# ROLE OF LYSOSOMES IN CHANGES IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN THE RAT LIVER DURING INTENSIVE EXERCISE

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Physical exercise leads to activation of lysosomes, and the degree of this activation depends on the intensity of the exercise. Changes in the lysosomes, it is assumed, are biologically useful and are aimed at adaptive reorganization of intracellular metabolism and structures.

In the investigation described below an attempt was made to study the concrete mechanisms of the role of lysosomes in this reorganization. For this purpose, changes in activity of glucose-6-phosphae dehydrogenase (G6PDH), the key enzyme of the pentose phosphate pathway, were studied in the rat liver during physical exertion and in the course of the recovery period after exertion in intact animals and after inhibition of certain functions of the lysosomes.

## EXPERIMENTAL METHOD

Female Wistar rats weighing 180-220 g were used. Physical exertion consisted of swimming carrying a load equivalent to 4% of the body weight for 3.5 h in water at a temperature of 30-32°C. The rats were decapitated immediately after swimming (group 1) and 6, 12, 16, 24, and 48 h after swimming (groups 2, 3, 4, 5, and 6, respectively). The liver was homogenized in 0.25 M sucrose in Tris-HCl buffer, pH 7.4 (ratio 1:5 w/v). The homogenate was subjected to differential centrifugation to obtain subcellular fractions [4]. Free and total (in the presence of Triton X-100) activity of marker lysosomal enzymes was determined in the total homogenate and in the subcellular fractions: acid phosphatase (by hydrolysis of β-glycerophospate) and cathepsin D (by degradiation of hemoglobin). The nuclear fraction obtained at 800g was again carefully washed by centrifugation in 2.4M sucrose [10]. To estimate the degree of this contamination by mitochondrial or cytoplasmic proteins, asuccinate dehydrodenase (SDH) [3] and tyrosine aminotransferase [5] activity was determined. G6PDH activity was determined [8] in the supernatant fraction (105,000 g). To block protein synthesis *de novo* actinomycin D (0.1 mg/kg) was used, and translocation of the lysosomes was inhibited by vinblastine (0.1 mg/kg) or colchicine (0.2 mg/kg); proteolytic activity of the lysosomes was inhibited by gordox (1 ml/100g). All preparations was injected intraperitoneally 1 h before swimming. The significance of differences was estimated by Student's test.

## EXPERIMENTAL RESULTS

Free acid phosphatase and cathepsin D activity increased significantly in the liver homogenate during physical exertion, but total activity was virtually unchanged (Table 1). Determination of acid hydrolase activity in the subcellular fractions showed that most activity in the liver of the control animals was bound with the fraction of "light" mitochondria, rich in lysosomes, and activity bound with the fraction of "heavy" mitochondria was rather lower. Immediately after swimming significant redistribution of activity was observed into the "heaviver" fraction. Under the influence of physical exertion, processes of autophagy are evi- laboratory showed that the fall in G6PDH activity in the liver during stress is effected through a cAMP-dependent mechanism [2]. This explains the fall in activity of this enzyme immediately after swimming.

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TABLE 1. Changes in Activity of Lysosomal Enzymes (in μmoles substrate/min/mg protein) in Subcellular Fractions of Rat Liver after Physical Exercise (M ± m)

| Fraction | Parameter studied | Acid phosphatase | | | | Cathepsin | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | control | R | experiment | R | control | R | experiment | R |
| Homogenate | Free activity | 2,36±0,12 (10) | | 4,36±0,26** (10) | | 0,225±0,02 (10) | | 0,314±0,027* (10) | |
| | Total activity | 20,4±0,92 (10) | 1,0 | 19,2±1,20 (10) | 1,0 | 1,092±0,143 (10) | 1,0 | 0,949±0,102 (10) | 1,0 |
| Nuclear | The same | 9,36±0,69 (6) | 0,46 | 12,3±1,02* (7) | 0,64 | 0,47±0,04 (6) | 0,43 | 0,71±0,06** (7) | 0,75 |
| "Heavy" mitochondria | » » | 27,3±0,71 (6) | 1,34 | 30,0±0,50** (7) | 1,56 | 1,97±0,17 (6) | 1,80 | 2,53±0,13* (7) | 2,67 |
| "Light" mitochondria | » » | 49,3±3,30 (6) | 2,42 | 34,9±1,82** (7) | 1,82 | 3,30±0,13 (6) | 3,02 | 2,19±0,18** (7) | 2,31 |
| Microsomes | » » | 5,6±0,09 | 0,27 | 5,1±0,05 | 0,27 | 0,22±0,05 | 0,20 | 0,19±0,03 | 0,20 |
| Cytosol | » » | 2,0±0,03 (6) | 0,10 | 2,0±0,07 (7) | 0,10 | 0,20±0,04 (6) | 0,18 | 0,17±0,03 (7) | 0,18 |

Legend. R) ratio of specific enzyme activity in fraction to activity in whole homogenate; number of animals shown in parenthesis.

*) Differences compared with control significant at $P < 0.05$ level; †) at $P < 0.01$ level.

TABLE 2. Changes in G6PDH Activity (in μmoles substrate/min/mg protein) during Physical Exercise and in Recovery Period After Swimming (M ± m)

| Experimental conditions | Control (k) | After swimming | | | | | |
|---|---|---|---|---|---|---|---|
| | | immediately after swimming (1) | 6 h (2) | 12 h (3) | 16 h (4) | 24 h (5) | 48 h (6) |
| Intact animals | 26,8±0,88 (44) | 22,4±1,41 (19) $P_{-1}<0,05$ | 34,2±2,63 (11) $P_{K-2}<0,05$ $P_{2-1}<0,01$ | 22,4±1,48 (9) $P_{-3}<0,05$ | 31,1±1,52 (18) $P_{-4}<0,05$ $P_{4-3}<0,01$ | 27,0±2,75 (15) | 24,6±1,90 (11) |
| Receiving actinomycin D (a) | — | — | 23,2±2,88 (5) $P_{2-2a}<0,05$ | — | 21,8±2,57 (7) $P_{4-4V}<0,01$ | — | — |
| Receiving vinblastine (v) | — | — | 25,6±2,54 (11) $P_{2-2V}<0,05$ | — | 20,8±2,34 (7) $P_{4-4C}<0,01$ | — | — |
| Receiving colchicine (c) | — | — | 24,3±3,60 (5) $P_{2-2C}<0,05$ | — | 21,8±2,46 (5) $P_{4-4}<0,01$ | — | — |
| Receiving gordox (g) | — | — | 21,1±2,16 (5) $P_{2-2}<0,01$ | — | 23,8±2,57 (7) $P_{4-4}<0,05$ | — | — |

Legend. Number of animals shown in parenthese.

idently intensified in the liver and the quanity of the "heavier" forms of secondary lysosomes is increased.

Particular attention must be paid to the fact that activity of acid phosphatase and cathepsin D in nuclei isolated from the rat liver after swimming was significantly higher than in the control. Meanwhile activity of the mitochondrial makrker enzyme SDH, and of the cytosol enzyme tyrosine aminotransferase in highly purified preparations of the nuclei were measured in trace amounts, and did not increase after swimming. Probably in this case specific translocation of lysosomes toward the nucleus took place. Besides an increase in permeability of the lysosomal membranes, shown by an incrase in free acid hydrolase activity in the homogenate, this state of affairs is also evidence that lysosomes participate in the induction of protein synthesis during physical exercise. This view is supported also by data showing that proteinases with a pH-optimum close to neutral, capable of hydrolyzing nuclear proteins, including histones [6, 7], were found in the lysosomes. Activation of the lysosomal apparatus during physical exercise may thus be aimed at initiating adaptive synthesis of intracellular proteins. This suggestion is confirmed by observations made by other workers also [9].

To study the role of lysosomes in the adaptive reorganization of metabolism, changes in G6PDH activity were studied in the course of the recovery period after physical exercise and during the action of specific inhibitors on the lysosomes. As Table 2 shows, G6PDH activity in the liver underwent phasic changes: Immediately after swimming a significant decrease in the activity of this enzyme was observed. By 6 h it was increased by 1.5 times, but by 12 h it had fallen again to minimal values. A second rise in G6Pdh activity was observed by 16 h, and by 24 h it had fallen again to the control level. Previous investigations in the writers'

Injection of actinomycin D 1 h before swimming prevented the appearance of both peaks of enzyme activity, evidence of its synthesis *de novo* (Table 2). To study the possible role of the lysosomal apparatus in induction of G6PDH synthesis, vinblastine or colchicine was injected in a series of experiments into the rats 1 h before swimming. The G6PDH activity of these animals fell by 15-18% relative to the control after swimming and remained at this level at all subsequent times of observation. A similar effect was produced by gordox, an inhibitor of lysosomal proteolytic activity. The inhibitors used in this investigation had no action on G6PDH in experiments *in vitro*.

It can thus be concluded from these results that intensive physical exercise leads to induction of synthesis of G6PDH, the key enzyme of the pentose phosphate pathway, in the rat liver. Initiation of this synthesis is preceded by activation of lysosomes and there translocation toward the nucleus. The effect of blockers of lysosomal translocation and of the inhibitor of proteolytic activity confirms the writers' hypothesis that lysosomes participate in the mechanism of induction of enzyme protein synthesis *de novo*. Since the actinomycin block abolishes the inducing effect of lysosomes, lysosomal enzymes most probably intervene at the transcription level.

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# SEROTONIN CONTENT IN DIFFERENT PARTS OF THE BRAIN, LIVER, INTESTINE, AND BLOOD OF RATS PREDISPOSED AND NOT PREDISPOSED TO ALCOHOL CONSUMPTION


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The contradictory nature of results obtaine during the study of the effect of ethanol on the serotoninergic system during exposure to the acute and chronic action of alcohol, and pharmacologic analysis of the role of this system in the regulation of voluntary alcohol consumption have not provided an unequivocal answer to the question of its role in the mechanisms of alcohol motivation [9]. The possiblity of preliminary selection of animals (noninbred albino rats) predisposed and not predisposed to alcohol consumption, on the basis of the duration of alcohol narcosis [3], discovered previously, makes it possible to study the


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