010; Imai et al., 2012a). Briefly, a commercially available pressure-sensitive sheet for dentistry (Dental Prescale 50H-R; Fuji Photo Film, Japan) was used. With the pressure-sensitive sheet positioned on the upper dental arch, the patient was instructed to clench the teeth with maximum force for 5 s. The sheet was then analyzed with a computer analyzing system FPD-707 (Fuji Photo Film, Japan) and the bite force was calculated.

2.4. Repetitive nerve stimulation and movement-related potentials recording

Detailed procedures of repetitive stimulation of the masseteric branch of the trigeminal nerve and recording of potentials have been described previously (Tsuda et al., 2010; Imai et al., 2012a). Briefly, trigeminal RNS was delivered via a cathode (bare-tipped monopolar needle) placed approximately 15 mm into the mandibular notch between the coronoid process and condyle, and an anode (surface electrode) placed on the ipsilateral zygomatic process (Fig. 1). CMAP was recorded from surface disc electrodes placed on the antero-inferior region of the muscle and over the mandibular angle, with a ground electrode placed on the neck. A 0.2-ms rectangular pulse was delivered with gradual increasing intensity to reach a supramaximal response. Once a supramaximal CMAP was obtained, a train of ten stimuli was given at a frequency of 3 Hz. We define abnormal decrement as over 10% reduction in amplitude on the fifth CMAP compared to the first, which indicates impairment of synaptic transmission at the endplate (Ozdemir and Young, 1971; Mayer and Williams, 1974).

The masseteric ECCT and acceleration of jaw movement were measured by Imai's methods, with the mouth opened gently with an inter-lip distance of 2.5 cm. The stimulating and CMAP recording techniques were the same as described above for trigeminal RNS. The mandibular MRP was recorded after a single supramaximal stimulation using an acceleration converter (SV1101; NEC, Tokyo, Japan) taped at the chin (Tsuda et al., 2010). The ECCT was defined as the difference in onset latencies between the mas-