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Proteolytic capacity in mouse cardiac muscle following strenuous exercise[1]

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Summary. Proteolytic capacity in mouse cardiac muscle was analyzed 1, 3, and 6 days after exhaustive intermittent or submaximal prolonged running. No significant changes were recorded in the activities of acid or alkaline proteases, β-glucuronidase or trypsin inhibitor. Similarly, no changes were found in the rates of acid or neutral autolysis.

Cell edema, mitochondrial changes and disintegration of myofibrillar structure are among the indications of ischemic myocardial or skeletal muscle injuries[2-4]. Similar ultrastructural phenomena are found in both muscle types after exhaustive exercise[5-7]. Ischemic damage may be reversible, or irreversible leading to cell necrosis. Some skeletal muscle fibres are necrotized both by exhaustive intermittent[8] and by submaximal prolonged running[9], but physical stress does not cause lethal lesions in the cardiac muscle of healthy animals[5,10].

A common sign of sublethal cell injuries are alterations in the lysosomal system, e.g. increased autophagic degradation[11]. In skeletal muscle, the post-exercise[8,12] or post-ischemia[13] lysosomal acid hydrolytic capacity of surviving fibres is strongly increased 3-7 days after the exercise, suggesting sublethal cell injuries. The aim of the present study was to determine whether similar changes in the cellular digestive mechanisms also occur in cardiac muscle.

Methods. 4-month-old NMRI mice were made to run to exhaustion on a motordriven treadmill by the intermittent running schedule described earlier[8]. In the submaximal prolonged running the mice ran for 9 h on the treadmill with 6° uphill tracks at a speed of 13.5 m/min. The mice were killed 1, 3, and 6 days after the exercise. The activities of acid protease[12], alkaline protease[14], β-glucuronidase[12], and trypsin inhibitor[15] and the rates of acid[16] and neutral[17] autolysis were analyzed.

Results and discussion. Acid hydrolase activities increase in reversibly injured skeletal muscle fibres after exhaustive exercise[8] or ischemia[13]. It has been suggested that this response reflects subcellular regenerative processes in muscle fibres[12]. However, in mouse cardiac muscle strenuous exercise did not affect the estimates of acid hydrolytic capacity (table). Only a small ($p < 0.05$) increase in acid protease activity in cardiac muscle was recorded after exhaustive running. However, the acid hydrolytic capacity

Estimates of proteolytic capacity in mouse cardiac muscle 1, 3 and 6 days after intermittent exhaustive or submaximal prolonged running

Variables		Intermittent exhaustive exercise			Submaximal prolonged exercise		
	Controls (n = 14)	1 day (n = 9)	3 days (n = 10)	6 days (n = 9)	1 day (n = 8)	3 days (n = 11)	6 days (n = 9)
Acid autolysis	148 ± 5	150 ± 6	148 ± 6	138 ± 4	154 ± 7	151 ± 5	144 ± 6
Acid protease	1110 ± 35	1180 ± 60	1240 ± 35*	1110 ± 30	1160 ± 64	1180 ± 39	1080 ± 40
β-Glucuronidase	11.9 ± 0.3	12.8 ± 1.7	11.4 ± 0.3	12.0 ± 0.6	11.7 ± 0.7	12.9 ± 0.7	13.1 ± 1.2
Neutral autolysis	5.8 ± 0.3	6.0 ± 0.3	6.0 ± 0.4	5.6 ± 0.3	5.8 ± 0.3	6.2 ± 0.2	5.4 ± 0.4
Alkaline protease	17.4 ± 0.7	15.6 ± 0.4	17.8 ± 0.8	16.6 ± 0.9	15.4 ± 0.6	17.2 ± 0.6	17.2 ± 0.8
Trypsin inhibitor	13.2 ± 0.9	12.9 ± 0.9	14.6 ± 0.9	13.0 ± 0.5	13.1 ± 0.9	14.2 ± 0.6	12.3 ± 0.8
Malate dehydrogenase	744 ± 15	742 ± 20	725 ± 19	742 ± 18	742 ± 18	753 ± 19	726 ± 24
Protein content	182 ± 2	182 ± 4	186 ± 2	183 ± 2	186 ± 2	187 ± 2	182 ± 3

Activities (means ± SE) are expressed as pmoles/min/mg fresh muscle, except malate dehydrogenase activity (nmoles/min/mg fresh muscle) and trypsin inhibitor activity ($\Delta E \times 10^3$/min/mg fraction protein). Protein content is given as μg/mg. * $p < 0.05$.

of the skeletal muscle strongly increased in the same exercised mice[9]. The lack of the hydrolytic response in cardiac muscle after exercise may indicate that mouse cardiac muscle has an inherent protective mechanism against overstrain. Such a mechanism can be induced in skeletal muscle by endurance training[18,19]. Species variation also influences the susceptibility of myocytes to cell damage[20]. Acid proteolytic capacity increases in cardiac muscle during ageing[21], starvation[22] or insulin deprivation[23].

In addition to lysosomal proteolytic enzymes there are also neutral and alkaline proteases[24] and protease inhibitors[25] in cardiac muscle. The activities of these nonlysosomal proteases have been found to increase very considerably during ageing[26], starvation[22,26], and some other conditions causing cardiac atrophy[26]. In mouse cardiac muscle the activities of alkaline protease, neutral autolysis and trypsin inhibitor were unaffected by strenuous exercise (table) as also in skeletal muscle[9]. Problems associated with inhibitors may, however, disturb the analysis of these nonlysosomal proteases[25].

In spite of some ultrastructurally similar alterations in reversibly damaged rat cardiac and skeletal muscle fibres[5-7] there seems to be no distinct enzymatic response to strenuous exercise in mouse cardiac muscle. This may be due to different responses between the lysosomal systems of cardiac and skeletal muscles or to species differences in the properties of the cardiac muscle.

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Alpha- and beta-adrenergic receptors in rat myocardium membranes after prolonged ethanol inhalation[1]

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Summary. After 3 weeks of continuous ethanol intoxication by inhalation, the maximal number and affinity of the α- and β-receptors of rat heart were unchanged. These data indicate that adrenergic receptor disturbances are not involved in the mechanism of chronic ethanol-induced triglyceride deposition in the heart.

Tremor, agitation, tachycardia and hypertension are characteristic features frequently observed after the discontinuation of long-term heavy ethanol consumption[2,3]. Similar symptoms also occur in hyperadrenergic states such as thyrotoxicosis[4], and propranolol, a β-adrenergic blocker, has been successfully used to control some of the clinical signs of ethanol withdrawal such as hypertension and tremor[5]. This led to the suggestion that the peripheral manifestations of increased adrenergic activity in ethanol withdrawal may be the result of increased β-adrenergic sensitivity[6].

In a recent study, Banerjee et al.[7] reported that the β-receptor density in rat brain membranes decreased after chronic ethanol-treatment and conversely increased during the withdrawal state. In addition, the same authors presented some partial data suggesting that the same alterations may affect the cardiac β-receptors as well[7]. The latter data, however, concerned experiments in which a single concentration of labeled ligand was used, thus allowing no conclusion about possible alterations in the maximal number and/or affinity of the measured binding sites. Therefore, and because no attempt was made by these authors[7] to investigate the cardiac α-receptors, we have studied both the α- and the β-receptors in heart membranes of rats submitted to a 3-week ethanol inhalation treatment[8].

Material and methods. Male Wistar rats weighing 300–350 g were exposed for 21 days to ethanol vapor according to Le Bourhis[8]. Food and water intake and total body weight were monitored daily. The ethanol concentration in the air was gradually raised from 15 mg/l on day 1 to 20 mg/l on day 21, resulting in blood ethanol concentrations of about 70 mg/100 ml on day 21. Animals were then sacrificed, and the hearts were quickly excised, rinsed and weighed.

Hearts were minced in cold buffer (0.25 M sucrose, 5 mM Tris/HCl, 1 mM $MgCl_2$, pH 7.4) and homogenized in a Potter-Elvejhem homogenizer. The homogenate was filtered through 1 layer of cheese-cloth and centrifuged at $1000 \times g$ for 10 min at 4 °C. The pellets were discarded and