The Effect of Aging on Glutathione and Cysteine Levels in Different Regions of the Mouse Brain (42879)

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Abstract. **A general glutathione (GSH) deficiency occurs in many tissues of the aging mouse. However, there is no information on GSH in the aging brain even though it has been involved in a number of neurobiologic reactions. To this end, C57BL/6 mice, 3–31 months old, representing the growth, maturation, and aging periods of the life-span were studied. Brain cortex, hippocampus, and stem samples were dissected, processed, and analyzed specifically for reduced and oxidized glutathione (GSH, GSSG) and cyst(e)ine using high performance liquid chromatography with dual electrochemical detection. The GSH content of each brain region varied in the order brain cortex > brain hippocampus > brainstem. However, the GSH profiles of all regions were the same through the life-span, namely, high values during growth dropping to a maturation plateau and then decreasing 30% during aging. In contrast to GSH, the order of cysteine levels was brain cortex < brain hippocampus < brainstem and no life-span changes occurred in any region. In addition, the brain GSSG and cystine contents of all regions were very low and did not change during the life-span. Thus, the GSH loss was not accountable by oxidation to GSSG or degradation to cyst(e)ine. Altogether these results demonstrated a GSH deficiency in brain tissues of aging mice like that found previously in other tissues. These findings suggest an increased susceptibility of the aging brain to oxidative damage.** [P.S.E.B.M. 1989, Vol 190]

Our previous results demonstrated that a glutathione (GSH) deficiency of aging occurs in different organisms such as the mosquito (1), mouse (2, 3), and humans (4, 5). In the mouse the deficiency was found in every tissue studied including liver, kidney, heart, and blood (2, 3).

The GSH deficiency in the aging mosquito was due to a decrease in GSH biosynthesis (6). Subsequent work showed that this was due to a lack of cysteine (Cys), for administration of Cys precursors enhanced GSH levels. This response could be elicited at all ages, indicating that the biosynthetic machinery was intact even at old ages (7).

No information has been available on the GSH and Cys status of the aging brain, although both compounds are required in many important brain reactions. For example, GSH plays an important role in the protection of brain cells against the effects of free radicals and reactive oxygen intermediates (8, 9). Also GSH has been shown to protect against the inhibitory effect of pyrithiamin on brain Na^+-K^+-ATPase (10). In addition, the neurotoxic effects of methyl mercury were shown to be closely related to the GSH-Cys status of brain (11). Furthermore, the GSH content of the substantia nigra region was found to be significantly lower in patients with Parkinson's disease than in normal subjects (12).

The objective of this study was to determine the GSH and Cys status of different brain regions in the mouse during its life-span.

Materials and Methods

Male C57BL/6 mice, a standard, well-characterized aging model, were obtained from the National Institute on Aging colony. Every shipment as well as daily experimental design included young and old mice. The ages ranged from 3 to 31 months and represented the growth (3, 6, and 12 months), mature (12 and 26 months), and aging (31 months) periods of the life-span. Each age group was comprised of 6–14 mice for the determination of GSH levels and 3–13 mice for Cys levels. All mice were clinically healthy and active upon

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receipt and were acclimated for a week in our animal care facility. During this time they were fed a stock diet and water *ad libitum*. At necropsy there was no evidence of tumors or other gross pathology in any of the mice.

The mice were killed by cervical dislocation, and brain cortex, brain hippocampus, and brainstem were identified, dissected quickly, and rinsed immediately in ice-cold 0.85% (w/v) NaCl solution. Homogenates (9% w/v) were prepared in ice-cold 5% (w/v) metaphosphoric acid using an all-glass Ten Broeck homogenizer and centrifuged at 11,000*g* for 1 min to yield acid-soluble, supernatant fractions for analysis.

Reduced and oxidized glutathione, cysteine, and cystine were determined simultaneously by a high performance liquid chromatographic method using a dual electrochemical detector of two Au/Hg electrodes in series (13). In brief, 40-μl samples were applied onto a 25-cm × 4.6-mm, 5-μm ODS column which was eluted isocratically using a mobile phase of 96% (v/v) 0.1 *M* monochloroacetic acid (pH 3.0), 4% (v/v) methanol, and an ion-pairing reagent of 2.0 m*M* heptane sulfonic acid with a flow rate of 1 ml/min. The resultant profiles were compared in each daily analysis with profiles obtained with authentic standards.

The data were analyzed by common statistical methods as described in Snedecor and Cochran (14).

Results

Tissue and body weight curves are useful criteria to delineate periods of growth and maturity. Thus, the body and brain region weights were determined in mice of different ages through the life-span. During the growth period from 3 to 12 months, the body weight increased as expected. Thereafter, during the mature and aging periods from 12 to 31 months, there was a slight, 10% decrease in weight (Table I), a common phenomenon of aging rodents. These data provide evidence that the animals were well-nourished and not starved. The brain weights of all three regions did not change during the entire 3- to 31-month life-span, indicating that maximal growth by this criterion was attained by 3 months of age.

The GSH levels ranged from 0.5 to 1.5 μmoles/g, and the concentrations in brain cortex > brain hippocampus > brainstem. However, the life-span profiles of GSH were the same for all three brain regions (Fig. 1). At 3 months there was a high GSH content which decreased by 6 months, reflecting a transition from growth to maturation. From 6 to 26 months, the levels were essentially constant and indicated a homeostatic plateau of maturation. Thereafter in the old (31-month) mouse, the GSH contents of all regions decreased about 30% ($P < 0.05$) from their 12-month maturation levels, reflecting an aging or senescent deficiency.

The Cys levels of all brain regions ranged from 100 to 200 nmol/g, which was only 10–20% of the GSH levels. The Cys concentrations were in the order of brain cortex < brain hippocampus < brainstem. In contrast to GSH the Cys levels in all regions fluctuated during aging but there were no statistical differences (Fig. 2).

The relationships between GSH and Cys were determined for the different regions and ages. GSH to Cys ratios were calculated and were in the order of brain cortex > brain hippocampus > brainstem. Also, in each brain region the ratios were near-constant from 3 to 26 months and then decreased in the 31-month group. These ratios demonstrate that the deficiency was due to a decrease in GSH and not to Cys which was unchanged.

The analytical results indicated that the GSSG and cystine contents were very low (<0.05 μmol/g) and did

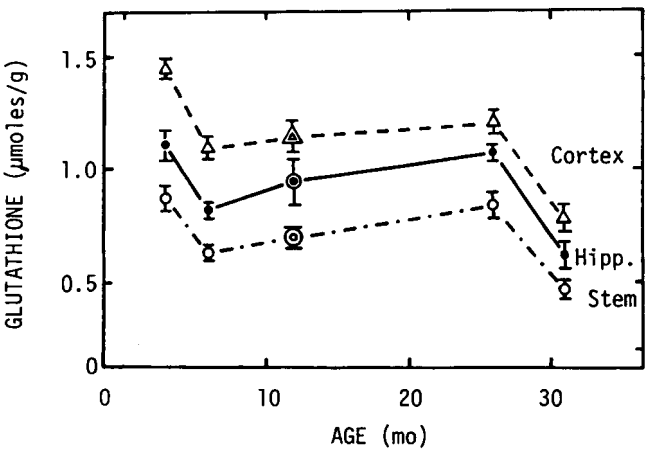

Figure 1. Brain glutathione profile during the mouse life-span. Glutathione concentrations of brain cortex, hippocampus, and stem were determined in samples from different ages throughout the life-span. Each point and bar represent the mean ± SEM of 6–14 mice.

Table I. Body and Brain Weights during the Life-Span of the Mouse[a]

Age (months)	Body weight (g)	Cortex (mg)	Hippocampus (mg)	Stem (mg)
3	27.1 + 0.402 (31)*	122 ± 4.58 (21)	22.9 ± 1.07 (21)	46.7 ± 1.57 (21)
6	31.5 ± 0.498 (34)*	111 ± 6.04 (14)	23.8 ± 0.695 (14)	53.0 ± 1.36 (14)
12	36.4 ± 1.13 (26)	121 ± 4.12 (17)	26.8 ± 1.29 (17)	51.7 ± 2.67 (17)
26	32.8 ± 0.658 (34)*	119 ± 3.45 (22)	24.6 ± 0.776 (22)	53.8 ± 3.62 (22)
31	32.0 ± 0.680 (26)*	126 ± 4.08 (24)	27.6 ± 2.28 (24)	57.5 ± 2.22 (24)

[a] Results are expressed as the mean ± SEM (*n*). * $P < 0.05$ vs 12-month values.

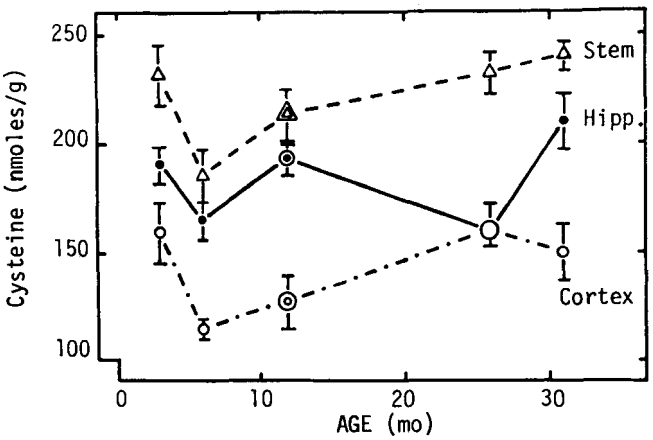

Figure 2. Brain cysteine profile during the mouse life-span. Cysteine concentrations of brain cortex, hippocampus, and stem were determined in samples from different ages throughout the life-span. Each point and bar is the mean ± SEM of 3–13 mice.

not change during the life-span. These results validated our sample processing methodology which was designed to minimize auto-oxidation of GSH and Cys to the disulfide forms.

Discussion

The life-span profiles of GSH in each brain region were parallel. Their general shape of a high GSH level during growth decreasing to a maturation plateau and then declining further during senescence verified our original hypothetical pattern of metabolic changes during the life-span (15). The GSH concentrations varied in different regions, but the significance and possible relationship to specific functions are unknown.

These results demonstrated for the first time that a GSH deficiency of aging occurs in different brain regions. Furthermore, these data extend our previous findings of a general GSH deficiency in other tissues of the aging mouse and other organisms (1–5). This evidence forms the basis of our belief that GSH status is a general biochemical marker of aging and longevity.

Age decrements in rat brain GSH have been reported by others, but the data focused on decreases during the growth period from 2.5 to 12 months of age (16), which confirm our mouse findings. However, their conclusion that an aging decrease occurred was unsupported by data or statistical analyses, and their results in 36-month-old rats were not compared with 12-month-old mature rats to demonstrate postmaturational aging changes.

The cause of the GSH deficiency in aging mouse brain is unknown but could be due to a decreased rate of GSH formation, an increased rate of degradation, or an increased utilization in the detoxification of endogenous peroxides and xenobiotics. Which of these possibilities is involved will require further investigation.

GSH plays a salient role in the regulation of intracellular redox status, and thus the lower level of GSH in the aging brain may lead to an increased susceptibility to oxidative injury. Indeed the recent 4th International Congress on Oxygen Radicals dealt primarily with oxidative damage of tissues in stroke and other ischemic conditions and with the importance of antioxidants such as GSH for protection.

There were lower GSH levels in the brain of the old mouse and, interestingly, also in Parkinson's disease (12). This decrement in both conditions may be more than coincidental. However, more information is needed on the extent and duration of the deficiency required to produce oxidative damage.

Regardless, a GSH deficiency may compromise a patient's ability to detoxify harmful peroxides and oxygen radicals and could lead to the loss of nigrostriatal dopaminergic neurons. This may be related to the finding that normal human aging is associated with a progressive loss of nigrostriatal neurons (17).

In contrast to GSH, Cys contents were unchanged during aging. Thus, brain differs from other aging mouse tissues (18) and the aging mosquito (7) where concomitant GSH and Cys deficiencies occur. The reason for this difference is unknown, but the data suggest that the brain may have a unique GSH metabolism that is independent of Cys availability.

Oxidized glutathione (GSSG) and cystine levels in all three brain regions were low and unchanged during aging, confirming the very low GSSG values of others in rat brain (19). Also the lack of changes indicated that the loss of GSH in aging is probably not due to its oxidation to GSSG or degradation to cystine, which otherwise might be expected to increase.

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