Physiol. Res. 47: 61–66, 1998

Glutathione-Dependent Antioxidant Enzyme Activities and Glutathione Content in the Rat Brain at Different Stages of Oestrous Cycle

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Received June 12, 1997
Accepted October 16, 1997

Summary

Enzymatic activities of glutathione peroxidase, glutathione-S-transferase, glutathione reductase and catalase, as well as the glutathione content were measured in the brain tissue of regularly cycling rats at dioestrus, proestrus and estrus. The activity of glutathione peroxidase was found to be suppressed at proestrus, whereas that of catalase was increased at dioestrus. Glutathione transferase and glutathione reductase activities, as well as the glutathione content appeared to be stable during the oestrous cycle. These results suggest that, in the female rat, glutathione peroxidase and catalase activities in the brain tissue are influenced by the ovarian hormone status.

Key words

Brain – Oestrous cycle – Glutathione – Glutathione-dependent enzymes – Rats

Introduction

In eucaryotic cells, H_2O_2 is generated in peroxisomes (Halliwell and Gutteridge 1985), mitochondria, microsomes (Nohl 1987, Halliwell and Gutteridge 1989) and the endoplasmic reticulum (Jones *et al.* 1978, Sies *et al.* 1978). H_2O_2 is produced by the action of oxidases such as urate oxidase, D-amino acid oxidase (Gaunt and De Duve 1976) and L-hydroxy acid oxidase, as well as during auto-oxidation of ascorbates, glutathione (GSH), catecholamines (Halliwell and Foyer 1976, Sinet *et al.* 1980) and thioles (Chance *et al.* 1979). The GSH redox cycle is the principal mechanism for reduction of endogenous hydroperoxides. Enzymes of the GSH redox cycle are selenium-containing glutathione peroxidase (GSH-Px), selenium-independent form of GSH-Px known as glutathione-S-transferase (GST) and glutathione reductase (GR), which converts the oxiform of glutathione (GSSG) to reduced GSH (Heffner and Repine 1989). In addition to the enzymes of the GSH redox cycle, excess H_2O_2 is removed from tissues by catalase (CAT).

GSH represents a major non-enzymatic antioxidant and the most abundant non-protein thiol source in the cell (Meister and Anderson 1983, Warner 1994). It serves as a substrate for GSH-Px and GST. Under physiological conditions, GR will rapidly reduce any GSSG to GSH, so that more than 98 % of intracellular glutathione appears as GSH, while the rest is present as GSSG and a mixed disulfide form GSSR.

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GSH has several major functions: it detoxifies reactive oxygen species under normal and impaired homeostasis, detoxifies drugs and pollutants, maintains an essential thiol status of proteins and other molecules, and provides the main molecular form in which cysteine can be stored within the organism and used for transfer between tissues (Meister and Anderson 1983, Meister 1988, Deleve and Kaplowitz 1991, Schnellmann 1991, Kannan *et al.* 1992, Julius *et al.* 1994).

Petrovic *et al.* (1991) have shown that dexamethasone significantly decreases the CAT and GSH-Px activities in the rat brain. According to Pinto and Bartley (1969), GSH-Px activity in the rat liver varies during the oestrous cycle. The highest value, found at oestrus, was 50 % higher than the lowest activity found at dioestrus. Smith *et al.* (1995) reported that, in the rat, the plasma selenium and GSH-Px activity fluctuate during the oestrous cycle. The plasma selenium concentration reached a peak during proestrus and plasma GSH-Px activity peaked during early proestrus and metoestrus. D'Almeida *et al.* (1995) demonstrated that the activities of superoxide dismutase (SOD), CAT and GSH-Px in red blood cells of female Wistar rats did not vary significantly during the oestrous cycle.

Ovarian hormones are known to act directly on CNS to modulate the electrical properties of neurones (Pfaff 1983) and certain important cellular functions, such as neurotransmitter uptake (Ghraf *et al.* 1983, Michel *et al.* 1987) and turnover (Di Paolo *et al.* 1985), calcium uptake (Nikezic *et al.* 1988, Horvat *et al.* 1991) as well as the activity of enzymes involved in neurotransmitter and energy metabolism (Luine and Rhodes 1983).

Pajovic *et al.* (1993) have shown that the activity of both forms of SOD in the rat brain tissue was steady during the oestrous cycle except at proestrus, when MnSOD activity was elevated significantly. In the present work, study of the influence of ovarian hormone status on the antioxidant enzyme activity in the rat brain has been extended to include enzymes of the GSH redox system and CAT. The enzymatic activities of GSH-Px (EC 1.11.1.9), GST (EC 2.5.1.18), GR (EC 1.6.4.2) and CAT (EC 1.11.1.6) as well as the total GSH content (GSH, reduced + GSSG, oxidized) in the brain of regularly cycling rats were measured at dioestrus, proestrus and oestrus.

Methods

Female Wistar rats aged 3.5 months and weighing 330 g on the average were used. The experiments were carried out in the summer. At sacrifice, the cycling rats were classified as dioestrous, proestrous and oestrous. They were kept in large open colony cages under controlled conditions of illumination (lights on: 05:00 h and off at 17:00 h) and temperature (23±2 °C), and were allowed free access to water and food.

All animals were sacrificed by decapitation with a guillotine (Harvard Apparatus), always between 08:00 and 10:00 h to avoid any possible rhythmic variations in the antioxidant enzyme levels. Fresh brains were dissected out and homogenized. Homogenization was performed with a Janke and Kunkel (Staufen, Germany) Ka-Werk Ultra-Turrax homogenizer at 0–4 °C using 0.25 M sucrose, 1 mM EDTA and 0.05 M Tris-HCl solution, pH 7.4 (Rossi *et al.* 1987, De Waziers and Albrecht 1987). The homogenates were sonicated for 30 s at 10 kHz on ice to release enzymes (Takada *et al.* 1982) and used to determine the content of total glutathione (GSH+GSSG). The remaining sonicates were centrifuged (90 min, 85 000 x *g*, 4 °C) and the supernatant was used for GSH-dependent antioxidant enzyme activity assays and total protein determination. All chemicals were Sigma (St. Louis, MO, U.S.A.) products.

GSH-Px activity was measured using t-butyl hydroperoxide as substrate (Paglia and Valentine 1967, as modified by Tamura *et al.* 1982) and the activity was expressed in nanomoles of NADPH oxidized/min/mg protein. For the determination of GST activity, 1-chloro-2,4-dinitro benzene (CDNB) was used as substrate (Habig *et al.* 1974) and the activity was expressed in nmol GSH used/min/mg protein. GR activity was assayed as suggested by Glatzle *et al.* (1974) and expressed in nmol oxidized NADPH/min/mg protein. CAT activity was assayed as suggested by Beutler (1982) and expressed as μmol H_2O_2/min/mg protein. The method is based on the rate of H_2O_2 degradation by the action of CAT contained in the samples, monitored spectrophotometrically at 230 nm in 5 mM EDTA, 1 M Tris-HCl solution, pH 8.0. For the GSH assay, the supernatants obtained after sonication were deproteinized by 5-sulfosalicylic acid (10 % w/v). After standing on ice, the samples were centrifuged (10 min, 3020 x *g*). Content of total glutathione (GSH + GSSG) was determined by glutathione reductase - 5,5'- dithiobis(2-nitrobenzoic acid) (DTNB) recycling assay (Tietze 1969, as modified by Griffith 1980) and expressed as nmol GSH/g wet mass. Protein content was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a reference.

The results were analyzed by Student's t-test and by ANOVA, followed by Scheffe test. Differences between means were considered significant at 5 %.

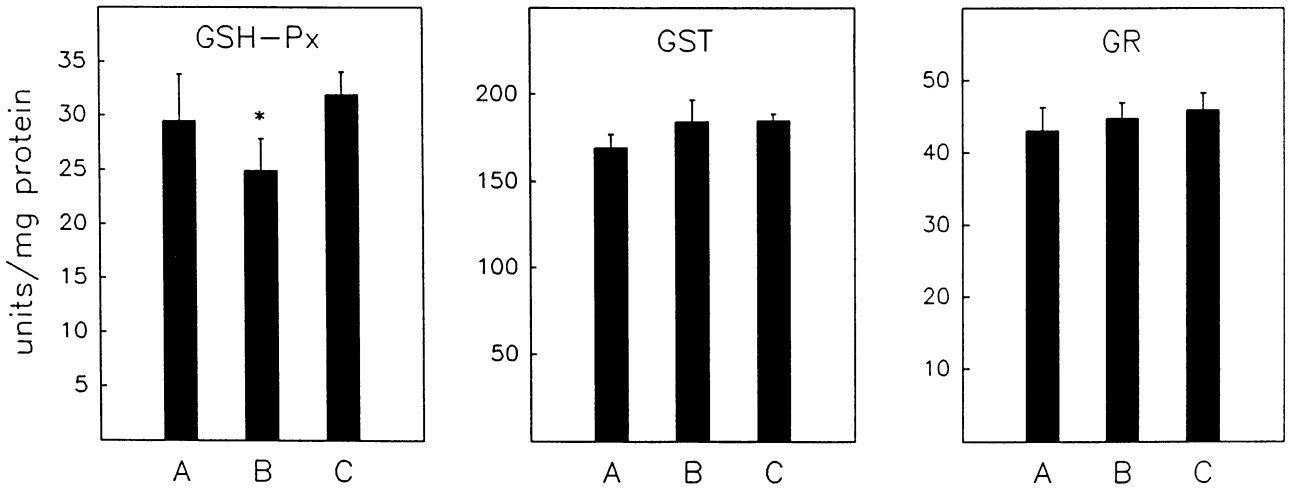

Fig. 1. *GSH-Px, GST and GR activities in brain homogenates prepared from rats at dioestrus (A), proestrus (B) and oestrus (C). Columns represent means of four animals and vertical bars are S.E.M., * $p<0.005$.*

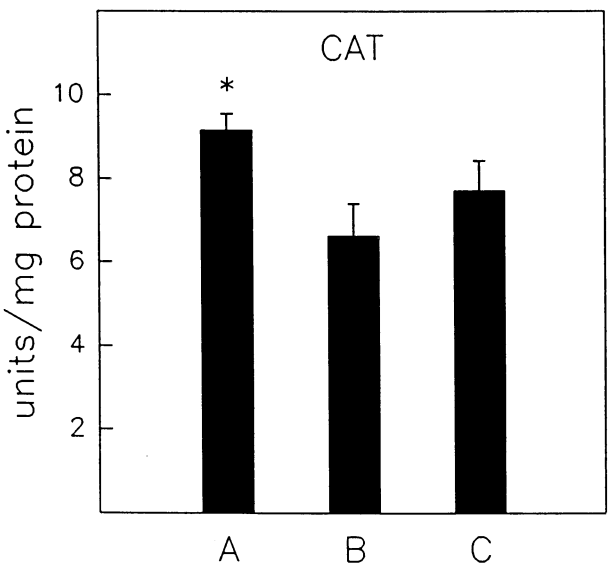

Fig. 2. *CAT activity in brain homogenates prepared from rats at dioestrus (A), proestrus (B) and oestrus (C). Columns represent means of four animals and vertical bars are S.E.M. * $p<0.005$.*

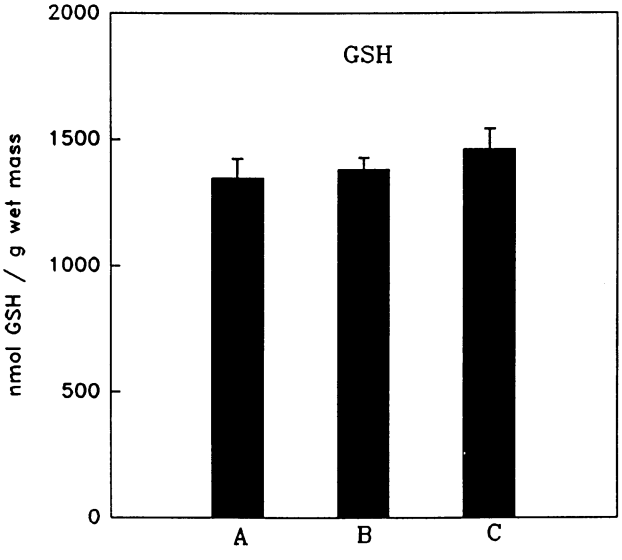

Fig. 3. *GSH content in brain homogenates prepared from rats at dioestrus (A), proestrus (B) and oestrus (C). Columns represent means of four animals and vertical bars are S.E.M.*

Results

The activity of brain GSH-Px was significantly decreased at proestrus (24.9±3.4 nmol NADPH/min/mg protein) in comparison to the activity at oestrus (31.9±2.5; $t_{(6)}=2.87$; $p<0.05$) (Fig. 1). At the same time, the activity of GST appeared to be steady during the oestrous cycle (169.3±8.9 nmol GSH/min/mg protein at dioestrus, 184.0±14.7 at proestrus and 184.8±4.5 at oestrus; $F_{(2,9)}=0.97$; $p>0.05$) (Fig. 1). Also, the activity of GR was not changed during the oestrous cycle (43.0±3.7 nmol NADPH/min/mg protein at dioestrus, 44.7±2.4 at proestrus and 45.9±2.8 at oestrus; $F_{(2,9)}=0.30$; $p>0.05$) (Fig. 1). However, the activity of CAT was significantly increased ($F_{(2,9)}=4.43$; $p<0.05$) at dioestrus (9.1±0.5 μmol H_2O_2/min/mg protein), in comparison to the activity at oestrus (7.5±0.8) and proestrus (6.6±0.9) (Fig. 2). The GSH content appeared to be stable during the oestrous cycle

(1347.3±77.1 nmol GSH/g wet mass at dioestrus, 1381.3±45.8 at proestrus and 1459.7±82.6 at oestrus; $F_{(2,9)}=0.89$; $p>0.05$) (Fig. 3).

Discussion

Changes in the GSH-Px and CAT activities during the oestrous cycle, as observed in this report, suggest that, in female rats, the enzymatic antioxidant activities in the brain depend on the ovarian hormone levels. Involvement of sexual hormones in the control of the antioxidant defense system has been investigated by other authors (Laloraya *et al.* 1991, Carlson *et al.* 1993). We have previously shown that oestradiol and progesterone suppress MnSOD and do not affect CuZnSOD activity in female (Pajović *et al.* 1993) and male (Pajović *et al.* 1996) rats. Sato *et al.* (1992) reported alterations in MnSOD activity and mRNA levels of the enzyme in ovarian tissue of rats during the ovulatory process, whereas CuZnSOD did not exhibit any difference either in the catalytic activity or in mRNA levels.

In addition, it is known that vitamins A, C and E are present in the corpus luteum and that their concentrations vary during the reproductive cycle (Capel and Smallwood 1983). Evidence that SOD may be regulated by the luteinizing hormone have been found by Verma *et al.* (1990) and Laloraya *et al.* (1991). The analysis of SOD levels in the thyroid gland of mice during the oestrous cycle revealed differences that result in a peak of enzyme activity at proestrus (Verma *et al.* 1990).

Reactive oxygen species (ROS) have been related to many physiological and pathological processes. ROS play a number of significant and diverse roles in reproductive biology (Riley and Behrman 1991). Superoxide, hydrogen peroxide and lipid peroxides might play a critical role in the physiology of ovulation (Laloraya *et al.* 1988, Pajovic *et al.* 1997). Hydrogen peroxide has been assigned the role of a second messenger (May and de Haen 1979) in hormonal systems and it is possible that SOD, in conjunction with peroxidase, in a sequential developmental process, may be involved in regulating follicular development, ovulation and luteal functions under the action of gonadotrophins. Hydrogen peroxide is converted to H_2O by CAT and GSH-Px. Therefore, a surplus of H_2O_2 is necessary for important physiological functions and this is in agreement with decreased GSH-Px activity and unchangeable CAT activity at proestrus, as observed in the present report. The upregulation of CAT activity at dioestrus might be the consequence of increased levels of hydrogen peroxide from previous cycle stages (Riley and Behrman 1991, Behrman *et al.* 1993).

In conclusion, GSH-Px and CAT activities in the female rat brain appear to be related to ovarian hormone status during the oestrous cycle, suggesting involvement of gonadal hormones in the control of H_2O_2 content in the brain, and thereby in the control of processes which protect the nerve tissue against oxidative damage.

Acknowledgements

This research was supported by the Ministry of Science and Technology of Serbia, Yugoslavia, grants No. 03E18 and 03E24.

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