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Age-Related Alterations in Superoxide Dismutase and Catalase Activities in Rat Brain

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Abstract: Active oxygen species have been proposed to be involved in the aging process of the brain, therefore alterations in the activities of enzymes involved in the defense system against free radicals and other active species could substantially influence the aging process.

This study is planned to determine the brain superoxide dismutase (SOD) and catalase activities which defend the components of brain cells against active oxygen species, in order to evaluate whether they play a role in the aging process.

The activities of SOD and catalase were measured in the cerebral cortex and cerebellum of Swiss male albino rats aged either 2 months (n=11) or 16 months (n=14). Tissue SOD activities were determined by Misra and Fridovich's method while catalase activities were measued by a modified method of Luck and Aebi.

When the results of this study were evaluated regardless of age, it is observed that catalase activity is higher in the cerebellum than the cerebral cortex. Whereas the cerebral cortex showed age-dependent decreases in both SOD (6.01 ± 0.94 vs 4.47 ± 0.34 U/mg protein, $p<0.05$) and catalase (0.235 ± 0.02 vs 0.145 ± 0.02 U/mg protein, $p<0.01$) activities, cerebellum showed a decrease only in the catalase activity (0.367 ± 0.05 vs 0.199 ± 0.04 U/mg protein, $p<0.01$).

Due to the fact that SOD and catalase have an important role in free radical detoxification of the brain, the age-related decrease in the expression of these enzymes might predispose this tissue to increased free radical damage.

Key Words: Aging, SOD, catalase, cerebral cortex, cerebellum, brain, rats.

Introduction

The changes that occur during aging may be attributed to environmental factors, inborn processes or underlying disease (1). The mechanisms responsible for the aging process are not throughly clarified (2). In recent years, many theories have been proposed to account for this process (1). Although no single theory has been generally accepted as yet, the free radical theory of aging by Harman et al (3) seems to be promising (1). This currently popular hypothesis predicts that the rate of aging is dependent on the level of oxidative stress, i.e., the balance between pro-oxidants and antioxidants and the consequent oxidative damage (2). Free radical induced damage is thought to be responsible, at least in part, for the degenerative effects of aging (4). Many investigators believe that free radical damage to cellular molecules and organelles is the primary cause of aging of the organism (5).

Superoxide anion is known to inactivate enzymes and initate the damaging chain reactions of lipid peroxidation. Cellular defense mechanisms against superoxides include a series of linked enzyme reactions which remove the toxic radicals and repair radical induced damage. The first of these enzymes is superoxide dismutase (SOD: EC 1.15.1.1) which converts superoxide anion to hydrogen peroxide. Hydrogen peroxide, also toxic to cells, is removed by catalase (EC 1.11.1.6) (4).

Cutler (6) has proposed that enzymes scavenging active oxygen species are key factors in determining the longevity of animals. Semsei I. et al (5) point to correlations between the levels of these enzymes and the maximum life span potential of different species.

So far, various age related changes have been investigated in lipid peroxidation and in antioxidant enzyme capacity in rat tissues. However it must be stressed that these experimental results are inconsistent (5, 7). Electron paramagnetic resonance studies have shown that free radical production increases with age in

Table 1. The mean±SEM values of the antioxidant enzyme activities and age-related decrease (%) in the enzyme activities in different brain regions. (CAT:catalase)

	2 months n=11		16 months n=14			
	U/mg protein	%	U/mg protein	%	Decrease %	
Cortex SOD	6.010±0.94	100	4.470±0.34	74.1	25.9	p<0.05
Cortex CAT	0.235±0.02	100	0.145±0.02	61.7	38.3	p<0.01
Cerebellum SOD	5.420±0.43	100	4.720±0.46	87.1	12.9	p>0.05
Cerebellum CAT	0.367±0.05	100	0.199±0.04	61.7	45.8	P<0.01

all organs, this elevation being more prominent in brain tissue compared to other tissues such as the heart or the liver. (8).

Based on all these points, the objective of this study has been to investigate the role of brain SOD and catalase activities in the aging process.

Materials and Methods

Reagents and solutions: All reagents were analytical grade and purchased from Sigma Chem Co (St Louis) and Merck Darmstadt (Germany).

Animals: Swiss male albino rats aged 2 months (n=11) and 16 months (n=14) were used in this study. Food and water were permitted ad libitum to the two different age groups. Two animals; one from each group, were guillotined on the same day, their brains being removed immediately and chilled in ice cold 0.9% w/v NaCl.

Assays: Cortex cerebri and cerebellum were dissected from the surrounding tissue on a chilled dissection board and rinsed in ice cold 0.9% w/v NaCl to remove blood. Tissue samples were blotted, weighed and afterwards placed into a phosphate buffer solution (KH_2PO_4 50 mM, Na_2HPO_4 50 mM, pH 7.0) in a ratio of 1/10 (w/v) and were homogenised at 0°C using a Braun homogenizer. After centrifugation at 600 g and 4°C for 10 minutes, the supernatants were removed and used for enzyme analysis and protein determination. Measurement of superoxide dismutase and catalase activities were made in duplicate with a LKB uv spectrophotometer.

Measurement of total SOD activity was performed according to Misra and Fridovich (9) based on the inhibition of autoxidation of epinephrine. The catalase mediated decomposition of H_2O_2 was followed directly at 240 nm with a modified method of Aebi (10) and Luck (11). For protein measurements, Lowry's method (12) was used.

Statistical analysis: The results reported represent the mean±SEM. Differences between the groups were evaluated using Student's t test. A p value <0.05 was considered to be statistically significant.

Results

The mean±SEM values of SOD and catalase activities and the age-related decrease (%) in the enzyme activities in different brain regions are summarized in Table 1. Whereas the cerebral cortex manifests age-dependent decreases in both SOD ($p<0.05$) and catalase ($p<0.01$) activities, the cerebellum manifests a statistically significant decrease only in the catalase activity ($p<0.01$). Meanwhile, in both groups catalase activity is higher in the cerebellum than in the cerebral cortex (Table 1).

Discussion

It has been suggested by many authors that oxidative stress is a possible aging-accelerating factor (7). During the aging process, tissues are damaged to some extent due to the oxidative processes primarily caused by reactive oxygen species. In particular, superoxide anion radicals are believed to be the major cause for such oxidative damages of living tissues (13-16).

Among various antioxidative mechanisms in the body, SOD is thought to be one of the major enzymes which protects against tissue damage caused by the potentially cytotoxic reactivities of radicals (13, 17). It is therefore possible that the decreases in SOD activities with age may be closely related to the aging of the organism. The reported decrease in SOD activities with age may further accelerate the aging process (5, 13, 18-23). Furthermore some authors even suggest a causal relationship between activities of antioxidant enzymes and the life span of animal species (24).

The effect of aging on the activities of SOD and catalase has been studied in a variety of organs and animals (1, 5, 25). Previous studies on the effects of age on the antioxidant enzyme activity of the brain have

yielded conflicting results and none of these studies have attempted to correlate the potential for oxidative metabolism with the enzyme activity (25, 26). Sawada and Carlson (27) report that superoxide radical formation increases with age, therefore a decreased protection againts toxic radicals may have serious consequences for the aging brain. Mizuno and Ohta (28) have found a direct correlation between the decrease in total activity of SOD and an increase in the level of lipid peroxidation in different regions of the aging rat brain. Benzi et al (18) also report a decline in the total SOD activity in different regions of the rat brain between 5 and 35 months of age.

The enzyme activity of catalase has also been shown to decrease during the development (1-40 week) of rat brain (25). Vertechy M et al (26) point out that in general the activity of catalase declines during the maturation of the animal to adulthood (15 months). As to the activity of SOD, no significant age-related changes have been found in any of the brain areas investigated. Similar to the results of Vertechy M et al (26), Carillo MC et al (13) and Matsuo M et al (7) imply that activities of catalase and SOD are relatively stable throughout the life span. In contrast Del Maestro R et al (25) indicate a marked and progressive decline in catalase activity in all of the brain regions studied, while Semsei I et al (5) point to a gradual decrease in the activity of both SOD and catalase with increasing age.

These discrepancies may be due to differences in methodologies for tissue preparation and enzyme determination. It is also possible that these discrepancies are due to differences in organs, sex, species and ages of animals studied. However, if these enzymes are directly related to the cellular aging process of the organism, there should be some common changes regardless of variables such as animal species or sex, at least in certain organs or tissues of the body (13).

In concordance with all of the findings (2, 5, 18, 25, 27, 28) supporting the role of oxidative stress in aging, our results point to an age-dependent decrease in the cerebral cortex SOD ($p<0.05$) and catalase ($p<0.01$) activities, while in the cerebellum only a decrease in the catalase activity ($p<0.01$) could be detected (Table 1).

In conclusion, our findings imply that SOD and catalase manifest age-related decreases in brain tissue. These changes may be contributory to the increased free radical damage of this tissue. However, accepting that the brain is a very heterogenous tissue with different cell types and function, further detailed studies shoud be carried in order to compare the expression of SOD and catalase in different regions of the brain tissue during aging.

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