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Aging affects but does not eliminate the enzymatic antioxidative response to hypoxia/reoxygenation in cerebral cortex

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Abstract

The effect of aging on basal and hypoxia/reoxygenation levels of both oxidative stress (protein carbonyl and TBARS) and antioxidative-enzyme activity (Cu/Zn-SOD; Mn-SOD; Catalase, CAT; Se-independent and Se-dependent glutathione peroxidase, GPX; glutathione transferase, GST and glutathione reductase, GR) has been studied in the cerebral cortex of adult and old rats. Oxidative stress markers increased with aging and show an age-dependent post-hypoxic response. Moreover, aging caused either no change (GST, GR and CAT) or an increase (Se-GPX, Cu/Zn-SOD, Mn-SOD) in the basal activity of the enzymes analysed. Only Se-independent GPX activity decreases. However, we detected an age-dependent response of SODs to the hypoxic injury. The early and sustained Cu/Zn-SOD activity rise in adult animals became late and weak in aged animals. Meanwhile, aging slowed the Mn-SOD post-hypoxic response although this activity was consistently higher in aged rats. Aging eliminated the post-hypoxic CAT response, but, perhaps offset by increased GPX activity, did not affect the GST response and slightly reduced post-hypoxic GR activity. In conclusion, aging rise basal ROS production, does not diminish or even increase the antioxidative-enzyme activity, and may slow but does not usually eliminate the enzymatic antioxidant response to the increased post-hypoxic ROS generation.

Keywords: Aging; ROS; Antioxidative enzymes; Cerebral cortex; Hypoxia/reoxygenation

1. Introduction

The free-radical or 'oxidative stress' theory holds that oxidative reactions are the main factors underlying the aging process (Harman, 1994). Moreover, age is known to be an important factor for the incidence and prevalence of hypoxic episodes, as well as for their outcome (Moulin et al., 2000; Shapira et al., 2002). In fact, among the complex metabolic reactions occurring during hypoxia/reoxygenation, many could be related to the formation of oxygen-derived free radicals (ROS) (Cazevieille et al., 1993; Globus et al., 1995; Saugstad, 1996), causing a wide spectrum of cell damage (Halliwell and Gutteridge, 1984; Yu, 1994).

Oxidative injury of the tissues involves the accumulation of damage due to persistent oxidative stress and/or the loss of proper balance of antioxidative mechanisms. The antioxidant system is composed of non-enzymatic substrates such as reduced glutathione (GSH), as well as enzymes such as copper/zinc-dependent superoxide dismutase (Cu/Zn-SOD), manganese-dependent superoxide dismutase (Mn-SOD), catalase (CAT), glutathione peroxidases (GPX), glutathione transferases (GST), and glutathione reductase (GR) (reviewed in Yu, 1994; Dringen et al., 2005). SOD enzymatically scavenges superoxide, converting it to H_2O_2, which is catabolised to water mainly by CAT, preventing the formation of the highly reactive hydroxyl radical. However, GPX may also catalyse the reduction of H_2O_2 and organic hydroperoxides. Two types of GPX have been described, selenium-dependent (Se-dependent GPX) and selenium-independent GPX (Se-independent GPX), with different substrate specificities. Se-dependent GPX exhibits a low capacity for H_2O_2 reduction while Se-independent GPX utilizes organic hydroperoxides as preferred substrates over H_2O_2. Another essential component of the antioxidative system is the GST family, involved in cell defence against peroxidation products of DNA and lipids (Ketterer and Coles, 1991). Finally, the maintenance of intracellular GSH levels is carried out by GR.

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The central nervous system is particularly vulnerable to oxidative damage because of its high energy requirements, high oxygen consumption, high tissue concentration of iron and relatively low levels of some antioxidant systems (Floyd, 1999). In fact, compared to others, CNS cells undergo a more pronounced functional decline with aging. However, few reports are available on the effect of age (Iantomasi et al., 1993; Tian et al., 1998; Floyd and Hensley, 2002; Martínez-Lara et al., 2003) and hypoxia/reoxygenation (Kimoto-Kinoshita et al., 1999; Liévre et al., 2000) on the antioxidative enzymes activity in the brain.

Therefore, the aim of the present work is to study the pattern of aging and hypoxia/reoxygenation-induced rat cerebral cortex changes in the production of ROS and in the main enzymatic defence mechanisms.

2. Materials and methods

2.1. Animals

A total of 24 adult (4-month-old) and 24 aged (28-month-old) male Wistar rats were kept under standard conditions of light and temperature and allowed ad libitum access to commercial rat chow and water until and after the exposure to the different conditions in the experimental chamber. All the experiments were carried out according to EU guidelines on the use of animals for biochemical research (86/609/EU).

2.2. Experimental procedure

For 30 min, 20 adult or aged rats were exposed to hypobaric hypoxia (8100 m, ≅30,000 ft) and killed at 0, 2, 24, 48 h or 5 days of reoxygenation. For the acute hypoxic hypobaric conditions, the experimental chamber was decompressed to 225 mmHg, resulting in a 48 mmHg O_2 partial press (PO_2) (López-Ramos et al., 2005). The ascent and descent speed was maintained below 1000 ft/min. Four animals from each age, maintained under normobaric normoxic conditions, were used as sham control animals.

2.3. Determination of protein carbonyl levels

The protein carbonyl contents were analyzed by 2,4-dinitrophenylhydrazine method as described by Levine et al. (1990). Results were expressed as nmol/mg protein.

2.4. Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances were determined as described by Buege and Aust (1978). Results were expressed as absorbance at 535 nm.

2.5. Enzyme assays

For the enzymatic assays, the cerebral cortex from four animals in each group was dissected, rinsed in saline solution and stored at −80 °C until used. Cerebral cortices were homogenized and sonicated in 1/9 (w/v) of 30 mM Tris–HCl, pH 7.4 containing protease inhibitors. After centrifugation at 10,000×g for 40 min, the supernatant were collected for protein determination (Bradford, 1976) and subsequent analysis. All the procedures were performed at 4 °C.

The GST activity towards the 1-chloro-2,4-dinitrobenzene (CDNB) was measured spectrophotometrically as described by Habig et al. (1974). CAT activity was studied by monitoring the decomposition of H_2O_2 at 240 nm, according to the method described by Beers and Sizer (1952). GR activity was measured by following the rate of NADPH oxidation at 340 nm (Carlberg and Mannervik, 1985). SOD activity was assayed by measuring the rate of inhibition of cytochrome c reduction by superoxide anions generated by a xanthine/xanthine oxidase system (Flohé and Ötting, 1984). For discrimination between Cu/Zn-SOD and Mn-SOD activities, the assay was additionally performed after incubation in the presence of KCN, which selectively inhibits Cu/Zn-SOD isoform. GPX activity was determined in a coupled assay with GR using cumene hydroperoxide (Se-independent) or H_2O_2 (Se-dependent) as substrates (Flohé and Günzler, 1984).

2.6. Statistical analysis

Data are expressed as means±SD for four rats in each group. The student's t-test was performed to evaluate the significance of differences between groups, accepting $P<0.05$ as the level of significance.

3. Results

3.1. Protein oxidation

Protein carbonyl content determination is commonly used for assessing the modification of proteins as a consequence of oxidative stress (Butterfield et al., 1998). As shown in Fig. 1, aging significantly increases protein carbonyl levels ($P<0.05$). Immediately after hypoxia (0 h) carbonyl content rises only in adult animals ($P<0.001$). However after 48 h of reoxygenation a second elevation, more significant in aged rat cerebral cortex, can be observed in both age groups ($P<0.01$ and $P<0.001$ in adult and aged groups, respectively).

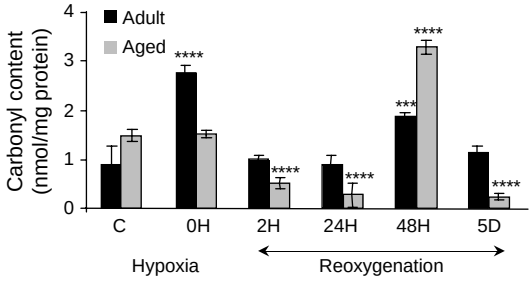

Fig. 1. Influence of hypoxia and reoxygenation on protein carbonyl content in the cerebral cortex of adult and aged rats. Protein carbonyl levels (nmol/mg protein) were determined as described in Section 2. Data are expressed as means±SD for $n=4$ per group. All measurements were run in triplicate. C, control; 0, 2, 24, 48 h and 5D, reoxygenation times. Statistically significant differences from the corresponding age control group: ***$P<0.01$, ****$P<0.001$.