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Sex-dependent Antioxidant Enzyme Activities and Lipid Peroxidation in Ageing Mouse Brain

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We investigated whether oxidant status and antioxidant enzyme activities during ageing of mouse brain are regulated in sex-dependent manner. In the homogenate from the brain of 1, 4, 10 and 18 months old male and female CBA mice, lipid peroxidation (LPO), total superoxide dismutase (tSOD), catalase (CAT) and glutathione peroxidase (Gpx) were determined. LPO was age- and sex-related, favoring males over females throughout the lifespan with the peak in both sexes at 10 months of age. Throughout ageing, no difference in tSOD activity between male and female brains was observed, except in immature 1 month old mice. Gender-related difference in Gpx activity was observed, with higher level in females comparing to males, reaching statistical significance in senescent (18 months old) animals. CAT activity was drastically changed with ageing in both the male and female brain. We found different age associated trends in CAT activity in males and females: decreased with age in males and increased with age in females. Taken together, the present findings indicate that brains of female mice have lower oxidant and higher antioxidant capacity mostly related to CAT and to a lesser extent to Gpx activity.

Keywords: Mouse; Brain; Ageing; Gender; Lipid peroxidation (LPO); Superoxide dismutase (SOD)

INTRODUCTION

The brain has an important oxidant metabolism; it consumes 20 percent of the oxygen received by the body and has the highest blood flow.[1] In addition, the brain contains large amounts of iron and polyunsaturated fatty acid and is relatively poor in antioxidant enzymes.[2] A recently published review by Beckman and Ames[3] summarized the data regarding the free radical theory of ageing postulated by Harman,[4] which claims both ageing and age-related diseases result from accumulated damage to cellular macromolecules caused by reactive oxygen species (ROS). High vulnerability of neural tissue to oxidant damage is due in part to its high lipid content[5] and therefore LPO is one of the prominent organic expressions of oxidant stress in the brain.[6] Cells contain antioxidant enzymes that can mitigate deleterious effects of ROS *in vivo*. It has been suggested that peroxidant damage occurring in the process of ageing is a consequence of the imbalance between formation and removal of free radicals. To the best of our knowledge, so far no such studies have been performed in mice.

On the other hand, although abundant data on age-related LPO exist,[7,8] little is known of gender associated oxidant and antioxidant status in experimental animals of different age. This study was undertaken to investigate LPO and activity of three major antioxidant enzymes: tSOD, CAT and Gpx in the brain of CBA mice of different age and gender.

METHODS

Animals

In our study, we used male and female CBA mice bred in the animal facility of the Ruđer Bošković

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Institute, Zagreb, Croatia. Food (Domžale, Slovenia) and tap water were given *ad libitum*. Animals were kept in conventional circumstances (light/dark rhythm 12/12 h, temperature 22°C and humidity 55%). In total, approximately 50 male and 50 female mice of 1, 4, 10 and 18 months of age were used in this study. Each group consisted of 10–15 animals.

Preparation of Brain Homogenates

Mice were sacrificed by decapitation and brains were quickly dissected on ice. For the determination of LPO and enzyme activity brain homogenates were prepared 5 or 20% wt/vol., respectively. Tissue samples were weighed, placed into 1.15% KCl or 50 mM phosphate buffer (pH = 7.8) and homogenized (5% wt/vol.) at 0°C using Potter S homogenizer (Braun, Biotech. Int., Germany). A portion of the homogenate 20% wt/vol. used to determine tSOD, CAT and Gpx activity prepared in 50 mM phosphate buffer was sonicated on ice for 30 s in three 10 s intervals and centrifuged at 20,000*g* for 15 min. Aliquots of the resulting supernatant were stored in plastic tubes at −70°C until assayed.

Lipid Peroxidation (LPO)

LPO was determined by measuring thiobarbituric acid reactive substances (TBARS). They were measured as decomposition products of peroxides (malondialdehyde and other aldehydes) which appeared during the heating of peroxidized material in an acidic environment using the method described by Ohkawa *et al.*[9] and is expressed as nmol/mg protein.

Total Superoxide Dismutase (tSOD)

tSOD activity was measured spectrophotometrically (550 nm) by the inhibition of xantine/xantine oxidase mediated reduction of cytochrome C as described by Flohé and Ötting.[10] One unit of tSOD activity is defined as the amount of enzyme required to give 50% inhibition in the typical calibration curve obtained with standard SOD. tSOD activity is expressed as U/mg protein.

Catalase (CAT)

CAT activity was determined spectrophotometrically according to Aebi[11] by measuring changes in absorbance in the reaction mixture using the final concentrations of 10 mM H_2O_2 and 50 mM phosphate buffer (pH = 7.0) at 240 nm during the time interval of 30 s after addition of the sample. The activity is expressed as U/mg protein.

Glutathione Peroxidase (Gpx)

Gpx activity was measured by glutathione peroxidase assay kit (RANSEL, RANDOX, San Diego, CA, USA) based on the method of Paglia and Valentine.[12] The absorbance was monitored for 3 min at 340 nm using an Camspec M330 equipped with M330Camspec software package (Camspec Ltd, Cambridge, UK) UV–Vis Spectrophotometer. To obtain the linearity of the assay, if the absorbance change per minute exceeded 0.1, the sample was diluted accordingly with the diluting agent. One unit of Gpx catalyzes the oxidation by H_2O_2 of 1 μM of reduced glutathione to oxidized glutathione per minute at pH 7.0 at 25°C. Gpx is expressed as U/mg protein.

Protein Measurement

Protein content in the supernatant was determined by the method of Lowry *et al.*[13] using bovine serum albumin as standard.

Chemicals

All reagents were obtained from Sigma (St. Louis, MO, USA) unless otherwise specified.

Statistical Analysis

Data were reported as mean ± SEM of n experiments each representing an individual animal and were analyzed using the statistical package SPSS for Windows (v.10.0.5). The effects of sex (male, female) and age (1, 4, 10 and 18 months) and their interactions were analyzed using a two-way ANOVA. Student's t-test was applied to define the difference between males and females of the same age. The level of significance was set at $p < 0.05$.

RESULTS

Effect of Age and Gender on Basal Levels of LPO

The results of measuring formation of TBARS representing LPO in the brain of male and female mice of several ages are presented in Fig. 1. In young (1 and 4 months) and old (10 months) females, LPO was significantly lower ($p = 0.05$; 0.02 or 0.01, respectively) than in males of the same age. Only senescent females (18 months old) had higher ($p = 0.04$) LPO than the corresponding males. The two-way ANOVA revealed significant effects on LPO of age ($p < 0.001$) and sex ($p < 0.01$) and also a significant sex × age interaction ($p < 0.01$).

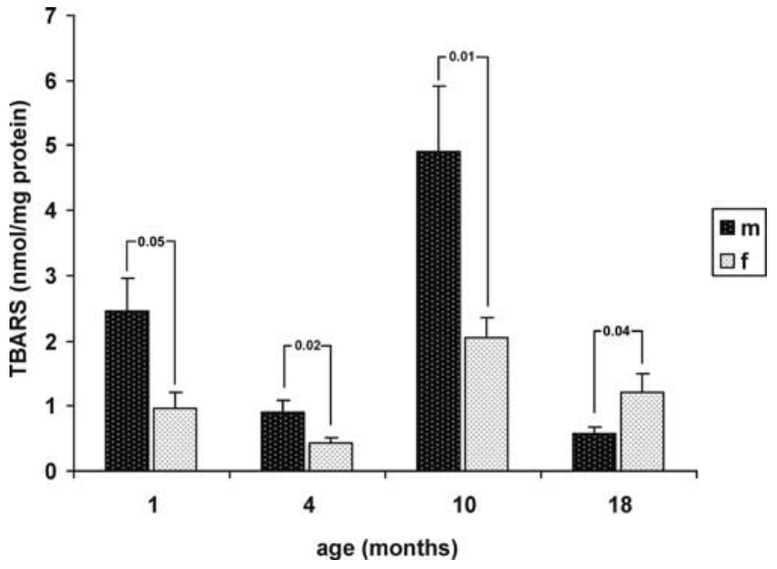

FIGURE 1 Effect of age and gender on lipid peroxidation (TBARS) in the brain of CBA mice. Data are means ± SEM of 10–15 mice per group. Male versus female mice of the same age group were compared using Student's *t*-test. Two-way ANOVA was performed with the effect of sex (m = male, f = female) as one factor ($F = 8.7$; $p < 0.01$), and age (1, 4, 10 and 18 months) as the other ($F = 15.9$; $p < 0.001$). Interaction: sex × age $F = 6.3$; $p < 0.01$.

Effect of Age and Gender on tSOD Activity

As shown in Fig. 2, statistically significant difference ($p = 0.001$) of tSOD activity was observed in males versus females only in young (1 month old) mice; female brains had only 30% tSOD activity as compared to males. As indicated by two-way ANOVA, both main effects (sex and age) significantly ($p < 0.001$) affected tSOD activity in the brain. The same level of significance was reached with interaction between these two factors ($p < 0.001$).

Effect of Age and Gender on CAT Activity

As demonstrated in Fig. 3, only in young (1 month old) females CAT activity was lower than in the corresponding males ($p < 0.03$), while in adult

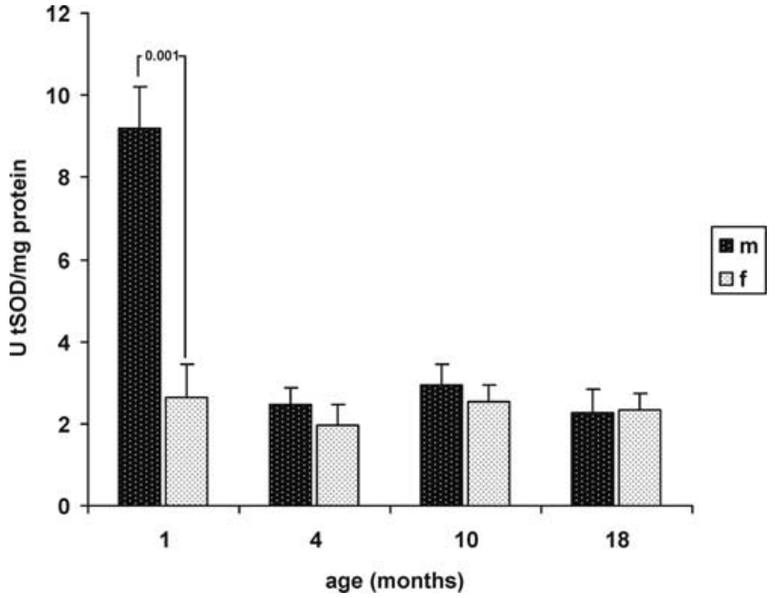

FIGURE 2 Effect of age and gender on total SOD activity in the brain of male and female CBA mice of different age. Values are means ± SEM of 10–15 mice per group. Male versus female mice of the same age group were compared using Student's *t*-test. Two-way ANOVA was performed with the effect of sex (m = male, f = female) as one factor ($F = 18.3$; $p < 0.001$), and age (1, 4, 10 and 18 months) as the other ($F = 14.3$; $p < 0.001$). Interaction sex × age $F = 11.9$; $p < 0.001$.

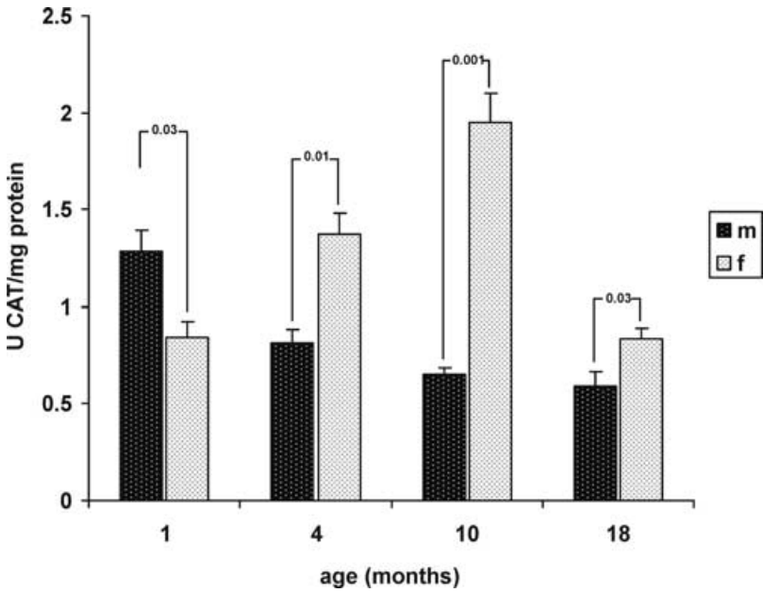

FIGURE 3 Effect of age and gender on catalase activity in the brain of male and female CBA mice of different age. Values are means ± SEM of 10–15 mice per group. Male versus female mice of the same age group were compared using Student's *t*-test. Two-way ANOVA was performed with the effect of sex (m = male, f = female) as one factor ($F = 11.2$; $p < 0.01$) and age (1, 4, 10 and 18 months) as the other ($F = 8.5$; $p < 0.001$). Interaction sex × age $F = 16.6$; $p < 0.001$.

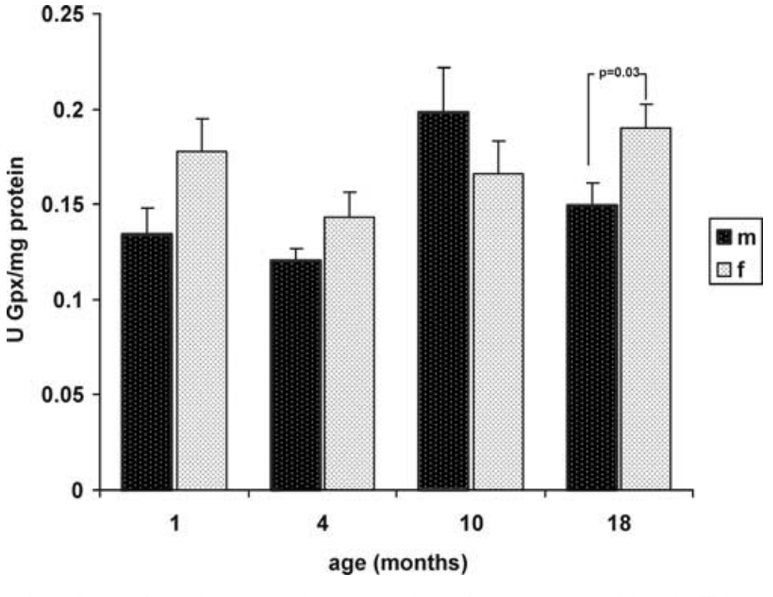

FIGURE 4 Effect of age and gender on glutathione peroxidase activity in the brain of male and female CBA mice of different age. Values are means ± SEM of 10–15 mice per group. Male versus female mice of the same age group were compared using Student's *t*-test. Two-way ANOVA was performed with the effect of sex (m = male, f = female) as one factor ($p = 0.83$) and age (1, 4, 10 and 18 months) as the other ($F = 3.2$; $p < 0.028$).

(4 months old $p < 0.01$; 10 months old $p < 0.001$) and senescence mice (18 months old $p < 0.03$) it was always significantly higher in females. As indicated by two-way ANOVA, sex and age significantly ($p < 0.01$ or $p < 0.001$, respectively) affected CAT activity with significant interaction of both factors ($p < 0.001$).

Effect of Age and Gender on Gpx Activity

Gpx activity in male and female mice is presented in Fig. 4. Young females (1 and 4 months old) showed 15–25% greater but insignificant Gpx activity comparing to males, reaching statistically significant difference in senescent mice ($p = 0.03$). As indicated by two-way ANOVA, age but not sex significantly affected Gpx activity ($p = 0.028$).

DISCUSSION

Our study was undertaken in the context of insufficient data about the possible gender related oxidant damage of the brain due to LPO and the brain's capacity to respond adequately with anti-oxidant enzymes. For our ageing population cohort we deliberately chose animals of <19 months old to avoid effects seen at later months, which may be due to individual variations in the parameters examined. We observed bimodal behavior of LPO upon ageing with 3–5 timed increase in TBARS level in both males and females at 10 months of age. However, this increase is always in favor of males over females, especially in 10 months old animals. Our results are in accordance to Navarro *et al.*[14], who showed changes in TBARS level of ageing mouse brain, that were both age-related and higher in males than in females. Observed difference in TBARS level might be due to beginning of tumor development, which appeared in 10% of 10 months old male mice. We also observed 56% appearance of tumors at 18 months old males, while at the same time females did not develop tumors at all (data not shown). In general, literature data showed an inverse relationship between LPO and tumor growth.[15,16] Other reasons for possible diversity of LPO in males and females might be (a) associated with well-documented sexual dimorphism of the nervous system[17] ascribed to steroid hormones[18]; (b) sex-differences in stress response associated with diverse activation of HPA axis;[19] (c) effect of gonadal steroids[20] and (d) induction of LPO via nitric oxide system,[21] reported higher in males than in females.[22]

Peroxidant damage occurring in the process of ageing might be a consequence of the imbalance between formation and removal of free radicals by antioxidant enzymes. Generally speaking (except in immature 1 month old mice) throughout ageing, we observed no difference in tSOD activity between male and female brains. We noticed 73% decline in tSOD activity in adult male mice comparing to immature 1 month old males. Hauck and Bartke[23] have demonstrated a significant reduction in Cu/Zn-SOD activity in old compared to young Ames dwarf male mice (38% decline), which is in accordance with our results. Compared to CAT and Gpx, SOD seems to be relatively inert antioxidant enzyme.[24] Among all other antioxidant enzymes, CAT activity was the main enzyme affected by gender in ageing mouse brain. Two different age-associated trends in CAT activity in male and female brains were observed. While in males it was negatively related to age, being decreased in adult and senescent animals, in females it was increased with age until senescence. In adult and old female mice CAT activity was always higher in female than in male mice. Mo *et al.*[25] also observed a decline of CAT activity in C57Bl/6N senescence (24 months old) male mice. Hamilton *et al.*[26] observed an age-related increase of CAT activity in the brain of female C56Bl/6 mice. These data are in accordance with our data of decreasing CAT activity in adult male mice and increasing CAT activity in adult female mice.

Changes in Gpx activity related to age and sex in rat liver have been already reported in 1968 by Pinto and Bartley.[27] We also observed differences in Gpx activity between sexes. Generally speaking, females had 15–25% higher Gpx activity compared to males of corresponding age. However, this difference reached statistical significance in senescent mice.

In general and in terms of sex differences, females had lower LPO and higher antioxidant enzyme activities (CAT and Gpx) than males. This means that throughout the majority of mouse lifespan male brains were more susceptible to LPO than females. The data presented might be relevant for the determination of different susceptibility to diseases between males and females.

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