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Effects of ovarian steroids on superoxide dismutase activity in the rat brain

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Abstract. The levels of manganese superoxide dismutase (MnSOD) and copper-zinc superoxide dismutase (CuZnSOD) were determined in appropriate subcellular fractions prepared from whole brain homogenates of cycling and long-term (3 week) ovariectomized (OVX) Wistar rats, and were compared to the levels found in corresponding samples prepared from OVX rats treated with progesterone (P) or estradiol 17B-benzoate (EB). The activity of both SODs was steady during the estrous cycle, except at proestrus, when MnSOD activity was elevated significantly. Bilateral ovariectomy resulted three weeks later in an increase of the MnSOD activity even higher than that recorded at proestrus. High post-castration MnSOD activity was lowered profoundly by exogenous P (2 mg) or EB (0.5 μg), given s.c. to OVX animals 2 h or 24 h before sacrifice. Neither removal of the ovaries nor the hormone treatments affected the activity of CuZnSOD. These results suggest suppressive effects of ovarian steroids on MnSOD activity in the rat brain.
Key words. Superoxide dismutase; brain; estradiol; progesterone.

The superoxide dismutases (SODs) dismutate superoxide radicals O_2^- into H_2O_2 plus O_2, thus participating, with other anti-oxidant enzymes, in the enzymatic defence against oxygen toxicity. In eukaryotic cells, the process of dismutation is catalyzed by two distinct enzymes. The MnSOD is found in the matrix of mitochondria, whereas the CuZnSOD occurs primarily in the cytosol (for review, see Fridovich[1]).

The specific activities and concentrations of SODs have been extensively studied in the brain and other tissues of mammals, including man, under different physiological and pathological conditions. The pattern of the brain enzyme activity has been reported to vary during ontogeny[2] and with aging[3-5] and to be altered in neurological disorders[6,7], as well as by heat[8] and cold[9] stress, and in vitamin E deficiency[10]. No attention has so far been paid, however, to a possible connection between brain SOD activity and endogenous ovarian steroids. Estradiol and progesterone are known to act directly on the CNS to modulate electrical properties of neurons[11,12] and certain important cellular functions, such as choline uptake and acetylcholine synthesis and release[13,14], neurotransmitter turnover[15,16] and uptake[17,18], calcium uptake[19,20] etc. The aim of the present study was to investigate whether specific effects of ovarian steroids on neuronal properties and functions, which are believed to underlie well-recognized central actions of the hormones in the feedback control of hypothalamo-hypophysial endocrine functions and sexual behavior, include modulation of MnSOD and CuZnSOD activities in the brain.

Materials and methods

Animals. Female Wistar rats aged 3.5 months and weighing 265 g on average, were used. Only animals with a regular 4–5 day estrous cycle were selected for the experiments. At sacrifice, the cycling rats were classified as proestrous, estrous or diestrous. In experiments with hormone treatment, the animals were subjected 3 weeks earlier to bilateral ovariectomy under ether anaesthesia. 2 h or 24 h before sacrifice, the OVX rats were injected s.c. with a single dose of 2 mg P suspended in 0.2 ml olive oil, or with 5 μg EB suspended in 0.1 ml olive oil. Controls received the vehicle alone. All animals were killed in the morning by decapitation with a guillotine (Harvard Apparatus) and their brains were dissected and pooled (2/pool).

Subcellular fractionations. The procedure as described by Bohnenkamp and Weser[21] was used with minor modifications. Fresh brain tissue was homogenized in 0.32 M sucrose containing 0.5 M EDTA, 3 mM $MgCl_2$ and 0.02 M phosphate buffer, pH 7.4. The homogenate was centrifuged at 800 × g for 10 min. The supernatant was decanted and centrifuged at 2100 × g for 5 min. The pellet was discarded and the supernatant was centrifuged at 8000 × g for 10 min. The pellet was washed with 0.25 M sucrose containing 3 mM $MgCl_2$ and 0.02 M phosphate buffer. The pellet was considered to be the mitochondrial fraction. It was suspended in 0.02 M phosphate buffer, repeatedly vortexed for 30 s several times while being cooled in ice, and finally kept frozen at −70 °C for 4 h before it was used for the enzyme assay. The supernatant was again centrifuged at 85,000 × g for 90 min and considered to be the cytosol. Protein concentrations were determined by the method of Lowry[22].

Enzyme assays. SOD activity in the cytosol (CuZnSOD) and mitochondrial fraction (MnSOD) was mea-

sured by the method of Misra and Fridovich[23]. The reaction of autoxidation of norepinephrine to adrenochrome was performed in 3 ml of 0.05 M Na_2CO_3 at pH 10.2. Inhibition of the autoxidation was monitored at 490 nm. The results were expressed in units of enzyme activity. One unit of SOD was defined as that amount of protein which caused 50% inhibition of the conversion rate between the 3rd and the 4th min of incubation.

Statistics. The results were analyzed by analysis of variance (ANOVA), in combination with Scheffe's test. Differences between means were considered significant at $p < 0.05$.

Results

MnSOD and CuZnSOD activities in cycling and ovariectomized rats. The results are summarized in figure 1. The activity of brain MnSOD was significantly increased ($F_{(2, 16)} = 14.53$; $p < 0.05$) at proestrus (93 ± 3.7 U/mg protein), in comparison to the activity at estrus (71 ± 3.9 U/mg) and diestrus (67 ± 3.3 U/mg). Bilateral ovariectomy resulted 3 weeks later in an increase of the MnSOD activity even higher than that recorded at proestrus (123 ± 10.2 U/mg protein; $F_{(3, 23)} = 16.02$; $p < 0.05$). At the same time, the activity of CuZnSOD appeared to be steady during the estrous cycle (60 ± 2.3 U/mg at estrus, 54 ± 1.6 U/mg at diestrus and 62 ± 3.1 U/mg at proestrus; $F_{(2, 16)} = 3.00$; $p > 0.05$) and was not affected by ovariectomy (65 ± 3.4 U/mg; $F_{(3, 23)} = 2.91$; $p > 0.05$).

Effects of EB and P on MnSOD and CuZnSOD activities. The high post-castration activity of brain MnSOD was lowered profoundly by 0.5 µg EB, given to OVX animals either 2 h or 24 h before sacrifice (fig. 2). The values for control OVX and OVX + EB treated animals were, respectively: at 2 h – 104 ± 5.6 and 50 ± 6.2 U/

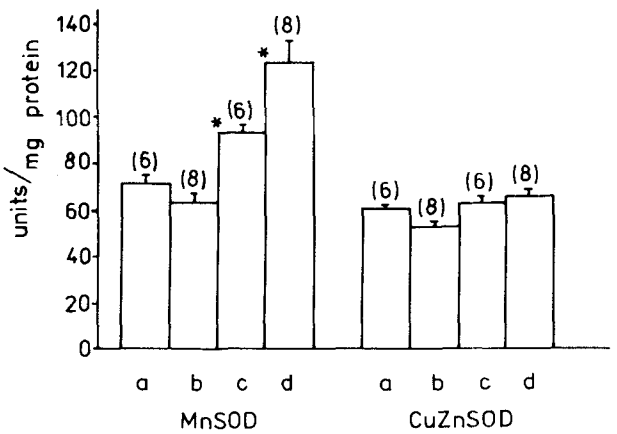

Figure 1. MnSOD and CuZnSOD activities in brain homogenates prepared from rats at estrus (a), diestrus (b), proestrus (c) and folowing long-term ovariectomy (d). Columns represent means of the number of samples (pools of brains) indicated in parentheses and lines represent SEM. Data analyzed by ANOVA and differences between groups tested by Scheffe procedure: * different from others and from each other, $p < 0.05$.

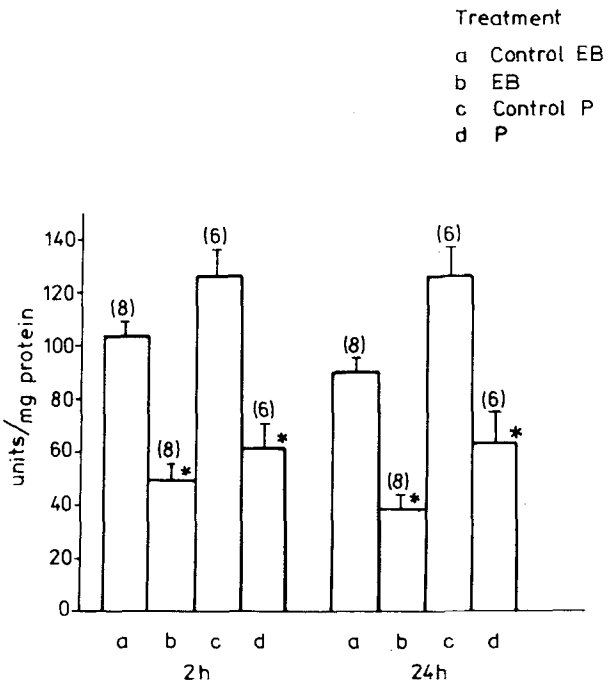

Figure 2. MnSOD activity in brain homogenates of long-term ovariectomized rats treated s.c. with 5 µg of estradiol-17B benzoate (EB) or with 2 mg of progesterone (P) 2 h or 24 h prior to sample preparations. Columns represent means of the number of samples (pools of brains) indicated in parentheses and lines represent SEM. Data analyzed by ANOVA and differences between groups tested by the Scheffe procedure: * different from corresponding control, $p < 0.05$.

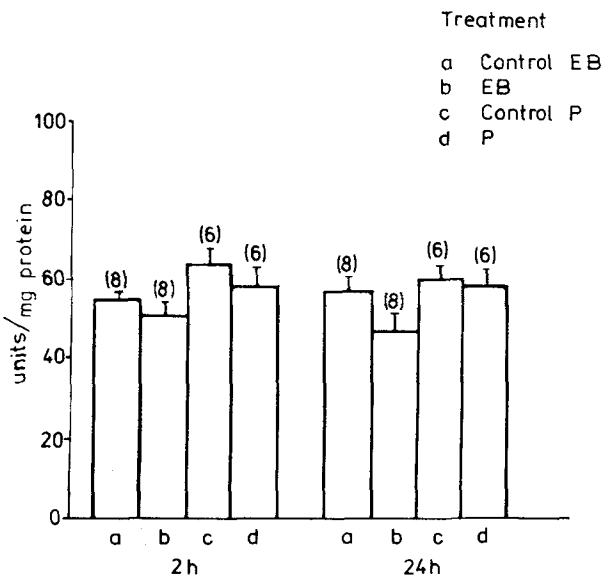

Figure 3. CuZnSOD activity in brain homogenates of long-term ovariectomized rats treated s.c. with 5 µg of estradiol-17B benzoate (EB) or with 2 mg of progesterone (P) 2 h or 24 h prior to sample preparation. Columns represent means of the number of samples (pools of brains) indicated in parentheses and lines represent SEM. Data analyzed by ANOVA and differences between groups tested by Scheffe procedure: no significant differences between corresponding groups, $p > 0.05$.

mg; at 24 h – 91 ± 5.4 and 39 ± 5.6 U/mg ($F_{(3, 28)} = 29.53$; $p < 0.05$). Similarly, a marked inhibition of MnSOD activity in OVX rats was obtained with 2 mg P (figure 2): at 2 h – 127 ± 9.7 and 62 ± 8.3 U/mg; at 24 h – 127 ± 10.6 and 64 ± 10.8 U/mg ($F_{(3, 20)} = 13.93$; $p < 0.05$). On the other hand, the low CuZnSOD activity in brain homogenates of OVX animals could not be affected by the hormone treatments (fig. 3); the respective values for control OVX and OVX + EB treated animals were 55 ± 1.4 and 51 ± 3.4 U/mg at 2 h and 57 ± 3.0 and 47 ± 4.1 U/mg at 24 h ($F_{(3, 28)} = 2.09$; $p > 0.05$), and for OVX controls vs OVX + P treated animals were 64 ± 3.8 and 58 ± 5.1 U/mg at 2 h and 60 ± 3.5 and 58 ± 4.4 U/mg at 24 h ($F_{(3, 20)} = 0.46$; $p > 0.05$) after hormone treatment.

Discussion

The experimental results presented suggest the existence of an inverse relation between MnSOD activity in the brain and endogenous progesterone or estradiol. At the same time, the CuZnSOD activity does not appear to be affected by these two hormones. A similar increase of the MnSOD activity at proestrus was found in the rat ovary[24]. This finding, and the report that MnSOD levels in the serum of patients with epithelial ovarian carcinoma correlate with the clinical picture and effects of steroid therapy[25], suggests that the observed relationship between MnSOD and ovarian steroids is not an artifact, although its biological meaning, if any, is difficult to understand.

Changes in the MnSOD activity observed in this work appear to coincide with a characteristic proestrus surge[26] or post-castration elevation[27,28] of circulating gonadotropins, and with the known decrease of high postcastration levels of these hormones following estradiol[29] or progesterone[30] injection in OVX rats in doses similar to those applied in this work. This raises the question of whether the effects of progesterone and estradiol on the brain MnSOD activity are directly on MnSOD expression or are mediated via LH and/or FSH action. Lutropin specifically induces SOD in the ovary of immature pseudo-pregnant rats primed with chorionic gonadotropin, and this process could be blocked significantly by anti-LH serum[24], and LH is present in numerous extrahypothalamic centers[31,32]. These findings imply that LH is a possible mediator of ovarian steroid effects on the brain MnSOD activity. This could explain the prolonged effects of the steroids on the enzyme activity seen in OVX animals 24 h after the treatment, whereas acute effects, seen 2 h after treatment, could be attributed to their direct action on MnSOD expression. A possibility that the effect of P is non-genomic cannot be excluded, since P-mediated effects in OVX rats are known usually to require E priming.

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