Protein synthesis and cyclic GMP content in rat cardiac muscle after swimming exercise

H. Kainulainen *, J. Komulainen, E. Ahomäki, T. Takala[a] and V. Vihko

Department of Cell Biology, University of Jyväskylä, SF-40100 Jyväskylä and [a] Department of Sport Science, Deaconess Institute of Oulu, SF-90100 Oulu (Finland)
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Summary. Rats were exercised for 6 h by swimming. Phenylalanine incorporation into myocardial proteins was increased when 2 h had elapsed after the termination of exercise. Cyclic GMP concentration did not change during the experiment, which indicates that cyclic GMP does not act directly as a trigger of myocardial protein synthesis in volume overload.
Key words. Protein synthesis; cyclic GMP; myocardium; exercise; rat.

Cardiac hypertrophy is a well-known consequence of increased pressure or volume overload of the heart. The total protein synthesis in the myocardium decreases during physical exercise [1, 2] and during the first hours of aortic constriction [3], but usually it increases in other models of experimental cardiac pressure overload [3]. The trigger mechanism(s) that accelerates protein synthesis is not known. Several factors have been proposed to be the trigger, e.g. fiber stretch or pressure on the nuclei, increased polyamine concentrations, elevated creatine and decreased ATP levels, catecholamines and cyclic AMP [3] or so far unknown intrinsic mediators [4, 5]. In a recent study by Nichols and Gonzales [6] an increased cyclic GMP (cGMP) content was found in the rat heart 4–96 h after aortic stenosis. They proposed that the renin-angiotensin system, by elevating the cGMP level, could be involved in the translation of mechanical stress into the biochemical events leading to myocardial hypertrophy. In order to test the role of cGMP in a volume overload or physiological model we measured protein synthesis and cGMP contents in rat hearts immediately after swimming exercise and after recovery periods of 2 and 4 h.

Materials and methods. 39 male Sprague-Dawley rats, aged 13 weeks, were divided into four groups, of which one served as a control group and the rest were exercised by swimming for 6 h (+ 32 °C, 15 min break after every 2 h). One group was killed immediately after the exercise, one after 2 h and one after 4 h of rest. One half of the animals in each group was used for the measurements of cardiac protein synthesis and the other half for cardiac cGMP measurements.

The protein synthesis from the hearts of anesthetized and heparinized rats was measured during Langendorff-perfusion as L(U-^{14}C)phenylalanine incorporation rate essentially as described by Takala [7] and Kainulainen et al. [8] in the atria and in the right and left ventricles. For cGMP measurements, ether-anesthetized and heparinized animals were also used, and the left ventricles were immediately dissected and frozen in liquid nitrogen. Cyclic GMP was measured by radioimmunoassay (cGMP RIA Kit, The Radiochemical Centre Ltd., Amersham, UK) from diethylether extracts of trichloracetic acid homogenates. Protein was assayed by the method of Lowry et al. [9]. The differences between group means were statistically compared by the Student's t-test.

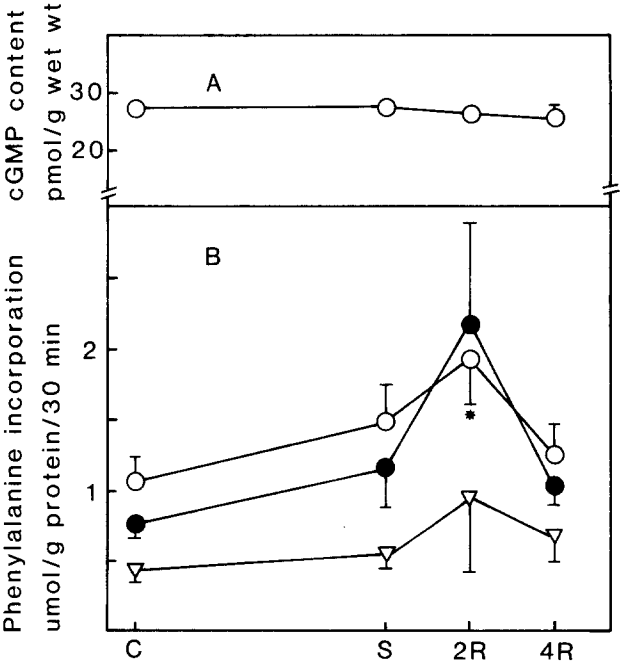
Results and discussion. There were no differences in the weights of ventricles, atria and body between the experimental groups (table).

Phenylalanine incorporation into the left and right ventricles and the atria and cGMP concentrations in the left ventricles are shown in the figure. Cyclic GMP content did not change during the experiment. Phenylalanine incorporation into the myocardium increased after 2 h rest, being significantly higher ($p < 0.05$) in the left ventricle.

In the study of Swartman et al. [1] – using phenylalanine as a marker of protein synthesis in isolated perfused rat hearts – the total myocardial protein synthesis was depressed after exhaustive swimming. The total protein synthesis returned to the control level in 2 h, but myofibrillar synthesis was at the control level after a 1-h recovery period and increased after a 2-h recovery period. Phenylalanine incorporation into the total myosin and into myosin heavy and light chains already increased immediately after the exhaustive exercise. All values of amino acid incorporation rates were at the control level after a 4-h recovery period. These results of Swartman et al. [1] show a preferential order for changes of the synthesis rates in different myocardial protein fractions. In our study, no depression in the protein synthesis was found immediately after the exercise, and the total protein synthesis was increased after 2 h rest in the left and right ventricles and atria, this increase was, however, only significant in the left ventricle. The rats swam for 6 h with two breaks and were not exhausted. This may explain the difference between our results and those of Swartman et al. [1]. The increased total protein synthesis after 2 h rest may focus on the synthesis of the myofibrillar proteins.

Group	n	Body (g)	Left ventricle (mg)	Right ventricle (mg)	Atria (mg)
C	10	408 ± 8	820 ± 22	251 ± 8	135 ± 5
S	10	403 ± 8	873 ± 24	248 ± 7	119 ± 7
2R	9	408 ± 7	870 ± 23	243 ± 11	139 ± 5
4R	10	406 ± 9	833 ± 41	244 ± 14	126 ± 9

Weight variables. C = sedentary controls, S = 6 h swimming, 2R = 2 h rest after swimming, 4R = 4 h rest after swimming; means ± SEM.



Panel A: cGMP levels in the left ventricles. Panel B: Phenylalanine incorporation into the different regions of the cardiac muscle. Experimental groups as in the table; (○) left ventricle, (●) right ventricle, (▽) atria; means ± SEM; * $p < 0.05$ compared with control.

Glycine and leucine incorporation into total myocardial proteins of the rat heart in vivo has been reported to decrease already after 1 h of swimming, and to return to the control level 2 h later[2]. The differences in the apparent protein synthesis rates may partially depend on the indicator amino acid used. In the measurements of cardiac muscle protein synthesis, phenylalanine is a very suitable monitor, because it e.g. penetrates the plasma membrane rapidly, it is non-metabolizable in myocardial tissue, and because the specific activity of the phe-tRNA reaches that of the extracellular phenylalanine when the latter is used at concentrations high enough[10,11].

The trigger mechanism of the protein synthetic processes involved in cardiac hypertrophy is not known, and several candidates have been proposed for it, as mentioned above. Nichols and Gonzales[6] proposed that elevated cGMP level could be the trigger in the hypertrophy caused by pressure overload. This is not probable in the case of volume overload, because we did not find any change in the cGMP content of the myocardial tissue after the exercise or during the 4-h recovery period, although the incorporation of phenylalanine into the total cardiac proteins transiently changed during that time.

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* Present address (for reprint requests); Department of Clinical Sciences, University of Tampere, P.O. Box 607, SF-33101 Tampere.

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The effect of hypoxia on hepatic cytochromes and heme turnover in rats in vivo

A. L. Jordi-Racine, E. Alvarez* and J. Reichen

*Department of Clinical Pharmacology, University of Bern, CH-3012 Bern (Switzerland), and *Department of Medicine, University of Colorado Medical School, Denver (Colorado, USA)*
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Summary. We evaluated the effect of hypoxia (7% v/v) on hepatic heme turnover in vivo and microsomal heme protein content in male Sprague-Dawley rats. Hepatic heme protein turnover, measured as ^{14}CO-production during continuous infusion of 5-^{14}C-aminolevulinic acid, a precursor of nonerythrogenic heme, was decreased 60% during hypoxia and returned to control levels promptly after reoxygenation. Hepatic cytochrome P-450 content was decreased in hypoxic and 24-h reoxygenated animals. We conclude that normobaric hypoxia decreases hepatic cytochrome P-450 which could contribute to decreased drug metabolism in hypoxia. This decrease is probably due to heme oxygenase-independent breakdown of hepatic heme.
Key words. Cytochrome P-450; heme oxygenase; cytochrome c reductase; hemoproteins; liver.

Hypoxia affects the handling of different drugs, e.g. of theophylline, in experimental animals[1] and man[2]. Hypoxia also prolongs hexobarbital sleeping time in the rat[3]. In the perfused liver a critical level of 45 mm Hg has been shown to be required before any changes in metabolism occur[4]. Normobaric hypoxia leads to a biphasic response of the hemoprotein class of cytochromes P-450, the main hepatic drug oxidizing system[5]. Hypobaric hypoxia, by contrast, induces cytochrome P-450 together with heme oxygenase, the main heme catabolizing enzyme[6].

How hypoxia affects hepatic heme metabolism is unknown, however. We therefore investigated hepatic heme turnover and cytochrome P-450 content by biosynthetically labeling the hepatic heme pool with 5-^{14}C-aminolevulinic acid and measuring ^{14}CO-output, a specific and sensitive method to measure heme oxygenase-dependent hepatic heme turnover[7,8].

Methods. Male Sprague-Dawley rats were obtained from the Charles River Breeding Laboratories, Wilmington, MA and maintained in temperature and humidity controlled animal quarters on a 12-h light-dark cycle. They were allowed access to standard rat chow and tap water ad libitum. The animals were acclimatized to the altitude of Denver (1610 m) for at least 3 weeks. At the time of the study, they weighed 260–280 g.

For the hypoxia studies, the animals were fitted with a chronic indwelling jugular catheter and kept in Bollman type metabolic cages as described previously[8]. Immediately after surgery, 5-^{14}C-aminolevulinic acid (sp. act. 48.9 mCi/mmol from Research Products International, Elk Grove, IL) was infused over 3 days to label the hepatic heme pool[8]. ^{14}CO-production was measured during continuous chronic infusion of 5-^{14}C-aminolevulinic acid; ^{14}CO was trapped in ethanolamine after catalytic conversion to $^{14}CO_2$ as previously described[8,9]. During a control period of 24 h compressed air (oxygen 21% v/v) was passed through the cages at a rate of 1 l/min. Thereafter, half of the animals were given a mixture of nitrogen/oxygen 93:7 (v/v) while an equal number of control animals continued on room air (n = 6/group). The gas composition was chosen after preliminary experiments had shown that at higher oxygen concentrations (8% v/v) no changes occurred, whereas the animals did not survive oxygen concentrations of 5 or 6% (v/v). Thereafter, ^{14}CO production was measured for a further 72 h. Then, the