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Mini review

Regulation of antioxidant enzyme activities in female rat brain by ovarian steroids

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Abstract

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 as well as the central nervous system (CNS). In our studies we have monitored the change of enzyme activity of the antioxidant (AO) system in the brain of female rats depending on the ovarian steroids. 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 AO system. The experimental results of our study indicate that the enzyme activity of the AO system in the brain tissue of female rats shows a certain dependence on the concentration of ovarian hormones, progesterone and estradiol, in the organism.

Study of the activity of the enzymes of the AO system in the brain of female 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.

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Bearing in mind these physiological roles of steroids, in relation to the sexual behavior 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 *(1)*. 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 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) *(2),* as well as tyrosine hydroxylase (TH) *(3)*. 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 and progesterone. In addition, there are some very interesting results that show that ovariectomy increases the TH activity 2–3 times and that estrogen and progesterone treatments have the opposite effect on the aforementioned enzyme activity in the hypothalamus. Sobočanes *et al.* (2003) investigated whether oxidant status and antioxidant enzyme

activities during ageing of mouse brain are regulated in sex-dependent manner. Throughout ageing, no difference in total superoxide dismutase (tSOD) 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 catalase (CAT) and to a lesser extent to glutathione peroxidase (GSH – Px) activity.

In our studies we have monitored the change of enzyme activity of the antioxidant (AO) system in the brain of female rats depending on the ovarian steroids *(5–7)*. 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 AO system. Study of the activity of the enzymes of the AO system in the brain of female 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 AO system in the brain tissue of female and rats shows a certain dependence on the concentration of ovarian hormones, progesterone and estradiol, in the organism *(8)*. 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 AO system in rat tissues *(9– 11)*.

Statistical analysis of the results shows that the activity of CuZnSOD, GST and GR remains stable during the estrous cycle, while the activity of the remaining three enzymes of the AO system – MnSOD (which removes superoxide anion radicals inside mitochondria), catalase and GSH-Px (which removes H_2O_2) changes depending on the status of ovarian hormones in the organism. During diestrus, catalase activity is increased, while during proestrus MnSOD activity increases but GSH-Px activity decreases. These changes can be understood bearing in mind that the concentration of superoxide anion radicals in proestrus is decreased *(12)* and that a certain concentration of H_2O_2 is necessary at this stage of the cycle, as it bears a certain physiological role *(13)*. 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 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 MnSOD and CuZnSOD gradually increase until the 15th day of the pregnancy, coinciding with the change in serum progesterone levels. The concentration of lipid peroxides 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 lipid peroxides play a prime role in the regulation of the luteal function during pregnancy.

In our studies, we have demonstrated that, compared to the enzyme activities inside the brain of intact female animals with normal cycles, bilateral ovariectomy was ineffective only regarding CuZnSOD activity. Prolonged absence of the ovaries leads to a significant increase of MnSOD, catalase, GSH-Px and GST activities, while the GR activity is decreased. The mid-day increase of MnSOD 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 LH and FSH is followed by a sudden release of these hormones into the bloodstream around noon and in the early afternoon during the proestrus *(15–17)*. 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 *(18–20)*. If the animals who have been subjected to ovariectomy are treated with exogenous progesterone, LH and FSH concentrations in the bloodstream decrease rapidly (24–48 hours) to the tonic levels *(21)*.

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 MnSOD 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 *(24)*. Regardless of its unexplained origin, the mere 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 MnSOD, but also on the activity of other antioxidative 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 MnSOD activity inside the ovary, while the CuZnSOD activity remains unchanged.

The question that arises next, whether the effects of progesterone and estradiol on the MnSOD 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 catalase activity, while hormonal treatments bear no effect on the activity of this enzyme. We can, therefore, assume that the effect of P and EB on catalase activity in the brain is achieved through an intermediary, i.e. through gonadotropin secretion. A significant increase in catalase activity during the estrous cycle has been observed in diestrus, which is understandable considering that catalase 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 *(24)*.

Unlike the catalase, 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 progesterone; 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 *(15)* and while the progesterone is at a lower level in comparison with the other phases of the estrous cycle *(17, 26)* seem to substantiate this hypothesis. On the other hand, in the case of selenium-independent GSH-Px, glutathione-S-transferase, 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 *(20)*. 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 EB 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 *(27, 28)*.

On the basis of these consideration, we showed that the important role of gonadal steroids in the modulation of prooxidant – antioxidant balance and alterations in antioxidant enzyme activities of central nervous tissue. Hormone-induced oxidative stress and modulations of antioxidant enzymatic defenses in the rat brain suggest that reactive oxygen species may play a role in hormone-induced pathogenesis *(29, 30)*.

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