Myofibrillar protein degradation after eccentric exercise

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Summary. Male rats were run downhill for 90 minutes (nonexhaustive). Following the exercise, muscle protein degradation was increased, as determined by urinary 3-methylhistidine. However, minimal changes were observed in the relative percentage of the minor myofibrillar proteins and in the protease calcium activated factor in the long head of the triceps brachii muscle (eccentrically exercised) following the exercise bout.

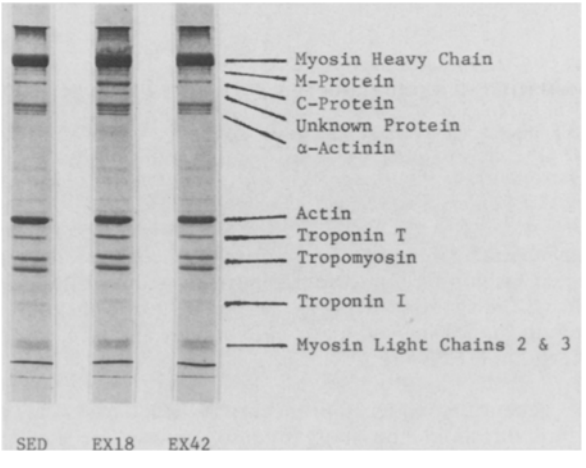
Net degradation of proteins[1] and ultrastructural disruption[2,3] in skeletal muscle has been demonstrated after severe physical exercise. There are reports that 0–48 h after extreme exercise there occurs an increase in urinary[4] and plasma[4,5] 3-methylhistidine and a decrease in muscle 3-methylhistidine[6], all indicators of actin and/or myosin breakdown. Muscles of animals sacrificed 2 days after exhaustive exercise exhibited degenerating fibers with edema, whereas necrotic and regenerating fibers appear 3 days after exercise[7]. Z-band disruption and disappearance along with fragmentation of myofilaments has been observed in electron micrographs of rat muscle after severe exercise[2]. Similar ultrastructural alterations have been observed in human muscle 48 h after a bout of nonexhaustive exercise which involved eccentric muscular contractions (force production in lengthening muscles)[3]. Eccentric contractions also produce more delayed muscle soreness[8] and inflammation[9] than concentric contractions with similar loads.

The mechanism underlying exercise-induced degradation of muscle proteins is unclear, but presumably involves increased activity of muscle proteases, one of which, the calcium activated factor (CAF), has been shown to degrade Z-disks[10]. The purposes of this study were: 1) to further characterize muscle protein degradation after exercise by assessing exercise-induced alterations in the relative percentages of the myofibrillar proteins and 2) to determine if increased protein degradation and/or changes in the relative percentages of the myofibrillar proteins were associated with increased CAF activity.

Methods. 60 male Wistar rats were randomly assigned to one of 3 treatment groups: a) sedentary control (SED), b) exercised and sacrificed 18 h later (EX18), and c) exercised and sacrificed 42 h later (EX42). The exercised animals ran for 90 min down a 16° decline on a treadmill at 16 m/min. This exercise regimen required eccentric contractions of the triceps brachii muscles. Animals were sacrificed by decapitation, and the long head of the triceps brachii muscle was removed immediately and weighed.

Urinary 3-methylhistidine (3MHIS) served as a marker for muscle protein degradation and was analyzed in samples of urine collected over an 18-h period prior to sacrifice and on a prior control day[11]. The degradation of the minor myofibrillar proteins was determined with the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis according to Laemmli[12]. Because Penny and Ferguson-Pryce[13] have shown that myosin and actin do not incorporate the stain in electrophoretic preparations in proportion to their concentrations, only the minor myofibrillar proteins, i.e., all proteins except actin and myosin, were used to determine relative percentages of each protein. Following isolation of the CAF enzyme by isoelectric precipitation, the activity of the enzyme was determined spectrophotometrically with the procedures of Dayton et al.[10]. Differences among group means for relative percentages of myofibrillar proteins and for CAF activity were tested with a one-way analysis of variance ($p < 0.05$) and Student-Newman-Kuels test when appropriate. Mean differences for 3MHIS were analyzed with an analysis of covariance with control day values for 3MHIS serving as the covariate.

Results. Final body weight, muscle weight and food intake were not affected by a single bout of exercise (table). Muscle protein degradation, as indicated by urinary 3MHIS levels, increased after the nonexhaustive eccentric exercise bout (table). Elevated levels of 3MHIS were observed in the urine at 18 and 42 h after the exercise bout, but only the 42 h value was significantly different from that recorded for the sedentary group. Except for the appearance of an unknown protein (mol. wt = 125,000 d), the increased muscle degradation did not significantly alter the relative percentages of any of the minor myofibrillar proteins (fig.). The unknown protein was found in very small amounts in the SED (0.6 ± 0.2) and EX42 (0.6 ± 0.2), but was 2.4-fold greater in muscles from the EX18 (1.5 ± 0.5) group. Small increases and decreases were observed in the percentages of the other minor myofibrillar proteins, but



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	SED	EX18	EX42
Body weight (g)	242 ± 4(20)	240 ± 3(19)	241 ± 4(20)
Muscle weight (mg)	814 ± 19(20)	812 ± 16(19)	811 ± 15(20)
Food intake (g)	20.0 ± 0.8(20)	18.3 ± 0.8(20)	20.6 ± 1.4(10)
3-Methylhistidine (nmoles/g b. wt/18 h)	5.03 ± 0.17(12)	5.46 ± 0.45(6)	6.20 ± 0.31(4)*
CAF activity (ΔABS/g muscle)	0.46 ± 0.02(11)	0.52 ± 0.04(11)	0.51 + 0.04(11)

All values are means ± SE (n). * $p < 0.05$.

none of these changes was statistically significant. The activity of the CAF protease (change in absorbance per g wet muscle weight) was not different among treatment groups (table). Similar nonsignificant increases were observed when CAF was expressed as the change in absorbance per mg of protein isolated or as the absolute change in absorbance (data not shown).

Discussion. The increased excretion of urinary 3MHIS observed after submaximal exercise is consistent with the hypothesis that muscle protein degradation was elevated. These results are in agreement with previous evidence of increased rates of muscle protein degradation after maximal exercise[1]. However, the only significant structural alteration that occurred in m. triceps brachii after this submaximal bout of eccentric exercise was the increased relative percentage of the 125,000 d protein 18 h after the exercise. The protein accounted for only 1.5% of the minor myofibrillar proteins. This minor change observed in the white triceps brachii is consistent with the data of Armstrong et al.[9] who showed an increased activity of glucose-6-phosphate dehydrogenase, a marker of muscle inflammation, in red muscles after an exercise bout similar to the one used in this study, but not in the triceps brachii. Similarly, Kuipers et al.[14] found degenerative changes to be greatest in red muscle fibers of rats 24–48 h after a submaximal nonexhaustive 1-h exercise, and Vihko and coworkers[15] found proteolytic lysosomal enzyme activity of rats to be increased more in red muscle than white following strenuous exercise. Finally, Dohm et al.[1] found no increase in the activity of CAF in rat gastrocnemius muscle, a white muscle, immediately following a run to exhaustion. Since the activity of CAF[16] and other proteolytic enzymes[17] has been found to be greater in red than white muscle, it appears likely that increased muscle protein degradation and structural alterations following exercise occur initially and preferentially in the red muscle fibers.

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Evaluation of monoaminergic receptors in the genetically epilepsy prone rat

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Summary. The intensity of sound-induced convulsions in the genetically epilepsy-prone rat (GEPR) was reduced in a dose related fashion by intracerebroventricular administration of dobutamine, (β_1 agonist), terbutaline (β_2 agonist) or phenylephrine (α_1 agonist). BHT-920 (α_2 agonist) did not cause a dose-related decrease in sound-induced convulsion intensity. Binding studies showed that whole brain α and β receptor densities (B_{max}) were normal while the K_d was increased for the β ligand in GEPR brain.

The genetically epilepsy-prone rat (GEPR) has a lower seizure threshold and more intense seizures for a given stimulus (electroshock, pentylenetetrazol or bicuculline) than do normal rats[3–5]. These rats are also characterized by susceptibility to sound-induced seizures[6] and to hyperthermic seizures[7]. Thus, like the human epileptic, the GEPR has a genetically determined decreased ability to suppress seizures once the process has been initiated[8].

Perturbation of biogenic amine neurotransmitter systems has been shown to alter seizures in a number of animal models although innate abnormalities in these systems have not been conclusively identified in most models (see Maynet et al.[9] and Chadwick[10] for reviews). In the GEPR, experimentally-induced perturbation of biogenic amines alters seizure characteristics and innate abnormalities in monoamine neurotransmitters have been demonstrated. Drug-induced decrements in CNS noradrenergic and serotonergic activity cause an increase in sound-induced seizure intensity in the GEPR[11–17]. Drug-induced increments in noradrenergic and/or serotonergic activity are associated with a decrease in the severity of sound-induced seizures[12,14,18–20]. Also, when compared with controls, the GEPR has a generalized abnormality in brain norepinephrine levels and turnover rate and widespread abnormalities in serotonin levels in the brain[20,21].

The purpose of the present investigation was 2-fold. First, it was to evaluate brain noradrenergic receptors by determining if selected noradrenergic agonists can decrease seizure