Effect of Acute Hypobaric Hypoxia on Spermatogenesis and Lactate Concentration in Testicular Tissue of Male Albino Rats

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Parameters of spermatogenesis in male albino rats was studied after experimental acute hypobaric hypoxia. The number of spermatogenic epithelial cells, Sertoli cells, and Leydig cells in testicular tissue significantly decreased in the posthypoxic period. Single exposure to hypoxia led to significant changes in lactate concentration in the testicular tissue. Cell composition of the testicles did not return to normal by the 60th day after treatment.

Key Words: *acute hypobaric hypoxia; spermatogenesis; lactate*

Germinative function consisting in division and differentiation of cells in the spermatogenic epithelium and production of spermatozoa from the spermatogonial stem cell is one of the main functions of the testicles [5]. Gametogenesis is an energy-dependent process. Testicular tissue is characterized by high-intensity glycolytic processes. Lactate, pyruvate, and glucose serve as energy substrates for maturating sex cells [8].

The concentration of lactate in the seminal fluid surpasses that of glucose and pyruvate [7]. Glucose is accumulated in the testicles, glycolytically utilized in Sertoli cells, and converted into lactate. Lactate is secreted into the lumen of seminiferous tubules [4].

Previous studies produced contradictory results regarding changes in reproductive function under conditions of acute hypobaric hypoxia.

Here we studied the effect of acute hypobaric hypoxia on spermatogenesis.

MATERIALS AND METHODS

Experiments were performed on 110 male outbred albino rats weighing 180-230 g. Experimental animals (n=80) were exposed to acute hypobaric hypoxia. Parameters of spermiogram and lactate concentration in testicular tissue were studied 40 min and 1, 3, 7, 14, 30, 45, and 60 days after hypoxia.

Thirty intact animals of the control group were examined on days 1, 30, and 60 after the start of the study.

Acute hypobaric hypoxia was modeled in a flow chamber. The rats "ascended" to a height of 11,500-12,000 m until the appearance of agonal breathing.

Activity of spermatogenesis was determined using a quantitative cytological study [2]. To this end, smears of homogenates from testicular tissue were stained by the method of Romanovsky—Giemsa. Various cells of the spermatogenic epithelium, Sertoli cells, and Leydig cells (n=500) were counted under a light microscope with an oil immersion objective. The spermiogram was constructed (percentage of testicular cells). The absolute number of cells was calculated from the absolute number of spermatozoids per 1 g testicular tissue. Spermatozoids were counted in a Goryaev chamber.

Lactate concentration in testicular tissue was measured enzymatically using lactate dehydrogenase [1]. Lactate concentration was estimated spectrophotometrically (by NADPH formation) at 340

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nm. Lactate concentration was expressed in μmol lactate per 1 g tissue.

The results were analyzed by Mann—Whitney *U* test (Statistica 6.0 software). The differences were significant at $p<0.05$.

RESULTS

We revealed an unambiguous relationship between spermatogenesis disturbances and extreme hypoxic exposure. On the 40th minute after hypoxia spermiogram of experimental animals did not differ from normal (Table 1). However, significant accumulation of lactate in testicular tissue reflected activation of glycolysis and/or decrease in utilization of pyruvic acid in the Krebs cycle after acute hypoxia (Fig. 1).

The absolute number of spermatocytes increased 2-fold on day 1 after hypoxia (Table 1). Activation of cell division was probably associated with an increase in lactate concentration in testicular tissue 40 min after hypoxia. It should be emphasized that lactate is the main metabolic substrate for dividing cells [4,6]. The counts of spermatogonia, spermatozoa, Leydig cells, and Sertoli cells in experimental rats did not differ from those in intact animals. We revealed a significant decrease in the number of early and late spermatids.

The number of various cells in the spermatogenic epithelium significantly decreased on days 3-7. A progressive decrease in lactate concentration was probably related to a reduction of Sertoli cells (Fig. 1). These cells utilize up to 95% glucose from the lumen of seminiferous tubules and serve as a major producers of lactate in germinative cells [7]. Early spermatids were not revealed on day 14 after

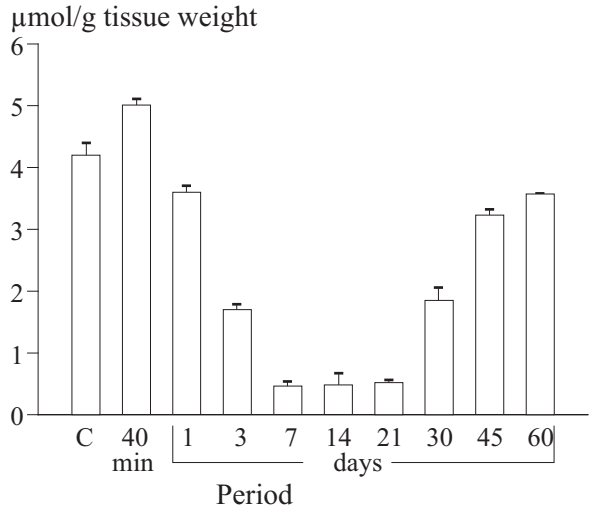

Fig. 1. Lactate concentration in testicular tissue of male albino rats at various periods after acute hypobaric hypoxia. C, control.

TABLE 1. Absolute Number of Spermatogenic Epithelial Cells, Sertoli Cells, and Leydig Cells in Testicular Tissue after Acute Hypobaric Hypoxia (*M*±*m*)

Parameter, million cells/1000 mg	Control (*n*=30)	40 min (*n*=10)	1 day (*n*=10)	3 days (*n*=10)	7 days (*n*=10)	14 days (*n*=10)	21 days (*n*=10)	30 days (*n*=10)	45 days (*n*=10)	60 days (*n*=10)
Spermatogonia	27.8±1.1	26.0±1.4	25.8±1.3	2.3±0.1*	1.2±0.1*	0.80±0.05*	2.1±0.1*	3.1±0.1*	4.2±0.2*	5.2±0.2*
Spermatocytes	132.2±7.5	124.0±4.8	260.7±10.0*	8.9±0.4*	4.6±0.3*	5.4±0.4*	7.5±0.5*	12.7±0.3*	19.5±1.6*	23.0±1.3*
Early spermatids	142.3±6.2	131.9±5.0	51.2±1.9*	1.1±0.1*	0.40±0.05*	0*	2.3±0.2*	3.6±0.4*	9.2±0.7*	13.5±0.5*
Late spermatids	147.9±5.5	141.6±3.2	73.7±1.9*	5.4±0.2*	2.8±0.1*	1.6±0.1*	4.1±0.3*	12.2±0.5*	18.9±1.1*	23.2±0.7*
Spermatozoa	81.0±1.6	79.5±1.2	78.5±1.8	32.0±1.7*	21.0±1.2*	12.5±0.8*	30.5±1.2*	33.5±1.3*	33.0±1.1*	39.0±1.2*
Sertoli cells	42.4±1.8	42.4±2.8	41.3±2.3	6.1±0.2*	3.4±0.2*	1.8±0.1*	4.3±0.3*	6.7±0.4*	8.1±0.5*	9.7±0.4*
Leydig cells	8.1±0.9	7.7±0.9	8.0±0.8	0.6±0.1*	0.30±0.06*	0.03±0.02*	0.3±0.1*	0.6±0.1*	1.0±0.2*	1.4±0.1*

Note. *$p<0.05$ compared to the control.

the incidence of acute hypoxia. Leydig cells were found only in 20% experimental animals.

A minor increase in the number of germinative cells on day 21 of the posthypoxic period was probably associated with a new stage of spermatogenic epithelium development and rise in the count of Sertoli cells providing metabolic activity of sex cells [3]. Division and maturation of testicular cells remained low on days 21-60, which was related to a small number of Sertoli cells and Leydig cells in the testicular tissue.

Our results show that acute hypobaric hypoxia is followed by pronounced and long-lasting changes in spermatogenesis. They are accompanied by a decrease in the number of spermatogenic epithelial cells, Sertoli cells, and Leydig cells.

Accumulation of lactate in testicular tissue 40 min after hypoxia maintains constant number of testicular cells and provides activation of cell division on day 1 of the posthypoxic period.

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