Lipoperoxidative Activities in the Cerebral Cortex and Medulla Oblongata, Related to Age, Sex, Oxygen Deficiency and Short-term Fasting

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Abstract: In albino rats (Wistar), aged 10 and 14-days of postnatal life, experiments were performed, in which the intensity of lipoperoxidative processes in cerebral cortex and medulla oblongata in four experimental series was measured and compared: a) between females and males (control group); b) between females and males exposed to the hypobaric hypoxia (corresponding to the altitude of 7000 m and lasting 20 minutes); c) between females and males exposed to the short-term starvation (for 24 h between the day 5^{th} and 6^{th} of postnatal life); d) between females and males exposed to the combination of short – term starvation and hypobaric hypoxia. No sex-dependent differences in control measurements could be detected. The hypobaric hypoxia evokes significantly greater increase in lipoperoxidative processes in brain tissue of males as compared with females. The short-term starvation affected more the oxygen radicals production in the brain of females. Finally – the short – term starvation and the subsequent hypoxic stress did not evoke the sex-dependent differences in the brain of 10-day-old rats; in 14-day-old females the higher values in brain cortex, in males higher values in medulla oblongata were established.
The different sensitiveness to mentioned stressors in males (more sensitive to oxygen deficiency) and in females (more sensitive to nutritional deficiency) was described.

Introduction

Several times in the past years we have dealt with the question of the activity of oxygen radicals in the brain, in its particular compartments and throughout the development of the individual [1, 2]. The findings included the fact that, apart from the other facts, lipoperoxidative processes in the brain of adult female rats (Wistar) following hypoxic distress show an undoubtedly smaller increase than in the male brain. There has been a wide and steady interest in the role of ROS (Reactive Oxygen Species). According to the available results, it is obvious that there is a causal relationship between the activity of oxygen radicals in the body and the occurrence of a variety of pathological changes and conditions. For instance, Morin et al. (2003) [3] showed that the destructive influence of ROS can be proved even at the mitochondrial level (due to their excessive activity not only the respiratory quotient but also their phosphorylating capacity decrease, which the authors ascribed to the damage to membranes, particularly internal ones, of the mitochondrial cristae). An increase in lipoperoxidative processes has been corroborated in current hypoglycaemia [4] as well as following an alcohol abuse [5]. An important the discovery was published by Sanchez-Alvarez et al. [6], showing that particularly lipoperoxidations in the nervous tissue of premature infants are responsible for numerous subsequent functional deviations which are later diagnosed in this risk group of children. As a matter of fact, several times we have pointed out these possibilities and facts before [7, 8].

Our earlier findings, indicating that production of oxygen radicals due to oxygen deficiency varies depending on particular compartments of CNS [9], were – much later – confirmed [10].

Recently, works have been appearing that found a causal relationship between the activity of oxygen radicals (lipoperoxidation) and development of some neurodegenerative or mental diseases [11]. In this connection we carried out a series of experiments to explore the total antioxidative capacity in patients with the diagnosis of depression. We learned that the depressives showed a lower total antioxidative capacity, compared to the control group [12]. Lehotský et al. [13] assumed that also an impairment of membranes caused by oxygen radicals can contribute to the post-ischemic damage, for example, Na^{+}-K^{+} or Ca^{++}-dependent ATPases. This, in turn, reminds of a rather old knowledge that action of a suitable detergent upon the membranes of nerve cells will eliminate the activity of cytochromoxidase [14]. It was then that this finding was ascribed by the authors to impaired relations between particular components of the membrane and transmembraneous localization of the above mentioned enzymes; Igo et al. (1960) [15] arrived to similar conclusions.

Material and method

Experiment were performed on rats of our own breed (Wistar), kept under conditions fully corresponding to EU standards, including the diet. Two age groups were chosen: 10-day-old animals and 14-day-old ones (considering the post-natal age). This choice was intentional: the two age groups, i.e. 10-day-old rats and 14-day-old ones (taken with the necessary spirit of criticism) most approximately correspond to human neonates born before and in full term, respectively [16, 17, 18]. The hypobaric hypoxia corresponded to the altitude of 7,000 m and lasted for 20 minutes (under euthermic conditions between 22 and 23° C, to exclude a considerable lowering of body temperature). The partial pressure of oxygen was at 8.6 kPa, the barometric pressure at 41.7 kPa. The rise as well as the descent to the set altitude was always performed within one minute. All the animals survived.

Immediately after the end of hypoxia, the animals were decapitated and both the grey matter of the cerebral cortex and the tissue of the medulla oblongata were separated in a cooled compartment. Lipoperoxidative activity was measured in a much similar way as in the case of our previous publications, i.e. using the method according to Ohkawa et al. (1979) [19], establishing the concentrations of malonyldialdehyde (MDA), stated in ng per 1 mg of wet tissue. The statistical evaluation was performed using T-test for two groups (at the Biocybernetic Section of the Institute of Physiology of the First Faculty of Medicine, Charles University).

The way of achieving the short-term fasting (including zero fluid intake) was precisely the same as in all our previous experiments (survey see [16]), particularly,

one half (i.e. four) of the young of the standardized litter (8 individuals) were separated from the mother into an environment which would not endanger their body temperature. This intervention was done between the 5th and 6th day of their postnatal life. Following the 24-hour separation, the young were returned to the mother (short-term starvation lowered the body weight approximate about 30–35% for a period of two weeks. All experimental puppies survived). To the proper experiment they were exposed either on the 10th or on the 14th day of their postnatal life.

The whole experiment, therefore, consists of four series of tests:

a) Comparison of lipoperoxidative activities in the cerebral cortex and medulla oblongata in females and males (control group).
b) Comparison of intensity of lipoperoxidative processes in the above parts of CNS in males and females exposed to the hypoxic conditions.
c) Comparison of intensity of lipoperoxidative processes in the above parts of CNS in males and females exposed to the short-term fasting.
d) Comparison of lipoperoxidative activities in the cerebral cortex and medulla oblongata in females and males exposed to the combined stress: first, between

Table 1 – Production of MDA in rat brain cortex and medulla oblongata of 10- and 14-day-old males and females

	n	cortex	p	n	medulla oblogata	p
M_{10}	10	18.01±1.17	p< 0.01	11	26.47±1.83	NS
F_{10}	10	20.46±0.82		11	27.04±1.86	
M_{14}	10	20.10±1.45	NS	11	17.67±0.61	NS
F_{14}	12	21.30±2.00		12	17.46±0.99	

$M_{10}F_{10}$ ($M_{14}F_{14}$) rats – Males, Females aged 10 and 14 – days of postnatal life
n = number of measurements
NS = the difference is not statistically significant
p = statistical significance
MDA = Malonyldialdehyde

Table 2 – The effect of hypoxia on MDA production in rat brain cortex and medulla oblongata of 10- and 14-day-old males and females

	n	cortex	p	n	medulla oblogata	P
M_{10}	10	25.51±0.99	p< 0.001	10	28.71±0.83	p < 0.01
F_{10}	10	23.14±0.69		10	27.20±0.63	
M_{14}	10	34.47±1.46	p< 0.001	12	33.03±3.10	p< 0.001
F_{14}	12	22.22±1.84		10	27.11±0.86	

$M_{10}F_{10}$ ($M_{14}F_{14}$) rats – Males, Females aged 10 and 14 – days of postnatal life
n = number of measurements
NS – the difference is not statistically significant
p = statistical significance
MDA –Malonyldialdehyde

the 5th and 6th day of their post-natal life, the young were exposed to short-term fasting (including zero fluid intake), and subsequently, on the 10th or 14th day, they were exposed to hypobaric hypoxia, as described above.

Results

In the first series we compare the initial levels of lipoperoxidations in the cortex and medulla oblongata in females and males of rats (Table 1). Only in 10-day-old individuals we found higher levels of lipoperoxidations in the cortex of the females. Any other levels did not substantially differ from each other. If hypobaric hypoxia was applied, however, then really considerable and statistically significant differences occurred. In both 10-day-old and 14-day-old individuals, the MDA levels in the respective tissues of CNS were remarkably lower in rat females, compared to the levels found in the males (Table 2).

The short-term fasting (including zero fluid intake) between the 5th and 6th day considerably more affected females than males (with the exception of the medulla

Table 3 – The effect of short-term starvation on lipoperoxidative processes in rat brain cortex and medulla oblongata of 10- and 14-day-old males and females

	n	cortex	P	n	medulla oblogata	p
M_{10}	12	23.19±1.21	NS	10	26.03±2.86	$p < 0.01$
F_{10}	12	25.48±1.35		12	29.90±1.83	
M_{14}	10	21.96±0.92	$p< 0.01$	10	29.58±1.20	$p < 0.01$
F_{14}	12	23.83±1.33		10	27.26±0.98	

M_{10} F_{10} (M_{14} F_{14}) rats – Males, Females aged 10 and 14 – days of postnatal life
n = number of measurements
NS = the difference is not statistically significant
p = statistical significance
MDA = Malonyldialdehyde

Table 4 – The action of previous (of short-term) starvation and following hypoxia on lipoperoxidative processes in rat brain cortex and medulla oblongata of 10- and 14-day-old males and females on the production of MDA

	n	cortex	p	n	medulla oblogata	p
M_{10}	12	27.07±2.91	NS	12	30.90±0.93	NS
F_{10}	12	25.35±0.97		12	31.23±1.23	
M_{14}	13	23.69±3.41	NS	10	26.24±0.91	$p < 0.05$
F_{14}	11	28.18±0.89		12	24.72±1.64	

M_{10} F_{10} (M_{14} F_{14}) rats – Males, Females aged 10 and 14 – days of postnatal life
n = number of measurements
NS = the difference is not statistically significant
p = statistical significance
MDA = Malonyldialdehyde

oblongata in the 14-day-old). The differences in this test series showed a lower level of statistical significance (Table 3).

Previous short-term fasting with the subsequent hypobaric hypoxia, applied on the 10^{th} or 14^{th} day of postnatal life, did not influence the differences between rat males and females in any statistically significant way. In the 14-day-old, the subsequent lipoperoxidative processes were in the cortex higher in females, while in the tissue of the medulla oblongata they were higher in males (Table 4).

Discussion

Several times we have dealt with the question of sex-dependent changes related to stress (application of hypobaric hypoxia, application of intraperitoneal adrenaline) [1, 12], considering – among other factors – the levels of ascorbic acid in the brain and in plasma. We found that there is a significant difference between the levels of ascorbic acid in the tissue of the cerebral cortex of rat females and males (i.e. adult individuals): in females the detected concentrations of ascorbic acid were considerably higher. Also the response to hypoxia was in this respect completely different in females and in males. While hypoxia or intraperitoneal application of adrenaline induced a rise of ascorbic acid levels in the tissue of the cerebral cortex in males (with a concurrent decrease in its concentration in plasma), in rat females, just the other way round, a decrease of the ascorbic acid contents (with a concurrent increase in plasmatic concentration of this acid) was detected.

The ascertained facts given in this report on lipoperoxidative activities in the brain of males and females – although at the very beginning of their post-natal development (and therefore in the period of intensive maturation and differentiation of CNS) – confirm and expand the facts that have been an object of intensive interest of neurochemists but – above all – also psychiatrists and psychopharmacologists in the past few years. The number of pronounced and significant differences in biochemistry of the male and female brain has been constantly increasing. In addition, our data point to the fact that sex-dependent differences can be present even since the earliest stage of development (as we proved, for instance, in the case of dopamine-β-hydroxylase [12]).

At the time, interpretation of the facts ascertained is very difficult. The established differences in concentrations and changes of flow of ascorbic acid, changes in the oxidative metabolism itself, contents of other anti-oxidative factors in the body (i.e. the state of anti-oxidative capacities and their efficiency), together with such factors as hyperglycaemia or shifts in pH, play a role, and may play certain – and somewhat different – roles in the two sexes.

Also short-term fasting (even in the hindsight, four or eight days later) proved to have increased the level of peroxidations. In this case, the rat females showed to be more “sensitive” (the increase being statistically significant in all four changes) than males (with only two changes statistically significant in terms of increase).

Fasting, causing a decrease in body weight and deceleration in development of the organism, including CNS, can also induce an increase in production of oxygen radicals with a subsequently increased lipoperoxidation. It may well be possible that concurrently with retardation of the general development also retardation in the development of anti-oxidative capacities, oxidative and energetic metabolism occurs, not only at the cellular but also at mitochondrial level.

At a time, however, our results show some difference in sensitivity and possible differences in the mechanisms of action (and reaction) of the stressors described above (hypoxia – short-term nutritional deprivation).

If females of laboratory rats at this stage of development in general show a higher resistance against oxygen deficiency (at medium level) but also a higher sensibility to short-time nutritional deficiency, this may indicate that resistance to various stressors (judged by production of oxygen radicals) is a result of a long phylogenic development with a deep biological sense.

The higher levels of MDA, produced in the medulla oblongata of 10-day-old males and females (compared to the cortex tissue), can be explained by the state of oxidative metabolism in these tissues: in particular, at the early stages of ontogenesis the phylogenically older compartments of CNS show, apart from other things, a higher oxygen consumption than the grey matter of the cortex, which only with the gradual maturation will increase its oxygen consumption (compared to that of the medulla oblongata tissue) more than twice (depending on the substrate) [18].

Combination of previous short-term fasting and subsequently applied hypobaric hypoxia (on the 10^{th} or 14^{th} day) induced the highest increase in MDA production in the 10-day-old, irrespectively of the sex and the compartment of CNS examined. Therefore we can suppose that deceleration in development (maturation) together with subsequent oxygen deficiency create conditions for a more intensive production of the radicals. In 14-day-old males this combined stress had a measurably lower impact than hypoxia alone. In 10-day-old and 14-day-old lab rat females, on the other hand, this impact was greater (with the exception of the medulla oblongata tissue).

In view of the generally accepted axiom of the toxic action of oxygen radicals in the body, our data pinpoint the possibility of their varying action upon CNS tissues in dependence on the sex and the fact that it can take place even since the early stages of postnatal development. At a time, we proved the difference in sensitivity (in lab rat males and females) to stressors with different ways of action.

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