Effects of Acute Muscle Contraction on Titin Stiffness-Related Contractile Properties in Rat Fast-Twitch Skeletal Muscle

Jiayu Shi

Graduate School of Integrated Arts and Sciences

Hiroshima University

March 2022

In a half sarcomere of a striated muscle, the filament known as titin runs from the Z-disk to the M-band. Passive force is the force of a muscle that is stretched but not activated. Most of the passive force is generated by titin. The contribution of titin to active force has been long thought to be negligible, because titin stiffness is much lower than that of the acto-myosin. However, recent studies have suggested the more important role of titin in active force than previously thought. In cardiac muscles, it has been shown that acute exercise resulted in an increase in passive force. Based on these findings, it might be expected that the changes in titin stiffness would affect active force. However, no studies have investigated whether titin stiffness can undergo changes with acute muscle contractions in skeletal muscles. The aim of this study was to elucidate acute muscle contraction on titin stiffness-related contractile properties in rat fast-twitch muscle. To this end, four separated experiments (1-4) were performed in this study.

In the experiment 1, the effects of isometric contraction (ISC) on passive force were examined. Intact gastrocnemius muscles of the rats were electrically stimulated *in situ* until the force was reduced to ~50% of the initial force. Immediately after cessation of the stimulation, the superficial regions of the muscles were dissected and subjected to skinned fiber analysis. The ISC resulted in a decrease in passive force. Protein kinase Cα-treatment increased the passive force in stimulated fibers to resting levels. The ISC had no effect on the maximum Ca^{2+}-activated force (max Ca^{2+} force) at a sarcomere length (SL) of 2.4-μm. Stretching the SL to 3.0 μm led to the augmentation of the max Ca^{2+} force. The extent of the increase was smaller in rested than in stimulated fibers.

In the experiment 2, the effects of ISC on length-dependent activation (LDA), residual force enhancement (RFE), and passive force enhancement (PFE) were examined. The electrical stimulation and the skinned fiber preparation were performed in a manner similar to those in the experiment 1. The ISC led to a decrease in myofibrillar (my-) Ca^{2+} sensitivity at 2.6-μm SL. Although a stretch of SL from 2.6 to 3.0 μm increased my-Ca^{2+} sensitivity in both rested and stimulated fibers, the extent of the increase was higher in the stimulated than in the rested fibers. To evaluate RFE, the fibers were stretched before activation (the force developed by the activation was referred to as M1 and the passive force after M1 was called P1) and during activation (the force was referred to

as M2 and the passive force after M2 was called P2). The ISC decreased M1 and M2 in both rested and stimulated fibers. A relative difference (the ratio of M2 to M1) between M1 and M2 did not differ between rested and stimulated fibers. P1, but not P2, decreased in stimulated fibers. In both rested and stimulated fibers, P2 was greater than P1. However, the degree of the difference differed between rested and stimulated fibers. Absolute (P2 minus P1) and relative (the ratio of P2 to P1) differences were greater in stimulated than in rested fibers.

In the experiment 3, the effects of eccentric contraction (ECC) on passive force were examined. Intact GAS muscles were electrically stimulated *in situ* to subject the muscles to 200 repeated ECCs. The skinned fiber preparation was performed in a manner similar to that in experiment 1. ECCs brought about a decrease in the max Ca^{2+} force at 2.4 μm SL. A stretch of the SL to 3.0 μm led to the augmentation of the max Ca^{2+} force in both rested and stimulated fibers. The degree of the augmentation in stimulated fibers resembled that in rested fibers. The ECC resulted in an increase in the titin-based passive force. Protein kinase A-treatment reduced the passive force in stimulated fibers to the resting levels.

In the experiment 4, the effects of ECC on LDA, RFE, and PFE were examined. The electrical stimulation and the skinned fiber preparation were performed in a manner similar to those in experiment 3. ECC tended to decrease my- Ca^{2+} sensitivity at 2.6-μm SL. Although a stretch of SL from 2.6 to 3.0 μm increased my-Ca^{2+} sensitivity in both rested and stimulated fibers, the extent of the increase was higher in the stimulated than in the rested fibers. M1 and M2 were decreased in stimulated fibers. An absolute difference or a relative difference between M1 and M2 did not differ between the rested and stimulated fibers. P1 and P2 were increased in stimulated fibers. An absolute difference between P2 and P1 was larger in the stimulated than in the rested fibers.

The results obtained from this study indicate that ISC suppresses the passive force, whereas ECC raises it. The decreased passive force may contribute to muscle fatigue. The altered passive force is ascribable, at least in part, to a reduction in phosphorylation levels by protein kinase Cα and protein kinase A for ISC and ECC, respectively. Both contraction modes are capable of potentiating LDA and

PFE, but not RFE. The potentiated LDA and PFE arguably help produce greater force compared to that without the potentiation. It is suggested that some of titin stiffness-based contractile properties may function to resist muscle fatigue in the muscles of the exercising body.