

ACTIVITY OF MONOAMINE OXIDASES IN RAT FEMALE GENITAL ORGANS DURING PREIMPLANTATION PERIOD OF PREGNANCY

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ABSTRACT

Our objectives in the present study were to determine the activity of monoamine oxidases A and B (MAO AB) in rat ovary, oviduct and uterus during preimplantation period of pregnancy. It should help us to clarify and better understand possible involvement of both MAO enzymes in the reproductive process. Pregnant females were killed employing a lethal dose of thiopental on the first (D1), on the third (D3), and on the fifth (D5) days of pregnancy. Rats were perfused transcardially with the PBS to rinse out of the body as much blood as possible. Ovaries, oviducts and uteri were immediately removed and stored until the measurement was done. MAO activity was determined by fluorescent monoamine oxidase detection kit. In the ovaries we have found the highest MAO activity at D3, followed by D1, and the lowest levels were recorded at D5 of pregnancy. In the oviducts, the highest MAO activity was detected again at D3, followed by D5, and by D1 of pregnancy. But statistical analysis did not reveal any difference between individual days of pregnancy nor in the ovaries, neither in uterine tubes. Uteri were the only organs, in which statistically significant differences were detected ($p < 0.001$). The highest activity of MAO was observed at D5, followed by D1, and by D3 of pregnancy. Potential mechanisms responsible for the changed MAO activity in gonads during preimplantation period of pregnancy are proposed.

Key Words: MAO, ovary, oviduct, uterus

INTRODUCTION

Monoamine oxidases (MAO) are flavoprotein enzymes located in the mitochondrial outer membrane. The enzymes exist as two forms, MAO-A and MAO-B, which are different gene products and have different substrate specificities. MAO-A preferentially oxidizes noradrenaline (NA) and serotonin (5HT), while MAO-B has a greater affinity for phenylethylamine and benzylamine. Dopamine (DA) is a common substrate of both MAO-A and MAO-B. The two MAO isoforms can also be differentiated according to their inhibition by synthetic compounds. MAO-A is selectively inhibited by clorgyline whereas MAO-B is selectively inhibited by L-deprenyl (selegiline). MAO are involved in many behavioral processes and their inhibition has a marked effect on brain function, blood pressure regulation and the detoxification of potentially harmful exogenous amines [1].

In attempt to understand the role of MAO enzymes in brain, many studies have investigated their distribution and cellular localization [2]. Recently, an increasing number of claims have been made about involvement of these enzymes in many psychiatric and neurological diseases, such as depression, bulimia, schizophrenia, Parkinson's disease, Alzheimer's disease, neurodegenerative diseases in general, etc. Although the molecular base of these diseases are often complex, the fact that most of them have been linked with abnormal MAO activity provides a biochemical rationale for further pursuit of their investigation [3]. In contrast, little is known about distribution of MAO in peripheral tissues. The enzymes have been identified outside the central nervous system in organs such as liver, kidney and intestine [4]. Studies of the biochemical properties of MAO are

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numerous, but information about the quantification of MAO in female gonads during pregnancy is limited.

Recently we have observed an antagonistic effect of chronic treatment with deprenyl, a potent MAO-B inhibitor, at the dose 0.25 mg/kg on embryo development in rats. Significantly less degenerated embryos were isolated from experimental females, but on the other hand, a decreased mean cell number in blastocysts was recorded [5]. Our objectives in the present study were to determine the activity of MAO in rat ovary, oviduct and uterus during preimplantation period of pregnancy, which should help us to clarify and better understand possible involvement of both MAO enzymes in the reproductive process.

MATERIAL AND METHODS

Animals

All procedures performed with animals adhered to the permission granted by the Committee for Ethical Control of Animal Experiments at Safárik University and the permission of the State Veterinary and Food Administration of the Slovak Republic (permission No. 715/08-221b). All efforts were made to minimize both the number of animals and their suffering.

Experiments were carried out on 15 young, virgin female Wistar rats (200-240 g, 85-90 days old) obtained from the animal facility of the University. The animals were given free access to standard diet and water and were maintained in a 12 h light/12 h dark cycle. Females were mated for two hours from 07:00-09:00 a.m. with males of the same strain. The first day on which a vaginal plug was present was designated as day 1 of pregnancy. Pregnant rats were killed by a lethal dose of thiopental (40 mg/kg; ICN Czech Pharma, Prague, Czech Republic) on the first (D1), on the third (D3), and on the fifth (D5) day of pregnancy.

After a lethal injection of thiopental rats were perfused transcardially with 100 ml of room-temperature PBS (pH 7.4) to rinse out as much blood as possible. Ovaries, oviducts, and uteri were immediately removed and stored in Eppendorf tubes at -80 °C until the measurement was done.

MAO AB activity

Activity of MAO was measured by fluorescent monoamine oxidase detection kit (Bachem; Cat. No. S-90092) based on detection of H₂O₂ released from the conversion of a substrate to its aldehyde via both forms MAO A and MAO B. H₂O₂ oxidizes the detection reagent in a 1:1 stoichiometry to produce the fluorescent product. A standard curve was prepared from resorufin to determine moles of product produced.

Briefly, organs were homogenized in five volumes (w/v) of the 25mmol/l TRIS-HCL (pH 7.4) mixed with 1mmol/l EDTA and subsequently were centrifuged at 10,000g for 15 min at 4 °C. To a black 96 well plate 100 ml of samples and 100 ml of reaction cocktail were added into individual wells to incubate at room temperature for 30-60 minutes. Reaction cocktail was prepared obeying the manufacturer's instructions and consisted of the detection reagent, horse radish peroxidase and dimethyl sulfoxide (DMSO). Samples were read using excitation at 570 nm and fluorescence was measured at 590-600 nm employing the fluorescence plate reader. Activity of MAO expressed as the $\mu\text{mol/l}$ resorufin was normalized on the basis of total protein content ($\mu\text{mol/l}$ of resorufin/mg of protein). Chemicals used for assessing of enzymes activity were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Statistical analysis

Data are expressed as the mean $\pm$ SD. Differences in the MAO AB activity were analyzed by the Kruskal-Wallis test for multiple comparisons. $P < 0.05$ was considered as significant. The data presented here are pooled from two independent replications of the same experiment.

RESULTS

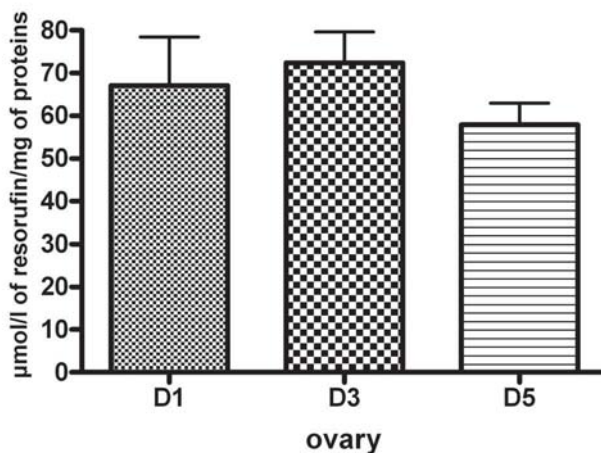


Fig. 1 Activity of MAO in the rat ovary on the first (D1), third (D3), and on the fifth (D5) day of pregnancy. Results are depicted as a $\mu\text{mol/l}$ of resorufin/mg of proteins $\pm$ S. D. ($p > 0.05$)

Concerning ovary (Fig. 1), we have found the highest MAO AB activity at D3 (72.44 ± 22.66), followed by D1 (67.10 ± 32.12) and the lowest activity was recorded at D5 (57.90 ± 17.60) of pregnancy. Kruskal-Wallis test did not reveal any significant difference compared individual days of pregnancy ($p > 0.05$).

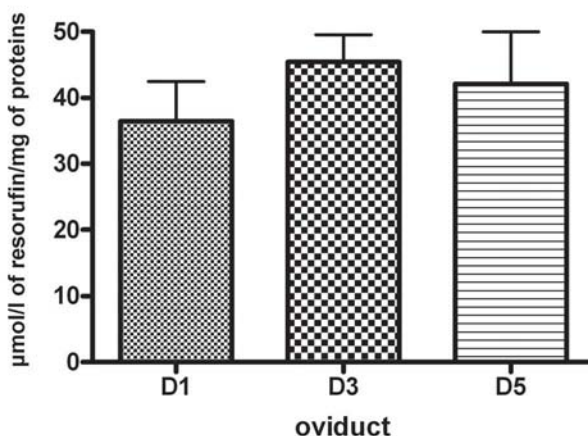


Fig. 2 Activity of MAO in the rat oviduct on the first (D1), third (D3), and on the fifth (D5) day of pregnancy. Results are depicted as the $\mu\text{mol/l}$ of resorufin/mg of proteins $\pm$ S. D. ($p > 0.05$)

In regard to oviduct (Fig. 2), the highest MAO AB activity was detected at D3 (45.44 ± 12.97), followed by D5 (42.09 ± 27.40) and D1 (36.49 ± 16.91) of pregnancy. Statistical analysis did not confirm any significant difference between individual days of pregnancy ($p > 0.05$).

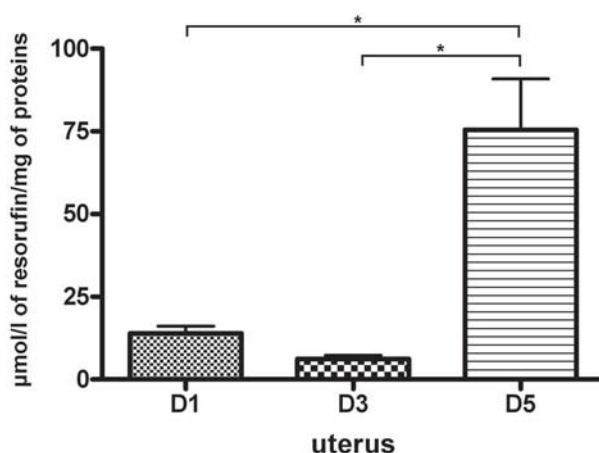


Fig. 3 Activity of MAO in the rat uterus on the first (D1), third (D3), and on the fifth (D5) day of pregnancy. Results are depicted as the $\mu\text{mol/l}$ of resorufin/mg of proteins $\pm$ S. D. (* it means $p < 0.001$)

Uterus (Fig. 3) was the only organ, in which statistically significant differences were detected ($p < 0.001$). The highest activity of MAO AB was observed at D5 (75.48 ± 53.24), followed by D1 (13.94 ± 6.09) and D3 (6.30 ± 3.19) of pregnancy.

DISCUSSION

We have found that monoamine oxidases activity undergo changes during early stages of pregnancy in all female reproductive organs examined, but their levels statistically differ only in the uterus.

Sympathetic nerve fibers are the largest source of NA in the ovary, but not the only one. Ovarian granulosa cells, after taking up catecholamines, can serve as an intra-ovarian catecholamine-storing compartment, releasing them in a regulated way [6]. High concentrations of both catecholamines NA and DA were found in the stroma of bovine ovaries and corpora lutea (CL), too [7]. NA given into the abdominal aorta affected the secretory function of the corpus luteum by stimulation of the luteal adrenoreceptors [8] in cattle and also in other species [9]. Tonic beta-adrenoreceptor stimulation of the CL ensures the basal secretion of progesterone, whereas acute noradrenergic activation supports the CL during stressful situations that could impair its function [10]. NA was also determined in the preovulatory follicular fluid of women undergoing *in vitro* fertilization. All of the follicular samples contained NA at concentrations substantially higher than those in the corresponding plasma samples. The data indicate that NA accumulates in follicular fluid, supporting the physiological significance of NA in the local regulation of human ovarian functions [11].

High levels of DA were described in the human ovary, raising question about its role in the female gonad [12]. It has been reported that monkey (*Macaca mulatta*) oocytes are able to take up DA and use it as a precursor for the synthesis of NA with the help of the dopamine- β -hydroxylase [13]. Bovine luteal cells are reported to perform synthesis of NA in a similar way [14], and presumably this occurs also in intraovarian nerve fibers, which likewise express dopamine- β -hydroxylase [15]. Recent study revealed dopamine 1,2,4,5 receptors expressed by endocrine cells of the follicle and the corpus luteum, which suggests a complex role of DA in the regulation of ovarian processes [16].

In the light of findings described above there is no surprise that the activity of MAO enzymes, which are responsible for the degradation of the catecholamines, was observed

in the CL of rat ovary employing enzymatic histochemical method [17]. In our work we found the highest MAO activity on the D3 of pregnancy, when corpora lutea are already formed. Similar results were recorded in women employing monoclonal antibodies. MAO-A was intensely expressed in CL of pregnancy, especially in large luteal cells [18]. MAO enzymes were also detected in the interstitial gland cells and in the blood vessels of rat ovary [17]. It has been recorded [19], that MAO activities in the vessels of ovarian pedicle of pigs were the highest on the 13-14 day of the estrous cycle. Authors suggest that high MAO activity in the vessels may be a significant factor in the regulation of the ovarian vasotone and might be responsible for increasing in the ovarian blood flow during the luteal phase of ovarian cycle. Interstitial glands are situated near to the blood vessels in the ovarian stroma, and arise from the follicles, which undergo atrophic process. Probably this is the reason why the further catecholamine accumulation is not needed and these substances are rather metabolized through MAO enzymes. Taken together, results suggest that MAO activity in the ovary might be involved in follicular development and progesterone metabolism.

High concentrations of NA have been detected in the human and cow oviductal compartments [20, 21]. The highest concentrations were found in the isthmus, where the adrenergic nerves are primarily related to smooth muscles [20]. Recently we have found that high doses of deprenyl (2.5 mg/kg), a potent MAO-B inhibitor, significantly slow down the movement of rat embryos through the female reproductive tract [22]. These findings need another investigation, because MAO-B and MAO-A are responsible for the DA degradation and DA was also detected in the human [20] and cow [21] oviduct compartments. Moreover, the addition of DA into the incubation bath significantly reduced the strength and frequency of spontaneous rhythmic contractions of the rat uterus [23]. NA was also identified in bovine oviductal fluid [24], and it could influence the oviduct epithelium via adrenergic receptors, which have been shown in the oviduct epithelial cells of several species [25, 26]. Recently, it was clearly demonstrated that mouse oocytes and embryos express α 2C- and β 2-adrenergic receptors, too [27]. As one could expect, MAO activity in the human oviduct has been detected on the same places, as their catecholamine substrates are located. It means in the epithelium and in the muscular layer [28]. Despite the fact that we have recorded the highest activity of monoamine oxidases on D3 and on D5, when embryo goes through the oviduct into the uterus, activity of MAO did not differ significantly compared the individual days of pregnancy. Probably, it could be the sign of similar metabolic MAO activity, as the oviduct almost permanently moves during the transport of spermatozoa in the time of fertilization and subsequently during the embryo passage down into the uterine cavity. Based on the works mentioned above, catecholamines play the pivotal role in this process.

Uterus is the only one reproductive organ, in which significantly different MAO activity during preimplantation period of pregnancy was recorded. In the time, when the oocytes and subsequently embryos are located in the oviduct, extremely low MAO activity in rat uterus was detected. However, on the D5, when embryos are present in the uterine cavity and their implantation into the uterine wall occurs, MAO activity significantly increased several times. Successful implantation depends both on the quality of the embryo and on the endometrial receptivity. The later depends on the progesterone-induced changes in gene expression. One of the genes whose transcription appears to be enhanced during the receptive period is probably gene for MAO. Similar results were obtained in women [29]. MAO-A transcript levels increased in human uterus between the pre-receptive and receptive phase with a median increase of 25-fold. Conversely, prior failure of embryo implantation was associated with a 29-fold decrease in MAO-A mRNA levels and a substantial reduction in MAO-A protein immunofluorescent label score. These results show a strong association between endometrial receptivity and MAO-A expression in the endometrial epithelium, suggesting an important role for this enzyme in normal implantation.

In the present study, we determined activity of monoamine oxidases in rat ovary, oviduct and uterus on the first, on the third and on the fifth day of pregnancy. We can conclude that significant elevation of MAO activity was recorded in the rat uterus in the time of embryonic implantation. The data obtained extend our knowledge about MAO enzymes in rat reproductive organs during early period of embryo development. To our knowledge this is the first paper describing the MAO activity in female reproductive organs during the whole preimplantation period of pregnancy in mammals.

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REFERENCES

1. Singer TP, Ramsay RR. Monoamine oxidases; old friends hold many surprises. *FASEB J* 1995; 9: 605-10.
2. Saura J., Bleuel Z., Ulrich J., Mendelowitsch A., Chen K., Shih J. C., Malherbe P., Da Prada M., Richards J. G. Molecular neuroanatomy of human monoamine oxidase A and B revealed by quantitative enzyme radioautography and in situ hybridization histochemistry. *Neuroscience* 1996; 70: 755-74.
3. Keung WM. Monoamine oxidase inhibitors. *Expert Opin. Ther Pat* 2002; 12: 1813-29.
4. Rodríguez M. J., Saura J., Billet E. E., Finch Ch. C., Mahy N. Cellular localization of monoamine oxidase A and B in human tissues outsider of the central nervous system. *Cell Tissue Res* 2001; 304: 215-20.
5. Mihalik J., Špakovská T., Prokopčáková L., Schmidtová K. Antagonistic effect of low deprenyl dose on the preimplantation embryo development in rat. *Bratislava Medical Journal* 2008a; 109: 151-154
6. Greiner M, Paredes A, Rey-Ares V, Saller S, Mayerhofer A, Lara HE. Catecholamine uptake, storage, and regulated release by ovarian granulosa cells. *Endocrinology* 2008; 149(10): 4988-96.
7. Denning-Kendall PA, Wild ML, Wathes DC. Regional differences in catecholamine concentrations in bovine ovaries analysed by high-performance liquid chromatography. *J Endocrinol* 1991; 129(2): 221-6.
8. Miszkziel G, Kotwica J. Mechanism of action of noradrenaline on secretion of progesterone and oxytocin by the bovine corpus luteum *in vitro*. *Acta Vet Hung* 2001; 49(1): 39-51.
9. Kotwica J. Role of the noradrenergic system in the secretory function of the corpus luteum. *J Physiol Pharmacol* 1992; 43(4 Suppl 1): 131-42.
10. Kotwica J, Bogacki M. Physiological importance of dopamine as a noradrenaline precursor in the corpus luteum. *Clin Exp Pharmacol Physiol Suppl* 1999; 26:S29-35.
11. Itoh MT, Ishizuka B, Kuribayashi Y, Abe Y, Sumi Y. Noradrenaline concentrations in human preovulatory follicular fluid exceed those in peripheral plasma. *Exp Clin Endocrinol Diabetes* 2000; 108(8): 506-9.
12. Bodis J, Bognar Z, Hartmann G, Török A, Csaba IF. Measurement of noradrenaline, dopamine and serotonin contents in follicular fluid of human Graafian follicles after superovulation treatment. *Gynecol Obstet Invest* 1992; 33: 165-7.
13. Mayerhofer A, Smith GD, Danilchik M, Levine JE, Wolf DP, Dissen GA, Ojeda SR. Oocytes are a source of catecholamines in the primate ovary: evidence for a cell-cell regulatory loop. *Proc Natl Acad Sci USA* 1998; 95: 10990-5.
14. Kotwica J, Bogacki M, Rekawiecki R. Neural regulation of the bovine corpus luteum. *Domest Anim Endocrinol* 2002; 23: 299-308.
15. D'Albora H, Anesetti G, Lombide P, Dees WL, Ojeda SR. Intrinsic neurons in the mammalian ovary. *Microsc Res Tech* 2002; 59: 484-9.
16. Rey-Ares V, Lazarov N, Berg D, Berg U, Kunz L, Mayerhofer A. Dopamine receptor repertoire of human granulosa cells. *Reprod Biol Endocrinol* 2007; 5: 40.
17. Yoshimoto Y, Sakumoto T, Arai R, Miyake A, Kimura H, Aono T, Tanizawa O, Maeda T. Monoamine oxidase in rat ovary during the estrous cycle. A histochemical study by a new coupled peroxidatic oxidation method. *Endocrinology* 1986; 119(4): 1800-4.
18. Takao Y, Fujiwara H, Yoshioka S, Fujii S, Ueda M. Monoamine oxidase A is highly expressed by the human corpus luteum of pregnancy. *Reproduction* 2008; 136(3): 367-75.
19. Dynarowicz I, Szumirski M. Monoamine oxidase activity in the uterine and mesenteric arteries, vessels of ovarian pedicle and myometrium of pigs during the oestrous cycle. *Arch Vet Pol* 1995; 35(1-2): 45-52.
20. Helm G, Owman CH, Rosengren E, Sjöberg NO. Regional and cyclic variations in catecholamine concentration of the human Fallopian tube. *Biol Reprod* 1982; 26: 553-8.
21. Kotwica G, Kurowicka B, Franczak A, Grzegorzewski W, Wrobel M, Mlynarczuk J, Kotwica J. The concentrations of catecholamines and oxytocin receptors in the oviduct and its contractile activity in cows during the estrous cycle. *Theriogenology* 2003; 60: 953-64.

22. Mihalik J, Kravčuková P, Špakovská T, Mareková M, Schmidtová K. Study of high deprenyl dose on the preimplantation embryo development and lymphocyte DNA in rat. *Gen Physiol Biophys* 2008; 27: 121-6.
23. Czerski A, Zawadzki W, Zawadzki M, Czerska Z. Influence of dopamine on rat uterine motility *in vitro*. *Acta Vet Brno* 2005; 74: 9-15.
24. Way AL, Barbato GF, Killian GJ. Identification of norepinephrine in bovine oviductal fluid by high performance liquid chromatography. *Life Sciences* 2001; 70: 567-76.
25. Tolszczuk M, Pelletier G. Autoradiographic localization of beta-adrenergic receptors in rat oviduct. *Mol Cell Endocrinol* 1988; 60: 95-9.
26. Einspanier R, Gabler C, Kettler A, Kloas W. Characterization and localization of B2-adrenergic receptors in the bovine oviduct: indication for progesterone-mediated expression. *Endocrinology* 1999; 140: 2679-84.
27. Čikoš Š, Veselá J, Ilková G, Rehák P, Czikková S, Koppel J. Expression of beta adrenergic receptors in mouse oocytes and preimplantation embryos. *Mol Reprod Dev* 2005; 71: 145-53.
28. Donnez J, Goenen E, Casanas-Roux F, Caprasse J, Ferin J, Thomas K. Monoamine oxidase reactivity in the human fallopian tube. *Fertil Steril* 1995; 43(3): 488-90.
29. Henriquez S, Tapia A, Quezada M, Vargas M, Cardenas H, Rios M, Salvatierra AM, Croxatto H, Orihuela P, Zegers-Hochschild F, Munroe DJ, Velasquez L. Deficient expression of monoamine oxidase A in the endometrium is associated with implantation failure in women participating as recipients in oocyte donation. *Mol Hum Reprod* 2006; 12(12): 749-54.

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