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NO EFFECT OF OXYGEN *IN VIVO* ON PLASMA OR TESTIS TESTOSTERONE IN RATS AND NO INDUCTION OF TESTICULAR SUPEROXIDE DISMUTASE

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SUMMARY

Male rats were exposed to 100% O_2 for 24 h in a temperature-controlled cage and compared to a control group, similarly exposed to air. No effect of O_2 was observed on plasma testosterone concentrations or on testis testosterone concentrations. The rat testis was found to contain a comparatively high level of superoxide dismutase activity, an enzyme believed to protect against the toxicity of oxygen. Oxygen did not induce the enzyme in testis under these conditions.

INTRODUCTION

Oxygen uptake and utilization by the testis bears many interesting physiological relations to the blood circulation and metabolism as well as to the exocrine and endocrine secretions of this organ[1]. In the ram a pronounced oxygen gradient of about 12 mm Hg was found, *in vivo*, between the blood leaving the testis and the tissue interstitial fluid, after having corrected for the temperature difference[2]. A short period of oxygen breathing doubled testicular oxygen tension while it was reduced by electrical stimulation of the sympathetic areas of the hypothalamus[3]. Warming the testis to body temperature, as in cryptorchidism, may damage spermatogenesis through an increase in oxygen tension[2]. Also, rats exposed to a 95–98% O_2 environment exhibited testicular degenerative changes after 2 days[4]. These changes were not affected by the administration of sodium bicarbonate although the survival rate increased. The absence of damage to the testicular exocrine function, after repeated exposure of rats to hyperbaric oxygen, led van der Brenk and Jamieson to conclude that the effect of oxygen was not related to oxygen toxicity[5]. To our knowledge no one has investigated the effect of oxygen on the testicular endocrine function, although Drosdowsky[6] showed an inhibition of the conversion of labelled progesterone to testosterone in rat testis *in vitro*.

It was previously shown in our laboratory that plasma testosterone levels decreased after surgery[7–9] in men. Factors connected with the anesthesia may be of importance for this reaction. Halothane has been implicated[10] although oxygen may protect against the toxic effects of halothane[11].

Oxygen alone may also be a factor of importance when breathing it at high concentration such as used in halothane anesthesia. High concentrations of oxygen is often used postoperatively in order to support the efficiency of ventilation.

The purpose of the present investigation was to study whether breathing a high concentration of oxygen might inhibit the endocrine function of the testis and thus, in part, explain the postoperative decrease of testosterone in plasma.

Rats were used for these experiments since prolonged oxygen exposure is toxic to this animal[4]. We chose 24 h exposures rather than just a few hours in order to obtain a clearcut answer without the damage observed after longer exposure. We have also studied the superoxide dismutase activity (SOD) in rat testis and the possible effect of oxygen breathing on the enzyme. For comparison, this activity was also determined in a number of other rat organs. The enzyme catalyzes the disproportionation of superoxide anion radicals $O_2 \cdot^-$ to H_2O_2 and O_2 ($2O_2 \cdot^- + 2H^+ \rightarrow H_2O_2 + O_2$)[12]. Superoxide dismutase is believed to exert an important protective function against the toxicity of oxygen. Thus the activity has been found in all cells capable of growing in O_2 and is lacking only in obligate anaerobes[13]. The induction of the enzyme by oxygen in a blue green algae[14], some bacteria[15] and the eucaryote, Sacharomyces cereviciae[16], has been paralleled by an increased resistance towards the toxicity of oxygen. Of seventeen obligatory anaerobic mutants of *E. coli* K12, ten were found to be defective in superoxide dismutase[17]. No reports on testicular superoxide dismutase activities have so far appeared in the literature to our knowledge.

MATERIALS AND METHODS

Male Wistar SPF rats were bought from Møllegaard-Hansen Laboratories (Ejby, Denmark) and kept in our laboratory for 6 weeks before the experiment in cages with 5 or 6 rats in each. They were fed standard rat pellets and water *ad libitum*. For the experiment the rats were randomly distributed between two large cages so that 35 rats were exposed to oxygen and 32 rats to air. The cages were firmly secured on the bottom of two plastic boxes of equal size (70 × 50 × 48 cm.). Cooling water was circulated around the cages from a water bath thermostated to keep the air temperature in the animal cages at 22–26°C. The boxes were closed by a tightly fitting plexiglass cover. The breathing gases were led into the boxes through a tube, ending close to the bottom of the rat cages, at a rate of 1.7 l/min (rate of exchange 15 times per 24 h). The oxygen concentration was determined in the effluent in an outled tube using Beckman oxygen analyzer model D 2. The steady state oxygen concentration thus determined was 98% from the oxygen box, and 20% from the box perfused with air.

After 24 h the rats were taken one by one, alternatively from the two boxes, and killed by decapitation. Blood was collected into heparin tubes for determination of plasma testosterone, and the testes were removed and weighed. In 10 rats from each group one testis was used for analysis of the SOD activity and the other was homogenized in physiological saline and a volume corresponding to 0.1 g of tissue was used for the determination of testosterone in duplicate. A small portion of this testis was used for histology, fixed in Bouin's solution and stained with the PAS reagent. The lungs were removed and appropriate pieces fixed in Bouin's solution and stained with haemotoxylin-eosin.

Table 1. Plasma and testis testosterone concentrations and testis superoxide dismutase activity (SOD) in rats after breathing 100% oxygen for 24 h. Control rats were breathing air. The breathing gases perfused thermostated plexiglass boxes that housed the animal cages

	Testes wet wt g (decapsulated) NS[1]	SOD units per g NS[2]	Testis testosterone conc. ng per g NS[1]	Plasma testosterone conc. ng per 100 ml NS[1]
Controls				
n	32	10	32	29
mean	2.9	509.9	177.1	351.5
SE	0.07	13.3	22.8	30.9
Oxygen				
n	35	10	35	33
mean	2.9	494.4	138.5	309.2
SE	0.06	8.6	16.1	35.2

[1] Significance according to Student's *t*-test.
[2] Significance according to Wilcoxon's two-sample *t*-test based on range[24].

Table 2. Superoxide dismutase activity in different rat tissues. Rats anesthetized with ether were killed by bleeding and the tissues homogenized in 5 vol. of 5 mM EDTA, pH 7.4. The homogenates were centrifuged after extensive dialysis (~48 h) against 50 mM Tris–HCl pH 7.4 and the supernatants assayed[1] for superoxide dismutase activity

Tissue	Superoxide dismutase activity (U/g wet weight)
Liver	1788
Kidney	849
Heart muscle	519
Testis	510
Brain	291
Thyroid gland	288
Spleen	280
Lung	267
Skeletal muscle	236
Fat tissue	32

Determination of testosterone concentration. Determination of testosterone was carried out using paper chromatography and competitive protein binding as previously described[8].

Determination of superoxide dismutase activity. The testes (~2 g each) were homogenized in 10 ml 5 mM EDTA, pH 7.4, the homogenate mixed for 1 h and then centrifuged (40,000 *g* × 30 min). The superoxide dismutase activity of the supernatant was then assayed with the pyrogallol autoxidation inhibition method[18]. One unit of SOD is taken as the amount required to inhibit by 50% the autoxidation of 0.2 mM pyrogallol in 50 mM Tris–cacodylic acid buffer, pH 8.20, containing 1 mM diethylenetriaminepentaacetic acid and 0.1 μM catalase in a total vol. of 3 ml. One unit corresponds to 0.30 μg bovine cupro-zinc superoxide dismutase[18].

In order to check the possibility of interference from reducing compounds in the homogenates, a few were also assayed after extensive dialysis. No change in activity was found.

RESULTS

Histology. No significant changes were observed using the light microscope when testes and lungs from the oxygen exposed rats were compared to those from the rats breathing air.

Testosterone. The testosterone concentration in plasma and testis from rats exposed to oxygen did not differ from rats exposed to air (Table 1).

Superoxide dismutase. The testicular superoxide dismutase activity of rats exposed to oxygen was not statistically different from rats exposed to air (Table 1). SOD activity of other rat organs are given in Table 2. The testes were found to contain a comparatively high level of this enzyme activity, next only to the activities found in the liver and the kidneys and comparable to that in the heart.

DISCUSSION

The blood flow through the testis as a whole is comparatively slow according to Setchell and Waites in the ram[19] and our own results in the dog (Carstensen and Näsman, unpublished). Since the tubular compartment is avascular, however, the capillary blood flow through the interstitial tissue must be high. Direct tissue measurements[3] and measurement on spermatic venous blood[2] indicate that the internal environment is normally somewhat hypoxic and this must be especially true for the tubular part in which the diffusion route is very long. It is likely that the spermatogenetic cells are normally adapted to hypoxia. Conversely they may not be very well adapted to high oxygen pressure. Thus breathing an atmosphere of high oxygen pressure for a long time (days) damages the testis as evidenced by impaired spermatogenesis[4,20,21]. The mechanism is unknown. However short time exposure (hours) did not appear to damage the germinal epithelium[5]. Studies on Leydig cell culture[6] shows that 95% O_2 – 5% CO_2 inhibited the formation of testosterone. We could not confirm this *in vivo*. However the oxygen pressure in the Leydig cells in rats breathing 100% oxygen is much lower than in the lungs. The relative importance of the 5-ene pathway may also explain the discrepancy.

Our results indicate that the disturbance of spermatogenesis on exposure to oxygen is not caused by derangement of the Leydig cell function at this early stage. Further studies are required in order to exclude damage to the Leydig cells, at later stages, after 24 h exposure to oxygen.

Unpublished results in our laboratory indicate that psychic trauma may be an important causative factor in the reduction of testosterone production, seen in rats as well as in dogs. Much care was therefore taken to treat the two experimental groups in this study identically and to avoid frightening the animals. We also performed the killing of the rats in a place remote from the cages to prevent the transmission of signals from the rats being killed.

The comparatively high level of superoxide dismutase activity in the testis is noteworthy. We did not, however, distinguish between tubules and interstitial tissue. Therefore, not knowing the level of activity in the Leydig cells, it is impossible to draw any conclusions with regard to the failure of oxygen to produce any acute effect on the hormone production. No induction of SOD activity was brought about by the exposure to pure oxygen for 24 h. Exposure to 85% oxygen has been reported to induce a moderate increase in SOD activity in rat lung[22]. No change in the SOD content of a number of other rat organs was found after exposure to 5 ATA oxygen for 1 h[23].

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