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Developmental Changes of Erythrocyte Catalase Activity in Rats Exposed to Acute Hypoxia

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Summary

The erythrocytes represent an important source of antioxidant capacity of the blood. Catalase (EC 1.11.1.6.) is one of the enzymatic components of their antioxidant defense system. The objective of this study was to follow erythrocyte catalase (CAT) in 7-, 15-, 21-, 35-, 60- and 90-day-old Wistar rats of both sexes in normoxia and after exposure to intensive acute hypobaric hypoxia. During the development CAT activity increases in both sexes, but the rise was usually higher in females. Hypobaric hypoxia increased CAT activity in all studied age groups of both sexes. However, higher CAT activity in females was less affected by hypoxia than the lower activity in males. This was true for nearly all age groups studied. It can be concluded that both ontogenetic aspects and sex differences play a major role in establishing the activity of CAT, which is an important part of the antioxidant defense of the organism.

Key words

Erythrocyte • Catalase • Development • Sex difference • Hypobaric hypoxia • Rat

Introduction

Erythrocytes are permanently in contact with potentially damaging levels of oxygen, but their metabolic activity is capable of reversing this injury under normal conditions. However, it is well known that variety of physiological and pathological factors may increase reactive oxygen species and induce the oxidative stress. In addition, hemoglobin is known to stimulate lipid peroxidation (Puppo and Halliwell 1988). Erythrocytes are equipped by many defense systems representing their antioxidant capacity (Kurata *et al.* 1993). This protective system includes superoxide dismutase (SOD), catalase (CAT), reduced glutathione, glutathione peroxidase (GPx), glutathione-S-transferase, and glutathione reductase. Two enzymes are shared in H_2O_2 detoxification: CAT (EC 1.11.1.6) and GPx (EC 1.11.1.9), but their relative significance in H_2O_2 scavenging is still not clear (Kurata *et al.* 1993, Gaetani *et al.* 1996, Guilivi *et al.* 1994, Mueller *et al.* 1997, Johnson *et al.* 2000, Nakababu *et al.* 2003).

In human subjects there is a considerable disagreement in age-related changes of erythrocyte CAT activity (Jozwiak and Jasnowska 1985, Guemouri *et al.* 1991, Bolzán *et al.* 1997, Inal *et al.* 2001). Lower activities of CAT (and SOD) were shown in premature

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infants during first 72 h of their life in comparison with full-term infants (Ochoa *et al.* 2003). Less than 10 % of normal erythrocyte CAT activity was observed in homozytes with inherited CAT deficiency – acatalasemia (Góth 2001), and less than 50 % in heterozygous subjects hypocatalasemia (Vitai and Góth 1997). As far as pathological processes are concerned, decreased CAT activities were found in erythrocytes from human patients of different ages with several types of aging brain disorders including dementia, stroke and Parkinson disease (de la Torre *et al.* 1996). Oxidative stress with alterations in profile of antioxidant enzymes in erythrocytes is also related to many others specific pathologies (Matés *et al.* 1999), e.g. diabetes (Góth and Eaton 2000, Sailaja *et al.* 2003), hypertension (Yuan *et al.* 1996, Johnson 2002) etc. Changes of erythrocyte CAT activity in dependence on oxygen consumption also occur in patients with hyperthyroidism (Kurasaki *et al.* 1986, Alicigüzel *et al.* 2001).

During the reoxygenation period after ischemia or hypoxia, the generation of highly oxidizing species in biological systems increases and evokes serious injuries with the possible loss of some cell functions. Such an example is hypobaric hypoxia. Our present study was undertaken to follow erythrocyte CAT activity in Wistar male and female rats of different ages and to investigate the effect of intensive acute hypobaric hypoxia on this activity.

Methods

The experimental animals were Wistar rats of both sexes aged 7 days (n=15 both males and females), 15 days (n=10), 21 days (n=10), 35 days (n=10), 60 days (n=9) and 90 days (n=10) from the breeding colony of the First Faculty of Medicine, Charles University, Prague. Animals were maintained under standard conditions of light (06:00-18:00 h) and temperature (22 °C) with a standard pelleted diet and water *ad libitum*. All experiments were performed in agreement with guidelines of the Animal Protection Law of the Czech Republic, fully compatible with the European Convention on Animal Protection. Control groups of rats were kept under a normobaric oxygen atmosphere. Experimental groups were exposed for 30 min to acute hypobaric hypoxia in a hypobaric chamber set at the pressure of 30.7 kPa (pO_2 = 6.4 kPa), simulating the altitude of 9 000 m. At the end of the exposure blood was sampled.

Blood (mixed with citrate) was centrifuged (at 3000 *x g* for 10 min) and the erythrocyte sediment was washed three times with isotonic saline solution. The hemolysate containing about 5 g Hb/100 ml was diluted with four volumes of water. Furthermore, the dilution 1:500 with 0.05 mM phosphate buffer (pH 7.0) was prepared. Hemoglobin (Hb) content was determined spectrophotometrically (at 540 nm) by the cyanmethemoglobin method (Drabkin and Austin 1935).

CAT activity was measured in hemolysates according to Aebi (1984). Decomposition of H_2O_2 was followed directly by monitoring the decrease of absorbance at 240 nm. Enzyme activity was calculated as catalytic content of a sample and expressed as k/g Hb.

Data are presented as means ± S.E.M. The results were statistically analyzed by one-way ANOVA followed by Student's t-test, where $p<0.05$ was considered as significant.

All chemicals were of the purest grades available commercially.

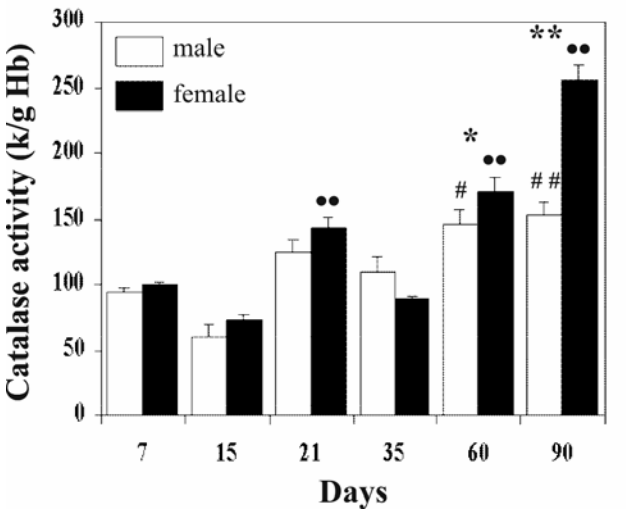

Fig. 1. Catalase activity in male and female rats under normoxic conditions during the ontogeny. * and ** indicate significant differences ($p<0.05$ and $p<0.01$) between male and female rats under normoxic conditions, # and ## indicate significant differences ($p<0.05$ and $p<0.01$) from 7-day-old males, •• indicate significant difference ($p<0.01$) from 7-day-old females.

Results

Figure 1 shows the erythrocyte CAT activity during ontogenesis in male and female rats under normoxic conditions. The enzyme activity increases with the age. CAT activity of 7-day-old male is significantly lower against 60-day-old ($p<0.05$) and 90-day-old ($p<0.01$) animals. CAT activity of 7-day-old female is

significantly lower against 21-, 60- and 90-day-old ($p<0.01$) animals. In all studied age groups CAT activity is slightly higher in females in comparison with males except of 35-day-old group. At this age the female CAT activity reached only 81 % of the activity in males. Significant differences between sexes were found in 60- and 90-day-old animals.

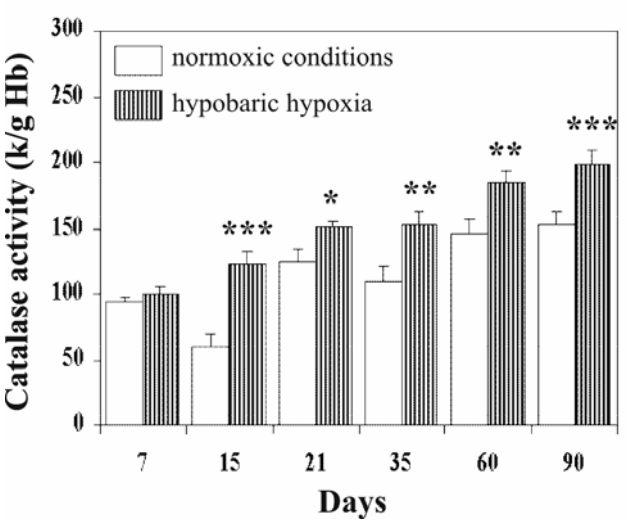

Fig. 2. Catalase activity in male rats under normoxic conditions and after the exposure to acute hypobaric hypoxia. *, ** and *** indicate significant differences ($p<0.05$, $p<0.01$ and $p<0.001$) from rats under normoxic conditions.

After the exposure to acute hypobaric hypoxia, CAT activity increased in all age groups of males (Fig. 2) with the exception of 7-day-old males. The most significant difference ($p<0.001$) was observed in 15-day-old males (an increase by 103 %).

In females with higher control values the exposure to acute hypobaric hypoxia did not increase CAT activity so markedly as it was seen in males. Similarly the most significant increase (by 102 %) was detected in the 15-day-old group (Fig. 3).

Discussion

During erythrocyte life span there are many changes in size and deformability, lipid and protein content in the membrane, ion permeability and enzyme activities. Contrary to SOD and GPx activities no relationships were found between the erythrocyte life span and glutathione concentration or CAT activity in various birds or mammals such as pigeon, duck, mouse, rat, cat, dog, hamster, rabbit, sheep, cattle, monkey and human (Maral *et al.* 1977, Kurata *et al.* 1993). However, Stagsted and Young (2002) showed very large species differences in H_2O_2-induced lysis of erythrocytes that were attributed to the differences in CAT activities.

Very little information is available on the effect of age on CAT activity in the rat. Significantly elevated CAT activity was observed in erythrocytes of 12-month-old male Wistar rats (Öztürk and Gümüslü 2004). In our experiments on Wistar rats we demonstrated the increase of CAT activity with age in both males and females. In both sexes the developmental rise in CAT activity was considerably slowed down at the age of 15 and 35 days. The age of 15 days is an important age-period characterized by eye opening and diet changing when the high-lipid diet of suckling pups (maternal milk) is supplemented with high-carbohydrate rat chow. The 35-day-old rats are in pubescent period where the different hormonal changes begin to develop.

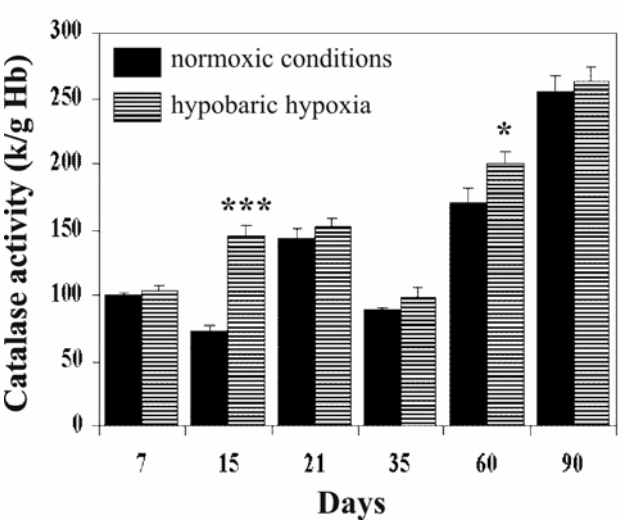

Fig. 3. Catalase activity in female rats under normoxic conditions and after the exposure to acute hypobaric hypoxia. * and *** indicate significant differences ($p<0.05$ and $p<0.001$) from rats under normoxic conditions.

We usually found higher CAT activity in females than in males, but this difference was significant only in 60- and 90-day-old animals. D'Almeida *et al.* (1995) did not observe any significant sex differences of erythrocyte CAT activity in male and female Wistar rats aged 3-4 months. Moreover, these authors did not find any alterations of CAT activity in relation to different stages of estrous cycle. Similarly, Massafra *et al.* (2000) showed that no significant cycle phase-dependent changes in erythrocyte CAT (and SOD) activity were observed in healthy eumenorrhoic women. They did not find any significant correlation between these enzyme

activities and plasma concentrations of follicle-stimulating hormone, luteinizing hormone, estradiol, progesterone, testosterone and androstenedione. In addition, Lutosławska *et al.* (2003) showed that mean CAT activity did not differ significantly in ovulating and non-ovulating women.

Erythrocytes as oxygen carriers are continuously exposed to high oxygen tension. Oxidative stress, which exceeds the antioxidant capacity, irreversibly damages erythrocytes, resulting in their ultimate loss by hemolysis and their removal from the circulation. Because mature erythrocytes are cells without nucleus and other cell organelles, they have no capacity to repair the damaged components. Celedón *et al.* (1998) showed that biochemical changes taking place during acute hypobaric hypoxia make erythrocytes particularly susceptible to oxidative injury. Kramer *et al.* (1987) investigated whether biochemical changes that occur in the rat brain tissue are also reflected in the erythrocytes. They found partial parallelism between changes in cerebral cortex homogenates and erythrocytes after complete brain ischemia (for 5 min). However, no parallelism was apparent after the chronic hypoxic conditions (3 weeks at 10 % O_2). Under the conditions of intensive acute hypobaric hypoxia we found an increased lipid peroxide formation in different parts of brain (cerebral cortex, subcortical structures, medulla oblongata and cerebellum) indicating the excess of reactive oxygen species production in the body (Koudelová and Mourek 1992, Rauchová *et al.* 2002). In our experiments hypoxia increased CAT activity in all studied age groups. The increase was most pronounced in 15-day-old groups (by 103 % in males and by 102 % in females), i.e. in the age period where we observed a transient stagnation of the developmental rise in CAT activity.

Our present study documented elevated CAT activity in female compared to male rats. This difference appears gradually during sexual maturation and might be the basis for higher antioxidant capacity of female rats. It is also evident that acute hypoxia increases CAT activity in rats of both sexes, but this effect is greater in males than in females and seems to be inversely proportional to the level of CAT activity before hypoxia exposure.

It can be concluded that both developmental aspects and sex differences play a major role in establishing the activity of CAT, which is an important part of the antioxidative defense of the organism. It remains to determine whether similar changes also occur in other tissues including brain and cardiovascular system.

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References

AEBI H: Catalase in vitro. *Methods Enzymol* **105**: 121-126, 1984.

ALICIGÜZEL Y, ÖZDEM N, ÖZDEM SS, KARAYALÇIN U, SIEDLAK SL, PERRY G, SMITH MA: Erythrocyte, plasma, and serum antioxidant activities in untreated toxic multinodular goiter patients. *Free Radic Biol Med* **30**: 665-670, 2001.

BOLZÁN AD, BIANCHI MS, BIANCHI NO: Superoxide dismutase, catalase and glutathione peroxidase activities in human blood: influence of sex, age and cigarette smoking. *Clin Biochem* **30**: 449-454, 1997.

CELEDÓN G, GONZÁLEZ G, SOTOMAYOR CP, BEHN C: Membrane lipid diffusion and band 3 protein changes in human erythrocytes due to acute hypobaric hypoxia. *Am J Physiol* **275**: C1429-C1431, 1998.

D'ALMEIDA V, HIPÓLIDE DC, DA SILVA-FERNANDES ME: Lack of sex and estrous cycle effects on the activity of three antioxidant enzymes in rats. *Physiol Behav* **57**: 385-387, 1995.

DE LA TORRE MR, CASADO A, LÓPEZ-FERNÁNDEZ ME, CARRASCOSA D, CASADO MC, VENARUCCI D, VENARUCCI V: Human aging brain disorders: role of antioxidant enzymes. *Neurochem Res* **21**: 885-888, 1996.

DRABKIN D, AUSTIN H: Spectrophotometric studies preparations from washed blood cells. *J Biol Chem* **112**: 51-55, 1935.

GAETANI GF, FERRARIS AM, ROLFO M, MANGERINI R, ARENA S, KIRKMAN HN: Predominant role of catalase in the disposal of hydrogen peroxide human erythrocytes. *Blood* **87**: 1595-1599, 1996.

GÓTH L: A new type of inherited catalase deficiencies: Its characterization and comparison to the Japanese and Swiss type of acatalasemia. *Blood Cells Mol Dis* **27**: 512-517, 2001.

GÓTH L, EATON JW: Hereditary catalase deficiencies and increased risk of diabetes. *Lancet* **356**: 1820-1821, 2000.

GUEMOURI L, ARTUR Y, HERBETH B, JEANDEL C, CUNY G, SIEST G: Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. *Clin Chem* **37**: 1932-1937, 1991.

GUILIVI C, HOCHSTEIN P, DAVIES KJ: Hydrogen peroxide production by red blood cells. *Free Radic Biol Med* **16**: 123-129, 1994.

INAL ME, KANBAK G, SUNAL E: Antioxidant enzyme activities and malondialdehyde levels related to aging. *Clin Chim Acta* **305**: 75-80, 2001.

JOHNSON P: Antioxidant enzyme expression in health and disease: effects of exercise and hypertension. *Comp Biochem Physiol* **133C**: 493-505, 2002.

JOHNSON RM, GOYETTE G Jr, RAVINDRANATH Y, HO Y-S: Red cells from glutathione peroxidase-1-deficient mice have nearly normal defenses against exogenous peroxides. *Blood* **96**: 1985-1988, 2000.

JOZWIAK Z, JASNOWSKA B: Changes in oxygen-metabolizing enzymes and lipid peroxidation in human erythrocytes as a function of age of donor. *Mech Ageing Dev* **32**: 77-83, 1985.

KOUDELOVÁ J, MOUREK J: Different degrees of lipid peroxidation in the CNS of young and adult rats exposed to short-term hypobaric hypoxia. *Physiol Res* **41**: 207-212, 1992.

KRAMER K, VOSS H-P, GRIMBERGEN JA, TIMMERMAN H, BAST A: The effect of ischemia and recirculation, hypoxia and recovery on antioxidant factors and ß-adrenoceptor density. *Biochem Biophys Res Commun* **149**: 568-575, 1987.

KURASAKI M, SAITO T, KAJI H, KOJIMA Y, SAITO K: Increased erythrocytes catalase activity in patients with hyperthyroidism. *Horm Metab Res* **18**: 56-59, 1986.

KURATA M, SUZUKI M, AGAR NS: Antioxidant systems and erythrocyte life-span in mammals. *Comp Biochem Physiol B* **106**: 477-487, 1993.

LUTOSŁAWSKA G, TKACZYK J, PANCZENKO-KRESOWSKA B, HUBNER-WOŹNIAK E, SKIERSKA E, GAJEWSKI AK: Plasma TBARS, blood GSH concentrations, and erythrocyte antioxidant enzyme activities in regulatory and anovulatory menstrual cycles. *Clin Chim Acta* **331**: 159-163, 2003.

MARAL J, PUGET K, MICHELSON AM: Comparative study of superoxide dismutase, catalase and glutathione peroxidase levels in erythrocytes of different animals. *Biochem Biophys Res Commun* **77**: 1525-1535, 1977.

MASSAFRA C, GIOIA D, DE FELICE C, PICCIOLINI E, DE LEO V, BONIFAZI M, BERNABEI A: Effects of estrogens and androgens on erythrocyte antioxidant superoxide-dismutase, catalase and glutathione-peroxidase activities during the menstrual cycle. *J Endocrinol* **167**: 447-452, 2000.

MATÉS JM, PÉREZ-GÓMEZ C, NÚÑEZ DE CASTRO I: Antioxidant enzymes and human diseases. *Clin Biochem* **32**: 595-603, 1999.

MUELLER S, RIEDEL HD, STREMMEL W: Direct evidence for catalase as the predominant H_2O_2-removing enzyme in human erythrocytes. *Blood* **90**: 4973-4978, 1997.

NAKABABU E, CHREST FJ, RIFKIND JM: Hydrogen-peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. *Biochim Biophys Acta* **1620**: 211-217, 2003.

OCHOA JJ, RAMIREZ-TORTOSA MC, QUILES JL, PALOMINO N, ROBLES R, MATAIX J, HUERTÁS JR: Oxidative stress in erythrocytes from premature and full-term infants during their first 72 h of life. *Free Radic Res* **37**: 317-322, 2003.

ÖZTÜRK O, GÜMÜSLÜ S: Changes in glucose-6-phosphate dehydrogenase, copper, zinc-superoxide dismutase and catalase activities, glutathione and its metabolizing enzymes, and lipid peroxidation in rat erythrocytes with age. *Exp Gerontol* **39**: 211-216, 2004.

PUPPO A, HALLIWELL B: Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Is haemoglobin a biological Fenton reagent? *Biochem J* **249**: 185-190, 1988.

RAUCHOVÁ H, KOUDELOVÁ J, DRAHOTA Z, MOUREK J: Hypoxia-induced lipid peroxidation in rat brain and protective effect of carnitine and phosphocreatine. *Neurochem Res* **27**: 899-904, 2002.

SAILAJA YR, BASKAR R, SARALAKUMARI D: The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. *Free Radic Biol Med* **35**: 133-139, 2003.

STAGSTED J, YOUNG JF: Large differences in erythrocyte stability between species reflect different antioxidative defense mechanisms. *Free Radic Res* **36**: 779-789, 2002.

VITAI M, GÓTH L: Reference ranges of normal blood catalase activity and levels in familiar hypocatalasemia in Hungary. *Clin Chim Acta* **261**: 35-42, 1997.

YUAN YV, KITTS DD, GODIN V: Heart and red blood cell antioxidant status and plasma lipid levels in the spontaneously hypertensive and normotensive Wistar-Kyoto rat. *Can J Physiol Pharmacol* **74**: 290-297, 1996.

Reprint requests

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