Lysosomal changes related to ageing and physical exercise in mouse cardiac and skeletal muscles[1]

A. Salminen, H. Kainulainen and V. Vihko

Division of Muscle Research, Department of Cell Biology, University of Jyväskylä, SF-40100 Jyväskylä 10 (Finland), 3 December 1981

Summary. Physical exercise increased the activities of arylsulphatase, cathepsin D and β-glucuronidase in mouse skeletal muscle but not in cardiac muscle. Exercise-induced lysosomal response was more prominent in young adult than in senescent mice. The lipofuscin content of cardiac and skeletal muscles increased markedly during ageing and was also found to increase slightly after exertion in young mice, but not in senescent ones.

Heavy physical exercice causes a myopathy in certain skeletal muscles[2-4]. This exercise myopathy is characterized by fiber necrosis and by the stimulation of the lysosomal system in surviving muscle fibers, especially in red oxidative fibers[5]. The lysosomal activation, which occurs 2-7 days after exertion[6], coincides with an autophagic response in muscle fibers[4] and most probably reflects sublethal injuries in surviving fibers.

Lipofuscin granules are regarded as the indigestible residue of lysosomal degradation[7,8]. Lipid peroxidation of cell organelles and constituents produces fluorescent lipofuscin pigments[8] which accumulate in animal cells, e.g. during ageing[7-9] and myocardial ischemia[10].

The capacity for cellular respiration is reduced during ageing in both skeletal and cardiac muscles[11,12]. The retardation in the efficiency of energy production may increase the susceptibility of muscle fibers to cellular damage. The present study was aimed at clarifying whether the lysosomal changes after exercise are age-related in cardiac and skeletal muscles.

Methods. Male NMRI-mice, aged 5 or 19 months, were made to run for 8 h with two 15-min pauses on a motor-driven treadmill[13]. The running speed of the young mice was 13.5 m/min and that of the senescent mice 9.0 m/min. The running ability of the aged mice is diminished and hence the exercise used was strenuous enough to cause a condition of exhaustion approximately similar to that in the young mice. The mice were killed 5 or 9 days after the exertion. The skeletal muscle sample was composed of the red parts of the quadriceps femoris muscle[13]. The apex of the myocardium was used for lipofuscin determination and the remainder of the ventricles for enzymatic assays. The lipid-soluble lipofuscin content was determined as described by Tappel[8]. The fluorescence was measured with a Farrand ratio fluorometer-2 using 7-60 as the primary and 3-74 as the secondary filter. Of the lysosomal enzymes the activities of arylsulphatase, cathepsin D and β-glucuronidase were assayed[6].

Results and discussion. The accumulation of lipofuscin granules, especially in muscle cells and neurons, is the most striking of the age-associated ultrastructural changes[14]. A considerable increase in the lipofuscin content was observed in both cardiac and skeletal muscles (table). During ageing, the activity of β-glucuronidase increased in both muscle types, but that of cathepsin D only in skeletal muscle. Arylsulphatase activity was unaffected. These results confirm our earlier observations[15], and also those of Wildenthal et al.[16] on rat myocardium, indicating that the age-related lysosomal changes are selective by nature. Certain variations may, however, exist between different species.

Prolonged running induced a typical lysosomal response to exertion in the skeletal muscles of young and senescent mice (table). The degree of the response was age-related as in our earlier study[15]. We then used a shorter period of exercise and no necrosis or inflammation was observed[15]. The present results show that the exercise-induced lysosomal response is also age-related after more prolonged, exhausting exertion, which causes necrotic and inflammatory changes in the skeletal muscles of adult mice[5]. Differences between the adult and senescent mice in the inflammatory responses may affect the degree of the lysosomal response after vigorous exertion, as in this study, but not after light exercise[15]. However, the activities of lysosomal enzymes in muscle fibers increase in parallel to the severity of the myopathy[5].

We have observed that the number of autophagic vacuoles increases in surviving muscle fibers during exercise myopathy[4]. Cytochemical studies have shown that autophagosomes contain acid hydrolase activities, e.g. in denervation myopathy[17]. Autophagocytosis is stimulated in many tissues in association with sublethal injuries probably to wall off damaged organelles[18]. Reduced lysosomal response in skeletal muscle of senescent mice may reflect a decrease in cellular repair process rather than increased resistance to injurious effects. However, the lack of the lysosomal re-

Lysosomal estimates in cardiac and skeletal muscles of young and senescent mice 5 and 9 days after heavy physical exercise

Variable	Young mice	Days after exertion		Senescent mice	Days after exertion	
	Control (n = 20)	5 (n = 12)	9 (n = 11)	Control (n = 12)	5 (n = 10)	9 (n = 8)
Cardiac muscle						
Arylsulphatase	1.08 ± 0.04	1.09 ± 0.07	1.11 ± 0.05	1.19 ± 0.08	1.36 ± 0.09	1.25 ± 0.10
Cathepsin D	21.5 ± 0.7	22.6 ± 1.3	22.2 ± 1.3	24.0 ± 1.6	24.7 ± 0.8	23.7 ± 2.3
β-Glucuronidase	0.32 ± 0.01	0.36 ± 0.03	0.35 ± 0.02	0.41 ± 0.02[f]	0.44 ± 0.03	0.40 ± 0.02
Lipofuscin	2.67 ± 0.18	3.42 ± 0.44	4.30 ± 0.57[b]	4.65 ± 0.39[f]	5.45 ± 0.59	4.89 ± 0.52
Skeletal muscle						
Arylsulphatase	0.42 ± 0.03	1.39 ± 0.18[c]	0.73 ± 0.09[c]	0.40 ± 0.03	0.90 ± 0.15[b]	0.50 ± 0.05
Cathepsin D	13.3 ± 0.3	22.6 ± 1.5[c]	18.5 ± 1.2[c]	15.5 ± 0.5[f]	19.1 ± 1.1[b]	17.7 ± 1.2
β-Glucuronidase	0.17 ± 0.01	0.91 ± 0.11[c]	0.42 ± 0.06[c]	0.20 ± 0.01[e]	0.52 ± 0.08[c]	0.29 ± 0.03[b]
Lipofuscin	2.40 ± 0.19	2.64 ± 0.35	3.34 ± 0.44[a]	3.58 ± 0.35[e]	4.56 ± 0.71	3.80 ± 0.64

Enzyme activities are expressed as μmoles assayed products $\times sec^{-1} \times kg^{-1}$ fresh muscle. Lipofuscin contents are given as lipofuscin units, 1 unit corresponding to fluorescence in procedure used $\times g^{-1}$ fresh muscle. Values are means ± SE. Statistical significance: [a]$p < 0.05$. [b]$p < 0.01$. [c]$p < 0.001$ (controls/exercised groups); [d]$p < 0.05$. [e]$p < 0.01$. [f]$p < 0.001$ (young control/old control).

sponse after exercise in cardiac muscle may indicate that mouse cardiac muscle has an inherent protective mechanism against overstrain due to exertion.

The changes in lipofuscin content did not coincide with the increases in lysosomal enzyme activities. After exercise there was only a slight increase in the lipofuscin content of cardiac and skeletal muscles in the young mice, and none at all in the senescent mice. The accumulation of fluorescent pigments after exercise could be caused by lipid peroxidation associated with heavy exercise. Further studies are necessary to verify this hypothesis.

1 This study was supported by the grants from the Ministry of Education and the Academy of Finland.

2 H.-J. Schumann, Zentbl. allg. Path. path. Anat. *116*, 181 (1972).
3 H.J. Hecht, H.-J. Schumann and D. Kunde, Med. Sport, Berl. *15*, 270 (1975).
4 A. Salminen and V. Vihko, unpublished observations.
5 V. Vihko, J. Rantamäki and A. Salminen, Histochemistry *57*, 237 (1978).
6 V. Vihko, A. Salminen and J. Rantamäki, Pflügers Arch. *378*, 99 (1978).
7 D.F. Travis and A. Travis, J. Ultrastruct. Res. *39*, 124 (1972).
8 A.L. Tappel, in: Pathobiology of cell membranes, vol. 1, p. 145. Eds B.F. Trump and A.U. Arstila. Academic Press, New York 1975.
9 B.E. Leibovitz and B.V. Siegel, J. Geront. *35*, 45 (1980).
10 H. David, Zentbl. allg. Path. path. Anat. *124*, 305 (1980).
11 A. Bass, E. Gutmann and V. Hanzlikova, Gerontologia *21*, 31 (1975).
12 R. Harris, in: The physiology and pathology of human aging, p. 109. Eds R. Coldman and M. Rockstein. Academic Press, New York 1975.
13 A. Salminen and V. Vihko, Pflügers Arch. *389*, 17 (1980).
14 J. Aune, Interdiscipl. Topics Geront. *10*, 44 (1976).
15 A. Salminen and V. Vihko, Acta physiol. scand. *112*, 89 (1981).
16 K. Wildenthal, R.S. Decker, A.R. Poole and J.T. Dingle, J. molec. cell. Cardiol. *9*, 859 (1977).
17 S. Schiaffino and V. Hanzlikova, J. Ultrastruct. Res. *39*, 1 (1972).
18 J.L.E. Ericsson, in: Lysosomes in biology and pathology, vol. 2, p. 345. Eds J.T. Dingle and H.B. Fell. North-Holland, Amsterdam 1973.

Regioselectivity in the oxidative deamination of 2-methyl-1,4-diaminobutane catalyzed by diamine oxidases[1]

E. Santaniello, Ada Manzocchi, P.A. Biondi and Tatjana Simonic

Istituto di Chimica, Facoltà di Medicina, Università di Milano, Via Saldini, 50, I-20133 Milano (Italy), and Istituto di Fisiologia Veterinaria e Biochimica, Università di Milano, Via Celoria, 10, I-20133 Milano (Italy), 30 October 1981

Summary. Diamine oxidase from pea seedlings (PDAO) catalyzes the oxidation of 2-methyl-1,4-diaminobutane in a regioselective fashion, whereas diamine oxidase from pig kidney (KDAO) shows no regioselectivity for the same reaction.

Diamine oxidases (DAO) (E.C. 1.4.3.6., diamine:oxygen oxidoreductase, deaminating) are enzymes which catalyze the oxidative deamination of a variety of diamines[2], including 1,4-diaminobutane (putrescine, Pu) and 1,5-diaminopentane (cadaverine, Ca):

$$H_2N\,CH_2(CH_2)_nCH_2NH_2 + O_2$$
$$\rightarrow H_2N\,CH_2(CH_2)_nCHO + NH_3 + H_2O_2$$
$$n=2: \text{Pu};\ n=3: \text{Ca}$$

Pea seedling DAO (PDAO) and pig-kidney DAO (KDAO) have been among the most extensively studied from plant and animal sources respectively.

Kinetic data on the oxidation of 2-hydroxyputrescine (OH-Pu) and 2-hydroxycadaverine have pointed out certain differences in the active site of these 2 enzymes[3,4]. Indirect findings concerning the regioselectivity came from experiments with OH-Pu, which suggested that both enzymes preferentially attack the amino group more distant from the centre of asymmetry[3].

The aim of this work has been to throw more light on PDAO and KDAO catalytic action, giving new information about their regioselectivity. For this purpose a branched chain diamine, 2-methylputrescine (MePu) was used; MePu was expected to be oxidized by DAO to 2 possible aminoaldehydes, 2- and 3-methyl-4-aminobutanal, both compounds being in equilibrium with the corresponding methyl-Δ^1-pyrrolines. In order to obtain stable derivatives of such reactive compounds, the method of Sakamoto and Samejima[5] seemed the most suitable. According to this procedure, the condensation with 2-aminobenzaldehyde (OAB) and the subsequent oxidation of the formed quinazolinium salt afforded 1'- or 2'-methyl-2,3-trimethylene-4(3H)quinazolone, which were analyzed by ^{1}H-NMR-spectroscopy.

Materials and methods. MePu was prepared from 3-methyladipic acid according to a previously described procedure[6]; OAB was obtained by reducing o-nitrobenzaldehyde with ferrous sulphate[7]. PDAO was extracted from pea seedlings grown in the dark for 10 days, following the method of Hill[8] up to step IV, and then stored at -20 °C in 0.01 M phosphate buffer pH 7.0; KDAO was purchased by Sigma Chem. Co. (USA) and used without further purification.

Enzyme activities were assayed using the colorimetric method introduced by Naik et al.[9], except that the calibration curve was obtained with synthetic Δ^1-pyrroline, produced by acid hydrolysis of γ-aminobutyraldehyde diethylacetal (Aldrich). Methyl-Δ^1-pyrrolines, used for NMR-studies, were obtained by preparative scale incuba-

Specific activity of DAO[a]

Substrate	Preparation	Enzyme source	
		Pea seedlings	Pig kidney
Pu	1	500	1.1
	2	420	2.2
MePu	1	155 (31%)[b]	0.24 (22%)
	2	117 (27%)	0.60 (27%)

[a] Specific activity is expressed as mU/mg; 1 unit is defined as that amount of enzyme which catalyzes the oxidation of 1 µmole of substrate per min at 37 °C. [b] Percent of specific activity with Pu.