REVIEW

Hypoxia-Induced Lipid Peroxidation in the Brain During Postnatal Ontogenesis

H. RAUCHOVÁ[1,2], M. VOKURKOVÁ[1,2], J. KOUDELOVÁ[3]

[1]Centre for Cardiovascular Research, Prague, Czech Republic, [2]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [3]Institute of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

Reactive oxygen species (ROS) are common products of the physiological metabolic reactions, which are associated with cell signaling and with the pathogenesis of various nervous disorders. The brain tissue has the high rate of oxidative metabolic activity, high concentration of polyunsaturated fatty acids in membrane lipids, presence of iron ions and low capacity of antioxidant enzymes, which makes the brain very susceptible to ROS action and lipid peroxidation formation. Membranes of brain cortex show a higher production of thiobarbituric acid-reactive substances (TBARS) in prooxidant system ($ADP.Fe^{3+}$/NADPH) than membranes from the heart or kidney. Lipid peroxidation influences numerous cellular functions through membrane-bound receptors or enzymes. The rate of brain cortex Na^+,K^+-ATPase inhibition correlates well with the increase of TBARS or conjugated dienes and with changes of membrane fluidity. The experimental model of short-term hypoxia (simulating an altitude of 9000 m for 30 min) shows remarkable increase in TBARS in four different parts of the rat brain (cortex, subcortical structures, cerebellum and medulla oblongata) during the postnatal development of Wistar rat of both sexes. Young rats and males are more sensitive to oxygen changes than adult rats and females, respectively. Under normoxia or hypobaric hypoxia both ontogenetic aspects and sex differences play a major role in establishing the activity of erythrocyte catalase, which is an important part of the antioxidant defense of the organism. Rats pretreated with L-carnitine (and its derivatives) have lower TBARS levels after the exposure to hypobaric hypoxia. The protective effect of L-carnitine is comparable with the effect of tocopherol, well-known reactive species scavenger. Moreover, the plasma lactate increases after a short-term hypobaric hypoxia and decreases in L-carnitine pretreated rats. Acute hypobaric hypoxia and/or L-carnitine-pretreatment modify serum but not brain lactate dehydrogenase activity. The obtained data seem to be important because the variations in oxygen tension represent specific signals of regulating the activity of many specific systems in the organism.

Key words

Hypobaric hypoxia • Reactive oxygen species (ROS) • Polyunsaturated fatty acids • Na^+,K^+-ATPase • Catalase • L-carnitine

Corresponding author

H. Rauchová, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 14220 Prague 4, Czech Republic. Fax: ++420 241 062 488. E-mail: rauchova@biomed.cas.cz

Brain energy metabolism

The brain belongs to organs with the very active energy metabolism. At rest it is responsible for 20 % of the total oxygen consumption in the body, although it represents only a small part of body weight. The brain needs the constant continual delivery of oxygen and glucose for the function of central nervous system. Its total energy reserves (including glycogen) are sufficient to maintain normal energy demands for about 80 seconds if the supply of substrates is completely interrupted (Ames 2000). The free energy of ATP is utilized for ion transport, synthetic work (synthesis of neurotransmitters) and a permanent rebuilding of various structural units.

Most of ATP in the brain is produced from glucose by oxidative phosphorylation in mitochondria and only 1-5 % is produced by glycolysis in cytosol (Erecińska and Silver 1989).

In the brain of suckling mammals ketone bodies (generated in the liver) appear to be at least as important as glucose as a source of metabolic fuel, because all developing mammals are fed with milk with high-fat content (Hawkins *et al.* 1971). The immature brain is able to consume, incorporate and metabolize a large number of organic metabolites such as lactic acid, acetoacetate, β-hydroxybutyrate, free fatty acids or amino acids if they are available in reasonable concentrations in blood and if there are appropriate enzymes for their degradation (Drahota *et al.* 1965, Hawkins *et al.* 1971, Vannucci and Vannucci 2000). On the other hand, the adult brain can also obtain its energy from ketone bodies when glucose is less available, e.g. during hypoglycemia, fasting or strenuous exercise (Hasselbalch *et al.* 1994, Vannucci and Vannucci 2000). The concentration of ATP in the brain is maintained under steady-state conditions but the ATP production can vary between cell types and/or brain regions (Erecińska and Silver 1989).

Reactive oxygen species formation

Common products of normal metabolism are reactive (chemically unstable) oxygen species (ROS), which include O_2-derived free radicals such as superoxide anion ($O^{2\bullet-}$), hydroxyl ($HO^{\bullet}$), peroxyl ($RO_2^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals, as well as O_2-derived non-radical species as hydrogen peroxide (H_2O_2) or singlet oxygen ($^1O_2$) (Naquie *et al.* 1986). ROS react quickly with the nearest stable molecule to capture the electron. The injured molecule loses its electron becoming a radical species itself. The most toxic reactive radical is $HO^{\bullet}$ with half-life of 10^{-9} seconds, which can interact only in the place of its formation. The major intracellular producers of ROS are mitochondria (Lenaz 2001, Turrens 2003, Bernacchia *et al.* 2004, Genova *et al.* 2004, Adam-Vizi 2005). In their classical study Chance *et al.* (1979) estimated that about 1-2 % of the oxygen is leaked from respiratory chain and forms $O^{2\bullet-}$. Later Fridowich (2004) reviewed this value because previous estimates were derived from rat liver and rat and pigeon heart mitochondria inhibited by cyanide but under normal conditions the fractional univalent reduction of oxygen in the respiratory chain is only 0.1 %. On the other hand, Nohl *et al.* (2003, 2004, 2005) showed arguments against mitochondrial ROS formation in the living cells based on the fact that bioenergetic alterations may result from the mechanical removal of mitochondria from their natural environment and from the general use of inappropriate methods. The question how much $O^{2\bullet-}$ mitochondria produce *in vivo* is still open (Kudin *et al.* 2004, 2008, Murphy 2009, Brown and Borutaite 2012).

The crucial role in mitochondrial ROS metabolism and oxidative stress response plays a protein $p66^{Shc}$ (Migliaccio *et al.* 1999, Nemoto *et al.* 2006, Orsini *et al.* 2006), which is involved in ROS production leading to mitochondrial damage and in aging dysfunction under oxidative stress conditions (Trinie *et al.* 2009, Ray *et al.* 2012). Mitochondrial origin of cellular ROS is not exclusive. Besides the mitochondrial respiratory chain there are other sources of ROS formation, e.g. flavin-containing monoamine oxidase located on the mitochondrial outer membrane, cytosolic xanthine oxidase or aldehyde oxidase or microsomal NADH cytochrome b_5 reductase and NADPH-dependent cytochrome P-450 reductase.

Under physiological conditions ROS production occurs continuously and ROS are necessary to the proper physiological functioning of the body. ROS operate as messengers in intracellular signaling and modulation of cell function including apoptotic process (Drőge 2002, Valko *et al.* 2007, Leloup *et al.* 2011, Ray *et al.* 2012). However, when ROS production is in excess to the capacity of cellular antioxidant systems, oxidative stress occurs. Oxidative stress damages cellular components, including DNA, proteins and lipids. Nucleic acid oxidation can result in strand breakage, nucleic acid-protein cross linking and nucleic base modifications, which can lead to the disruption of transcription, translation or DNA replication (Aust and Eveleigh 1999, Blair 2008, Winczura *et al.* 2012). Protein oxidation leads to their conformation modifications, possible denaturation, aggregation or precipitation (Sayre *et al.* 1992, Smith *et al.* 1992, Winczura *et al.* 2012). Oxidative damages have the crucial role in many neurological disorders, neurodegenerative diseases and injuries of central nervous system (Adibhatla and Hatcher 2010).

Membrane unsaturated fatty acids and lipid peroxidation in the brain

The brain contains a high concentration of polyunsaturated fatty acids in membrane lipids, which are suitable targets for free radical attack (Tappel 1973, Reed

2011). In addition, the brain has low levels of antioxidant enzymes, such as catalase and glutathione peroxidase as compared to other organs (Shohami *et al.* 1997, Halliwell 2001).

To evaluate the maximum capacity of biological membranes to react with oxygen radicals we measured lipid peroxide formation using *in vitro* membrane preparations from rat cerebral cortex, heart and kidney where all cytosolic antioxidant defense systems were eliminated. We confirmed the highest quantity of thiobarbituric acid-reactive substances (TBARS) in cerebral cortex in comparison with heart and kidney membranes in both induced lipid peroxidation systems (60 min at 37 °C) – enzymatic, ADP.Fe^{3+}/NADPH and non-enzymatic, ADP.Fe^{2+}/ascorbate (Rauchová *et al.* 1993). Higher production of TBARS in cerebral cortex membranes (Fig. 1) was the result of higher content of lipids, which correlated well with the estimated content of lipid phosphorus. Similarly, Piergiacomi *et al.* (2001) observed higher values of lipid peroxidation measured by chemiluminiscence in mitochondria and microsomes obtained from rat brain in comparison with mitochondria and microsomes obtained from rat kidney in peroxidation-induced system *in vitro* (120 min at 37 °C in the presence of ADP.Fe^{2+}/ascorbate). According to their experiments, the most sensitive fatty acid for peroxidation was docosahexaenoic acid (C22:6 n3) in brain mitochondria.

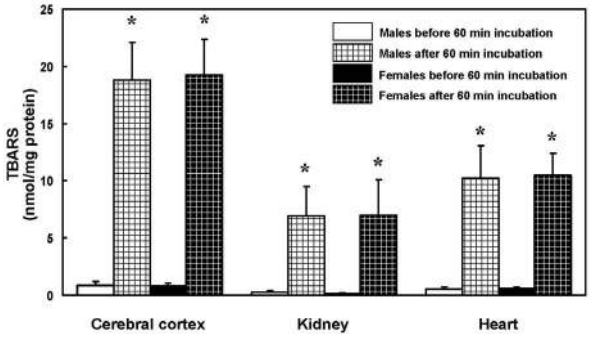

Fig. 1. Lipid peroxidation expressed as levels of TBARS in isolated membranes from cerebral cortex, kidney and heart of 21-day-old Wistar rats. Isolated membranes (0.5 mg protein) were incubated 60 min in the presence of 150 mmol/l KCl, 20 mmol/l Tris-HCl buffer (pH 7.4), 0.05 mmol/l $FeCl_3$, 3 mmol/l ADP and 0.225 mmol/l NADPH. The results are given as the means ± S.D. Significantly different: * $p \leq 0.001$ *vs.* controls before incubation.

Specific parts of the brain (e.g. substantia nigra) contain high levels of transition metal, such as iron (Drayer *et al.* 1986, Snyder and Connor 2009). Iron accumulation occurs in a number of neurodegenerative disorders of the central nervous system such as Parkinson's, Alzheimer's and Huntington's diseases, Friedreich's ataxia or amyotrophic lateral sclerosis (Nuñez *et al.* 2012). Iron can be subsequently converted *via* the Fenton reaction and Haber-Weiss reaction to the extremely toxic hydroxyl radical (Halliwell and Gutteridge 1984, Winterbourn 1995). Thereafter these free radicals may elicit cellular damage, lipid peroxidation and eventually cytotoxic processes such as apoptosis or necrosis leading to cellular destruction (Gutteridge 1994).

Lipid peroxidation

Above mentioned factors (the high rate of oxidative metabolic activity, high concentration of polyunsaturated fatty acids in membrane lipids, presence of iron ions and low capacity of antioxidant enzymes) make the brain very susceptible to lipid peroxidation involving the interaction of oxygen-derived free radicals with polyunsaturated fatty acids (Reed 2011). Double bonds of polyunsaturated fatty acids in biological membranes are easily attacked by free radicals, they undergo degradation by a chain reactions and form lipid hydroperoxides. This complex process yields a mixture of epoxy-fatty acids, alkanes, alkenes, alkanals, alkenals, 4-hydroxyalkenals, and aldehydes including malondialdehyde (MDA), which represents about 70 % of the total aldehydes produced during the membrane lipid peroxidation (Esterbauer *et al.* 1991). One of the oldest, simplest and most widely used methods is the determination of MDA with thiobarbituric acid (Ohkawa *et al.* 1979, Janero 1990, Esterbauer *et al.* 1991, Ďurfinová *et al.* 2007, Catalá 2009, Guéraud *et al.* 2010). However, thiobarbituric acid reacts with several substances that are not related to lipid peroxidation. This may contribute to the overall absorbance, which is not corresponding to the concentration of MDA *in vivo* but rather to a range of products appropriately termed TBARS. Thiobarbituric assay is often criticized because of low specificity for MDA (Esterbauer 1996, Liu *et al.* 1997, Dalle-Donne *et al.* 2006, Lykkesfeldt 2007). Nevertheless, the assay is still frequently used at present time (Seminotti *et al.* 2011, Weis *et al.* 2011, Sumathi *et al.* 2012). To detect and measure biologically relevant lipid peroxidation products many other methods have been developed (Kohen and Nyska 2002, Soh 2006, Wardman 2007, Yin 2008, Vladimirov and Proskurnina 2009, Spickett *et al.* 2010).

Physiological functions of cell membranes change because lipid peroxidation modifies properties of

membrane bilayer such as membrane potential, fluidity or permeability to different substances. Consecutively the properties of membrane receptors or membrane-bound enzymes could be altered. For our studies we chose rat cerebral cortex Na^+,K^+-ATPase. Membrane-bound Na^+,K^+-ATPase (EC 3.6.1.9.) plays an essential role in cellular ion homeostasis because it is responsible for generation of the membrane potential through the active transport of sodium and potassium ions (Kaplan 1985, 2002, Lingrel and Kuntzweiler 1994). In the brain this enzyme (moving 3 Na^+ from inside to outside against 2 K^+ in the opposite direction with a hydrolysis of 1 ATP molecule) is necessary to maintain neural excitability and cellular volume control. We evaluated the time course of its inactivation after the induction of lipid peroxidation by ADP.Fe^{2+}/ascorbate prooxidant system (Rauchová *et al.* 1999). Our data indicated that membrane fluidity changes (evaluated by steady-state anisotropy measuring using DPH and TMA-DPH probes) participate in Na^+,K^+-ATPase inhibition during the initial period of lipid peroxidation process, whereas during the following period the enzyme changes inversely correlates with levels of TBARS and conjugated dienes. The influence of lipid peroxidation on Na^+,K^+-ATPase is known for 40 years but the different aspects are studied till present time (Sun 1972, Svoboda *et al.* 1984, Mishra *et al.* 1989, Jamme *et al.* 1995, Kaplán *et al.* 1997, Kurella *et al.* 1997, Khan *et al.* 2003, Stefanello *et al.* 2007, Ribeiro *et al.* 2011).

Hypoxia

Hypoxia (insufficient oxygen supply to tissue) was defined by Connett *et al.* (1990) as oxygen availability not coping with aerobic ATP requirements. This can be paradoxically one of the reasons of increased lipid peroxidation as documented by increased production of indicators of oxidative stress in breath, blood, urine and tissue of laboratory rats or in humans (Askew 2002, Maiti *et al.* 2006, Behn *et al.* 2007). During hypoxia less oxygen is available to be completely reduced to water at mitochondrial cytochrome oxidase. Thus, reduced equivalents are accumulated in respiratory chain. The accumulation leads to ROS formation by the auto-oxidation of mitochondrial complexes with direct reduction of oxygen to superoxide and hydroxyl radical (Kehrer and Lund 1994). Besides mitochondrial respiratory chain other enzymes may contribute to an increased ROS formation under hypoxia, e.g. nitric oxide synthase (Turrens 2003) and xanthine oxidase (Dosek *et al.* 2007). Hypoxic cells are associated not only with biochemical alterations (Nakanishi *et al.* 1995, Li and Jackson 2002, Solaini *et al.* 2010) but also with different morphological changes (Oechmichen and Meissner 2006, Titus *et al.* 2007, Ježek and Plecitá-Hlavatá 2009). ROS released during hypoxia are key signals that trigger transcriptional regulators such as hypoxia-inducible factor-1 (HIF-1), nuclear factor κB (NF-κB), activator protein 1 (AP-1) and some mitogen-activated protein kinases (MAPK) (Semenza 2000, 2012, Li and Jackson 2002, Poyton *et al.* 2009). The brain is very complicated organ containing metabolically heterogeneous cell populations (Kalous *et al.* 2001). There are large variations in the vulnerability of different parts of the brain to hypoxia (Ikonomidou *et al.* 1989, Wang and Michaelis 2010). Moreover, the resistance to oxygen deficiency changes during the ontogenesis (Trojan 1978).

In our experimental model of short-term hypoxia simulating an altitude of 9000 m (air pressure = 30.7 kPa, pO_2=6.4 kPa) for 30 min we followed the changes in TBARS in four different parts of the rat brain: cortex, subcortical structures (including the thalamus, hypothalamus and basal ganglia), cerebellum and medulla oblongata during the postnatal development from 3-day-old till 90-day-old Wistar rats of both sexes. The increase of TBARS was estimated immediately after removing animals from the barometric chamber. We found the higher TBARS increases following acute hypoxia in the immature brain tissue than in the brain of adult rats (35- and 90-day-old). There were no significant changes in 35-day-old rats, whereas in 90-day-old rats we found significant increase of TBARS level only in the phylogenetical youngest brain region, i.e. cortex (Fig. 2).

Combination of different conditions (duration of hypoxia, type of hypoxia, duration of reoxidation) showed also age-dependent results: young rats were more sensitive to any hypoxic damage in comparison with the older ones studied after the end of weaning period (Koudelová and Mourek 1992, Koudelová *et al.* 1992). Paradoxically, we found that lipid peroxidation in the mature brain was lower as compared with lipid peroxidation in the immature brain in spite of the higher content of polyunsaturated fatty acids in mature brain (Šmídová *et al.* 1984). This fact could be explained by the lower activities of antioxidant enzymes in immature rats as we showed for erythrocyte catalase (Rauchová *et al.* 2005). We also found that the male brain tissue was more sensitive to hypoxia-induced lipid peroxidation in comparison with the female one.

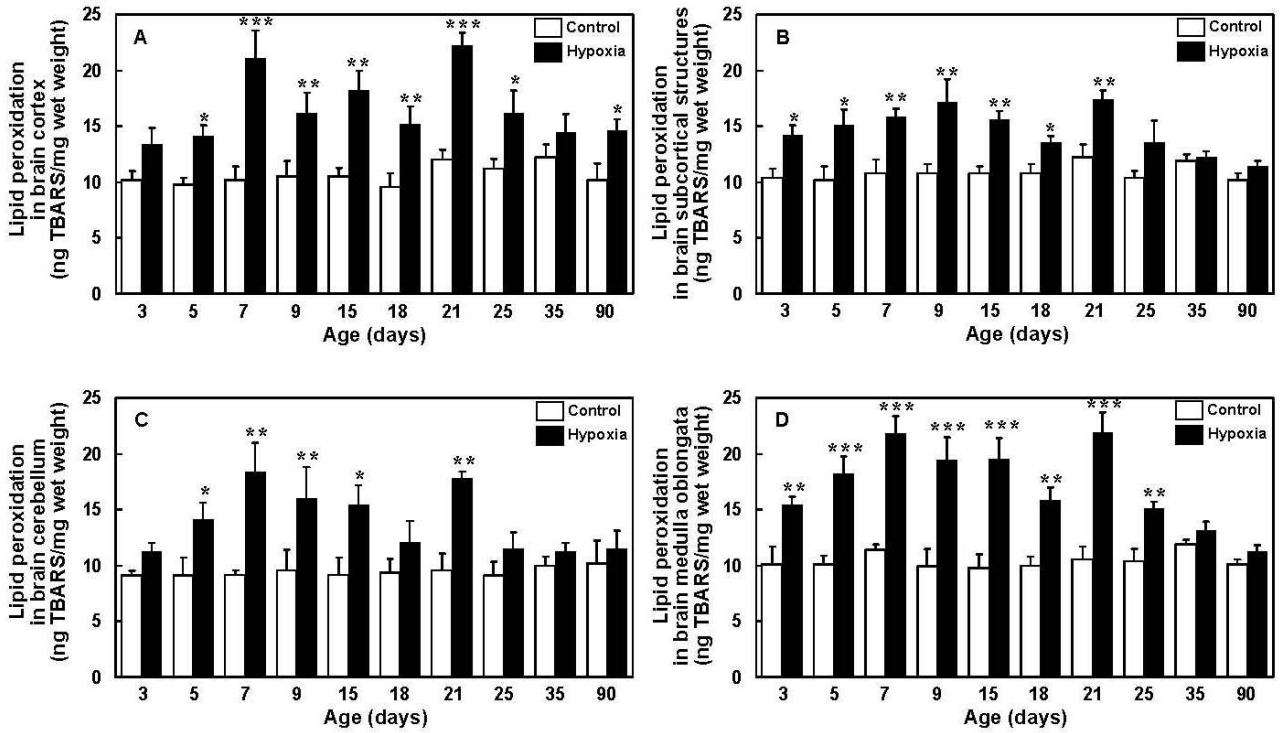

Fig. 2. Lipid peroxidation expressed as levels of TBARS in cerebral cortex **(A)**, subcortical structures **(B)**, cerebellum **(C)** and medulla oblongata **(D)** in controls and in Wistar rats of different age after 30 min hypobaric hypoxia. The results are given as the means ± S.D. Significantly different: * p≤0.05, ** p≤0.01 and *** p≤0.01 *vs.* controls.

Table 1. The effect of hyperoxia on lipid peroxidation expressed as levels of TBARS (ng/mg wet weight) in the different part of the brain in 7-day-old, 21-day-old and adult rats.

		7-day-old rats		21-day-old rats		Adult rats	
	Sex	Controls	100 % Oxygen	Controls	100 % Oxygen	Controls	100 % Oxygen
Cortex	M	7.8±0.5	18.4±2.0**	11.3±0.5	16.4±0.2**	9.3±0.6	11.6±0.4*
	F	6.9±1.1	9.5±0.2*##	10.8±0.4	11.1±0.4##	9.0±0.4	9.3±0.8
Subcortical	M	9.1±0.3	18.1±1.0**	11.2±0.6	14.7±0.2*	9.3±0.9	12.7±0.4*
structures	F	9.5±0.5	10.5±0.3##	11.0±0.4	11.8±0.3#	9.6±0.7	7.7±0.4#
Medulla	M	9.3±0.2	20.4±0.9**	13.5±0.3	18.4±0.7**	10.1±1.0	11.7±0.9
oblongata	F	13.4±1.2#	12.8±0.9##	11.8±0.7	11.9±0.2##	7.8±0.7#	5.9±0.3#
Cerebellum	M	7.2±0.2	17.8±1.2**	10.4±0.3	12.3±0.3*	9.2±1.0	9.1±0.6
	F	7.0±1.6	7.9±0.13##	9.6±0.5	7.1±0.2##	8.8±0.4	7.4±0.8

Data are presented as means ± S.E.M. M – males, F – females. Significantly different: * p≤0.05 and ** p≤0.01 *vs.* controls; # p≤0.05 and ## p≤0.01 *vs.* males.

Significantly higher resistance of female brain tissue to induce lipid peroxidation was considerable when rats were exposed to normobaric pure oxygen atmosphere for 30 min (Koudelová and Mourek 1994) as shown in Table 1. A similar model of normobaric 100 % hyperoxia with similar values of lipid peroxidation in brain tissue of adult and aged (30-month-old) rats was reported by Tong *et al.* (2002) who further demonstrated the attenuation in the DNA binding activity of the AP-1 and NF-κB transcription factors in aged rats. There are important components of stress response signal transduction pathways, which can determine the shifts in cellular commitments to necrotic death, apoptotic death or survival in the brain (Tong *et al.* 2002).

Antioxidant defense

Production of oxygen radicals is under the control by efficient cellular and extracellular defense systems. The defense against free-radical-induced damage includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). In our experiments we followed erythrocyte CAT (EC 1.11.1.6.) in normoxia and after the exposure to intensive acute hypobaric hypoxia (9000 m for 30 min) of Wistar rats. During the development CAT activity increased 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 its lower activity in males. We 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 organism (Rauchová *et al.* 2005).

A non-enzymatic defense includes water-soluble antioxidants such as glutathione, ascorbic acid, uric acid, bilirubin and lipid-soluble antioxidants such as α-tocopherol, β-carotene, melatonin or lipoic acid. Some of antioxidants are synthetized by the cells (glutathione, uric acid, bilirubin, melatonin) but some vitamins are derived from the diet. Several natural antioxidants, such as flavonoids isolated from plants (green tea polyphenols, red wine anthocyanins or soy isoflavone) have radical scavenging activity (Weisburger 1999, Lionetto *et al.* 2011). Moreover, there are also endogenous agents with radical scavenging activity, such as pyruvate and carnitine. Nontoxic and freely diffusible pyruvate (abundantly present in mammalian cells) is able to scavenge H_2O_2 in different cellular compartments (Desagher *et al.* 1997, Jagtap *et al.* 2003, Paquin *et al.* 2005, Wang *et al.* 2007). A quaternary ammonium compound L-carnitine (a vital cofactor for the mitochondrial oxidation of fatty acids) belongs to natural agents present in all mammalian tissue. L-carnitine (and its derivatives) possess unique neuroprotective, neuromodulatory and neurotrophic properties and are able to decrease the brain injury following an ischemic attack (Matsuoka and Igisu 1992, Zanelli *et al.* 2005, Picconi *et al.* 2006) or after hypoxia-ischemia in newborn rats (Wainwright *et al.* 2003, 2006, Onem *et al.* 2006) or to function as free radical scavengers in aging (Rani and Panneerselvam 2001, 2002, Muthuswamy *et al.* 2006).

We found that L-carnitine pretreatment (30 min before hypobaric hypoxia exposure) contributed to lowering of brain lipid peroxidation (Koudelová *et al.* 1994). In our experiments we also compared the protective effect of L-carnitine with that of well-known lipophilic reactive species scavenger, α-tocopherol (Chow 1991). Rats pretreated with α-tocopherol or L-carnitine had lower TBARS levels after the exposure to hypobaric hypoxia in comparison with untreated rats (Rauchová *et al.* 2002). Similarly, rats pretreated with phosphocreatine had lower TBARS levels after the exposure to hypobaric hypoxia. As a marker of hypoxia we followed lactate/pyruvate ratio. Lactate/pyruvate ratio was improved to control value only in rats pretreated with L-carnitine or phosphocreatine. We conclude that protective effect of L-carnitine or phosphocreatine was due to their regulation of metabolic reactions during hypobaric hypoxia rather than to their scavenger activity (Rauchová *et al.* 2002). Similarly, pretreatments with L-carnitine derivatives, acetyl-L-carnitine or propionyl-L-carnitine (intraperitoneal administration 30 min before hypobaric hypoxia exposure) prevented TBARS formation in four different parts of brain (Table 2). The decrease of free radical generation, lipid peroxidation and protein oxidation, which resulted in augmenting of the energy status and amelioration of memory impairment induced by 14-day-hypobaric hypoxia, was reported by Barhwal *et al.* (2009).

The plasma level of lactate increased after a short-term hypobaric hypoxia and significantly decreased in L-carnitine pretreated rats. One of the possibilities, why plasma lactate was decreased by L-carnitine pretreatment, could be the influence on the activity of lactate dehydrogenase (LDH; EC 1.1.1.27.), which catalyzed a mutual conversion between lactate and pyruvate. The hypoxia exposure increased serum LDH activity of 21-day-old rats only. Pretreatment of rats with L-carnitine decreased serum LDH activity in 21- and 90-day-old rats probably due to membrane stabilizing role of L-carnitine (Di Lisa *et al.* 1985, Arduini *et al.* 1990). However, we did not find any significant difference of LDH activity in the brain after the exposure to hypobaric hypoxia or L-carnitine pretreatment (Koudelová *et al.* 2006). Thus, acute hypobaric hypoxia and/or L-carnitine pretreatment modified serum but not brain LDH activity.

Table 2. The effect of hypoxia on lipid peroxidation expressed as levels of TBARS (ng/mg wet weight) in the different parts of the brain in 21-day-old rats.

	Sex	Before hypoxia	After hypoxia
CORTEX			
Controls	Males	12.1 ± 0.3	18.5 ± 0.7**
	Females	11.8 ± 0.2	17.8 ± 0.3**
L-carnitine	Males	12.0 ± 0.3	13.5 ± 0.5##
	Females	12.6 ± 0.3	11.3 ± 0.2##
Acetyl-carnitine	Males	18.3 ± 2.3	13.5 ± 0.6##
	Females	15.4 ± 0.9	14.9 ± 1.0##
Propionyl-carnitine	Males	18.7 ± 1.0	16.2 ± 1.4
	Females	16.9 ± 0.8	13.7 ± 0.8##
SUBCORTICAL STRUCTURES			
Controls	Males	12.5 ± 0.2	18.1 ± 0.6**
	Females	11.6 ± 0.2	17.9 ± 0.2**
L-carnitine	Males	12.4 ± 0.4	12.5 ± 0.3##
	Females	12.4 ± 0.5	11.5 ± 0.6##
Acetyl-carnitine	Males	16.3 ± 1.3	14.3 ± 0.6##
	Females	14.2 ± 0.7	13.0 ± 0.5##
Propionyl-carnitine	Males	18.2 ± 0.8	14.3 ± 1.0##
	Females	16.2 ± 0.9	13.9 ± 0.7##
MEDULLA OBLONGATA			
Controls	Males	11.7 ± 0.3	18.6 ± 0.4**
	Females	12.1 ± 0.2	17.1 ± 0.3**
L-carnitine	Males	11.9 ± 0.4	13.4 ± 0.5##
	Females	12.4 ± 0.7	11.5 ± 0.5##
Acetyl-carnitine	Males	16.2 ± 1.1	14.2 ± 0.7##
	Females	14.4 ± 0.7	13.5 ± 0.6##
Propionyl-carnitine	Males	14.7 ± 1.6	12.9 ± 0.8##
	Females	15.0 ± 0.5	13.0 ± 0.5##
CEREBELLUM			
Controls	Males	9.6 ± 0.4	18.4 ± 0.8**
	Females	9.0 ± 0.4	16.1 ± 0.4**
L-carnitine	Males	9.7 ± 0.2	9.1 ± 0.2##
	Females	9.8 ± 0.2	8.6 ± 0.3##
Acetyl-carnitine	Males	13.9 ± 1.6	10.1 ± 0.9##
	Females	11.7 ± 0.9	10.2 ± 0.9##
Propionyl-carnitine	Males	12.9 ± 1.7	11.5 ± 0.8##
	Females	13.4 ± 0.8	10.3 ± 1.2##

Data are presented as means ± S.E.M. Significantly different: ** $p \leq 0.01$ *vs.* the status before hypoxia; ## $p \leq 0.01$ *vs.* controls.

Conclusions

Our model of hypobaric hypoxia is associated with a paradoxical increase of ROS formation and lipid peroxidation in the brain. The resistance to reactive oxygen species in different parts of brain is dependent on the sex and age of animals. The necessity for fundamental and clinical research aimed to the development of the preventive or therapeutic help using a variety of antioxidant nutritional and pharmacological interventions is increasing. L-carnitine and its derivatives belong to promising natural antioxidants with minimal toxicity and

excellent tolerability. Their protective effect may be medicated through the regulation of metabolic reactions during hypobaric hypoxia.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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