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The Lipid Peroxidation in Various Parts of the Rat Brain: Effect of Age, Hypoxia and Hyperoxia

J. KOUDELOVÁ, J. MOUREK

Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

The effect of normobaric oxygen atmosphere on hypoxia-enhanced lipid peroxidation in the brain cortex, subcortical structures, medulla oblongata and in the cerebellum was observed in 7- and 21-day-old and adult rats. The production of free oxygen radicals causing lipid peroxidation was assessed by the method described by Ohkawa *et al.* (1979). The rats were exposed for 30 min to 100 % oxygen atmosphere which significantly stimulated the production of malondialdehyde (MDA) in all the studied regions of the brain in 7- and 21-day-old male rats, and in the brain cortex and subcortical structures of adult males. Higher levels of MDA were found in the brain cortex of 7-day-old female rats only. Reoxygenation with pure oxygen after 30 min hypobaric hypoxia corresponding to 9000 m increased MDA production in all studied parts of the brain on both male and female rats 7- and 21-day-old. In adult rats significantly increased MDA production was only found in the brain cortex of male and female rats and in the subcortical structures of males. The exposition to hypobaric hypoxia followed by reoxygenation by atmospheric air enhanced MDA production in all studied regions of the brain in 7-day-old males and in the cerebellum of females; in 21-day-old rats of both sexes a significant increase of MDA was detected in all parts of the brain. In adult rats were found higher MDA levels in the cerebral cortex of both males and females.

Key words

Lipid peroxidation – Rat brain – Postnatal development – Brain hypoxia – Brain hyperoxia

Introduction

Free oxygen radicals produced in small amounts under physiological conditions in the mammalian tissues participate in various regulatory mechanism (Halliwell and Gutteridge 1984). However, it is very important to exclude the redundant production of oxygen radicals and to maintain a fine balance between the systems producing these radicals and the systems providing protection against them, i.e. to prevent toxic effects of oxygen radicals (Frank and Massaro 1980, Harman 1984).

One of the main toxic effects of oxygen radicals is lipid peroxidation which damages cellular and subcellular structures and functions. The reaction of free oxygen radicals with the polyenoic (polyunsaturated) fatty acids linked to hydrophobic phospholipid layers is of the highest interest and importance (Demopoulos *et al.* 1982, Cross 1987).

Hypoxic and ischaemic states in patients are treated effectively by immediate supply of oxygen. The positive therapeutic effects of such treatment need not necessarily compensate the concomitant negative consequences of such therapy (Kihlström et al. 1989, McCord 1985. McCall et al. 1987). From this point of view, neonatology and pediatry are the most involved fields. The relationship between bronchopulmonary dysplasia (Huber and Drath 1985, Hansen and Gest 1984), early retinopathy (Stuart *et al.* 1990) and necrotic enterocolitis following oxygen therapy has been undoubtedly provided (Saugstad 1990).

Extreme hyperoxia may also evoke pathological symptoms of the brain functions, e.g. convulsions, auditory halucinations, involuntary movements, nausea, paraesthaesia in clinical practice (Schaefer 1985).

In our laboratory, we have been studying for a long time the maturation of the brain and so-called "risk factors" influencing brain development.

In the present paper we investigated the effects of normobaric hyperoxia on lipid peroxidative processes in various parts of the brain in rats of different ages. This paper is related to our previous work concerning the effects of age and hypoxia on lipid peroxidative processes (Koudelová and Mourek 1992, Rauchová *et al.* 1993).

Material and Methods

Wistar rats of our own breeding colony both males and females were used for our experiments. The animals were fed with a standard diet and kept under standard conditions. Animals aged 7 and 21 days as well as adults (90-day-old) were divided into four groups:

1. Control groups
2. Animals exposed for 30 min to a normobaric pure oxygen atmosphere
3. Animals exposed for 30 min to hypobaric hypoxia corresponding to an altitude of 9000 m (air pressure = 30.7 kPa, pO_2 = 6.4 kPa) and then for 30 min to a pure oxygen atmosphere (reoxygenation)
4. Animals exposed for 30 min to hypobaric hypoxia (see 3) and then reoxygenation occurred in a normal atmosphere (30 min)

Rats after having been subjected to the above mentioned procedure were rapidly decapitated, the brain was removed and divided on a cooled block into the brain cortex, subcortical structures (including thalamus and basal ganglia), the medulla oblongata and the cerebellum. Lipid peroxidation was measured by the method of Ohkawa *et al.* (1979), i.e. estimation of malondialdehyde (MDA), one of the secondary products of peroxidation. The method is based on the reaction of MDA with thiobarbituric acid (TBA) which creates the colour change. This reaction occurs optimally at 90 °C in an acid environment (pH=3.5).

The coloured samples were measured spectrophotometrically at 530 nm (Vitatron). As the standard for calibration malondialdehyde-bis-acetal (Merck) was used. The amount of produced MDA was expressed in $ng.mg^{-1}$ w.w. and the results were statistically evaluated by Student's t-test in the Biocybernetic Department of our Institute.

Table 1
Lipid peroxidation in the brain of 7-day-old rats. The effect of hypoxia and hyperoxia

		Controls	Oxygen	Hypoxia + Oxygen	Hypoxia + Air
Cortex	M	7.80±0.54	18.36±1.95^{xx}	12.10±1.35^{x}	13.90±1.70^{xx}
	F	6.84±1.09	9.54±0.20^{x}	14.55±1.62^{xx}	5.80±0.32
Subcortical structures	M	9.60±0.26	18.14±1.04^{xx}	12.60±1.17^{x}	16.50±2.51^{x}
	F	9.46±0.49	10.50±0.27	14.90±1.53^{xx}	9.22±1.11
Medulla oblongata	M	9.26±0.16	20,44±0.93^{xx}	17.15±1.25^{xx}	12.21±0.75^{x}
	F	13.44±1.22	12.84±0.85	18.25±1.04^{x}	14.82±1.75
Cerebellum	M	7.18±0.20	17.82±1.22^{xx}	10.44±0.87^{xx}	16.40±0.85^{xx}
	F	7.02±1.55	7.90±0.13	14.90±1.53^{xx}	11.90±1.08^{x}

Oxygen = 30 min exposure of animals to normobaric oxygen atmosphere. Hypoxia = 30 min lasting stay under hypobaric conditions corresponding to 9000 m altitude. Hypoxia + Oxygen = 30 min lasting altitude hypoxia followed by 30 min of reoxygenation in pure oxygen (30 min). Hypoxia + Air = 30 min lasting altitude hypoxia followed by 30 min reoxygenation in a normal atmosphere. M = males, F = females. Concentration of malondialdehyde = ng/mg^{-1} w.w. The values are presented as arithmetical means ±S.E.M. Statistical significance of differences compared with the controls: $x = p<0.05$, $xx = p<0.01$.

Results

Table 1 presents the effect of 30 min normobaric pure oxygen atmosphere on lipid peroxidation on the brain of 7-day-old rats. The production of MDA was increased in all parts of the

brain in male and female brain cortex only. Hypoxia followed by 30 min of reoxygenation (using pure oxygen) significantly increased the MDA production in males as well as in females in all brain parts. Reoxygenation using atmospheric air created practically the same effect in males as pure oxygen. On the other hand, reoxygenation with air was less effective in female rats.

Table 2 shows the results in 21-day-old rats. Thirty-minute exposition to the oxygen atmosphere significantly increased MDA production in all parts of the brain in males but not in females.

Hypoxia followed by 30 min of reoxygenation with pure oxygen and air also enhanced MDA production in brain of both male and female rats.

In adult animals (Table 3), the nervous tissues of males was significantly more vulnerable. This especially concerned the cerebral cortex and subcortical structures and was manifested by an increased content of MDA. The preceding hypoxia followed by 30 min stay in oxygen atmosphere raised the MDA production (lipid peroxidation) in cerebral cortex of both sexes and in subcortical structures of males. The reoxygenation by a normal atmosphere raised the lipid peroxidation only in cerebral cortex of both male and female rats.

Table 2
Lipid peroxidation in the brain of 21-day-old rats. The effect of hypoxia and hyperoxia

		Controls	Oxygen	Hypoxia + Oxygen	Hypoxia + Air
Cortex	M	11.34±0.48	16.44±0.21^{xx}	19.94±1.41^{xx}	16.27±0.61^{xx}
	F	10.80±0.35	11.14±0.39^{NS}	18.41±1.97^{xx}	19.13±0.45^{xx}
Subcortical	M	11.16±0.60	14.68±0.21^{x}	18.37±1.21^{xx}	17.14±0.45^{xx}
Structures	F	10.97±0.42	11.82±0.27	17.89±1.22^{x}	17.67±0.58^{xx}
Medulla	M	13.54±0.28	18.36±0.71^{xx}	18,87±1.15^{xx}	18.53±1.04^{xx}
oblongata	F	11.78±0.66	11.88±0.22	18.24±1.37x	18.50±0.88^{xx}
Cerebellum	M	10,40±032	12.32±0.32^{x}	17.04±2.02^{xx}	14.06±0.77^{x}
	F	9.64±0.52	7.14±0.16	15.60±1.24^{x}	16.50±1.54^{xx}

For further explanation see footnote of Table 1

Table 3
Lipid peroxidation in the brain of adult rats. The effect of hypoxia and hyperoxia

		Controls	Oxygen	Hypoxia + Oxygen	Hypoxia + Air
Cortex	M	9.25±0.57	11.63±0.39^{x}	14.82±1.03^{x}	11.22±0.37^{x}
	F	9.02±0.40	9.32±0.84	10.89±0.66^{x}	11.90±0.89^{x}
Structures	M	9.25±0.92	12.65±0.38^{x}	13.82±0.68^{x}	11.86±0.37
Structures	F	9.56±0.65	7.67±0.36	10.31±0.39	10.91±0.71
Medulla	M	10,10±0.99	11.73±0.94	12.46±0.59	10.08±0.29
oblongata	F	7.82±0.70	5.92±0.31	8.85±0.88	9.46±0.88
Cerebellum	M	9.20±1.02	9.13±0.61	12.14±0.44	9.16±0.61
	F	8.76±0.38	7.35±0.81	9.69±0.49	10.42±1.02

For further explanation see footnote of Table 1

Discussion

Even very small deviations from normal development and maturation of the brain processes during early ontogeny might induce serious deleterious consequences especially for the evolution of regulatory mechanisms and behaviour. It is known that the morphological recovery of even sligthly damaged nervous tissue is very limited because neurones in the postmitotic phase are unable to compensate their reduced number by subsequent proliferation. The influence of any damage during early ontogeny could be demonstrated in adults as "the late consequences of early injury" (Křeček 1977).

Compared with other tissues the brain contains high amounts of polyunsaturated fatty acids, especially of such fatty acids as arachidonic and docosahexaenoic. The proportion of polyunsaturated fatty acids in the brain during the maturation significantly raises (Šmídová *et al.* 1984). The high content of polyunsaturated fatty acids represents large amounts of double bonds which could be easily attacked by free oxygen radicals.

We established previously (Koudelová and Mourek 1991, 1992) that the peroxidation of lipids in the mature brain is low (in spite of the high content of polyunsaturated fatty acids) as compared with lipid peroxidation in the young immature brain.

In our experiments, we established that there is a 25 % increase of lipid peroxidation in the brain of adult males as a consequence of their previous exposure to a pure oxygen atmosphere. This finding is in agreement with the published data of Noda *et al.* 1983. These authors desribed a 22 % increase of lipid peroxidation in the cerebral cortex and substantia nigra in adult male rats under similar experimental conditions.

In young animals a comparison with these results is not available. The present experiments have demonstrated significantly higher production of MDA in all parts of the immature brain as compared with the adult brain. This confirmed our previous data concerning the influence of hypobaric hypoxia on lipid peroxidative processes in rats of various ages (Koudelová and Mourek 1991, 1992). The higher vulnerability of the immature brain tissue could be plausibly explained by the still low activities of antioxidant enzymes (Mavelli *et al.* 1982). The catalase activity during maturation increases six times and the activity of glutathion-peroxidase and Cu-Zn superoxide-dismutase rose to twofold values. The major increase of these activities occurs between the 20th and 40th day of postnatal life in rats. This fact correlates well with our own findings about lipid peroxidation in the brain of 21- or 35-day-old rats (Koudelová *et al.* 1992).

An important result appears to be the difference in lipid peroxidation in the cerebral tissue of male and female rats. The females exhibited a significantly higher resistance against the stressors stimulating MDA production. This difference could be associated with a different arsenal of antioxidant factors (enzymes and natural scavangers), with different concentrations of iron in the brain tissue and lastly with the different metabolic rate of MDA in the brain (Horton 1970, Bird and Draper 1982). Different concentration of ascorbic acid in 21-day-old male and female rats in cerebral cortex only (Koudelová and Mourek 1991).

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Reprint Requests

Dr. J. Koudelová, Institute of Physiology, First Faculty of Medicine, Charles University, 128 00 Prague 2, Albertov 5.