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Effects of progesterone and estradiol benzoate on superoxide dismutase activity in the brain of male rats

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Abstract. The activities of mitochondrial, manganese-containing superoxide dismutase (MnSOD) and cytoplasmic, copper-zinc-containing superoxide dismutase (CuZnSOD) were measured in subcellular fractions of whole brain homogenates prepared from intact and gonadectomized (GDX) male rats, untreated or treated subcutaneously (sc) with a single dose of 2 mg progesterone (P) and/or 5 μg estradiol benzoate (EB). Neither MnSOD nor CuZnSOD was affected by the removal of the testes. Similarly, CuZnSOD activity was steady following systemic administration of P and/or EB to intact and GDX animals 2 h or 24 h prior to sacrifice. On the other hand, both P and EB suppressed MnSOD in the brain of either intact or GDX rats. These results suggest involvement of P and EB in the control of MnSOD activity in the brain of male rats.
Key words. Superoxide dismutase; brain; progesterone; estradiol; rats.

The activity of antioxidant (AO) enzymes in different tissues 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. Murakoshi et al.[1] have found that castration leads to profound inhibition of glutathione peroxidase (GSH-Px) synthesis in the rat prostate; the inhibition could be removed by treating males with testosterone alone or in combination with estradiol. Differences between the sexes with respect to AO enzyme activities in different tissues and organs, especially in the liver, have been described by several groups of authors. Pinto and Bartley[2,3] reported that the activity of GSH-Px and catalase (CAT) in the rat liver is higher in males than in females and that, in the female rat, GSH-Px activity is higher in estrus than in diestrus. Capel and Smallwood[4] have shown that the activity of GSH-Px in the rat liver is considerably higher in females than in males, whereas no difference between the sexes was found when the activity was measured in the brain. Finley et al.[5] found higher blood selenium (Se) levels and increased GSH-Px activity in the plasma and kidney of male rats, whereas the enzyme activity and Se content were higher in the female liver. Zarida et al.[6] reported higher GSH-Px and glutathione reductase (GR) activities in the liver of female rats, and higher glutathione-S-transferase (GST) activity in the male liver. Rikans et al.[7] reported that the activity of CuZnSOD and GSH-Px, as well as vitamin E and malondialdehyde concentrations, are lower in the liver of male rats whereas CAT and GR activities are higher suggesting that, in addition to AO enzymes, nonenzymatic AO defense mechanisms in the rat liver are sex-dependent. Prohaska and Sunde[8] demonstrated that, in the rat liver, the GSH-Px activity is considerably higher in females than in males, as a consequence of increased levels of mRNA for GSH-Px and of Se concentration, whereas no sex difference was detected with respect to CuZnSOD activity. We have recently demonstrated that, in the female rat, MnSOD activity in the brain is increasing following long-term ovariectomy and that high post-castration enzyme activity can be suppressed by exogenous progesterone (P) and estradiol benzoate (EB), in contrast to brain CuZnSOD activity, which appeared not to be under the influence of the ovary[9]. In this report, the study of the influence of P and EB on the activities of cytoplasmic SOD in the rat brain has been extended to include male animals.

Materials and methods

Intact and bilaterally gonadectomized male Wistar rats, aged 3.5 months and weighing 328 g on average, 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. Gonadectomy was performed under ether anaesthesia two weeks before hormone treatment.

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 on 0.1 ml olive oil, was given subcutaneously (sc) to intact and GDX animals. Controls received the oil alone.

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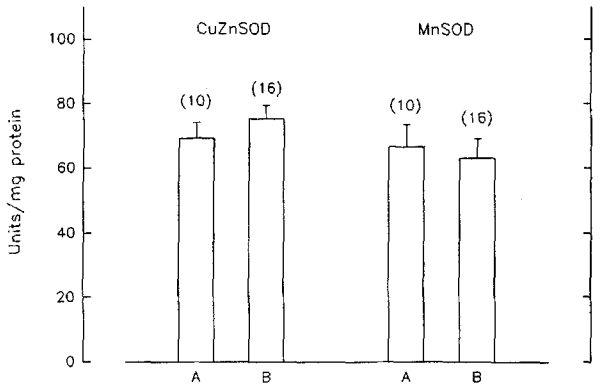

Figure 1. CuZnSOD and MnSOD activities in brain homogenates prepared from intact (A) and gonadectomized (B) male rats. Columns represent means of the number if samples indicated in parentheses, and lines represent ±SEM. Non-significant differences (Students t-test).

Animals were killed by decapitation with a guillotine (Havard Apparatus) and fresh brains were dissected out and subjected to subcellular fractionation. The procedure of Bohnenkamp and Wester[10] was employed as described elsewhere[9] to prepare cytosolic and mitochondrial fractions. Protein concentrations were determined by the method of Lowry et al.[11]

SOD activity in the cytosol (CuZnSOD) and mitochondrial (MnSOD) fractions was measured by the method of Misra and Fridovich[12]. Autoxidation of epinephrine to adrenochrome was performed in 3 ml of 0.05 M Na_2CO_3 at pH 10.2. Inhibition of autoxidation was monitored at 480 nm. The results were expressed in units of enzyme activity. One unit of SOD was defined as the amount of protein which caused 50% inhibition of the conversion rate between the third and fourth minute of incubation.

The results were analyzed by Student's t-test and by ANOVA in combination with Scheffe's procedure. Differences between means were considered significant at 5% level.

Results

Bilateral gonadectomy two weeks prior to experiments did not significantly ($p > 0.05$) affect the activity of brain MnSOD (63 ± 6 units/mg protein) in comparsion to intact animals (67 ± 7) (fig. 1). Similarly, the activity of CuZnSOD appeared to be steady after castration (69 ± 5 in intact vs. 75 ± 4 in GDX; $p > 0.05$) (fig. 1). CuZnSOD activity in brain homogenates of intact animals could not be affected by the hormone treatments (fig. 2). Thus, the respective values for intact controls and intact + P and/or EB treated animals, 2 h after hormone treatment, were 70 ± 5.2, 76 ± 3.7, 87 ± 15.2, and 81 ± 8.7 units/mg protein ($F_{3,32} = 0.62$; $p > 0.05$). At the same time, the activity of brain MnSOD was profoundly lowered by 2 mg P and 5 µg EB, given alone or in combination to intact animals 2 h before sacrifice (fig. 3). The values for controls and intact rats treated with P, EB and P + EB were, respectively, 68 ± 8, 24 ± 3, 24 ± 3 and 31 ± 4 ($F_{3,32} = 19.75$; $p < 0.05$).

Brain CuZnSOD activity in GDX animals was not changed following sc injection of 2 mg P and/or 5 µg EB (fig. 2); at 2 h, the respective values for GDX controls and GDX treated with P and/or EB were 72.1 ± 5.3, 74 ± 8.2, 62.9 ± 7.3, and 75.9 ± 8.2 units/mg protein ($F_{3,32} = 0.59$; $p > 0.05$); at 24 h, the corresponding values were 83.3 ± 6.9, 83.8 ± 7.7, 76 ± 4.4, and 82 ± 4.5 units/mg protein ($F_{3,20} = 0.36$; $p > 0.05$).

The high post-castration activity of brain MnSOD was depressed profoundly by 2 mg P, given to GDX animals either 2 h or 24 h before sacrifice (fig. 3). The respective values for control GDX and GDX + P treated animals at 2 h, were 64 ± 5.7 and 27.3 ± 2.5 ($p < 0.05$), and at 24 h were 63.2 ± 14.7 and 25.5 ± 7.2 ($p < 0.05$). Sys-

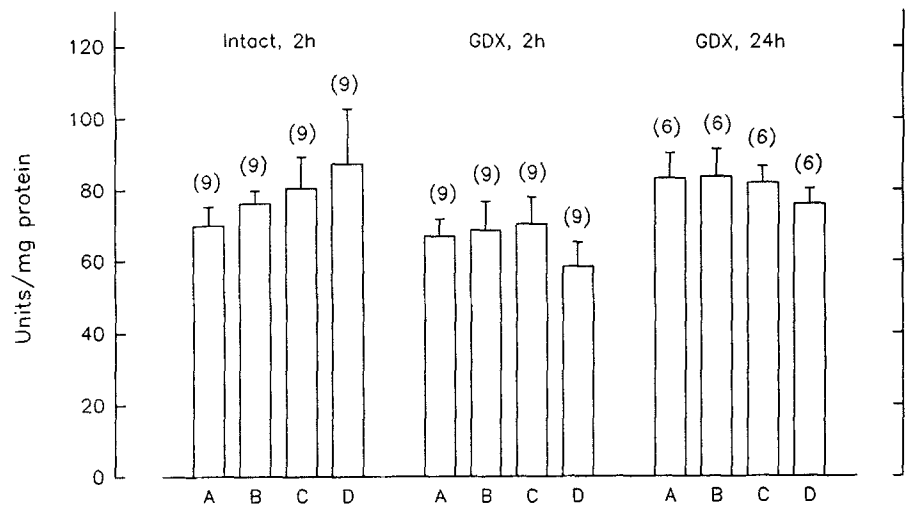

Figure 2. CuZuSOD activity in brain homogenates from intact and gonadectomized (GDX) rats prepared 2 h and 24 h following treatment. A: controls; B: treated with progesterone (P); C: treated with estradiol 17β-benzoate (EB); and D: treated with P + EB. Columns represent means of the number of samples indicated in parentheses, and lines represent ±SEM. Non-significant differences (ANOVA).

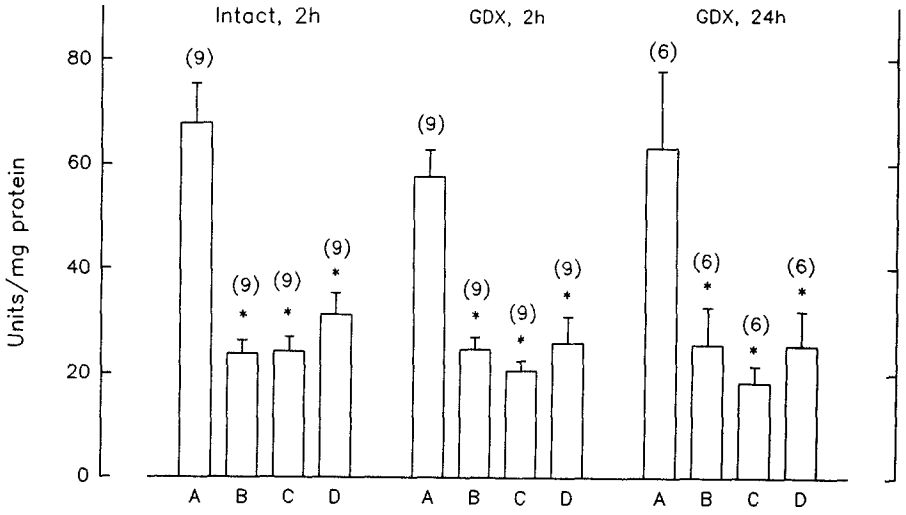

Figure 3. MnSOD activity in brain homogenates from intact and gonadectomized (GDX) rats prepared 2 h and 24 h following treatment. A: controls; B: treated with progesterone (P); C: treated with estradiol 17β-benzoate (EB); and D: treated with P + EB. Columns represent means of the number of samples indicated in parentheses, and lines represent $\pm$SEM. *: $p < 0.05$ (ANOVA followed by Scheffe procedure).

temic administration of 5 µg EB to GDX rats evoked a significant decrease of MnSOD activity in the brain (23 ± 2) 2 h later, in comparison to GDX controls (64 ± 6) ($p < 0.05$). Low MnSOD activity was also found 24 h following EB treatment (18 ± 3 vs 63 ± 15; $p < 0.05$). Similarly, a marked inhibition of MnSOD activity in GDX rats was obtained with 2 mg P and 5 µg EB (fig. 2); the respective values were: at 2 h, 64 ± 6 and 29 ± 6 ($p < 0.05$); at 24 h, 63 ± 15 and 25 ± 7 ($p < 0.05$).

Discussion

The results presented in this work show that exogenous P and EB suppress enzymatic activity of MnSOD in the brain of male rats. These effects, observed in both intact and GDX animals, are comparable to previously described suppressive effects of ovarian hormones on brain MnSOD activity in female rats[9]. Thus, suppression of brain MnSOD activity by P and EB appears to be a phenomenon common to rats of both sexes. The possibility that, in the female rat, gonadotropins are mediators of ovarian steroid effects on brain MnSOD activity has been suggested earlier[9], since in the rat ovary, hCG and PMSG decrease MnSOD and do not affect CuZnSOD[13], and lutropin induced total SOD activity[14]. In contrast to female rats, in which bilateral ovariectomy resulted in increased MnSOD activity[9], gonadectomy proved to be ineffective in the male. This is somewhat unexpected, since removal of the testes should suppress (via the aromatase system present in the male rat brain) the formation of neuroactive estrogens, which are obviously inhibitory, and suggests that the mechanisms of P and EB action to modulate brain MnSOD activity differ between the sexes. It is possible that in the male rat the adrenal androgens, in addition to estrogens, act centrally to hold in check the enzyme in the brain. Since gonadectomy, which is known to increase serum LH levels[15], did not affect MnSOD activity, and since GDX animals responded equally to exogenous EB and/or P by a decrease in the enzyme activity, it appears that in the male rat, P and EB act non-synergistically on nerve cells to suppress MnSOD expression directly, possibly via common receptors[16] or molecular pathways. In contrast to MnSOD, the activity of brain CuZuSOD remained constant following hormone treatments. This dissociation between the two enzymes, also found in female rats, could be explained by the higher inducibility of MnSOD in comparison to CuZuSOD, as detected earlier by Petrović and his group in different tissues of rats exposed to paraquat and cold or after hormonal treatment[17,18,19].

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