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Low glutathione levels in brain regions of aged rats

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Glutathione (GSH) was measured in 6 regions of brain and liver of young adult, middle-aged and aged rats. GSH levels were significantly lower in cortex, cerebellum, striatum, thalamus and hippocampus of aged rats, while no changes were observed in liver as compared to young adult rats. On the other hand, lipid peroxidation as measured by thiobarbituric acid-reactive products increased significantly in all the regions of brain examined and in the liver of aged rats. Since GSH plays an important role as a cellular protectant against oxygen radical-mediated injury, decreased levels of GSH in aged rat brain are indicative of the vulnerability of the aged cerebral tissue to oxidative injury.

Aging brings about impairment of the function of the central nervous system (CNS), which is manifested by changes in memory and cognitive performance, concomitant with the aging process is the neuronal loss in certain brain regions [3]. A possible role for free radicals, particularly the oxygen free radicals, in age-related changes and in the etiopathogenesis of certain neurodegenerative diseases has been postulated [7–9]. Defense against oxygen radicals comprises of the enzymes superoxide dismutase and catalase which inactivate superoxide (O_2^-) and hydrogen peroxide respectively [4]. Peroxides (including hydrogen peroxide and those derived from oxidation of membrane lipids) are detoxified by glutathione peroxidase, for which the limiting factor is the level of glutathione (GSH); enzymatic oxidation of GSH to the disulphide (GSSG) accompanies the reduction of the peroxides. In order to maintain a high GSH/GSSG ratio in the cells, GSH is regenerated from GSSG by glutathione reductase using NADPH as a cofactor [10]. Nearly 90% of the non-protein sulphydryls in the cells is comprised of GSH, which also maintains the thiol status of proteins in cells. It is present in the brain in significant amounts (1.7–2 mM) and plays an important role in the detoxification of hydrogen peroxide generated during oxidative metabolism of dopamine and serotonin [10, 13].

The present study was carried out to determine if there were changes in the GSH

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levels in the brain as a result of aging. Since lipid peroxidation is one of the more deleterious effects of oxygen radical-induced damage, thiobarbituric acid (TBA)-reactive products, which are indicative of malondialdehyde levels, were also measured [6]. The hepatic levels of both TBA-reactive products and GSH were also measured in young adult, middle-aged and aged rats in order to determine if the changes observed were specific to the brain.

Female Sprague–Dawley rats, 3 months old (young adults), 15–18 months old (middle-aged) and 29–30 months old (aged), were used for the study. The body weights of the animals were as follows: 170–190 g (3 months old), 240–250 g (15–18 months old) and 350–390 g (29–30 months old). Animals had free access to food and water. Animals were anesthetized with ether and the brain was perfused transcardially with normal saline prior to decapitation and removal of the brain. The brains were dissected into different regions [5] and frozen immediately in liquid nitrogen. The main lobe of the liver was also removed. The samples were weighed while frozen and homogenized in 10 volumes of 0.1 M phosphate buffer containing 1 mM EDTA (pH 7.4). An aliquot of the homogenate was immediately mixed with an equal volume of 10% (v/v) perchloric acid, centrifuged and the supernatant was assayed for total GSH [1]. Another aliquot of the homogenate was assayed for TBA-reactive products [14].

The levels of TBA-reactive products in brain regions and liver of young adult, middle-aged and aged rats is given in Table I. Significantly higher amounts of TBA-reactive products were observed in all regions of the aged rat brain that were examined, as compared to those in the 3-month-old animals. The levels of TBA-reactive products increased in the liver, indicating that this change was not specific to the brain alone. The amount of TBA-reactive products in all regions of the brain was significantly higher than that in the liver in rats of all ages.

TABLE I

THIOBARBITURIC ACID REACTIVE PRODUCTS IN YOUNG ADULT, MIDDLE-AGED AND AGED RAT BRAIN REGIONS

All data are mean ± S.D. ($n = 6$–9). TBA-reactive products are expressed as nmol/g wet tissue. Statistical analysis was carried out against TBA-reactive products in young adult rats in respective brain regions and liver.

	Young adult (3 months)	Middle-aged (5 18 months)	Aged (29–30 months)
Cortex	41.74 ± 10.4	63.16 ± 7.88**	58.48 ± 9.28**
Cerebellum	55.76 ± 15.85	47.22 ± 11.80	79.02 ± 5.33*
Brainstem	48.41 ± 7.21	55.65 ± 6.41	56.38 ± 10.64*
Striatum	51.19 ± 7.35	67.04 ± 14.64*	75.61 ± 12.17**
Hippocampus	36.67 ± 7.09	47.92 ± 7.72**	48.62 ± 5.94**
Thalamus	43.71 ± 4.03	53.18 ± 7.79*	61.55 ± 6.16*
Liver	26.82 ± 2.66	34.84 ± 10.23	37.75 ± 8.66**

*$P < 0.01$; **$P < 0.001$.

Regional variations were observed in the cerebral GSH levels in all age groups examined. These changes were however, more pronounced in the 3-month-old rats (Table II). GSH levels were very low in the brainstem region (45%) as compared to the cerebral cortex. Age-associated changes were observed in the GSH levels of cortex, cerebellum, striatum, thalamus and hippocampus. GSH levels were decreased significantly in these regions in aged rats as compared to young adults. These changes were noticeable in the middle-aged animals (15–18 months old) also, but were more significant in the aged rats. The age-associated change was maximal in the cerebral cortex, cerebellum and hippocampus, where it declined by 42 and 43%. In the striatum and thalamus it decreased by 35 and 39%, respectively, while no change was observed in brainstem. No change was observed in the hepatic GSH level as a result of aging.

Several investigations have been carried out earlier, to determine the relationship between aging and oxygen toxicity-protecting enzymes [2, 12, 16–18]. Conflicting reports have emerged, which have been attributed to the inhomogenous age-related profile of enzyme activities [2]. However, in addition to these enzymes, cellular levels of GSH play an important role in protection against oxygen toxicity [11]. The present study demonstrates, for the first time, that GSH levels are substantially decreased as a result of aging in brain regions only and not in the liver; while TBA-reactive products are increased in both liver and brain regions.

The GSH levels in substantia nigra have been observed to be considerably lower as compared to the cerebral cortex in human brain and this deficiency is enhanced in Parkinson's disease [15]. It has been postulated that the decreased level of GSH in the substantia nigra could lead to insufficient protection of nigral neuronal cell bodies from cytotoxic effects of oxidants. The GSH levels are also substantially lower in the brainstem as compared to other regions in the rat brain. In aged rats, the GSH levels in the other regions are decreased to the levels observed in brainstem indicating that the aged brain as a whole would be susceptible to injury by oxygen radicals and

TABLE II

TOTAL GLUTATHIONE LEVELS IN YOUNG ADULT, MIDDLE-AGED AND AGED RAT BRAIN REGIONS

All data are mean ± S.D. ($n = 6$–9). Glutathione levels are expressed as μmol of GSH/g wet tissue. Statistical analysis was carried out against GSH levels of young adult rats in respective brain regions.

	Young adult (3 months)	Middle aged (15–18 months)	Aged (29–30 months)
Cortex	1.75 ± 0.34	1.62 ± 0.34	1.02 ± 0.13**
Cerebellum	1.65 ± 0.67	1.57 ± 0.24	0.95 ± 0.27**
Brainstem	0.79 ± 0.36	0.81 ± 0.23	0.72 ± 0.31
Striatum	1.56 ± 0.36	1.28 ± 0.34	1.02 ± 0.46**
Hippocampus	1.49 ± 0.29	1.47 ± 0.25	0.85 ± 0.56**
Thalamus	1.62 ± 0.46	1.47 ± 0.22	0.99 ± 0.68*
Liver	5.09 ± 0.74	6.56 ± 1.48	5.60 ± 1.85

*$P < 0.05$ and **$P < 0.001$.

hydrogen peroxide, that are produced either during normal neuronal processes or by exogenous agents.

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