HYDROPEROXIDE AND O_2^- UTILIZING ENZYMES IN THE RAT MYOCARDIUM AT DIFFERENT AGES

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KEY WORDS: myocardium; aging; glutathione peroxidase; superoxide dismutase; catalase.

A key factor in the mechanisms of disturbance of functions of biological membranes during aging [8, 11] and in the development of cardiovascular pathology [2, 8] is lipid peroxidation (LPO). The intensity of this process in the cell is under the control of superoxide dismutase (SOD) and enzymes of hydroperoxide destruction [11], as well as the system of lipid-solubie antioxidants [1]. It was shown previously that the intensity of LPO in rat heart homogenates diminishes with age [5]. Meanwhile during aging the antioxidant activity of lipid extracts from myocardial tissue falls considerably [1]. It can accordingly be postulated that age changes in the intensity of LPO in the myocardium are mainly determined by the state of the antiperoxide enzyme system [6].

The aim of the present investigation was to obtain further experimental confirmation of the above hypothesis by studying activity of SOD and hyperoxide utilizing enzymes in the rat myocardium at different ages.

EXPERIMENTAL METHOD

Male Wistar rats aged 1, 3, 12, and 24 months were used in experiments during the fall. After decapitation of the animals the heart was cooled in ice-cold 0.25 M sucrose. The myocardium was cut into small pieces with scissors, washed to remove blood, and squeezed through a press. The resulting mince (about 0.5 g) was homogenized in 10 ml of medium consisting of 0.3 M sucrose, 10 mM Tris-HCl buffer, 1 mM EDTA, pH 7.4. The homogenate was centrifuged for 1 h at 105,000g on a BAK-601 centrifuge (East Germany). The supernatant (cytosol) was immediately frozen in liquid nitrogen in 0.5-ml polyethylene ampules. All the experimental material was obtained in the course of 3 days, so that possible variability of the data due to seasonal changes and other factors could be ruled out. Before measurement of enzyme activity the ampules were heated in a waterbath at 37°C.

SOD activity of the cytosol was measured as inhibition of reduction of neotetrazolium in a xanthine–xanthine oxidase O_2^--generating system [9]. Glutathione peroxidase (GP) activity was determined spectrophotometrically at 340 nm [3] in 50 mM Na,K-phosphate buffer, pH 7.4, 1 mM EDTA, 0.15 mM NADPH, 0.5 unit of yeast glutathione reductase, 3 mM Na azide, 0.04 mg protein in 1 ml. tert-Butyl and cumene hydroperoxides were added in a concentration of 1.2 mM and H_2O_2 in a concentration of 0.8 mM. Catalase activity was measured spectrophotometrically at 240 nm [12] in 10 mM K-phosphate buffer, pH 7.4, 0.1 mM EDTA, 15 mM H_2O_2, about 0.2 mg protein in 1 ml. All measurements were made on a Specord UV VIS spectrophotometer (East Germany) at 37°C.

EXPERIMENTAL RESULTS

Since the experiments were carried out on specimens of heart cytosols, preserved by freezing, data are given in Table 1 to show the effect of freezing on enzyme activities studied. Cytosol from animals aged 1 month was used in these experiments. The conditions of freezing and thawing selected had no harmful action on GP, SOD, or catalase.

Only the catalase activity of the myocardium was shown to remain virtually unchanged with the rats' age (Table 2). SOD activity, which did not change significantly between the ages of 1 and 3 months, increased

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TABLE 1. Effect of Freezing and Thawing on Activity of SOD, GP, and Catalase from Cardiac Cytosol of Rats Aged 1 month (M±m)

Enzyme	Activity, %	
	control	experiment
SOD	100±10	103±9
GP (H_2O_2)	100±6	99±3
GP (cumene hydroperoxide)	100±3	93±5
Catalase	100±4	104±6

Legend. n = 6.

TABLE 2. Age Changes in Catalase, SOD, and GP Activity of Rat Myocardial Cytosol (M±m)

Enzyme	Age, months			
	1	3	12	24
Catalase, μmoles H_2O_2/mg protein/min	22,8±1,6	26,4±0,9	26,3±1,3	25,5±1,3
SOD, units/mg protein	70,7±7,5	73,6±13,0	103,0±9,3*	109,9±6,9*,**
GP, nmoles NADPH/mg protein/min				
with H_2O_2	426±10	606±27*	707±27*,**	644±23*
with cumene hydroperoxide	473±31	656±36*	753±24*,**	709±29*
with tert-butyl hydroperoxide	416±27	603±27*	722±28*,**	647±27*

Legend. *P < 0.05 compared with animals aged 1 month, **P < 0.05 compared with animals aged 3 months; n = 6-7.

in the later period of development and during aging. GP activity, when hydroperoxides of a different nature were used, increased significantly in the pubertal period, and this tendency continued until middle age. GP activity in old rats did not statistically differ significantly from that in middle age or in rats aged 3 months. The velocity of enzymic glutathione-dependent hydroperoxide destruction thus reaches a maximum in the myocardium of middle aged rats, and remains virtually unchanged during subsequent aging.

These data are evidence of enhanced enzymic protection of myocardial membranes against the harmful action of hydroperoxides and of superoxide radicals during postnatal development of rats. An increase in GP and catalase activities in cardiac mitochondria of rats aged 24 months compared with their activity in animals aged 3 months, and a relatively unchanged level of SOD activity were demonstrated in [13]. No significant change was observed in SOD activity in the heart of rats and mice during aging in [10], and likewise in man between the age of 20 and 80 years [14].

The increase in activity of the enzymic antiperoxide system of rats with age revealed by these experiments agrees well with data showing the fall in the intensity of LPO in myocardial homogenates toward middle age and continuation of this low level in old animals [5]. A similar relationship also was demonstrated previously for the liver [3, 6, 7, 13].

The results of this investigation thus confirm the view that activity of the enzymic antiperoxide system and its role in the protection of biomembranes against free-radical injury increase with age [6].

LITERATURE CITED

1. E. B. Burlakova, E. M. Molochkina, N. P. Pal'mina, et al., Vopr. Med. Khimii, 22, 541 (1976).
2. V. Z. Lankin, Kardiologiya, No. 8, 42 (1980).
3. V. Z. Lankin, A. K. Tikhaze, A. L. Kovalevskaya, et al., Dokl. Akad. Nauk SSSR, 261, 1467 (1981).
4. V. V. Lemeshko, P. A. Kaliman, and Yu. V. Nikitchenko, Biokhimiya, 46, 620 (1981).
5. V. V. Lemeshko and Yu. V. Nikitchenko, Byull. Eksp. Biol. Med., No. 11, 12 (1982).
6. V. V. Lemeshko, Yu. V. Nikitchenko, and P. A. Kaliman, in: Molecular and Cellular Mechanisms of Aging [in Russian], Kiev (1981), p. 101.
7. V. V. Lemeshko, Yu. V. Nikitchenko, and P. A. Kaliman, Ukr. Biokhim. Zh., 55, 523 (1983).
8. L. K. Obukhova and N. M. Emanuel', Usp. Khim., 52, 353 (1983).

9. C. Beauchamp and I. Fridovich, Anal. Biochem., 44, 276 (1971).
10. E. W. Kellogg and I. Fridovich, J. Gerontol., 31, 405 (1976).
11. B. E. Leibovitz and B. V. Siegel, J. Gerontol., 35, 45 (1980).
12. S. Marklund, I. Nordensson, and O. Bäck, J. Gerontol., 36, 405 (1981).
13. H. Nohl, D. Hegner, and K.-H. Summer, Mech. Ageing Develop., 11, 145 (1979).
14. J. M. Tolmasoff, T. Ono, and R. G. Cutler, Proc. Nat. Acad. Sci. USA, 77, 2777 (1980).

CHANGES IN THE PROTEIN-SYNTHESIZING SYSTEM OF HeLa CELLS IN CULTURE IN THE PRESENCE OF TRACE ELEMENTS

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Zinc, nickel, cobalt, cadmium, and fluorine belong to the group of trace elements, because their concentration in the body does not exceed 10^{-3}-10^{-12}%. Of these elements zinc, nickel, cobalt, and fluorine are considered essential for life. For instance, zinc activates about 120 enzymes concerned with different aspects of cell metabolism [15]. There is evidence that nickel can increase and depress activity of certain enzymes both in vivo and in vitro. Cobalt is a component of vitamin B_{12}. In addition, within a certain concentration range, this trace element can block sulfhydryl groups of certain structural proteins and enzymes [3]. The action of fluorine on the cell varies, for it behaves not only as an inhibitor of many enzyme systems, but also as a powerful activator, unique among the trace elements, of the metabolically important enzyme adenylate cyclase [2]. Cadmium belongs to a group of trace elements which are toxic for the body even in very low concentrations [4]. It often behaves as an antagonist of zinc, for they compete with each other for certain intracellular ligands. However, it must be remembered that the subdivision of trace elements into essential, neutral, and toxic is very conventional, for all essential elements become toxic when they reach certain concentrations, and the differences between doses in which they are useful and dangerous may be small.

The aim of this investigation was to study the state of the protein-synthesizing system of HeLa cells in culture in the presence of certain trace elements.

EXPERIMENTAL METHOD

The cytopathic action of zinc, nickel, cobalt, cadmium, and fluorine was studied in the presence of maximal allowable concentrations (MAC) adopted for liquid media, in the form of $ZnSO_4$, $NiCl_2$, $CoCl_2$, $CdCl_2$, and NaF. These concentrations, calculated on the basis of the content of the ion of the trace element in the molecule, were (in μg/ml): Zn^{++} 1, Ni^{++} 0.1, Co^{++} 1, Cd^{++} 0.01, and F^- 1.5. Sodium sulfate and chloride, in equimolar concentrations, calculated as sulfate and chloride ions, were added to the control cultures. Cultures without addition of salts also were studied (background control). The action of the trace elements on the cell culture was investigated for 2, 4, and 24 h. Charactistics of the method of culture, treatment of the glassware, and conduct of the experiments were described previously [1]. The dynamics of RNA and protein synthesis were studied by autoradiography. For this purpose, 30 min before the end of incubation with the elements to be studied, [^{3}H]uridine (80 mBq/ml, specific radioactivity 1040 GBq/mmole) or [^{3}H]leucine (200 mBq/ml, specific radioactivity 4 GBq/mmole) was added to the cultures of HeLa cells. Autoradiographs were prepared in the usual way and the number of tracks counted above the whole cell ([^{3}H]leucine) or above a unit of area ([^{3}H]uridine). To determine the content of ribosomal RNA (rRNA) and total protein two series of experiments were carried out with staining for RNA by Einarson's method with gallocyanin and chrome alum, and with Naphthyl Yellow for total protein. Staining for rRNA was preceded by treatment of the cells with DNAse (from Reanal, Hungary) for 2 h at 37°C. Concentrations of metabolites were determined by the logarithmic screen method on an MIF integrating photometric microscope at wavelengths of 547 nm (rRNA) and 436 nm (total protein). All numerical data were analyzed on the Nairi computer.

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