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The Influence of Superovulation Preparations on the Levels of Catecholamines in Eminentia Mediana, the Pituitary and Pineal Gland of the Sheep

B. PÁSTOROVÁ

Department of Normal Physiology, University of Veterinary Medicine, Košice, Slovak Republic

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Summary

The influence of hormonal superovulation preparations of FSH (450 IU) or PMSG (1500 IU), on the levels of catecholamines (dopamine, norepinephrine and epinephrine) was studied in the oestrus period using radioenzymatic methods. The administration of FSH caused a significant increase in the concentrations of norepinephrine (NE) and epinephrine (EPI) in eminentia mediana (EM) of sheep ($p<0.001$ and $p<0.01$, respectively). The pituitary gland exhibited an increase in the level of norepinephrine after administration PMSG while no marked changes were recorded for epinephrine and dopamine (DA). The administration of FSH affected the increase in pituitary epinephrine ($p<0.01$). The hormonal stimulation by FSH resulted in a marked decrease of dopamine ($p<0.05$) as well as in , a significant increase of norepinephrine ($p<0.05$) and epinephrine ($p<0.05$) in the epiphysis. The comparison of the effect of hormonal preparations on the changes in catecholamine levels showed that the effect of FSH was observed mostly in eminentia mediana and the pituitary gland while that of PMSG was recorded in the epiphysis.

Key words

Catecholamines – FSH and PMSG superovulation – Eminentia mediana – Pineal gland – Pituitary gland – Sheep

Introduction

The hormonal preparations generally used for inducing superovulation in farm animals act on steroidogenesis and influence hypothalamic nuclei and their gonadotropic receptors through a feedback mechanism (Deaver and Dailey 1983). High concentrations of circulating oestrogens affect adrenergic receptors and the levels and metabolism of catecholamines in the central and peripheral adrenergic systems (Fernandez-Pardal *et al.* 1986, Pástorová and Várady 1996a,b). Simultaneously with the changes in the metabolism of catecholamines, some changes in the activity of catecholamine-degrading enzymes in the brain of sheep were observed after hormonal treatment (Chevillard *et al.* 1981, Pástorová and Várady 1996a).

With regard to the sporadic information about the effect of hormonal preparations that are commonly used in the biotechnology of controlled reproduction on the catecholaminergic system in the hypothalamus, pineal and pituitary gland of sheep, we studied changes in catecholamines in the regions which regulate the reproductive system of sheep after administration of FSH and PMSG.

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Fax +420 2 293 894
E-mail physres@sun1.biomed.cas.cz
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Materials and Methods

Examinations were carried out on brain samples from 18 Slovak Merino sheep, 2–3 years old, of average body weight 42±3.8 kg, in their oestric period (September – October). Sheep were fed standard melasse feed with vitamin additives twice daily. The oestrus of all sheep was synchronized with intravaginal polyurethane Ageline sponges (20 mg, Agelin Spofa, Prague). On day 13 after the instillation, the sponges were removed and FSH *ad usum vet.* (Folistiman, Spofa, Prague) was administered to the first experimental group (n=6) three times daily for 2 days in overall doses of 450 IU. After complete synchronization of the oestrus, the sheep of the second experimental group (n=6) were hormonally stimulated by the administration of 1500 IU PMSG (Bioveta, Ivanovice na Hané) and the remaining 6 sheep served as controls. The animals were slaughtered 104–110 hours after the first dose of FSH or PMSG.

The brains for radioenzymatic determination of catecholamines were rapidly removed, and samples were taken from the median eminence, pineal and pituitary gland. Tissues were immersed into liquid nitrogen and stored in the frozen state until further processing. Samples for radioenzymatic determination of catecholamines were homogenized in microhomogenizers in cooled $HClO_4$ (0.4 $mol.l^{-1}$) with an addition of 1 μl reduced glutathione (0.05 $ml.l^{-1}$) per milligram of tissue, and they were centrifuged at 15 000 x $g.min^{-1}$ at 0 °C for 30 min. Catecholamines were determined by the radioenzymatic method according to Johnson *et al.* (1980) in 50 μl of supernatants (in parallel samples). The radioactivity of catecholamine derivatives was measured on a scintillating spectrometer Packard TriCarb in a ^3H channel. The results were expressed as catecholamine nmol.mg $protein^{-1}$. Proteins were determined in identical tissue homogenate according to Lowry *et al.* (1951). Due to the higher concentrations of catecholamines in the brain, the tissue supernatants were diluted with redistilled water in the ratio 1:20. The coefficient of methodical variation calculated from 10 repetitions of one sample was 4.2 % for norepinephrine and 4.1 % for dopamine. The results were statistically evaluated by the non-paired t-test and are given as means ± S.E.M. in nmol.mg $protein^{-1}$.

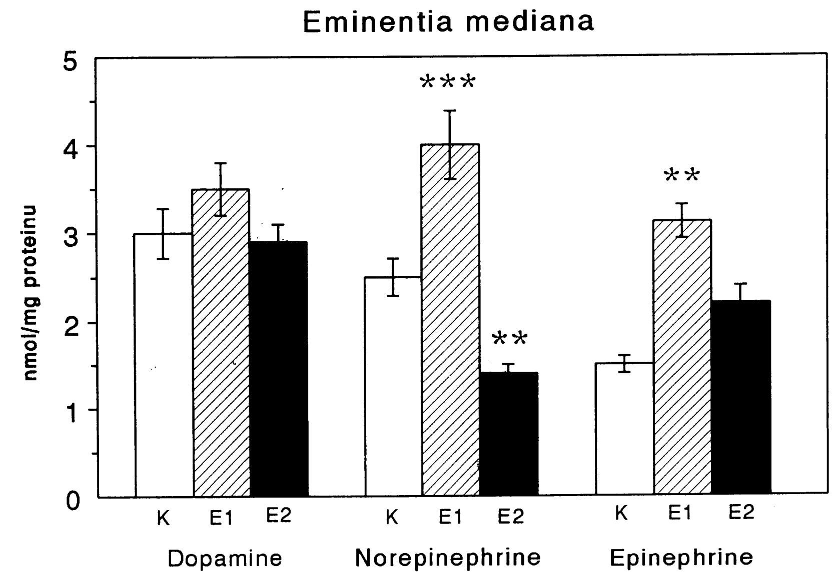

Fig. 1. *The effect of oestrus synchronization and hormonal stimulation by 450 IU FSH and 1500 IU PMSG on the dopamine, norepinephrine and epinephrine levels in the eminentia mediana. The results are expressed in nmol.mg protein*$^{-1}$ *(means ± S.E.M.). K = control group with synchronized oestrus (20 mg chlorsuperlutin), E1 = group with synchronized oestrus and stimulated by administration of FSH (450 IU), E2 = group with synchronized oestrus and stimulated by 1500 IU PMSG. Significant differences from controls:* * $p<0.05$; ** $p<0.01$.

Results

Results obtained are presented in Figs 1–3. The administration of 450 IU FSH and 1500 IU PMSG caused no difference in the level of dopamine in comparison with the controls (Fig. 1). The levels of norepinephrine in eminentia mediana (Fig. 1) after the administration of FSH were considerably increased ($p<0.001$). FSH stimulation resulted in a similar significant increase ($p<0.01$) in the level of epinephrine (Fig. 1). On the contrary, the administration of PMSG reduced the concentration of norepinephrine in eminentia mediana of sheep ($p<0.01$) while the levels of epinephrine were not altered in comparison with the control animals. The levels of dopamine in the pituitary gland of sheep (Fig. 2) were not affected by hormonal stimulation with FSH or PMSG. The norepinephrine concentrations recorded after the PMSG stimulation were enhanced ($p<0.05$). The administration of FSH significantly increased ($p<0.01$) epinephrine levels in the pituitary gland.

The response of the pineal gland to administration of superovulation preparations (Fig. 3) differed from that exhibited by the pituitary gland. A decrease in the level of dopamine (Fig. 3) and an increase in the concentration of norepinephrine ($p<0.01$) and epinephrine (Fig. 3) were recorded after the administration of 450 IU FSH. The administration of 1500 IU PMSG did not cause any appreciable changes in the levels of DA and EPI in the pineal gland but the concentrations of the NE were significantly decreased ($p<0.001$).

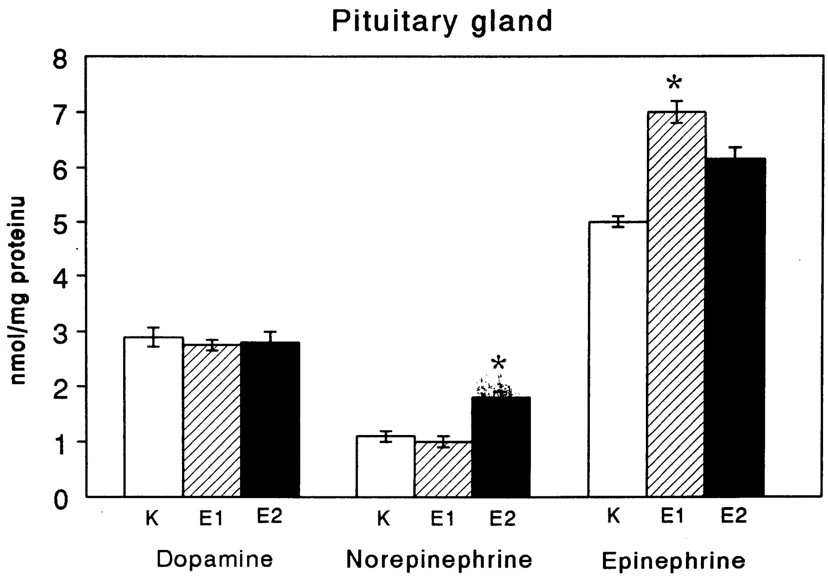

Fig. 2. *The effect of oestrus synchronization and hormonal stimulation (450 IU FSH; 1500 IU PMSG) on the catecholamine levels in the pituitary gland of sheep. For further details see text to Fig. 1.*

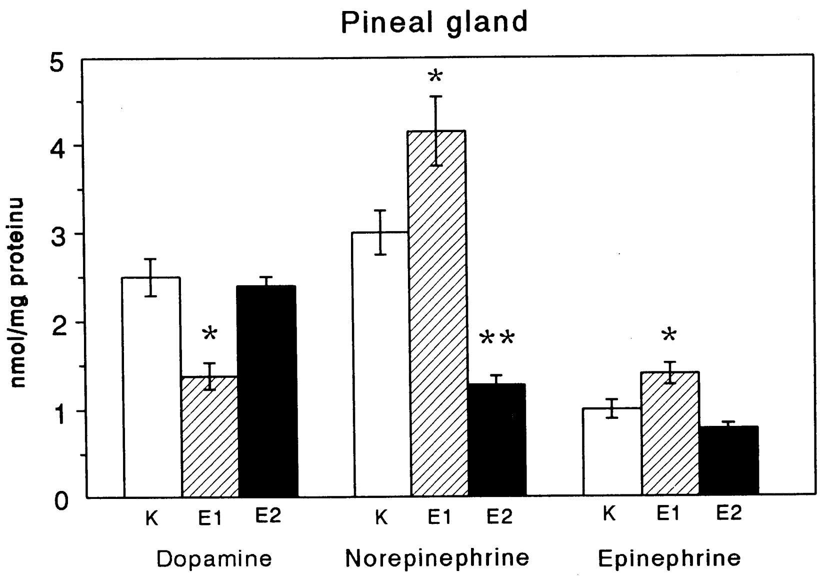

Fig. 3. *The effect of oestrus synchronization and hormonal stimulation (450 IU FSH and 1500 IU PMSG) on the catecholamine levels in the pineal gland of sheep. For further details see text to Fig. 1.*

Discussion

The administration of the pituitary hormone FSH is associated with luteolysis 48 hours after its administration, which is followed by the polyovulation oestrus (Schiewe *et al.* 1991). At present, most authors (Moor *et al.* 1985, Schiewe *et al.* 1991, Driancourt and Fry 1992) prefer FSH preparations to serum gonadotropins (PMSG) in biotechnically directed reproduction because FSH is a better regulator of the superovulatory process. This ability is based on the short half-life of FSH in the organism and the more stable gonadotropic effect of FSH (Moor *et al.* 1985, Schiewe *et al.* 1991), although it has to be administered several times daily. The extrahypophyseal hormone PMSG, which shows LH and FSH activity, has a long half-life in the organism and its administration is accompanied with a marked increase of oestrogens in the blood plasma. High concentrations of circulating oestrogens recorded after administration of superovulation preparations (Moor *et al.* 1985) act on adrenergic receptors and influence both the levels and metabolism of catecholamines in the central and peripheral adrenergic systems (Fernandez-Pardal *et al.* 1986, Pástorová and Várady 1996a,b).

Eminentia mediana (EM), which connects the hypothalamus with the pituitary gland, is a place where the nerve signals sent from the brain to the blood are integrated. Dopaminergic and noradrenergic nervous endings are located throughout eminentia mediana reaching higher concentration in the palisade zone and subependymal region of EM (Zoli *et al.* 1986). The studies of dopamine turnover in eminentia mediana indicate that the feedback effect of oestrogens on the secretion of LH takes place in the lateral palisade zone by means of dopaminergic nervous paths.

In our study we observed statistically different changes from the control in the levels of dopamine in eminentia mediana after the administration of FSH, although the concentrations of norepinephrine and epinephrine exhibited a significant increase ($p<0.001$ and $p<0.01$, respectively) in the above region. The high levels of oestrogens observed after the administration of hormonal preparations (Arita *et al.* 1987) increase the turnover of catecholamines, while progesterone inhibits the activity of tyrosine-β-hydroxylase, the limiting enzyme of biosynthesis of catecholamines (Arita and Kimura 1981, Rasmussen 1986). Supposedly, the increased level of oestrogens affects the increase in the levels of catecholamines, norepinephrine and epinephrine, in eminentia mediana of sheep by means of a feedback mechanism.

Anatomical and functional interactions between oestrogens and catecholamines are also described in the pituitary gland. Saavedra *et al.* (1975) observed changes in the balance of pituitary catecholamine levels and an increase dopamine turnover after application of 17-β-oestradiol to rats, particularly in pars intermedia of the pituitary gland.

No significant changes in the levels of dopamine were observed following the administration of PMSG and FSH in the pituitary gland of sheep. A significant increase ($p<0.01$) was observed in the levels of pituitary epinephrine. After administration of FSH the concentrations of norepinephrine remained unchanged after PMSG in comparison with the control ($p<0.01$).

Catecholamines participate in the regulation or modulation of melatonin synthesis in the pineal gland. The dominant neurotransmitter in the pineal gland is norepinephrine which acts on α- and β-receptors of pinealocytes and stimulates the activity of serotonine-N-acetyltransferase (NAT), the basic enzyme of melatonin synthesis (Charron *et al.* 1991). The administration of hormonal preparations carried out in our study resulted in a significant increase of norepinephrine and epinephrine ($p<0.01$), and a decrease of dopamine ($p<0.05$). The turnover and actual levels of catecholamines in the nervous tissue depend on more factors, such as the synthesis and degradation, storage and uptake, transneural flux and interactions with autoreceptors. Alterations in some of these factors with hyperoestrogenisation lead to changes in the concentration and function of catecholamines in the nervous tissue.

We thus propose that the given changes in the levels of catecholamines in the pituitary and pineal gland and eminentia mediana of sheep after the administration of PMSG or FSH are related to steroid alterations after hormonal stimulation.

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Reprint requests
B. Pástorová, Department of Physiology, University of Veterinary Medicine, Komenského 73, Košice, Slovak Republic.