CHANGES IN ACTIVITY OF ANTIOXIDATIVE ENZYMES DURING SPERMATOGENESIS

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The need to transmit intact hereditary material to future progeny requires the presence of an effective system to protect spermatogenic cells against various harmful factors. Free radicals formed during cell function have a cytotoxic action and, in partiuclar, lipoperoxides inhibit cell division [8]. Meanwhile lipid peroxidation (LPO) may play an essential role in the regulation of cell metabolism, through its influence on transport of substances into the cell, on reception of external signals, and on oxidative phosphorylation processes [1]. Spermatozoa characteristically have a high content of one of the principal LPO substrates, namely polyunsaturated docosahexaenic acid [9], and for that reason these cells are highly sensitive to the harmful action of oxygen [7, 10]. Complex biochemical processes accompanying differentiation of the spermatogenic epithelium may be closely linked with considerable changes in the intensity of LPO and in activity of enzymes which participate in the detoxication of the products formed.

Antioxidant protective enzyme systems such as superoxide dismutase (SOD) and glutathione peroxidase (GP), which are responsible for detoxicating O_2^- anion-radicals, H_2O_2, and lipoperoxides, play a part in the regulation of LPO *in vivo* [2, 5]. Recently an active role in the utilization of lipoperoxides *in vivo* has been ascribed also to various glutathione-S-transferases (GT) [12, 13]. In view of the facts described above, it was decided to study changes in SOD, GP, and GT activity during differentiation and maturation of the spermatogenic epithelium in mice.

EXPERIMENTAL METHOD

Experiments were carried out on mice weighing 30 ± 2 g. To obtain a cell suspension the seminiferous tubules were subjected to enzyme treatment with collagenase, trypsin, and DNase by the method in [14]. Isolated cells were fractionated in a "STAPUT" system [11] in a 1-3% gradient of human serum albumin for 4 h. Fourteen fractions each of 50 ml were collected through the bottom of the system. Cells composing the fractions were identified by the thymidine test [6] and by light and electron microscopy.

As a result of fractionation of the spermatogenic epithelium of the mice cell suspensions containing mainly spermatogonia, early primary spermatocytes, and Sertoli cells (fractions 4-6), late pachytene spermatocytes (fractions 1-3), early spermatids (fractions 7-9), middle and late spermatids (fractions 10-12), and residual bodies (fractions 13, 14) were obtained.

Ripe spermatozoa were obtained from the epididymis of the animals and washed once in distilled water and twice in Dulbecco—Vogt salt medium.

To determine enzyme activity the cells were disintegrated by rapid freezing and thawing and the membranes were sedimented by centrifugation at 6000 g for 20 min. SOD activity was determined as inhibition of reduction of nitro-BT in a xanthine—xanthine oxidase system [3], and GP activity was determined as oxidation of NADPH in a coupled glutathione reductase system, using tert-butyl hydroperoxide as substrate [3]. GT activity was determined by the formation of conjugates of reduced glutathione with 1-chloro-2,4-dinitrobenzene [4]. The unit of SOD activity was taken to be the quantity of enzyme needed for 50% inhibition of nitro-BT reduction under experimental conditions; the unit of GP activity was taken to be the quantity of enzyme needed to oxidize 1 micromole of reduced glutathione in 1 min; the unit of GT activ-

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TABLE 1. SOD, GP, and GT Activity During Spermatogenesis (M ± m)

Cell composition of suspension	Fractions	SOD	GP	GT
Spermatogonia	4+5+6	51,5±0,7	8,9±0,8	32±6,5
Early primary spermatocytes	»	39,9±2,7	7,9±0,45	22±6,4
Sertoli cells	»	40,6±3,6	7,9±0,6	13±2,0
Late pachytene spermatocytes	1+2+3	53,5±5,8	13,24±1,0	62±18,0
		53,2±1,6	12,1±2,7	38±8,0
Early spermatids	7+8+9	19,2±0,59	6,7±0,2	11±2,0
		16,4±2,1	5,6±0,1	9±1,0
		15,1±0,94	6,4±0,55	12±2,0
Middle and late spermatids	10+11+12	19,0±1,9	7,8±0,35	26±7,0
		21,3±1,35	8,7±0,06	26±7,0
		20,8±0,5	8,4±0,3	26±7,0
Residual bodies	13+14	21,1±0,1	9,1±0,2	46±4,0
		29,7±0,8	12,2±1,0	55±1,4
Spermatozoa	—	19,8±0,5	9,9±0,7	20,0±1,4

ity was taken to be the quantity of enzyme required to conjugate 1 nanomole of reduced glutathione per minute.

The kinetics of the enzyme reactions was studied on an LKB (Sweden) 2086 reaction velocity analyzer at 25°C. Protein in the enzyme preparations was determined by Lowry's method. The experiments were repeated three times, observing the experimental conditions specified above.

EXPERIMENTAL RESULTS

SOD activity in spermatogenic cells was comparable with activity of this enzyme in the liver — a tissue with a high level of antioxidant enzyme activity [2]. Meanwhile GP activity in the spermatogenic epithelium was more than an order of magnitude lower, and GT activity more than an order of magnitude higher than in the liver [4]. The high GT activity can maintain effective detoxication and removal of endogenous and exogenous hydrocarbons from the spermatogenic epithelium, some of which are known to be carcinogens. By means of GT, endocrine regulation of spermatogenesis may also be effected, for this enzyme participates in the binding and elimination of certain steroids. Utilization of lipoperoxides in these cells may perhaps take place mainly by a glutathione transferase, and not a glutathione peroxidase mechanism.

The results showed that activity of the enzymes studied during spermatogenesis varied considerably. In premeiotic cells (spermatogonia, early primary spermatocytes, fractions 4-6) and, in particular, in late pachytene spermatocytes (fractions 1-3), in which complex processes connected with exchange of genetic material between homologous chromosomes take place and the cells are prepared for meiotic division, high activity of these enzymes was observed (Table 1). In the postmeiotic period (early spermatids, fractions 7-9) their activity fell sharply (by 2.8-3.5, 1.9-2.3, and 4.2-5.6 times, respectively for SOD, GP, and GT). In the period of spermatogenesis considerable morphological and biochemical reorganization of the spermatogenic cells (middle and late spermatids, fractions 10-12) was accompanied by an increase in activity of SOD, GP, and, in particular, GT. Significant enzyme activity was found in all fractions containing cytoplasm removed during spermatogenesis from spermatids (the fraction of residual bodies). Enzyme activity in the ripe sex cells was comparable with their activity in late spermatids.

The results thus point to the existence of a highly active system protecting the spermatogenic epithelium against the cytotoxic action of active forms of oxygen, lipoperoxides, and xenobiotics, and they also indicate considerable changes in activity of antioxidant enzyme systems, observed during differentiation and maturation of spermatogenic cells.

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DYNAMICS OF NITRITE-INDUCED METHEMOGLOBIN FORMATION AFTER TOTAL GAMMA-RAY IRRADIATION OF RATS

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One trend in the design of atomic power stations (APS) based on fast neutrons is the development of APS in which dissociating nitrogen tetroxide is used as heat carrier and as working heat. The multifactor nature of the radiational and toxic action (ionizing radiation, nitrogen oxides and their transformation products, mainly nitrates and nitrites) determines the need for solution of a number of medical biological problems, one of which is the study of the combined action of ionizing radiation and of sodium nitrite on methemoglobin formation, associated with the action of nitrites as a metabolic product of nitrogen oxide metabolism.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. The animals were kept under animal house conditions on a standard diet. Single whole-body irradiation was given on the UGU-420 gamma-ray source in doses of 77.4 and 180.6 mCi/kg body weight with a dose rate of $1.64 \cdot 10^{-4}$ A/kg. Sodium nitrite (chemically pure) dissolved in physiological saline was injected intraperitoneally (7 mg/100 g body weight) 1, 3, 7, and 15 days after irradiation. The animals were killed under superficial ether anesthesia by bleeding from the heart. The methemoglobin concentration was determined 15, 45, 60, 90, and 180 min after injection of sodium nitrite [1].

The dynamics of methemoglobin formation and reduction was determined in hemolysates [2]. Oxidation of hemoglobin to methemoglobin was carried out with glucose and methylene blue [9-11].

The dynamics of changes in the methemoglobin concentration was monitored on a VSU-2-P spectrophotometer at 540-630 nm. The hemoglobin content in the hemolysates was determined by the cyanmethemoglobin method, the methemoglobin content by the method of Austin and Drabkin and by spectrophotometry [2].

EXPERIMENTAL RESULTS

Methemoglobin is present in small quantities (1-4%) in the blood of intact animals. The methemoglobin concentration in the blood of the irradiated rats increased with the time elapsing after iation in a dose of 180.6 mCi/kg, and was 4.45, 6.56, 12.2, and 8.3% on the

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