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Modulation effects of cordycepin on the skeletal muscle contraction of toad gastrocnemius muscle

Li-Hua Yao [a,*,1], Wei Meng [a,1], Rong-Feng Song [b], Qiu-Ping Xiong [b], Wei Sun [a], Zhi-Qiang Luo [c], Wen-Wen Yan [d], Yu-Ping Li [a], Xin-Ping Liu [a], Hai-Hang Li [d], Peng Xiao [d]

[a] School of Life science, Jiangxi Science & Technology Normal University, Nanchang, Jiangxi 330013, PR China
[b] Internal Medicine Department 3, Jiangxi Province Tumor Hospital, Nanchang, Jiangxi 330029, PR China
[c] Department of Neurology, The Second Hospital of Nanchang City, Nanchang, Jiangxi 330003, PR China
[d] School of Life Science, South China Normal University, Guangzhou 510631, PR China

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ABSTRACT

Isolated toad gastrocnemius muscle is a typical skeletal muscle tissue that is frequently used to study the motor system because it is an important component of the motor system. This study investigates the effects of cordycepin on the skeletal muscle contractile function of isolated toad gastrocnemius muscles by electrical field stimulation. Results showed that cordycepin (20 mg/l to 100 mg/l) significantly decreased the contractile responses in a concentration-dependent manner. Cordycepin (50 mg/l) also produced a rightward shift of the contractile amplitude-stimulation intensity relationship, as indicated by the increases in the threshold stimulation intensity and the saturation stimulation intensity. However, the most notable result was that the maximum amplitude of the muscle contractile force was significantly increased under cordycepin application (122 ± 3.4% of control). This result suggests that the skeletal muscle contractile function and muscle physical fitness to the external stimulation were improved by the decreased response sensitivity in the presence of cordycepin. Moreover, cordycepin also prevented the repetitive stimulation-induced decrease in muscle contractile force and increased the recovery amplitude and recovery ratio of muscle contraction. However, these anti-fatigue effects of cordycepin on muscle contraction during long-lasting muscle activity were absent in Ca^{2+}-free medium or in the presence of all Ca^{2+} channels blocker (0.4 mM $CdCl_2$). These results suggest that cordycepin can positively affect muscle performance and provide ergogenic and prophylactic benefits in decreasing skeletal muscle fatigue. The mechanisms involving excitation-coupled Ca^{2+} influxes are strongly recommended.

1. Introduction

Cordyceps militaris is a rare caterpillar fungus in traditional Chinese medicine. This fungus is widely used in oriental countries as a tonic for anti-aging and nourishment (He et al., 2010) and for the prevention and treatment of various diseases, including those in the circulatory, immune, respiratory, and glandular systems (Bueters et al., 2008; Lin et al., 2011; Yao et al., 2011). Cordycepin (3-deoxyadenosine), a major component of *C. militaris* (Paterson, 2008), has been shown to have anti-tumor (Lee et al., 2013; Pao et al., 2012), anti-ageing (Ramesh et al., 2012), antioxidant (Chang et al., 2008; Ramesh et al., 2012), anti-inflammatory (Seo et al., 2013), anti-diabetic (Shin et al., 2009), and neuroprotection effects (Cheng et al., 2011; Yao et al., 2011, 2013). Evidence also indicated that cordycepin may have an important regulation effect in muscle tissue. Cordycepin was reported to inhibit the proliferation of vascular smooth muscle cells (Chang et al., 2008; Jung et al., 2012). However, few reports demonstrate the effects of cordycepin on the skeletal muscle thus far. Cordycepin is also widely used as an ergogenic aid for anti-fatigue and nourishment in sports health care market. Thus, further studies are necessary to address these pharmacological differences.

The gastrocnemius muscle is a typical skeletal muscle tissue that is frequently used to study the motor system because it is an important component of the motor system (Vukova et al., 2010; Park et al., 2013). Therefore, the modulating function of cordycepin on the motor system was first investigated in the present study, and the effects of cordycepin were assayed on the skeletal muscle contractile function in an isolated toad gastrocnemius muscle via an electrical field stimulation (EFS) technique (Ziganshin et al., 2005).

2. Materials and methods

2.1. Drug preparation

Chemicals used for making Ringer's solution (RS) were purchased from Sigma Co. (St. Louis, MO, USA). Toad RS provides the equivalent physiologic condition of the toad and is composed of

* Corresponding author. Tel.: +86 18079110719; fax: +86 79183815794.
E-mail address: yaolh79@yahoo.com (L.-H. Yao).
[1] These authors contributed equally to this study.

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112 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 0.1 mM NaH_2PO_4, and 2.38 mM $NaHCO_3$. The pH of the RS was adjusted to 7.2, and all measurements were recorded with the preparations equilibrated at room temperature (22–25 °C).

Cordycepin with a purity of more than 98% was provided by the South China Normal University (Ni et al., 2009; Yao et al., 2013). Cordycepin was dissolved in RS at concentrations of 20, 50, 100, and 200 mg/l, and its effects were tested via a superfusion system (5 ml/min).

2.2. Tissue preparation

The care and use of animals and the experimental protocol of this study were approved by the Institutional Care and Use Committee of our university. A total of 71 toads were used in this study. Toads were euthanized by decapitation and destruction of the spinal brain. The hind limbs were skinned, and the resting length of both gastrocnemius muscles was measured to the nearest millimeter. Each muscle was carefully separated from the tibia after inserting a hook through the Achilles tendon and cutting the tendon distal to the hook. The tibia was then severed below the knee and the femur was cut near the pelvis. The muscles were then allowed to equilibrate in bath conditions for 15 min. The muscles were immersed in the RS during preparation and throughout the experiment.

In separate experiments in a Ca^{2+}-free medium, the muscles were immersed in Ca^{2+}-free RS during preparation and throughout the recordings (Pandey and Deshpande, 2012). In another set of experiments, the muscles were immersed in RS supplemented with the all Ca^{2+} channels blocker $CdCl_2$ (0.4 mM) (Dong and Xu, 2002; Yang et al., 2005) during preparation and throughout the recordings.

2.3. Electrophysiological techniques and performance testing

Muscle performance characteristics were measured using a modification of the procedures described previously (Ziganshin et al., 2005; Costanzo et al., 2008). Briefly, EFS was applied and responses of the tissue were recorded and analyzed using a Powerlab data acquisition system (ADInstruments, Inc., Colorado Springs, CO, USA). Each muscle was suspended vertically in 10-ml organ baths for isometric recording of mechanical activity by securing the truncated femur in a clamp and attaching the distal tendon, via the hook and a thread ligature, to the blade of a force displacement transducer (ADInstruments, Inc., model FT-100). The muscle was gently stretched to the measured resting length under optimal tension (~2 g). A pair of stimulating electrodes was inserted into the muscle belly at right angles to each other, such that virtually all fibers could be stimulated simultaneously.

An individual muscle contractile response was elicited by applying single rectangular impulses. Typically, the stimulation intensity was adjusted initially to produce the maximum amplitude of contractile response using single square pulses with 5 ms in duration. Sampling of individual muscle contractile response was carried out using pulses delivered once every 30 s. Unless otherwise noted, intervals of at least 30 s were allowed between each pulse stimulation to prevent unstable responses. In the experiment for the testing of the contractile amplitude-stimulation intensity relationship, the stimulation intensity of the single square pulse was gradually increased from the subthreshold stimulation intensity to the maximum stimulation intensity (100 mV increments; 5 ms duration; 30 s intervals). The threshold stimulation intensity was determined by gradually increasing the intensity of the single square pulse stimulation voltage until a contraction was elicited. Gradually increasing the single-pulse stimulation intensity further increased the contractile force until the maximum amplitude was reached. This stimulation intensity was determined as saturation stimulation intensity.

To characterize the effects of cordycepin on the long-lasting exercise-induced decrease in muscle contractile force, a repetitive stimulation with saturation stimulation intensity was delivered at 1 Hz; the times to reach 50% and 10% of the maximum amplitude (initial value) were labeled as T50% and T10%, respectively. For the postexercise strength recovery test, a testing single-pulse (using the same stimulation intensity impulse) was subsequently applied every 30 s to 10 min when the muscle contractile force decreased to 10% of the maximum amplitude (initial value) induced by the pre repetitive stimulation. The amplitude of the last single muscle contraction of the 10th min was employed to analyze the muscle recovery status. A contractile amplitude recovery of up to 60% of the maximum amplitude (initial value) was set as an eligible recovery data threshold. To avoid tissue death, EFS was maintained constant only until the tension from muscle contraction declined to 10% of the initial recording.

All recordings were collected until a stable baseline was present for at least 3 min; if this condition did not occur, the specimen was excluded from the study.

2.4. Data analysis

The effects of cordycepin on the skeletal muscle contractile responses evoked by using EFS were characterized. The mean of the individual contraction amplitudes recorded under stable conditions at the start of the experiment was taken as the baseline, and the amplitudes following drug application were expressed as a percentage of the baseline. In the experiment of the contractile amplitude-stimulation intensity relationship testing, recordings were carried out before and after the addition of a single concentration of cordycepin. The maximum contractile force elicited by the corresponding saturation stimulation intensity in the presence of cordycepin was normalized to the values prior to cordycepin application. To study the effect of cordycepin on the exercise-induced decrease in muscle contractile force and postexercise recovery of strength of the muscle contraction, the maximum amplitude (initial value) was scaled to 100%. The amplitude following the repetitive stimulation or test impulse stimulation was expressed as a percentage of the maximum amplitude.

All numerical data were presented as mean ± S.E.M., unless otherwise indicated. Statistical significance of difference was calculated using Student's paired *t*-test. The recovery ratio of muscle contractile force was expressed as a percentage and evaluated using the chi-square test. A level of confidence of $P < 0.05$ was employed for statistical significance.

3. Results

3.1. Reduction in muscle contractile responses to external stimulation by cordycepin in isolated toad gastrocnemius muscle

The effects of cordycepin on the contractile responses elicited via the single pulse stimulation on isolated toad gastrocnemius muscles are illustrated in Fig. 1. Application with cordycepin profoundly suppressed the amplitude of muscle contraction in a concentration-dependent manner. The cordycepin-mediated decrease in muscle contractions appeared within 1 min after application with cordycepin. This decrease peaked at 1–2 min and recovered gradually to the baseline level after a washout of 3–10 min, indicating that the effects of cordycepin were reversible. At concentrations of 20, 50, 100 and 200 mg/l, the resultant percentages of contractile amplitudes were decreased to 89.1 ± 13.68% ($n=8$), 50.19 ± 11.98% ($n=10$),