

Reactive Oxygen Species and Antioxidant Enzymes in the Developing Brain

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ABSTRACT

Reactive oxygen and nitrogen species can regulate cellular processes fundamental for development such as proliferation, migration, differentiation and apoptosis. Brain cells at various stage of development can exhibit discrete changes in their parameters of oxidation and antioxidant defence. This review will focus on the role of reactive oxygen and nitrogen species and antioxidants in brain development. The following topics will be discussed: critical periods of the brain development, control of physiological functions by free radicals, especially the development process, participation of free radicals and antioxidants in brain development. Emphasis will be on changing in the activity and expression of antioxidant enzymes.

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Key words: Free radicals; Antioxidant enzymes; Brain development

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INTRODUCTION

The developing brain is very susceptible to oxidizing agents such as

free radicals. The vulnerability of the developing brain to oxidative stress is due to high metabolic activity, sufficiently high concentration of iron ions and relatively low antioxidant system. One of the main pathogenic factors of the brain damage is perinatal hypoxia-ischemia leading to oxidative stress. On the other hand, free radicals such as reactive oxygen or reactive nitrogen species can regulate fundamental cellular processes during development. This review will focus on the role of such oxygen derivatives and antioxidants in the developmental processes.

FREE RADICALS AND CONTROL OF PHYSIOLOGICAL FUNCTIONS

A free radical can be defined as a molecule with one or more unpaired electrons in its outer orbital. This unpaired electron usually gives a considerable degree of reactivity to the free radicals. Main radicals generated in the mammalian organism are oxygen derivatives called reactive oxygen species (ROS). The most common ROS are superoxide anion, hydrogen peroxide and their derivatives, e.g. hydroxyl radical. There are also reactive nitrogen species (RNS); the best known among them are nitric oxide (NO) and peroxynitrite. However, there exist other special "reactive species" (abbreviation "RS" introduced by Halliwell^[1]) manifesting biological effects, e.g. reactive chlorine species (hypochlorite) or reactive sulfur species^[1-4]. RS are highly reactive oxidants and their uncontrolled production may destroy cellular function. The harmful effect of free radicals causing potential biological damage is termed "oxidative stress"^[5]. In order to avoid oxidative stress in the cells, special mechanisms have developed during evolution. Compounds that prevent cellular damage may act in a number of ways – from prevention of ROS and RNS formation, to reparation of oxidative molecules. The antioxidant system involves various types of antioxidants, which are usually found in cells and tissues. Antioxidants can be classified as enzymatic (superoxide dismutase, catalase, glutathione-dependent enzymes, glutaredoxins, peroxiredoxins, thioredoxin system) and non-enzymatic (vitamins C, E, glutathione, carotenoids, flavonoids

and so on)^[6,7]. Under normal conditions there is a balance between the intensity of free radical oxidation and the activity of antioxidant defense. When there is a deficiency of antioxidants on the one side and overproduction of free radicals on the other, oxidative stress occurs in the cells^[8,9].

The steady state level of oxygen radicals and related “reactive species” plays an important physiological roles. Due to their high reactivity, the location where they are produced is essential for manifestation of their biological functions^[3,10]. ROS play a key role in the defence of organisms against pathogens^[2]; it is now well accepted that ROS are signaling molecules, which are involved in the intracellular signaling cascade and the regulation of signal transduction^[10,11,12]. Nitric oxide is involved in vascular tone, innate immunity, and neuronal signal transduction. Moreover, RS can play a role in development^[10,13]. At least the ability of ROS to regulate cellular processes fundamental for development such as proliferation, migration, differentiation and apoptosis was shown^[14,15]. Cells and tissues at various stages of differentiation can exhibit discrete changes in their parameters of oxidation and antioxidant defence^[13]. Control of cell proliferation and differentiation involves changes in gene expression and is characterized by the appearance of tissue-specific enzymes and other proteins. The mechanisms by which RS can regulate these processes generally involve changes in the redox status (the ratio of oxidizing and reducing equivalents – $R_{ox/red}$) of cells. It includes $R_{ox/red}$ for the thiol groups of peptides and proteins involved in almost all signaling pathways known to date^[2,16,17]. These oxidative modifications result in changes in the structure and/or function of the proteins including protein conformation, enzyme activity, transporters and receptors modification, protein–protein interactions, protein–DNA interactions and so on^[18].

CRITICAL PERIODS OF THE BRAIN DEVELOPMENT

Nervous system development is marked by several periods, each of them defined by major events in brain growth and structural maturation^[19,20,21,22]. In general, these processes are remarkably parallel for rodents and humans, but the temporal windows are significantly different^[22]. In the rat central nervous system (CNS) there is a considerable period of postnatal development. Neurogenesis (process that includes proliferation, migration and differentiation of neurons) starts in embryogenesis (in Embryonic Day (ED) 12–15), but the maximal rate of proliferation occurs in Postnatal days (PD) 10–15, including an increase of cell size, growth of axons and dendrites^[19]. It should be noted that timing of neurogenesis varies in different brain regions.

The most important period in postnatal ontogenesis of the brain is the process of myelination. Formation of myelin begins in different animals in different ways: some neurons in humans begin to be covered by myelin quite early in the prenatal period and at the age of 3–5 years the brain is almost completely myelinated; in rats and other nest-building animals myelination occurs mainly in the postnatal period: beginning in about 10–12 days and reaching the maximal rate of myelin accumulation in about 20–21 days^[19,22,23].

The period of maximal myelination and gliogenesis coincides with other important changes in the CNS. Myelination dramatically alters the lipid composition of the brain^[24]. Brain weight increases more than four times and reaches 90–95% of adult weight. It is known as “brain growth spurt”. The critical period of synaptogenesis in rats occurs during the first three postnatal weeks with the changes in many neurotransmitter systems^[25]. These processes need energy.

The rate of glucose metabolism and energy utilization as well as in oxygen uptake significantly increases (from 3 to 7 times according to various estimates) between PD7–10 and PD21 days^[20]. Within the cells the number of mitochondria increases. Acceleration in mitochondrial respiration occurs immediately after birth and then the number of mitochondria per cell quadruples from PD1 to PD21; changes also occur in the density, structure and functional activity^[26].

Another important event in the developing brain is the programmed cell death (apoptosis). The excess of neuronal cells, forming during the brain development, is eliminated by apoptosis that is triggered by internal signal (e.g. from ROS). In certain brain regions more than half of neurons die due to apoptosis during normal brain development^[27]. This process occurs during pre- and postnatal development. The earlier apoptosis occurs in proliferative zones and the later one – in neuronal and glial post mitotic cells^[19].

ROS/RNS, ANTIOXIDANTS AND BRAIN DEVELOPMENT

Several metabolic features of the adult brain suggest that it is sensitive to oxidative damage. These features include enhanced oxygen metabolic rate (brain uses 20% of O_2 body consumption), leading to excessive levels of ROS; high content of polyunsaturated fatty acids and the presence of redox-active metals (Cu^{2+} and Fe^{2+}) in some brain regions; an inadequate defense system against oxidative stress^[28]. At the same time, the brain can use the increased production of ROS to perform specific neuronal functions.

Oxygen derivatives generated metabolically may be a cause of the initiation of certain developmental events in the brain^[29]. It has been shown that ROS can influence multiple aspects of neurogenesis, including the proliferation of neural precursors, their differentiation into specific neuronal cell types. For example, high levels of ROS are present in newborn neurons^[30,31]. In cell cultures from embryonic rat cortex (isolated from ED 14–15) ROS were generated as soon as neurons differentiated from multipotent progenitor cells and are not associated with cell death. Worth noting that a high level of ROS persists in the adult brain only in neurogenic regions such as the hippocampus and olfactory bulb. These neurons differentiate in the clonal culture into two types of cells: large pyramidal-like neurons and smaller neurons that express nuclear calretinin. Treatment with antioxidants, lowering ROS level, does not alter number of neuron but dramatically changes the ratio of formation of these two types shifting differentiation towards an increase in the number of smaller neurons^[31]. These data indicate that the level of ROS in the cells can influence neuronal differentiation.

As previously mentioned the mechanisms by which ROS affect these processes involve changes in redox state. Redox state has been shown to be essential for NGF (nerve growth factor)-induced differentiation of pheochromocytoma PC12 cells^[32,33]. It may influence differentiation of mesencephalic precursors^[34] and neural crest stem cells^[35]. In addition, redox state modulates differentiation of glial precursor cell (oligodendrocyte/type-2 astrocyte progenitors)^[36].

RNS can be involved in the development and maturation of the nervous tissue too. It has been hypothesized that messenger NO may affect axonal growth, differentiation and migration process during embryonic development of the rat brain and play an important role in neuronal maturation and cortical plasticity in early postnatal development^[37,38]. It is suggested also that NO can mediate the switch from proliferation to differentiation during neurogenesis^[39]. Transcription of all three NO-synthase isoforms (neuronal - nNOS,

endothelial- eNOS, and inducible - iNOS) take place in brain cells from early embryonic period. Expression of nNOS in rats was first observed on ED14 in the marginal zone of the cortical mantle – in cells responsible for migration of neuronal precursors to their appropriate positions in the cortical plate^[40]. This activity decreased from ED20. At the same time expression of iNOS during embryonic development is significantly lower than that of nNOS. During early postnatal development cortex of rats exhibits a high expression of nNOS and iNOS. The expression of iNOS increases from PD1 to PD10 and then declines to a very low level during the adult period, while nNOS maintains comparatively high expression throughout adult life^[41,42].

Expression and activity of the antioxidant enzymes also change during the maturation of the brain. Despite limited and contradictory data on this issue, the most considerable changes in enzyme activity in mammals are observed immediately after birth (in response to changes in oxygen tension), whereas the expression of antioxidant enzymes takes place in embryogenesis.

One of the main antioxidant enzymes is superoxide dismutase (SOD). It catalyzes the dismutation of superoxide anion to hydrogen peroxide. In mammals there are three SOD isoforms: the Cu/ZnSOD – abundant cytosolic enzyme coded by the *Sod1* gene, the MnSOD – mitochondrial enzyme coded by the *Sod2* gene and minor extracellular Cu/ZnSOD coded by the *Sod3* gene^[43]. The activity and expression of Cu/Zn SOD in the rodent brain were shown at ED15–18^[44,45]. An increase in Cu/Zn SOD expression was demonstrated around the time of birth in rats^[45]. Activity of SOD in guinea pigs remained constant throughout the ED30–60 period^[46].

In the postnatal period Cu/Zn SOD levels are reported either to reach a peak around the second week of life and then declines to adult level^[44,47] or increases up to adult level with some variations^[48,49,50,51]. On the other hand the enzyme activity remained relatively constant whereas Cu/Zn SOD protein level increased from PD1 to PD21 that may be related to copper availability^[52].

In the immature rat brain widespread expression of Cu/Zn SOD is observed primarily in neurons: in cortical layers II, III and V, in the sub-plate, in the pyriform cortex, in the hippocampus, and in the hypothalamus^[53], with the lack of expression in glial cells. Very little is known about the presence of this enzyme in progenitor cells in the immature brain. It is suggested that Cu/Zn SOD is expressed in certain populations of these cells at certain stages of maturation^[54].

The activity of mitochondrial superoxide dismutase (MnSOD) gradually increased during the first month of life^[44,50]. It is suggested that MnSOD plays a critical role in protection of neuronal cells against oxidative stress and is essential for animal survival^[55]. Complete knockout of MnSOD (*Sod2*–/–) in mice results in early neonatal death^[56,57]. Over expression of Cu/ZnSOD does not prevent oxidative imbalance and neonatal lethality in mice that lack MnSOD^[58], but over expression of MnSOD decreases death of differentiated neuroblastoma cells with mutation in *Sod1*^[59]. More over in the immature brain over expression of Cu/ZnSOD induced tissue damage after perinatal hypoxia-ischemia^[60].

The two main enzymes involved in H₂O₂ detoxification are catalase and glutathione peroxidase. In the adult brain the major systems responsible for this process are peroxidase systems, including glutathione/glutathione peroxidase and thioredoxin/peroxiredoxins^[61]. Activity of catalase and glutathione peroxidase in guinea pig brain has been demonstrated to remain constant from ED30 to 45 and to increase from ED45 to ED60^[46]. Catalase activity in the developing rat brain is apparently higher than in the adult one^[49,62]. It should be noted that the specific activity of catalase is significantly lower in the

adult brain than in other tissues^[28]. According to various estimates the maximum enzyme activity in different brain regions of rats is observed from birth to PD5 - PD10, thereafter decreasing to the adult level around day 30^[44,49]. Our data (not published) suggest that the activity of catalase in the cytosolic fraction of rat brain cortex is two times higher in PD5 than in PD10. On the other hand catalase expression and activity were relatively constant during the maturation of oligodendrocytes^[63]. However, the complete absence of enzyme is not critical for survival. Catalase knockout mice develop normally, but their brain mitochondria are more susceptible to trauma-induced oxidative stress^[64].

Glutathione peroxidases (GPxs) are several-times more abundant than catalase in the adult brain^[28]. The GPx family includes eight different isoforms (GPx1 – 8), which are encoded by different genes and have different substrate specificities. In addition to other enzymes, such as thioredoxin reductases and deiodinases of thyroid hormones, GPxs (except for GPx5, rodent GPx6, and GPx7) are members of a large group of selenium-containing proteins^[65,66,67]. Main GPxs of the brain are classical GPxs (cGPx or GPx1), this enzyme has also been called cytosolic or cellular GPx, and phospholipid hydroperoxide glutathione peroxidase (PHGPx or GPx4)^[28].

The total GPx activity was very similar in different brain regions, it decreased after birth, but then increased again after 10 days reaching the maximum activity by 11th week^[49]. Data of other authors demonstrated continued elevation in GPx activity up to the P45^[44]. Our data showed that the activity of cytosolic GPxin the rat brain cortex did not substantially change during the first 20 days of life, but it was significantly higher at the PD90^[68].

Among others glutathione peroxidases – GPx4 is a unique member playing a key role in brain development and function, presumably due its impact on proliferation^[69,70]. Gpx4 knockout mice show abnormalities in brain development. Complete absence of the enzyme leads to the death of the embryo^[70]. GPx4 exists as cytosolic, mitochondrial, and nuclear isoforms^[71]. Comparative expression analysis showed that the onset of expression Gpx4 in rats had been observed during gastrulation^[72]. At ED6 all three isoforms are expressed in extra-embryonic and embryonic structures, including the ecto-, meso- and endoderm. During the somite stage of development, mitochondrial and cytosolic isoforms of GPx4 mRNA were expressed in the forebrain, midbrain and hindbrain and in the developing eye. Despite the fact that the expression of nuclear isoforms was found during embryogenesis, it is very poorly expressed in the neuroepithelium and expression disappears at ED16–17 until birth. During postnatal development, GPx4 is mainly found in the cortex, hippocampus and cerebellum. Expression of the enzyme reaches peak values in the PD15 and gradually decreases thereafter^[69,73].

Little is known about the expression and function of glutaredoxins (Grxs), peroxiredoxins (Prxs), thioredoxins (Trxs) during normal brain development. Glutaredoxin and thioredoxin are present in many tissues of mouse embryo at organogenesis stage (ED11–13) and increase later in development^[74]. These oxidoreductases may control the cellular redox state^[66] which, as mentioned above, is very important for brain embryonic development. Most vertebrates contain four Grxs: Grx1, Grx2 (dithiol) and Grx3, Grx5 (monothiol)^[75]. The developing zebrafish brain depends on the enzymatic activity of Grx2, loss of Grx activity during development is associated with apoptotic neuronal death^[76].

All these data indicate that the steady state level of ROS/RNS plays an important role in the development of mammalian brain.

Besides that there seems to be a general trend towards increasing antioxidant capacity in the brain during maturation. Accordingly, a decrease in antioxidants (in some pathological states) can have negative consequences for developmental processes. However, addition of antioxidants can disturb the natural redox homeostasis in the brain and have unpredictable deleterious consequences. It should be concluded that experimental data about antioxidant system of the brain during embryonic and early postnatal periods are still insufficient and contradictory.

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CONFLICT OF INTERESTS

The author has no conflicts of interest to declare.

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