Effects of Progesterone and Estradiol Benzoate on Glutathione Dependent Antioxidant Enzyme Activities in the Brain of Female Rats

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Abstract. The activities of glutathione dependent antioxidant enzymes were measured in subcellular fractions of whole brain homogenates prepared from ovariectomized (OVX) female rats, untreated or treated 2 h or 24 h prior to sacrifice with a single dose of 2 mg progesterone (P) or 5 μg estradiol benzoate (EB) Glutathione peroxidase (GSH-Px) activity was not changed following systemic administration of EB, but P increased GSH-Px in the brain of OVX rats 24 h after the treatment The activity of glutathione reductase (GR) was suppressed by EB short time, only 2 h following treatment, whereas P increased the enzyme activity 24 h after treatment On the other hand, the activities of catalase (CAT) and glutathione-S-transferase (GST) were not changed following systemic administration of EB or P The present work was carried out to study the involvement of ovarian steroids, especially P, in the control of GSH-Px and GR activities, and our results suggest that oxidative stress in the brain of female rats may be modulated by the level of progesterone

Key words: Brain — Antioxidant enzymes — Estradiol — Progesterone

Introduction

The brain is particularly susceptible to free radical attack because it generates more of these toxicants per gram of tissue than any other organ (Reiter 1995) It is a highly oxygenated structure, responsible for almost one fifth of the body's total oxygen consumption In addition, the brain contains large amounts of iron and

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polyunsaturated fatty acids and is relatively poor in many antioxidant enzymes such as catalase (CAT) and glutathione peroxidase (GSH-Px) (Halliwell 1989)

On the other hand, we and others have reported that the activities of a number of enzymes involved in neurotransmittion, energy metabolism and antioxidant defense system are affected in the brain by gonadal hormones (Luine and Rhodes 1983, Becker et al 1984, Petrović et al 1991, Pajović et al 1993) Luine and Rhodes (1983) reported gonadal hormone-dependent changes in activities of type A monoamine oxidase (MAO) and acetyl-choline esterase (AChE), enzymes responsible for monoamine and acetylcholine degradation, respectively, and in glucose-6-phosphate dehydrogenase (G6PDH), a regulatory enzyme of the pentose phosphate pathway of glucose oxidation The activity of antioxidant defense enzymes in the brain has been shown to vary during the estrous cycle, to be altered following gonadectomy, and to differ between the sexes, suggesting involvement of gonadal hormones in the control of processes which protect cells and tissues against oxidative damage (Pinto and Bartley 1969, Smith et al 1995) Petrović et al (1991) have shown that the injection stress induces an increase in copper- zinc containing superoxide dismutase (CuZn SOD) activity and a decrease in CAT and GSH-Px activities in the brain of rats Dexamethasone treatment induced a further increase in CuZn SOD, an increase in the manganese containing superoxide dismutase (Mn SOD) activity, but did not restore control levels of the CAT and GSH-Px activities Pajovic et al (1993, 1996) have recently demonstrated that, in the brain of gonadectomized female and male rats, Mn SOD activity can be suppressed by exogenous progesterone (P) or estradiol benzoate (EB), in contrast to brain CuZn SOD activity, which appeared not be under the influence of the ovary

In the present work, the study of the influence of progesterone and estradiol benzoate on the glutathione dependent antioxidant enzyme activities was extended The enzymatic activities of CAT (EC 1 11 1 6), GSH-Px (EC 1 11 1 9), GST (EC 2 5 1 18) and GR (EC 1 6 4 2) in the brain of female rats were examined

Materials and Methods

Bilaterally ovariectomized (OVX) female Wistar rats, aged 3 5 months, were used They were kept in large open-colony cages under controlled conditions of illumination (lights on 5 a m 5 p m) and temperature (23 ± 2 °C), and were allowed free access to water and food Ovariectomy was performed under ether anaesthesia three weeks before the hormone treatment All chemicals were Sigma (St Louis, USA) products

Two hours or 24 h before sacrifice, a single injection of 2 mg P (progesterone, Sigma), or 5 μg EB (β-estradiol-3-benzoate, Sigma) suspended in 0 1 ml olive oil, was given subcutaneously (sc) to OVX animals Controls received the oil alone

The animals were killed by decapitation with a guillotine (Harvard Apparatus)

and fresh whole brains were dissected out and homogenized in a Janke and Kunkel (Staufen, Germany) Ka-Werk Ultra-Turrax homogenizer at 0–4 °C using 0.25 mol/l sucrose, 1 mmol/l EDTA and 0.05 mol/l Tris-HCl solution, pH 7.4 (Rossi et al. 1983; De Waziers and Albrecht 1987). The homogenates were centrifuged (90 min, 85,000 × *g*, 4 °C) and the supernatant was used for GSH dependent antioxidant enzyme activity assays and total protein determination.

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 mmol/l EDTA, Tris-HCl solution, pH 8.0. GSH-Px activity was measured using *t*-butyl hydroperoxide as a substrate (Paglia and Valentine 1967 as modified by Tamura et al. 1982) and activity was expressed as nmol of NADPH oxidized/min/mg protein. For determination of GST activity, 1-chloro-2,4-dinitro benzene (CDNB) was used as a substrate (Habig et al. 1974) and the activity was expressed as nmol GSH consumed/min/mg protein. GR activity was assayed as suggested by Glatzle et al. (1974) and expressed as nmol NADPH oxidized/min/mg protein. Protein concentrations were determined by the method of Lowry et al. (1951).

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

Results

Effects of P and EB on CAT activity

The post-castration activity of brain CAT appeared to be steady after treatments with 2mg P or 5μg EB, given to OVX animals 2 h or 24 h before sacrifice (Fig. 1 *a* and *b*). The values for control OVX and OVX + P treated animals were, respectively: at 2 h – 5.3 ± 0.9 and 6.7 ± 1.3 U/mg protein ($t_{(6)}=0.97$; $p > 0.05$); at 24 h – 9.3 ± 1.3 and 9.4 ± 0.5 U/mg ($t_{(6)}=0.015$; $p > 0.05$). Similarly, the values for control OVX and OVX ± EB treated animals were, respectively: at 2 h – 5.3 ± 0.9 and 6.5 ± 0.6 U/mg protein ($t_{(6)}=1.25$; $p > 0.05$); at 24 h – 9.3 ± 1.3 and 8.1 ± 0.5 U/mg protein ($t_{(6)}=0.99$; $p > 0.05$).

Effects of P and EB on GSH-Px activity

The GSH-Px activity in brain homogenates of OVX animals could not be affected by the EB treatments (Fig. 2*a*, *b*). The respective values for control OVX and OVX ± EB treated animals were 23.9 ± 3.3 and 21 ± 4.7 U/mg protein at 2 h and 20.8 ± 2.2 and 23.8 ± 3.2 U/mg protein at 24 h ($F_{(3,12)}=0.35$; $p > 0.05$). The GSH-Px activity was steady 2 h after administration of P (control OVX – 23.9 ± 3.3 and OVX + P – 19.5 ± 3.8 U/mg protein; $t_{(6)}=1.01$, $p > 0.05$) (Fig. 2*a*). On the other hand, a marked increased of GSH-Px activity in OVX rats was obtained with 2mg

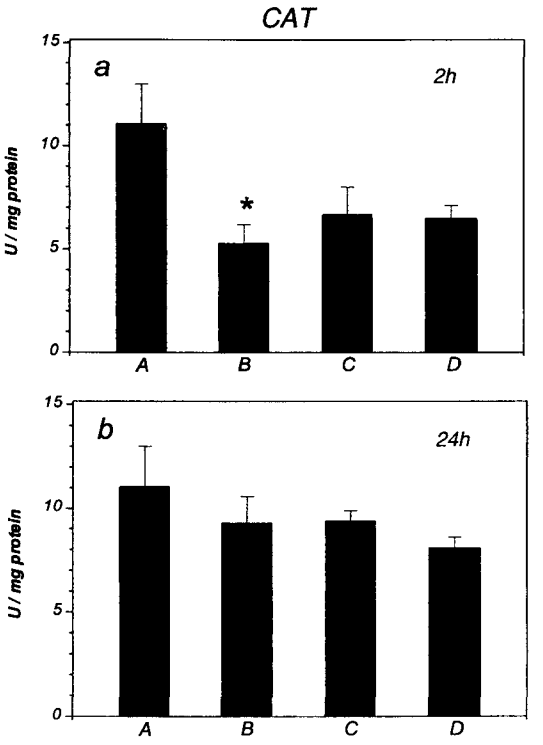

Figure 1. CAT activity in brain homogenates of long-term ovariectomized rats treated 2 h and 24 h prior to sample preparation, *A* nontreated controls ($n = 5$), *B* controls treated with olive oil ($n = 4$), *C* treated with 2 mg P ($n = 4$), and *D* treated with 5 μg EB ($n = 4$) Columns represent mean values, and vertical bars are S E M *a*, *A* *B* - $^{*}p < 0\ 05$

P at 24 h after treatment (control OVX – 20.8 ± 2.2 and OVX ± P – 30.2 ± 4.8 U/mg protein; $t_{(6)}$=2 05, $p < 0.05$) (Fig. 2*b*).

Effects of P and EB on GST activity

In OVX rats, the activity of GST was not influenced by 2mg of P given to OVX animals either 2 h or 24 h before sacrifice; at 2 h. control OVX – 154.4 ± 18.1 and OVX + P – 159.3 ± 12.3; at 24 h: control OVX – 155.5 ± 17 7 and OVX ± P – 179.9 ± 6.4; ($F_{(3,12)}$=0.912, $p > 0.05$) (Fig. 3*a*, *b*). Also, the activity of brain GST was not affected by EB; the respective values for control OVX and OVX + EB treated animals were 154.4 ± 18.1 and 154.8 ± 13.6 U/mg protein at 2 h and 155.5 ± 17.7 and 138.4 ± 15.6 at 24 h ($F_{(3,12)}$=0.34, $p > 0.05$) (Fig. 3*a*, *b*)

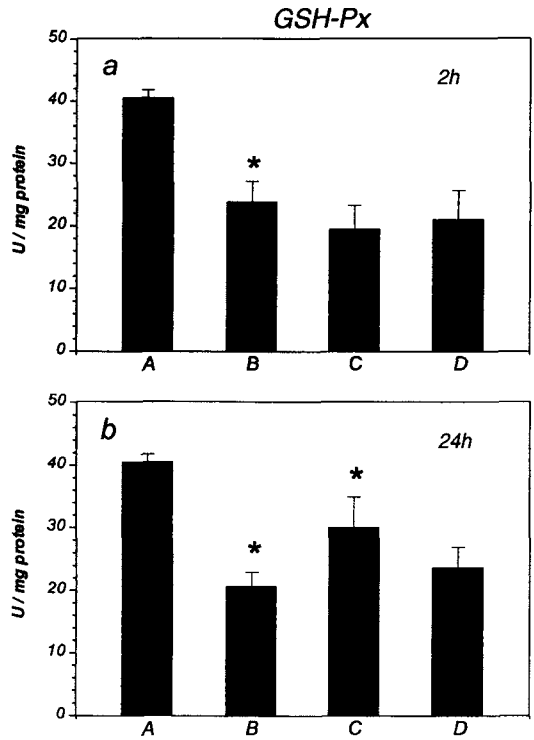

Figure 2. GSH-Px activity in brain homogenates of long-term ovariectomized rats treated 2 h and 24 h prior to sample preparation, *A* nontreated controls ($n = 5$), *B* controls treated with olive oil ($n = 4$), *C* treated with 2 mg P ($n = 4$) and *D* treated with 5 μg EB ($n = 4$) Columns represent mean values and vertical bars are S E M *a*, *A B* – $^*p < 0\ 05$, *b*, *A B* and *B C* – $^*p < 0\ 05$

Effects of P and EB on GR activity

Systemic administration of 5μg EB to OVX rats evoked 2 h later a significant decrease of GR activity in the brain (control OVX – 40.5 ± 2.5 and OVX + EB – 31 9 ± 4 5 U/mg protein) ($t_{(6)}$=1.94, $p < 0.05$) (Fig. 4*a*). GR was not significantly decreased 24 h after EB administration (control OVX – 38.5 ± 2.8 and OVX + EB – 33.8 ± 3 3 U/mg protein) ($t_{(6)}$=1.26, $p > 0.05$) (Fig. 4*b*). The activity of GR was not affected by 2mg of P 2 h after the treatment (control OVX – 40.5 ± 2.5 and OVX + P – 38.6 ± 3 7 U/mg protein) ($t_{(6)}$=0.50; $p > 0\ 05$) (Fig. 4*a*). However, 24 h after the treatment the activity of GR was significantly elevated by P (control OVX – 38.5 ± 2.8 and OVX ± P – 45 6 ± 2.3 U/mg protein) ($t_{(6)}$=2.35, $p < 0.05$) (Fig 4b)

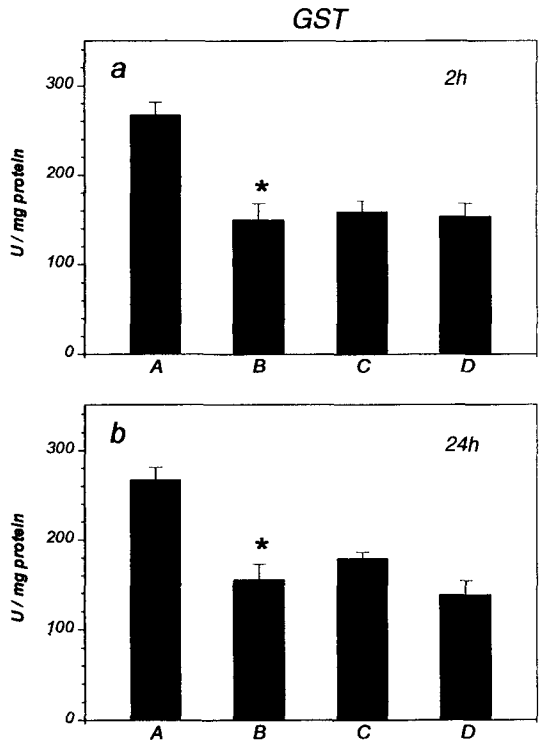

Figure 3. GST activity in brain homogenates of long-term ovariectomized rats treated 2 h and 24 h prior to sample preparation, *A* nontreated controls ($n = 5$), *B* controls treated with olive oil ($n = 4$), *C* treated with 2 mg P ($n = 4$), and *D* treated with 5 μg EB ($n = 4$) Columns represent mean values, and vertical bars are S E M *a*, *A B* – $^{*}p < 0\ 05$, *b*, *A B* – $^{*}p < 0\ 05$

Discussion

The role of glutathione and glutathione related enzymes in the cellular metabolism and detoxification of cytotoxic and carcinogenic compounds is well established (Hendrich and Pitot 1987). Cell membrane protection is also provided by the activity of GSH-Px which reduces both H_2O_2 and other peroxides using GSH converted to oxidized glutathione (GSSG). Therefore, maintenance and regulation of the intracellular GSH are important as a defence against cytotoxic compounds. The activities of glutathione related enzymes in rats have been reported to be influenced by age and sex (Igarashi et al.1983). Zarida et al. (1993) have shown that the activities of glutathione related enzymes on the plasma and the liver of rats

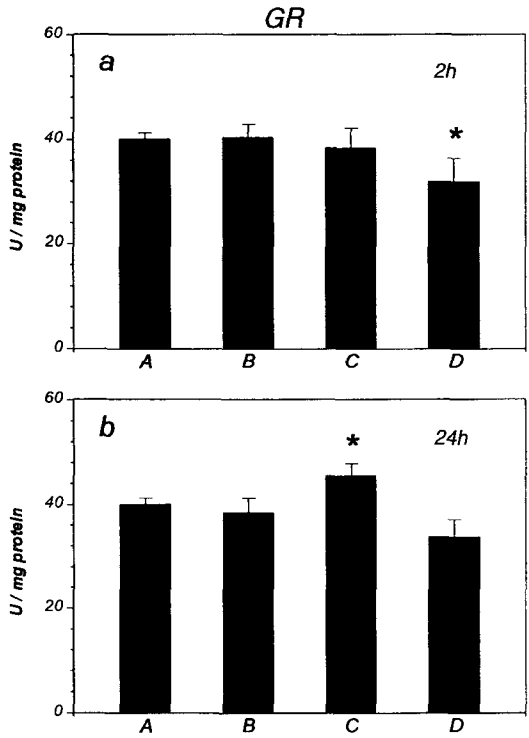

Figure 4. GR activity in brain homogenates of long-term ovariectomized rats treated 2 h and 24 h prior to sample preparation, *A* nontreated controls ($n = 5$), *B* controls treated with olive oil ($n = 4$), *C* treated with 2 mg P ($n = 4$), and *D* treated with 5 μg EB ($n = 4$) Columns represent mean values, and vertical bars are S E M *a*, *B* *D* – $^*p < 0\ 05$, *b*, *B* *C* – $^*p < 0\ 05$

subjected to gonadectomy and sex hormones replacement (either estradiol or progesterone, or both in females, and testosterone in males). Kume-Kick et al. (1996) found enhanced oxidative stress in female rat brains after gonadectomy. These findings suggest that loss of ovarian hormones can significantly enhance oxidative stress during ischemia in females, while oxidative stress in males was relatively unaffected by gonadectomy (Weiner et al. 1994; Pajović et al. 1996).

Our results show that 2mg P increased the activities of GSH-Px and GR, 24h following the treatment. GSH is generally accepted to have an important function in chemical detoxication processes. Its two most widely known biological roles include inactivation of H_2O_2 via GSH-Px with the concomitant oxidation of GSH to its disulfide (GSSG) (Sies and Ketterer 1988). The balance between oxidation of GSH

and reduction of GSSG is keept by GR. Therefore, parallel increases in activities of GSH-Px and GR by P were observed.

In the central nervous system autooxidation of intracellular flavins, quinones, and other substances, as well as uni- and divalent electron transfer to O_2 by cytosolic enzymes or membrane-bound electron transport systems, are the predominant sources of intracellular $O_2^{-\cdot}$ and H_2O_2 during normal metabolism (Fridovich 1978; Chance et al. 1979), and can be further reduced by transition metals to generate the highly reactive secondary radical $^{\cdot}OH$ (McCord and Day 1978) In the brain, a greater production of free radicals is a consequence of progesterone treatment as it was shown that estrogen treatment *in vivo* and *in vitro* results in increased NOS activity and NOS mRNA expression (Weiner et al. 1994). Steroids increase the accumulation of reactive oxygen species in primary neuronal culture (McIntosh and Sapolsky 1996). According to Kume-Kick et al. (1996), after gonadectomy increased ascorbate loss was seen in all female brain regions, indicating enhanced oxidative stress. The generation of H_2O_2 causes increased lipid peroxidation and increased GSH oxidation (Zirkle et al. 1965; Dirks and Faiman 1982). The up-regulation of GSH-Px and GR might be a consequence of increased levels of H_2O_2 and oxidation of GSH in the nerve cells. It is necessary, because the brain contains relatively low specific activities of H_2O_2 scavenging enzymes, including GSH-Px (Sinet et al 1980) to be able to have adequately respond.

Acknowledgements. This work was supported by Research Science Fonds of Serbia, Yugoslavia, grants No 03E24 and 03E18

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Final version accepted February 20, 1999