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THE INFLUENCE OF PMSG AND ANTISERGON ON MONOAMINE OXIDASE ACTIVITY IN SOME HYPOTHALAMIC-HYPOPHYSIARY STRUCTURES AND IN THE EPIPHYSIS OF SHEEP IN THE ESTRUS PERIOD

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ABSTRACT

Hormonal influence resulting from serum gonadotropin was investigated by radiochemical activity studies of the degradation enzyme of monoamine oxidase catecholamines (MAO) in certain regions of the hypothalamus (*area preoptica, corpus mamillare, infundibulum*), which participates in the regulation of sheep sexual functioning, as well as in *nucleus caudatus,* in the hypophysis and epiphysis. We attempted to eliminate the negative effect of PMSG by application of anti-PMSG serum (Antisergon) administered 24 and 58 hours after PMSG. The administration of 100 IU PMSG resulted in statistically significant ($p < 0.01$) increases in MAO activity (by 25.9%) in the *area preoptica* of the hypothalamus of sheep, in comparison to a control group with synchronized estrus. After the administration of Antisergon (after 58 hours) this increase was adjusted to the level of MAO activity of the control group. The most pronounced increase in MAO activity (by 82.8%) was recorded in *infundibulum*. After the administration of Anti-PMSG, 24 and 58 hours post PMSG, MAO activity in *infundibulum* did not differ from that of the control group. MAO activity in the hypophysis and epiphysis of sheep after administration of PMSG, showed a significant decrease ($p < 0.01$), persisting even after administration of Antisergon. Our results indicate that hormonal stimulation with serum gonadotropin causes an increase in MAO activity in the observed regions of sheep hypothalamus. The changes are less pronounced after administration of Antisergon. Hypophysis and epiphysis react to the hormonal preparation by a decrease in MAO activity which persists even after administration of anti-PMSG serum.

Key words: sheep; monoamine oxidase; superovulation; PMSG; brain

INTRODUCTION

Extrahypophysiary gonadotropins, used to induce superovulation in farm animals, affect steroidogenesis of the ovaries and influence hypothalamic nuclei and their gonadotropic receptors through feedback mechanisms. The application of the hormonal preparation PMSG is accompanied with hyperestrogenization. High concentrations of circulating estrogens act specifically upon the adrenergic receptors and affect the levels and metabolism of catecholamines in the central and peripheral adrenergic system (Fernandez-Pardal et al., 1986; Pástorová et al., 1992). The direct administration of estrogens to ovariectomized rats affects the turnover of norepinephrine in hypothalamic nuclei with a subsequent decrease in LH in blood plasma, despite an increase

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in activity of the synthesizing enzyme of monoamine thyrosine-β-hydroxylase and dopamine turnover in the hypothalamus (Deaver and Dailey, 1983). Concurrent with the changes in metabolism of catecholamines, some changes in activities of monoamine metabolism enzymes were recorded after hormonal induction (Yoskimoto et al., 1986).

Monoamine oxidase (MAO) is an enzyme which plays an important role in the degradation of catecholamines by oxidative deamination, and participates in the regulation of a functionally active pool of monoaminergic neurotransmitters in the nervous tissue. Some authors (Chevillard et al., 1981) reported that estrogens also modify the enzymatic activity of degradation enzymes of such catecholamines – as MAO and catechol-o-methyl-transferase (COMT) – in the hypothalamus and striate region. Mušicki et al. (1987) observed changes in the activity of monoamine oxidase and adenylate cyclase, together with a simultaneous increase in c-AMP after application of the hormonal preparations hCG and LH.

Our experiment focused on the study of the influence of hormonal preparation of serum gonadotropin upon activity of MAO-degradation enzymes of catecholamines in regulative organs of sheep reproduction (hypothalamus, hypophysis, epiphysis), as well as the striate region. We attempted to eliminate those negative effects of PMSG, connected with hyperestrogenization and their influence upon ovarian population, by means of administration of anti-PMSG serum (Antisergon) at two different time intervals.

MATERIAL AND METHODS

Twenty sheep of the Slovak Merino breed, 3-4 years old, mean live weight 44 kg, in estrus period (October-November) were used in the experiment. Synchronization of estrus in all sheep was achieved by polyurethane sponges containing 40 mg fluorogestonacetate (FGA, Chrono-gest, Intervet International B.V., Boxmeer, The Netherlands) being inserted for 12 days.

Sheep were divided into four groups (n=5). The first was the control group (C). Immediately after interruption of synchronization, PMSG (Bioveta, Ivanovice na Hané, Czech republic) was applied to all experimental animals at a dosage of 1000 IU. Sheep of the third and fourth groups received 3.3 ml of Antisergon (Bioveta, Ivanovice na Hané, Czech republic) 24 and 58 hours after application of PMSG, respectively.

Sheep were sacrificed by bleeding within a time interval of 104-120 hours after administration of PMSG. Brains were divided by a segmental analysis according to Welento et al. (1968). The experiment included determination of activity of the degradation enzyme of metabolism of monoamine oxidase (MAO) catecholamines in the *area preoptica, corpus mamillare, infundibulum, nucleus caudatus* of the hypothalamus, hypophysis and epiphysis. Immediately after removal, the tissues were submerged in liquid nitrogen in which they were stored frozen for further processing.

The radiochemical method of Wurtman and Axelrod (1963) was used to determine MAO activity. The ^{14}C-S-adenosyl-l methionine of specific activity $18.5.10^{-7}$ Bq.pmol^{-1} (Amersham, England) was used as a substrate specific for determination of MAO A and MAO B activities. The final concentration of 6.25 nmol substrate was used for the sample, and the activity of MAO was measured using a Packard TRICARB scintillation spectrometer on the ^{14}C channel. Proteins were determined in identical tissue homogenates according to Lowry et al. (1951). The results were expressed in pmol of the product (^{14}C-dihydroxyphenyl acetaldehyde) per mg of proteins per min. Statistical analysis of the results was carried out using the Student`s t-test.

RESULTS

PMSG resulted in a significant ($p < 0.01$) increase in MAO activity (by 25.9%) in the *area preoptica* region of sheep hypothalamus as compared to the control group with synchronized estrus. After the administration of PMSG in combination with Antisergon (24 h post PMSG), the increase in MAO activity persisted. A decrease in MAO activity to the level of the control group was observed after application of Antisergon 58 hours post PMSG.

The *corpus mamillare* region of the hypothalamus showed no changes either after the administration of PMSG alone, or in combination with Antisergon, applied at above mentioned intervals.

The most significant ($p < 0.001$) increase in MAO activity (by 82.8%) was recorded in *infundibulum* of superovulated sheep, this activity not differing from that of the control group after application of Antisergon (post 24 and 58 hours).

Unlike the hypothalamus, the hypophysis showed a decrease in MAO activity ($p < 0.05$), which did not return to the levels of the control group, even after administration of Antisergon thus remaining at a decreased level.

The administration of serum gonadotropin resulted in a similar increase in MAO activity in the epiphysis of sheep. After administration of 1000 IU of PMSG, a statistically important decrease ($p < 0.01$) in MAO activity was recorded in sheep in sheep epiphysis, as compared to the control group. This decrease persisted even after application of Antisergon, 24 and 58 hours post PMSG.

Table 1: Activity of MAO in the studied areas of the brain

Area	Control	Ageline 1000 IU PMSG	Ageline 1000 IU PMSG+Antisergon (24 h)	Ageline 1000 IU PMSG+Antisergon (84 h)
Area preoptica	280 ± 3.5	350 ± 3.5	330 ± 4.5	285 ± 2.5
Corpus mamillare	170 ± 1.7	150 ± 1.8	175 ± 2.2	153 ± 1,9
Infundibulum	138 ± 2.0	250 ± 2.8**	150 ± 1.8	130 ± 1.1
Hypophysis	95 ± 0.1	60 ± 0.9*	58 ± 1.2*	56 ± 0.7*
Epiphysis	310 ± 3.2	205 ± 3.0*	138 ± 1.5**	152 ± 1.4**
Nucleus caudatus	150 ± 1.55	230 ± 2.7*	120 ± 1.6	165 ± 1.6

* $P < 0.01$; ** $P < 0.001$
Results are expressed in pmol MAO.mg.protein^{-1}.min^{-1} as arithemetic means ± S.E.M.

DISCUSSION

Administration of hormonal preparation of serum gonadotropin, commonly used in controlled reproduction of farm animals, has some negative effects associated with hyperestrogenization, and uneven development of oocytes and follicular cells; however, such changes were not observed after administration of other superovulation preparations, such as Folistiman (Grave et al., 1988).

Anti-PMSG serum (Antisergon) suppresses extensive development of follicles, significantly increases the number of normal oocytes, and at the same time decreases estrogen levels in blood plasma (Moor et al., 1980). The species and inter-breed differences are significantly affected by the administration of PMSG, in combination with Antisergon (Kim et al., 1991; Sopková et al., 1999). Moyaert et al. (1985) revealed a marked increase in estrogens and progesterone in the peripheral blood, after application of serum gonadotropin. However, after application of anti-PMSG serum, the second wave of plasmatic estradiol was suppressed. Some authors (Yoskimoto et al., 1986) discovered that estradiol in the blood decreased to its basic level, 24 hours after administration of Antisergon, and remained at the control level during the subsequent days. Our previous report (Molnárová et al., 1991) showed that average estradiol values ($p < 0.02$) decrease 24-48 hours after administration of Antisergon to superovulated sheep.

MAO is an enzyme of the external membranes of mitochondria and the microsomal fraction. Intraneuronal MAO plays an important role in regulation of a functionally active pool of catecholamines and serotonin, and in the maintenance of their cytoplasmic concentration at the lowest neuron point (Chevillard et al., 1981).

Hormonal preparations used to induce superovulation in sheep markedly affect the concentration of hypothalamic catecholamines (Pástorová et al., 1989; 1991; 1992), serum gonadotropins having the most pronounced effect. The application of anti-PMSG serum (Antisergon) results in some adjustment of catecholamine levels in the hypothalamus of superovulated sheep, approaching the level of control values. However, the effect of Antisergon is not clear in all areas studied.

In our experiment a significant increase in MAO activity was recorded in the *area preoptica* ($p < 0.01$) and *infundibulum* ($p < 0.001$), and in the *nucleus caudatus* ($p < 0.01$) of the sheep brain, after the administration of 1000 IU of PMSG. Hypophysis and epiphysis of superovulated sheep exhibited a decrease in MAO activity as compared to the control values.

Administration of Antisergon 24 and 58 hours after PMSG resulted in a decrease in MAO activity in the preoptical region and *infundibulum* of the sheep hypothalamus, to the level of control values. It is likely that the neutralization effect of Antisergon manifested itself in the mentioned regions and, additionally, a decrease in estrogen levels in the peripheral circulation was observed. An additional decrease in monoamine oxidase activity was recorded in the epiphysis of sheep after application of Antisergon at both administration intervals.

Evaluation of the results of the influence of PMSG, in combination with Antisergon, on the catecholaminergic system of hypothalamic-hypophysiary structures and epiphysis, and MAO activity, indicates the suitability of administration of Antisergon which causes partial supression of the negative effects of PMSG on the controlling centers of sheep reproduction. The application of PMSG has the opposite effect on hypophysis and epiphysis, as on the hypothalamus, i.e. it causes a decrease in MAO activity. It is presumed that this is related to a marked increase in concentrations of dopamine and epinephrine observed after administration of serum gonadotropin in previous experiments (Pástorová et al., 1991).

REFERENCES

DEAVER, D. R. – DAILEY, N. 1993. Effect of ovarian secretion and dopamine secretion of luteinizing hormone and prolactin in ewes. *J. Anim. Sci.,* vol. 57, 1993, p. 978-984.

FERNANDEZ-PARDAL, J. – RIMERA, W. P. – GIMERA, A. L. 1986. Catecholamine in sow graafian follicles at proestrus and at diestrus. *Biol. Reprod.,* vol. 34, 1986, p. 439-445.

GRAVE, T. – BAK, A. – SCHMIDT, M. 1988. Superovulation of dairy cattle: Effect of PMSG-antiserum treatment. *Theriogenology*, vol. 29, 1988, p. 252.

CHEVILLARD, C. – BARDE, N. – SAAVEDRA, J. M. 1981. Estradiol treatment decreases type A and increases type B monoamine oxidase in specific brain areas and cerebellum of ovariactomized rats. *Brain Res.,* vol. 222, 1981, p. 177-181.

KIM, H. N. – RORIE, R. W. – YOUNGS, C. R. – WHITE, K. L. – GODKE, R. A. 1987. The use of anti-PMSG antibodies with PMSG for superovulating beef cattle. *Theriogenology*, vol. 27, 1987, p. 243.

MOLNÁROVÁ, M. – ARENDARČIK, J. – MESZAROSOVÁ, T. 1991. Antiproteolytic activities of blood plasma and follicular fluid of ewes after serum gonadotrophin (PMSG) stimulation and Antisergon administration. *Vet. Med. (Praha),* vol. 36, 1991, p. 497-507.

MOOR, R. M. – CAHILL, L. P. – STEWART, F. 1980. Ovarium stimulation of egg production as a limiting factor of egg transfer. In: *Abstract book from 9th Int. Congr. Anim. Reprod.* Madrid, 1980, p. 42-58.

MOYAERT, I. – BOUTERS, R. – SCHONHERR, O.T. – WILDERBLEK, A.T.M. – COERT, A. – CORYN, M. – VANDEPLASSCHE, M. 1985. The use of a monoclonal PMSG antibody in cows superovulated with PMSG. *Zuchthygiene,* vol. 20, 1985, p. 49-53.

MUŠICKY, B. – LONČAR-STEVANOVIČ, H. – OLOVERA, K. – IVANIŠEVIČ-MILOVANOVIČ, K. 1987. Concentration of circulating steroid hormones and catecholamines in the ovaries and uteri of rats with hypothalamic lesion. *Periodicum Biologorum,* vol. 89, 1987, p. 251-254.

PÁSTOROVÁ, B. – STANÍKOVÁ, A. 1989. Biochemical and histological changes in the hypothalamus of sheep after hormone stimulation. In: BOĎA, K. *Endocrinology of Farm Animals.* Košice : Institute of Physiology of Domestic Animals, 1989, p. 123-128.

PÁSTOROVÁ, B. –VÁRADY, J. – KOZÁČKOVÁ, M. 1991. Elevated catecholamine levels and monoaminooxidase activity in the hypophysis of ewes following hormonal stimulation with serum gonadotropin. *Homeostasis.,* vol. 33, 1991, p. 256-262.

PÁSTOROVÁ, B. – VÁRADY, J. 1992. Vplyv superovulácie na katecholamíny v krvnej plazme oviec v estrickom období. [The effect of superovulation on the plasma catecholamine levels in the sheep of estric period]. *Vet. Med. (Praha)*, vol. 37, 1992, p. 101-107.

SOPKOVÁ, D. – VÁRADY, J. – ELIÁŠ, V. – MAKOVÁ, Z. 1999. Stress and biocatalysis. *Physiol. Res.,* vol. 48 (3), 1999, p. 21.

WELENTO, J. S. – SZTEYN, S. – MILART, A. 1969. Observation on the stereotaxic configuration of the hypothalamus nuclei in sheep. *Anat. Anz.,* vol. 124, 1969, p. 1-30.

WURTMAN, J. – AXELROD, J. A. 1963. A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem. Pharmacol.,* vol. 12, 1963, p. 1434-1440.

YOSKIMOTO, Y. – SAKUMOTO, T. – RYCHACHI, A. – MIYAKE, A. – KIMURA, H. – AONO, T. – TANIZAWA, O. – MAEDA, T. 1986. Monoamine oxidase in rat ovary during the estrous cycle. A histochemical study. *Endocrinology,* vol. 119, 1986, p. 1800-1804.