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The effect of prior exercise on thermal sensitivity of malignant hyperthermia susceptible muscle

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Abstract

Malignant hyperthermia (MH) episodes may occur upon exposure to halogenated anesthetics, during resistance and endurance exercise, and in response to thermal stress. The purpose of this study was to investigate the effects of prior eccentric and concentric (i.e., wheel running) exercise on the thermal sensitivity of isolated MH susceptible mouse muscle (RyR1^{Y522S/wt}). Eccentric, but not concentric, exercise attenuated the thermal sensitivity of MH susceptible muscle.

Keywords

Malignant hyperthermia; eccentric contractions; muscle injury; thermal sensitivity; RyR^{Y522S/wt}; mouse; Junctophilin 1

Introduction

Malignant hyperthermia (MH) has traditionally been described as a pharmacogenetic disorder, whereupon genetic mutations, mostly of the sarcoplasmic reticulum (SR) Ca²⁺ release channel (ryanodine receptor; RyR1), confer hypersensitivity to halogenated anesthetics.¹ MH susceptible (MHS) individuals have been reported to experience episodic symptoms such as acidosis, rhabdomyolysis and even death after exposure to halogenated anesthetics. Symptoms also are reported to occur after endurance and resistance exercise and in response to thermal stress,^{2,3,4,5} raising the possibility that MH, environmental heat illness, and exertional rhabdomyolysis are related conditions.⁶ In support of this conclusion, MH episodes may be induced in MHS mice (RyR1^{Y522S/wt}) solely by thermal stress,^{7,8} and these episodes have been attributed to an enhanced post-translational modification of the RyR1.⁸ We have reported that RyR1^{Y522S/wt} mice can perform voluntary wheel running and eccentrically-biased exercise under non-stressful thermal conditions without inducing episodic symptoms.^{9,10} Because some individuals with MH likely participate in resistance and/or endurance training, it is important to understand the effect of prior eccentric or concentric exercise on the thermal sensitivity of MHS muscle.

Methods

Animals

The generation of MHS mice with heterozygous expression of Y522S mutation in RyR1 (RyR1^{Y522S/WT}) has been described previously.⁷ Wild type (WT; C57BL6) mice served as genetic controls. All animal care and use procedures were approved by the institutional animal care and use committee.

Experimental Design & Methodology

Mice were allotted to the following experimental groups: Single bout of 150 eccentric contractions, four bouts of 150 eccentric contractions (one per week for four weeks), or four weeks of voluntary wheel running. Eccentric contractions were performed with the left anterior crural muscles [e.g., tibialis anterior (TA) and extensor digitorum longus (EDL) muscles] using an *in vivo* injury model.^{10,11,12,13} Core body temperature during the eccentric contractions was ~35°C, and wheel running was performed at an ambient temperature of ~22°C. A description of the effects of eccentric contractions and wheel running on RyR1^{Y522S/WT} *in vivo* anterior crural muscle functional capacity is provided elsewhere.^{9,10}

Immediately, three days, and 14 days after a single eccentric bout (i.e., Ecc-0d, Ecc-3d, & Ecc-14d), three days after repeated eccentric bouts (Ecc-T), and two days after cessation of voluntary wheel running (Run), the left EDL muscles were dissected from anesthetized mice (100 mg·kg⁻¹ pentobarbital sodium) and studied *in vitro*.^{10,11,12,13} Muscles were incubated at 30°C and then at 35°C for 30 minutes at each temperature, while resting tension was monitored at three-minute intervals. Peak isometric force (300 Hz; 200-ms trains of 0.2-ms pulses) was measured at the end of each incubation period. Caffeine (50mM) contracture force was assessed after the 35°C incubation. Junctophilin 1 (Invitrogen 40-5100) protein content, a protein involved in the apposition of transverse tubule and SR membranes, was normalized to tubulin content as determined via Western blot.¹³

Statistics

Comparative analyses were performed using a variety of ANOVAs. Post-hoc means comparisons tests were performed with Bonferroni correction. α was set at 0.05. Values are reported as means $\pm$ SEM.

Results

When incubation temperature was increased from 30° to 35°C, neither resting tension integral nor active force values (e.g., Control WT 30 vs. 35°C; 23.1 $\pm$ 0.6 vs. 22.0 $\pm$ 0.7 N·cm⁻²) were significantly altered for WT mice under any condition. In contrast, control RyR1^{Y522S/WT} muscle exhibited an ~3.1-fold increase in resting tension integral and an ~65% decrease in active force (30 vs. 35°C; 21.4 $\pm$ 0.9 vs. 7.7 $\pm$ 1.0 N·cm⁻²) when incubation temperature was increased from 30° to 35°C. The temperature-associated increase in resting tension integral and decrease in active force production were attenuated in MHS EDL muscle after a single or multiple bouts of eccentric contractions, but not after 4 weeks of voluntary wheel running (Fig. 1a, b). MHS EDL muscle caffeine contracture force was qualitatively similar to values we have reported previously for uninjured and injured RyR1^{Y522S/WT} EDL muscle, and it was greater than WT values under each condition (Fig 1c).¹⁰ Serving as an indirect marker of triad disruption and then repair,¹³ JP1 was reduced three days after a single eccentric bout in WT and MHS muscle but was only recovered to baseline values in MHS muscle (Fig. 1d).

Discussion

These preliminary results indicate that high-force eccentric contractions (resistance-like exercise) performed under non-thermally stressful conditions, but not voluntary wheel running, attenuate the thermal stress-induced loss of function in MHS muscle with the Y522S RyR1 mutation for a prolonged period of time. Therefore, exercise-induced muscle injury may blunt the severity of a thermally related MH episode in humans. Furthermore, this finding may have implications on the interpretation of diagnostic contracture tests used to assess MH susceptibility in individuals who routinely engage in strength training exercise.

How eccentric contractions attenuate the thermal stress-induced loss of function of MHS muscle is unknown. However, because enhanced MHS muscle thermal sensitivity in RyR1^{Y522S/wt} mice has been ascribed to an altered RyR1 stability due to enhanced nitrosylation and oxidation,⁸ it is likely that an attenuated thermal response in MHS muscle after eccentric contractions is due to an improved RyR1 stability. One possible explanation for the attenuated thermal response after eccentric contractions is that triad re-structuring, as is indicated by the loss and recovery of JP1 after eccentric contractions,¹³ improves RyR1 stability in MHS muscle. With triad re-structuring following injury, it is likely that a number of accessory triadic proteins that are known to modulate RyR1 channel activity (e.g., JP-45 & MG-29) are damaged and replaced and that this re-structuring may provide improved stability to RyR1 in MHS muscle. For example, it has been shown that JP1 binds to RyR1 in a redox sensitive manner that may increase RyR1 open probability,¹⁴ indicating that the loss of JP1 after eccentric contractions may partly explain early time-point reductions in thermal sensitivity. Further study is currently being conducted to improve our understanding of the effects of eccentric contractions on triad structure and how these effects are related to early and prolonged eccentric contraction induced-attenuation of thermal sensitivity in MHS muscle.

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Abbreviations

Cav1.1	Voltage-gated L-type Ca ²⁺ channel
EDL muscle	Extensor digitorum longus
JP1	Junctophilin 1
JP-45	Junctional Protein 45
MHS	Malignant hyperthermia (MH) susceptible
MG-29	Mitsugumin 29
RyR1	Ryanodine receptor 1
SR	Sarcoplasmic reticulum
TA muscle	Tibialis anterior
WT	Wild type

Figure 1.

Effects of prior exercise on temperature sensitivity and functional capacity of MH susceptible muscle. **(a)** The total tension produced over the 30-minute incubation periods (i.e., resting tension integral) were compared between WT (top panel) and RYR1^{Y522S/wt} (bottom panel) mice and among conditions. See methods section for definition of groups. There were no significant differences between WT and RYR1^{Y522S/wt} values for any condition at 30°C, while only E-0d and E-3d values were similar (†) between genotypes at 35°C. **(b)** Peak isometric specific force of WT and RYR1^{Y522S/wt} muscle produced at 35°C, expressed as a ratio of force produced at 30°C, was compared between genotypes and among conditions. **(c)** Caffeine contracture force for WT and RYR1^{Y522S/wt} muscle produced at 35°C. * denotes a significant main effect of genotype ($p < 0.001$). **(d)** JP1:Tubulin values for WT and RYR1^{Y522S/wt} tibialis anterior muscles. Within each panel, values with different letters are significantly different ($p < 0.05$). All values are expressed as means $\pm$ SEM; sample sizes (n) are similar for (a & b).