MINIREVIEW

MODULATION OF ANTIOXIDANT ENZYME ACTIVITIES BY SEXUAL STEROID HORMONES

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This minireview is dedicated to the memory of the Academician Prof. Dr Vojislav M. Petrović

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Summary

Taking into consideration the biological importance of interaction between antioxidant defense (AD) enzymes and sexual steroid hormones it was deemed important to survey recent our achievements in the field with the state of current knowledge. The main goal of the present review was to investigate the changes of AD enzyme activities: superoxide dismutases, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase in the brain of female and male rats depending on the ovarian steroids (progesterone and estradiol). The sex steroids produce their effects by acting on numerous target tissues and organs, such as the reproductive organs, bone tissue and cartilage, peripheral blood vessels and the central nervous system (CNS). We have chosen it, as a new parameter that might represent an important indicator of the changes within the CNS, bearing in mind the biological importance of the enzymes of the AD system. Our experimental results indicate that the AD enzyme activities in the brain tissue of female and male rats show a certain dependence on the concentration of progesterone and estradiol. The presented review suggests that the modulation of the oxidative and antioxidative capacity by sexual steroid hormones is mediated through antioxidant metabolising enzymes.

Key words: Antioxidant defense enzymes ● Sexual steroid hormones ● Rat ● Brain

Introduction

Reactive oxygen species (ROS), generated as by-products of oxidative metabilism in mitochondria, can interact with biomolecules and damage various cellular components. Aerobic organisms developed a complex network of antioxidant defense (AD) system as a protection against harmful effects of ROS and maintenance of tissue homeostasis. The AD system primarily includes the AD enzymes such as: superoxide dismutases (both copper zinc, CuZn SOD and manganese containing, Mn SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutatahione-S-transferase (GST, EC 2.5.1.18) and glutathione reductase (GR, EC 1.6.4.2). As low molecular weight components we can highlight reduced glutathione (GSH), vitamins E and C, flavonoids, etc. (Halliwell and Gutteridge, 1999). However, when ROS generation exceeds the antioxidant capacity of cells, oxidative stress develops, potentially causing tissue damage (Araujo *et al.* 2006). Organization of the AD system is species-, organ- and tissue- specific and susceptible to alterations in response to both oxidative stress and changes of the tissue metabolic activity (Kasapović *et al.* 2001, Marković *et al.* 2006, Ognjanović *et al.* 1995, 2003, Žikić *et al.* 1996, 1997, 2001, Pajović *et al.* 1997, 2003, 2006, Saičić *et al.* 1998, 2006). Specificities of AD system in the brain can be seen not only at the level of its organization, but also at the level of its regulation. In the brain as a central regulatory tissue, balance between free radical production and AD system is of utmost importance for several reasons. The brain requires about one-fifth of total oxygen demand of the body (Nistico *et al.* 1992). This tissue is characterized by a high rate of oxidative metabolic activity, numerous membranes and a high concentration of readily oxidizable substrates (e.g., membrane lipid poyunsaturated fatty acids), high amounts of iron, especially in some regions (Weber, 1994) and endogenous generation of reactive oxygen metabolites by specific neurochemical reactions (monoamine oxidase [MAO; EC 1.4.3.4] catalyzed oxidation of catecholamines), prostaglandin metabolism (Evans, 1993), activation of macrophage-type microglial cells (Hall and Broughler, 1993) and nitric oxide generation by endothelial cells and neurones (Snyder, 1992). In addition, low levels of AD enzymes have been detected in the rat brain in comparison with other tissues (Hothersall et al. 1981, Herman, 1983).

Biological effects of the sexual steroid hormones are at the base of many physiological and pathophysiological processes. These hormones, as a group of regulatory molecules produced by the endocrine system, regulate sexual behaviour, prepare the organism for reproduction, enable it to adapt to the changes in its

environment and regulate ion concentrations in the body fluids. By their selective influence on gene transcription and production of ribonucleic acid (RNA) molecules, these molecules control the biosynthesis of specific proteins, thus realizing their biological effects at a physiological level (Ing 2005). Steroid hormones can exert their influence even independently of the genome transcription (Kane 1976, Prange *et al.*, Klaiber *et al.* 1979). The ovarian steroids produce their effects by acting on numerous target tissues and organs, such as the cerebral blood vessels (Stirone *et al.* 2005).

Bearing in mind these physiological roles of steroids, in relation to the sexual behaviour and reproduction, it is easy to see the significance of an intimate understanding of the effects of steroid hormones on the functions of the CNS and the mechanisms behind these effects, which is one of the central aspects of the contemporary neuroendocrinology. A wide range of events, such as the induction of specific receptors, neurotransmitter metabolism, ionic transport, enzyme activity and the like are studied as parameters of interactions between steroid hormones and the brain.

The information on the effects of sexual steroid hormones on the enzyme activity inside the brain in the scientific literature is scarce and usually refers to the monoamine oxidase (MAO), acetylcholinesterase (AChE), glucose-6-phosphate dehydrogenase (G-6-PDH),(Luine and Rhodes, 1983), as well as tyrosine hydroxylase (TH),(Kritzer and Kohama, 1998). It has been demonstrated that the activities of MAO, AChE and G-6-PDH in the preoptical region of the hypothalamus change differently following the treatments with estrogen (E) and progesterone (P). In addition, there are some very interesting results that show that ovariectomy increases the TH activity 2-3 times and that E and P treatments have the opposite effect on the afore mentioned enzyme activity in the hypothalamus. Sobočanes *et al.* (2003) investigated whether oxidant status and AD activities during ageing of mouse brain are regulated in sex-dependent manner. Throughout ageing, no difference in total superoxide dismutase (t SOD) activity between male and female brains was observed, except in immature 1 month old mice. Taken together, the their finding indicate that brains of female mice have lower oxidant and higher antioxidant capacity mostly related to CAT and to a lesser extent to GSH-Px activity.

In our studies we have monitored the change of enzyme activity of the AD system in the brain of male and female rats depending on the ovarian steroids (Pajović *et al.* 1993, Pajović *et al.* 1996, Pajović *et al.* 2003, Pejić *et al.* 2003). We have chosen it as a new parameter that might represent an important indicator of the changes within

the CNS, bearing in mind the biological importance of the enzymes of the AD system. Study of the activity of the AD enzymes of the AD system in the brain of female and male rats depending on the influence of ovarian hormones can answer whether the action of ovarian steroids on the CNS includes maintenance of a dynamic equilibrium of free radicals in the neurons.

The experimental results of our study indicate that the enzyme activity of the AD system in the brain tissue of female and male rats shows a certain dependence on the concentration of ovarian hormones, P and E in the organism (Pajović *et al.* 2003, Michos *et al.* 2006). These complement the already available information on the effects of hormone action in general and steroid hormones in particular on the enzyme activity of the AD system in rat tissues (Pereira *et al.* 1994; Zarida *et al.* 1993, Schmidt *et al.* 2005). The first information on the influence of steroid hormones on these enzymes in the brain that we found was in the studies by Petrović *et al.* (1991) and Saičić *et al*. (1991) investigating the influence of dexamethasone on the activity of AD enzymes of the AD system in certain tissues, including the rat brain tissue.

Effects of ovarian hormones on the antioxidant enzyme activities in the female rat brain

Statistical analysis of the results shows that the activity of CuZn SOD, GST and GR remains stable during the estrous cycle, while the activity of the remaining three enzymes of the AD system: Mn SOD, which removes superoxide anion radicals ($O_2^{\cdot -}$) inside mitochondria, CAT and GSH-Px, which removes hydrogen peroxide (H_2O_2) changes depending on the status of ovarian hormones in the organism. During diestrus, CAT activity is increased, while during proestrus Mn SOD activity increases but GSH-Px activity decreases. These changes can be understood bearing in mind that the concentration of $O_2^{\cdot -}$ in proestrus is decreased (Laloraya *et al.* 1988) and that a certain concentration of H_2O_2 is necessary at this stage of the cycle, as it bears a certain physiological role (Laloraya *et al.* 1989). According to Laloraya *et al.* (1989) the H_2O_2 plays the role of a second messenger within the hormonal system which regulates the development of the follicles, the ovulation and the luteal function. Additionally, the results by Sugino *et al.* (1993) from a study monitoring the change of the SOD activity as well as the change in lipid peroxide (LP) concentration in the corpus luteum of pregnant rats indicate that there is a certain dependence of the enzyme activity on the concentration of ovarian hormones. They have shown that the activities of Mn SOD and CuZn SOD gradually increase until the 15th day of the pregnancy coinciding with the change in serum P levels. The concentration of LP is decreased until the 15th day of pregnancy only to rise sharply between

the 15th and 21st day which is expected considering that the peroxides have an essential role in the regression of the corpus luteum. Hence, the authors came to the general conclusion that SOD and LP play a prime role in the regulation of the luteal function during pregnancy.

In our studies, we have demonstrated that compared to the AD enzyme activities inside the brain of intact female animals with normal cycles, bilateral ovariectomy was ineffective only regarding CuZn SOD activity. Prolonged absence of the ovaries leads to a significant increase of Mn SOD, CAT, GSH-Px and GST activities, while the GR activity is decreased. The mid-day increase of Mn SOD activity in proestrus of intact females and animals that have been subjected to an ovariectomy, as well as the decrease of the activity of this enzyme in the brain of animals subjected to ovariectomy but treated with ovarian steroids, coincide with the changes characteristic for the secretion of gonadotropins by the pituitary gland during the estrous cycle and following a bilateral ovariectomy. It is, therefore, necessary to examine these observed changes as possible consequences of indirect action of ovarian hormones, effectuated through the change in gonadotropin secretion. It is well known that the tonic secretion of luteinizing hormone (LH) and foliclle stimulating hormone (FSH) is followed by a sudden release of these hormones into the bloodstream around noon and in the early afternoon during the proestrus (Brown-Grant *et al.* 1970, Kalra *et al.* 1971, Butcher *et al.* 1974). Preovulatory release of gonadotropins in phases during proestrus is an essential requirement for the rupture of mature follicles and ovulation. It is also known that the inhibitory effect on secretion of gonadotropins results in the tonic circulatory concentrations of these hormones; with the removal of ovaries this inhibition is removed and the concentrations of LH and FSH in the circulation gradually rise to reach a ten-fold value and a plateau 2-3 weeks after the ovariectomy (Tapper *et al*. 1974). If the animals who have been subjected to ovariectomy are treated with exogenous P, LH and FSH concentrations in the bloodstream decrease rapidly (24-48 hours) to the tonic levels.

It is known that gonadotropins can act as intermediaries in the influence of the ovarian hormones on enzyme activity. For example, Laloraya *et al.* (1988) have demonstrated a specific induction of SOD activity by lutropin in rat ovaries. Considering that the effects of lutropin can be blocked using anti-LH serum, it is clear that lutropin is a functional analogue of LH. New research showing the presence of LH in the extrahypothalamical structures seems to support the hypothesis of an indirect effect of ovarian steroids, achieved through LH on the Mn SOD activity in the brain of the female rats. Using radioimmunological analysis and chromatography Emanuele *et*

al. (1983) have shown the presence of LH in the anterior lobe of the pituitary gland, amygdala, thalamus, cerebellum, hippocampus, nucleus caudatus and cortex. Immunoreactive LH in the extrahypothalamical structures has also been detected by Hostetter *et al.* (1987). The question as to whether LH inside the extrahypothalamical structures originates from the hypothalamus (reaching it via retrograde transport from the pituitary gland) or if it is synthetised *de novo* inside these structures remains unanswered. The fact that the concentration of LH inside the extrahypothalamical structures does not change following hypophysectomy, while it decreases in serum until it is no longer detectable, seems to, indirectly, corroborate the thesis of *de novo* synthesis (Kalra and Kalra 1977). Regardless of its unexplained origin, the presence of LH in brain tissues outside of hypothalamus indicates that it might have an intermediary role in the realization of the effects of ovarian steroids, not only on the activity of Mn SOD, but also on the activity of other antioxidant defense enzymes. Aside from the available information on LH, research describing the effects of other gonadotropins on the SOD activity can be found as well in the scientific literature. For example, Sato *et al.* (1992) have shown that treating rats with serum gonadotropin of a pregnant mere and human chorionic gonadotropin results in a significant decrease of the Mn SOD activity inside the ovary, while the CuZn SOD activity remains unchanged.

The question that arises next, whether the effects of P and E on the Mn SOD activity in the brain are direct, or are achieved through an intermediary, i.e. through LH or FSH secretion, is a logical one, since the changes in the activity not only coincide with the described changes in the gonadotropin secretion, but are also of the same direction.

After a certain time period, bilateral ovariectomy results in a significant increase in CAT activity, while hormonal treatments bear no effect on the activity of this enzyme. We can, therefore, assume that the effect of P and E on CAT activity in the brain is achieved through an intermediary, i.e. through gonadotropin secretion. A significant increase in CAT activity during the estrous cycle has been observed in diestrus, which is understandable considering that CAT is predominantly located in microperoxisomes in the hypothalamus (which is the target tissue for LH in a short positive feedback mechanism) and that the changes of LH concentrations in the serum, hypothalamus and extrahypothalamical structures do not necessarily have to be in the same direction (Kalra and Kalra 1977).

Unlike the CAT, GSH-Px is found in all parts of the CNS. Since ovariectomy increases the GSH-Px activity,

as does the treatment with 2 mg of P after 24 hours, the activity of this enzyme can be modulated in two ways: 1) by changing the level of P and 2) by changing the level of gonadotropins. The results that show that the activity of GSH-Px is decreased in the mornings during proestrus, before the sudden LH and FSH release (Brown-Grant *et al.* 1970) and while the P is at a lower level in comparison with the other phases of the estrous cycle (Butcher *et al.* 1974, Sodersten *et al.* 1981) seem to substantiate this hypothesis. On the other hand, in the case of selenium-independent GSH-Px, GST a significant increase of activity has been observed only in the brain of the animals that have been subjected to ovariectomy, which coincides with the drastic change in the level of gonadotropins (Tapper *et al.* 1974). Therefore, contrary to the selenium-dependent GSH-Px, GST activity can be modulated only in one way by changing the level of gonadotropins. In neither of the phases of the estrous cycle, not even during early proestrus, will there be such a drastic increase of gonadotropins (like the one that occurs a certain time after an ovariectomy) which would influence GST activity.

In the case of GR, the third enzyme in the glutathione redox cycle, ovariectomy results in a significant decrease of activity. The results of a hormonal treatment are very interesting, since they point to a divergence in the effects that the hormones have on the activity of GR. The effects are opposite, the inhibitory effect of the E on GR activity is detectable after 2 hours and will have passed after 24 hours, while the stimulatory effect of P is achieved only 24 hours after the treatment. Bearing in mind this difference in hormonal treatments, as well as the fact that the removal of the primary source of ovarian hormones results in a decrease of GR activity, it can be assumed that GR activity can be modulated directly by changing the level of ovarian hormones.

Sexual dimorphism in the antioxidant enzyme activities in the rat tissue

There are some particularly interesting studies that refer to the investigation of the presence of sexual dimorphism in the activity of AD enzymes in different rat tissues (Prohaska and Sunde 1993, Kasapović *et al.* 1997, Azevedo *et al.* 2001, Kasapović *et al.* 2001, Tam *et al.* 2003). Pinto and Bartley (1969) have demonstrated that the CAT activity in rat liver depends on the sex of the animal and that it is significantly higher in males than in females. Capel and Smallwood (1983) have examined whether there are any sexual differences in the GSH-Px activities in rat brain, liver and blood. Their results have shown that there is no significant difference in GSH-Px activity in the brain between males and females, while in the liver the activity of this enzyme is significantly higher in females than in males. The observed difference in GSH-Px activity in the

blood of male and female rats bears no physiological consequence. Prohaska and Sunde (1993) have also demonstrated that the GSH-Px activity in rat liver is higher in females than in males, which is understandable considering that their results show that messenger ribonucleic acid (mRNA) levels, as well as selenium concentrations, are much higher in female than in male rats. In a further study, in a series of experiments on males, both intact and subjected to orchidectomy, untreated and treated with ovarian steroids, we have attempted to find out whether there is a difference in the response to the administered hormonal treatment between the sexes. Unlike the ovariectomy in females, the orchidectomy has no effect on the activity of either cytosolic or mitochondrial SOD. CuZn SOD activity does not change even after the hormonal treatment. However, 2 mg of P or 5 μg of estradiol benzoate (EB) decrease the Mn SOD activity both 2 hours and 24 hours after the treatment, but do not display a synergistic effect. Since it is known that the orchidectomy results in an increase of serum LH concentration in comparison with intact animals and that it has no effect on the SOD activity, it is logical to assume that the ovarian hormones influence the activity of the mitochondrial SOD in the brain of a male rat directly. The results of hormonal treatments support this hypothesis. Special attention should be paid to the absence of a synergistic effect of hormones in relation to the enzyme activity, which is understandable considering that these hormones induce an effect directly, through the same receptors. It has been demonstrated, in rats and guinea pigs, that there is an overlap in the autoradiographic maps of receptors for E and P (Sar and Stumpf 1973) in the hypothalamus of these animals.

Comparisons of the hormonal modulation of antioxidant enzyme activities in the male and female rat brain

The effect of the ovarian hormones on the SOD activity in the brain of males and females is, thus, uniform. However, the analysis of hormonal treatments, ovariectomy or orchidectomy effects, indicates that the mechanisms of action of ovarian steroids on the SOD activity in the brain of male and female rats are different. It appears that the effects of the ovarian steroids on the enzyme activity in the brain of male animals are direct, while in the brain of the female animals these effects are achieved indirectly, through the change in the gonadotropin levels. No sexual dimorphism was observed in the comparison of the specific SOD activity in the brain of intact female and male rats. Prohaska and Sunde (1993) studied the activity of CuZn SOD in the liver depending on the sex and have come to the conclusion that there is no sexual dimorphism in the level of CuZn SOD activity either.

Since the orchidectomy does not influence the CAT activity and ovarian steroids increase it (EB 2 hours

and P 24 hours following the treatment) it can be concluded that this enzyme is more sensitive to the direct influence of the sexual steroids in the brain of the males compared to the same enzyme activity in the brain of the females which is only changed after a certain time following bilateral ovariectomy resulting in a drastic increase in gonadotropin levels. Modulation of the SOD and CAT activity in the CNS is extremely important considering that the presence of these two enzymes controls the level of the ROS, which, in turn, directly affect the permeability of the blood-brain barrier (Armstead *et al.* 1992). Pinto and Bartley (1969) have shown that the CAT activity is higher in the liver tissue of male animals than in the same tissue of female animals. Following a statistical analysis of the results we have shown that this conclusion applies to the brain tissue as well. Specific CAT activity is significantly increased in the brain of the male rats compared to that of the intact female rats regardless of the phase of the estrous cycle.

As is the case with CAT orchidectomy bears no effect on the GSH-Px activity. Treatments with P does not affect it either, while EB suppresses the activity 2 hours after the treatment, but this effect will not be maintained 24 hours later. Being that orchidectomy has no effect in this case either, unlike ovariectomy, which significantly increases the activity in the brain of female animals, it is probable that the GSH-Px activity is modulated directly by the changes in EB levels. This effect is short-lived. It can thus be concluded that the GSH-Px in the brain of female rats is more sensitive to the changes in gonadotropin levels compared to the same enzyme in the brain of male animals. Statistical comparisons of the specific GSH-Px activity in the brains of intact female and male rats have shown that the GSH-Px activity in the brain of the males was significantly increased compared to the same enzyme activity in the brain of the intact females regardless of the phase of the estrous cycle (Saičić *et al.* 1998, Pajović *et al.* 1999). Finley and Kincaid (1991) studied the dependence of the selenium content and plasma GSH-Px activity in plasma, erythrocytes and kidney cytosol on the sex of the animal. They have demonstrated that the selenium content and GSH-Px activity are increased in the plasma and kidney citosol, while being significantly decreased in the liver cytosol of the male animals compared to the same tissues in female animals.

Unlike the other enzymes of the AD system, GST activity is significantly increased following orchidectomy. Treatments with P have no influence, while EB only decreases GST activity 24 hours after the treatment. Therefore, unlike GSH-Px activity, which can only be modulated by modifying EB levels, GST activity in the brain of male animals can be modulated in two ways: by modifying EB levels and by modifying gonadotropin levels. This

is different than the GST activity in the brain of female animals which changes only after a bilateral ovariectomy, when the LH and FSH levels increase severely over the 2-3 weeks. Comparisons of the specific activities of cerebral GST in intact females and males show that this enzyme activity does not depend on the sex of the animal. Al-Turk *et al.* (1987) have shown that the content of GSH, GST and GR activities in the erythrocytes and lymphocytes of men and women do not depend on the sex. Our results also show that the GR activity in the brain of a male rat is not significantly different than the GR activity in the brain of an intact female rat. Orchidectomy does not influence the GR activity, while it takes 24 hours for EB to show a significant suppression of the said enzymatic activity. Therefore, GR activity in the brain of male rats, as well as the brain of female rats, is modulated directly by modifying steroid levels. However, it should be emphasized that the GR in the brain of female rats is sensitive to the changes in levels of both types of ovarian steroids, unlike the GR in the brain of male rats, which only changes depending on the EB levels. Also, the effect that EB has on GR activity in the brain of the females is clearly visible just 2 hours following the treatment, but it is short-lived and is not present 24 hours later. On the other hand, the effect that EB has on GR activity in the brain of the males is not as fast, and can only be clearly detected 24 hours after the treatment.

It has been said that the dependence of the enzymatic activity of the AD system in the brain of female rats on the level of ovarian hormones, P and E is probably based on the indirect effects of the ovarian steroids achieved through the changes in gonadotropin levels. On the other hand, enzymatic activity of the AD system in the brain of the male animals is also dependent, to a certain extent, on the ovarian hormone levels, although the direct modulation of enzymatic activity is more pronounced in males. This can be explained by the differences in gonadotropin secretion between the sexes. It is well known that the change of LH and FSH concentrations in the serum is an estrogen-dependent process in female and estrogen-independent process in male rats. This phenomenon was described by Saade *et al.* (1987) in their study of the effect of 17β-EB on the hypothalamic-pituitary system of intact males in which they noticed that LH response to luteinizing hormone releasing hormone (LHRH) was not modulated by E, not even 7 days after the treatment, as well as by Chen (1988) who demonstrated that all secretory cells of female rats containing LH secrete the LH under the influence of gonadotropin releasing hormone (GnRH) while a small number of pituitary cells in males secrete LH. Chen (1988) assumed that the increased number of pituitary cells secreting LH under the influence of GnRH in female rats,

compared to male rats, was a result of the E influence on the hypothalamic-pituitary system.

When the sensitivity of certain enzymes of the AD system to certain hormones is observed, the following conclusions become visible: a) in the brain of the females, only Mn SOD and GR show sensitivity to both hormones. Thus, in the case of Mn SOD, P and EB act in the same manner, suppressing the activity of the mitochondrial SOD. On the other hand, in the case of GR, as was previously explained, the effects of P and EB are different. GSH-Px activity is changed only by modulating the concentrations of P and b) in the brain of male animals, MnSOD and CAT activity changes depending on the levels of both hormones. Both P and EB act on the Mn SOD and CAT activity in the same manner-both decrease the activity of the mitochondrial SOD and increase the CAT activity in the brain. GSH-Px, GST and GR activities are suppressed by EB.

If the AD system of the female animals is examined to see in which direction each of the hormones modulates the activity of individual AD enzymes a certain logic in their relationships emerges. Being that Mn SOD activity decreases under the influence of P the production of $O_2^{\cdot-}$ radicals and H_2O_2 is increased explaining the increase of the GSH-Px activity (which removes excess H_2O_2) under the influence of P. As GSH-Px uses reduced GSH the induction of GR as a consequence of the treatment with P is also understandable. However, it is interesting that EB suppresses Mn SOD activity (thus increasing the concentration of $O_2^{\cdot-}$ radicals), but has no effect on any of the enzymes which remove the excess H_2O_2 (CAT, GSH-Px and GST), while it even reduces the GR activity, probably because of the decreased consumption of its substrate, GSH. Perhaps this effect of EB on the enzymes of the AD system is one of the causes of the development of tumors in the animals that have been chronically treated with EB (Crooke *et al.* 2006).

Within the AD system of a male, P decreases the Mn SOD activity just as it does in females, but at the same time increases the CAT activity (and not the activity of GSH-Px, as in females), which removes the excess H_2O_2. Progesterone does not influence other enzymes in the AD system in the brain of male animals. On the other hand, EB has the same effect on the Mn SOD and CAT activity as does P, but it also causes a significant suppression of the enzymes of the GSH redox cycle, which similarly to the situation in females increases the probability of the formation of excess H_2O_2.

The difference in effects of P and EB on the activity of the mitochondrial and cytosolic SOD that has been observed during this study has raised the question of the possible site of ovarian steroid action in the control of the

activity of the observed enzymes (Pajović *et al.* 1994a). We attempted to find the answer in an experiment on an isolated synaptosomal fraction as synaptosomes, isolated nerve endings are rich in mitochondria (Alnaes and Rahamimoff 1975, Pajović *et al.* 1994b) the site of the intensive oxidative processes (Turrens 2003). We assumed, as was later confirmed, that the inhibitory effect of the observed hormones on the SOD activity will be more pronounced in the synaptosomal fraction in comparison to the effect observed in the homogenate of the whole brain. In these experiments on the isolated synaptosomal fraction of the whole brain of a female rat we have demonstrated that the mentioned dependency applies not only to the Mn SOD but to the cytosolic SOD, CuZn SOD as well. During these experiments we found that both ovarian hormones suppress the Mn SOD activity, as well as that they show a significant synergistic effect on the said enzymatic activity. In the same way, EB modulates the activity of the cytosolic SOD, while P has no influence on the CuZn SOD activity if it is used on its own, but only if it is used on animals who have previously been treated with EB. This effect of the EB is analogue to the well know "priming" effect (Thornton *et al* 1986, Jones *et al.* 1987) and shows that this hormone is a regulatory factor in the P action in the case of AD enzyme activity in the CNS of female rats as well. Considering that our results show that the suppressing effect of the ovarian steroids in the purified synaptosomal fraction, rich in mitochondria, on both mitochondrial and cytosolic SOD is very pronounced it is reasonable to assume that the mitochondria is an important site of action, not only for glucocorticoids (Demonacos *et al.* 1993), but for ovarian steroids as well (Pajović *et al.*1994b, Duckles *et al.* 2006, Duckles and Krause 2007).

Conclusions

The data presented in this review suggest endogenous pathern of antioxidant defense enzyme expression which could be modulated by sexual steroid hormones. Under the normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and antioxidant defense system used by organisms to deactivate and protect themselves against free radical toxicity. The pro-oxidant/antioxidant balance and detoxification of potentially damaging ROS is crucial for cellular homeostasis. The modulation of antioxidant enzymes activities during and after hormonal treatment is related to the changes on the level of neuro- endocrine and oxidative status in the brain tissue.

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Abbreviations

AChE (acetylcholinesterase), AD (antioxidant defense), CAT (catalase), CNS (central nervous system), CuZn SOD (copper zinc containing superoxide dismutase), E (estrogen), EB (estradiol benzoate), FSH (foliclle stimulating hormone), G-6-PDH (glucose-6-phosphate dehydrogenase), GnRH (gonadotropin releasing hormone), GR (glutathione reductase), GSH (reduced glutathione), GSH-Px (glutathione peroxidase), GST (glutatahione-S-transferase), H_2O_2 (hydrogen peroxide), LH (luteinizing hormone), LHRH (luteinizing hormone releasing hormone), LP (lipid peroxide), MAO (monoamine oxidase), Mn SOD (manganese containing superoxide dismutase), mRNA (messenger ribonucleic acid), $O_2^{\cdot -}$ (superoxide anion radicals), P (progesterone), RNA (ribonucleic acid), ROS (reactive oxygen species), TH (tyrosine hydroxylase), t SOD (total superoxide dismutase)

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