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The Effect of Hormonal Superovulatory Preparation FSH on the Levels of Catecholamines in the Blood Plasma of Sheep

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Summary

The effect of hormonal stimulation with FSH injection in the doses of 18 mg (360 IU) and 24 mg (480 IU FSH) on the levels of plasma catecholamines (dopamine, norepinephrine and epinephrine) was studied by radioenzymatic methods during synchronized oestrous cycles of the sheep. Catecholamines were determined in the blood plasma before and 24, 48, 96 and 120 hours after application of FSH. It follows from the results that the levels of plasma dopamine increased significantly ($p<0.001$) 24 and 48 hours after FSH application. Furthermore, the levels of dopamine (DA) during the other time intervals observed, compared with those of controls before hormonal stimulation, remained at a higher level. A lower dose of the hormone (18 mg) had a more pronounced effect on changes in the levels of plasma dopamine. Norepinephrine (NE) did not exhibit any significant changes in comparison with the plasma levels of dopamine in the sheep after hormonal stimulation with FSH. A statistically significant increase in plasma norepinephrine was recorded 24 hours after administration of 18 or 24 mg FSH. During the other time intervals observed, its levels did not differ from the control values. Plasma epinephrine (E) showed a significant increase 24 and 48 hours after FSH application but not later. The effect of FSH on plasma catecholamine levels was not dose-dependent and their increase was pronounced especially in the period of ovulation.

Key words
Plasma catecholamine – FSH – Hormonal stimulation – Sheep

Introduction

The levels of catecholamines in the blood plasma reflect the activity of the adrenergic nerve system. Both plasma norepinephrine and dopamine are released from presynaptic endings of vascular neurones of the sympathetic system and from the adrenal medulla (Saavedra *et al.* 1984).

Miyake *et al.* (1987) found changes in plasma catecholamines and endorphines after i.m. administration of oestrogens. Rausch *et al.* (1989) described the changes in norepinephrine levels during ovulation in the blood plasma of women.

Changes of plasma catecholamines during regulated oestrous cycles and after induced superovulation in animals with gonadotropic hormones, occur sporadically in the available literature (Pástorová and Várady 1992). After the administration of pregnant mare serum gonadotropin (PMSG) to sheep a significant increase in the levels of plasma dopamine and norepinephrine was found during anticipated ovulation (Pástorová and Várady 1992) as well as significant changes in the levels of catecholamines and monoaminooxidase activity were observed in the central regions of the hypothalamus which regulate sexual functions in sheep (area preoptica, eminentia mediana) (Pástorová *et al.* 1994, 1995).

This work was aimed to study changes in the levels of catecholamines in the blood plasma at the time intervals 24–120 hours after administration of superovulatory FSH preparation in the luteal phase of the regulated sexual cycle of sheep.

Material and Methods

Twenty-five Slovak Merino female sheep with average body weight of 41±2.3 kg, aged 3–4 years, in oestrus (September – October) were used. Sheep were fed 2 times daily standard melasse feed with vitamin additives. Oestrus in sheep (n=20) was synchronized by the application of intravaginal polyurethane sponges containing 20 mg of chlorsuperlutine (Agelin Spofa, Prague). After 13 days, the sponges were removed and FSH inj. *ad usum vet.* (Léčiva Prague) was administered i.m. to sheep 3 times daily for 2 days in overall doses of 18 and 24 mg. The animals were killed at the time intervals of 120–140 hours after the first dose of FSH.

The concentrations of catecholamines were determined in the blood plasma of 25 sheep after synchronization of the oestrus in two experimental groups (n=10) before i.m. administration (0) of FSH (18 and 24 mg) and 24, 48, 72, 96 and 120 hours after administration of the first dose of FSH. The control group consisted of sheep (n=5) which had received a physiological solution instead of FSH. Catecholamine levels in the control group were determined at the same time intervals as in the experimental animals. Blood samples were always withdrawn from the jugular vein catheter between 0800 and 0900 h.

Glutathione (0.05 mol.l^{-1}) in the amount of 20 μl per 1 ml of blood was used as an antioxidative and anticoagulant agent. Blood was centrifuged at 2000 x *g* at 4 °C for 20 min. The clear, unhaemolysed plasma was stored for short time period at −70 °C until processed.

Catecholamines were determined in duplicate samples by the radioenzymatic method according to Johnson *et al.* (1980) using 50 μl of plasma. The radioactivity of catecholamine derivatives was measured on a scintillating spectrometer Packard Tri Carb in a ^{3}H canal. The sensitivity of the method in 50 μl sample is from 0.37–4.00 pmol.ml^{-1} for NE and E and from 0.53–4.00 pmol.ml^{-1} for dopamine. A coefficient of variation for DA is 4.1 % and 4.2 % for NE and E. The levels of catecholamine in the blood plasma are expressed as means ± S.E.M. and statistically evaluated by the unpaired t-test.

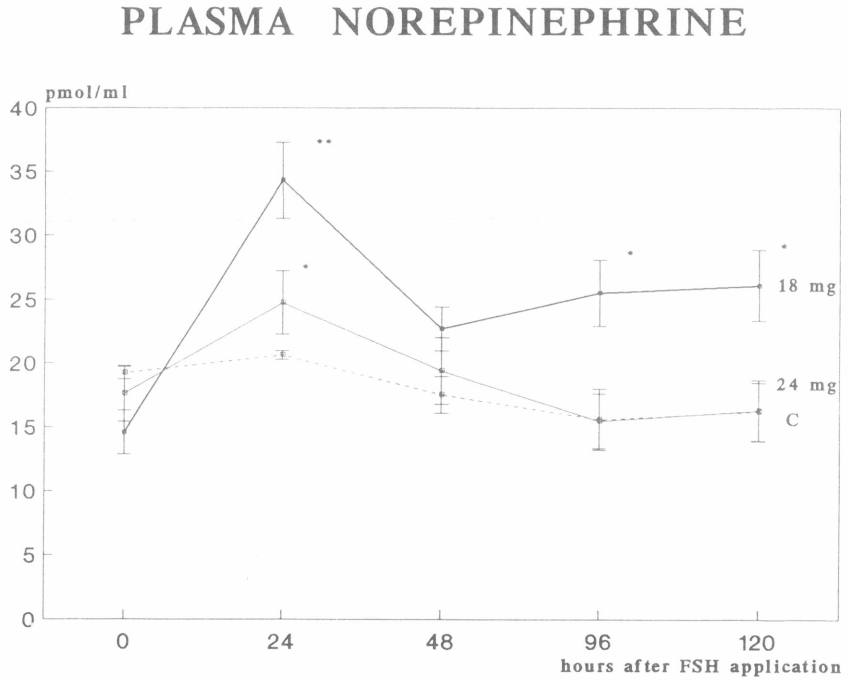

Fig. 1
*The effects of oestrus synchronization and FSH hormonal stimulation (18 and 24 mg FSH) on the levels of plasma norepinephrine in control samples (group 0) and 24, 48, 96 and 120 hours after hormone administration. The results are given in pmol.ml^{-1} of blood plasma, (n=10). Significant differences: **p<0.001; *p<0.01. Full line – 18 mg FSH; broken line – 24 mg FSH; dotted line – control group.*

Results

Plasma catecholamine levels did not markedly change during 0–120 hours in the control vehicle-treated group (Figs 1–3). Concentration of norepinephrine in the control blood plasma (Fig. 1) before the onset hormonal stimulation with FSH (sampling 0) of 14.58±1.70 pmol.ml^{-1}. Twenty-four hours after the administration of 18 mg FSH (p<0.001) plasma norepinephrine significantly increased to 34.30±2.99 pmol.ml^{-1} (by 29 %). After 96 and 120 hours of gonadotropin administration, the levels of norepinephrine remained to be significantly increased (p<0.01) in comparison with those before hormonal stimulation. Administration of 24 mg of FSH had a similar effect as 18 mg FSH at the 24 h time interval, when a significant increase in plasma NE was observed (p<0.01). Ninety-six hours after administration of 24 mg FSH an insignificant decrease in NE levels was recorded in the blood plasma of sheep. This decrease also persisted after 120 hours.

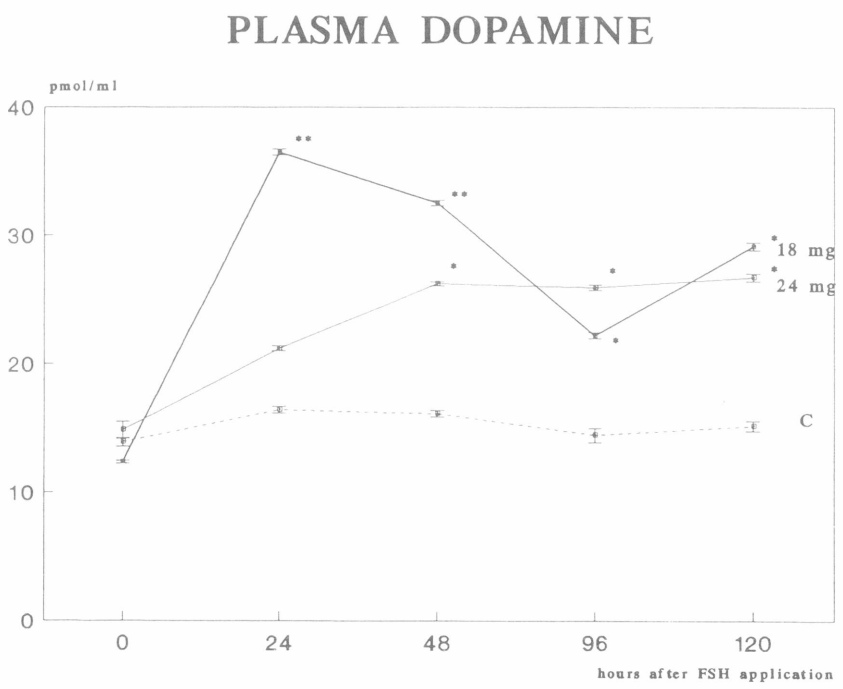

Fig. 2
The effects of oestrus synchronization and FSH hormonal stimulation (18 and 24 mg FSH) on the levels of plasma dopamine in control samples (group 0) and 24, 48, 96 and 120 hours after hormone administration (n = 10). For other details see Fig. 1.

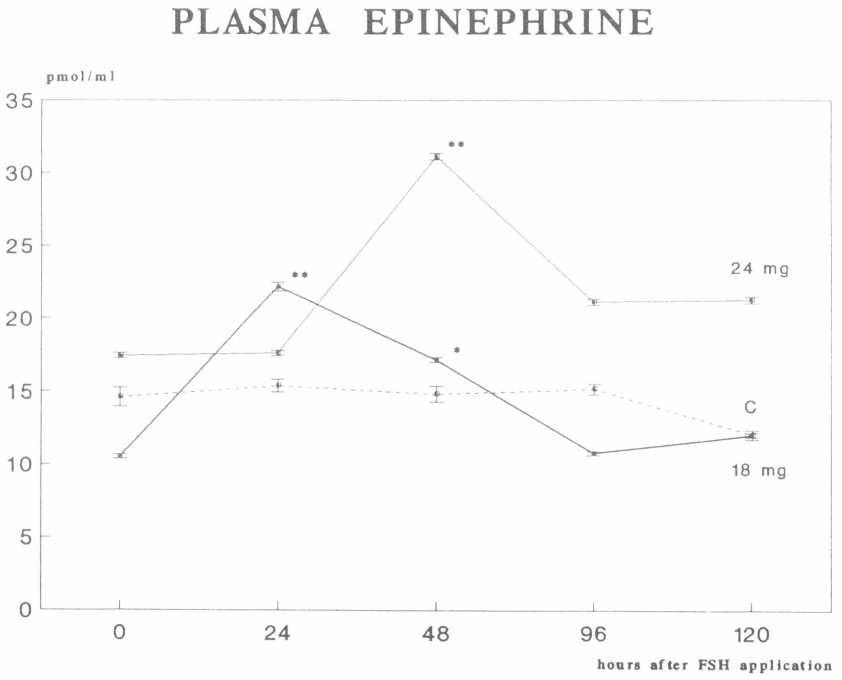

Fig. 3
The effects of oestrus synchronization and FSH hormonal stimulation (18 and 24 mg FSH) on the levels of plasma epinephrine in control samples (group 0) and 24, 48, 96 and 120 hours after hormone administration (n = 10). For other details see Fig. 1.

Dopamine (Fig. 2) showed a marked increase ($p < 0.001$) 24 and 48 hours after administration of FSH (by 196 and 164 %, respectively) compared with the control values. Ninety-six and 120 hours after the onset of ovulation, the levels remained significantly increased ($p < 0.01$; by 75 and 78 %, respectively) in comparison with sampling 0. Twenty-four and 48 hours after administration of 24 mg FSH there was an increase in plasma dopamine levels by 40 and 73 %, respectively. This significant increase in plasma dopamine levels ($p < 0.01$) also persisted during the next few days of the experiment, similarly as after administration of 18 mg FSH (Fig. 2). Plasma epinephrine (Fig. 3) followed a different time course. Twenty-four hours after administration of 18 mg FSH, an increase in its levels ($p < 0.001$) was observed which partially persisted at 48 h ($p < 0.01$) and returned to control value at subsequent time intervals. Twenty-four and 48 hours after administration of 24 mg FSH an increase in epinephrine levels was observed ($p < 0.001$ and $p < 0.01$, respectively). At other time intervals studied, the epinephrine levels did not differ from the control values.

Discussion

Catecholamines present in the blood participate in the regulation of various physiological mechanisms and reflect temporary (physical and psychical stress, drug action), or persistent changes (hypertension, nervous diseases) in activity of the peripheral adrenergic nerve system (Tobias *et al.* 1983, Rausch *et al.* 1989).

The pituitary hormone FSH induced luteolysis 48 hours after its administration and this was followed by polyovulatory oestrus. At present, most authors (Driancourt and Fry 1992, Donnelly and Dailey 1991) prefer FSH preparations to serum gonadotropins (PMSG) in biotechnically directed reproduction. These preparations are expected to exert better regulation of the superovulatory process. This regulatory ability is due to the short half-life of FSH in the organism, and a more stable gonadotropic effect of FSH (Moor *et al.* 1985) although it has to be applied several times daily.

The hormonal preparations (PMSG) used for inducing superovulation in sheep influence the levels of catecholamines and the activity of monoamine oxidase in the hypothalamus and hypophysis (Pástorová *et al.* 1994). It has been found (Yoshimoto *et al.* 1986, Arbogast *et al.* 1987) that pronounced direct changes of these parameters occur in the sexual apparatus of various animals species. Some authors (Mušicki *et al.* 1987, Nehr 1991) have reported that, in ovariectomized HCG and LH treated rats, changes occur in the activity of monoamine oxidase and catecholamine levels in the ovaries and uterus, and that the levels of c-AMP increase. They correlated these changes with an increase in steroidogenesis after hormonal stimulation.

Schiewe *et al.* (1991) found an 8–10 fold increase in 17-oestradiol with the peak at 24–36 h after FSH administration. We observed the same in our previous experiments (Arendarčík *et al.* 1990). Furthermore, the FSH levels were increased in the blood serum with a peak at 26.1 ± 1.2 h after administration of a follicle-stimulating hormone pituitary extract (Schiewe *et al.* 1991). The high levels of oestrogens act on adrenergic receptors and influence both functions and levels of catecholamines in tissues and plasma (Tobias *et al.* 1983, Miyake *et al.* 1987, Havern *et al.* 1991).

The administration of FSH had a more pronounced effect on plasma dopamine which showed a significant increase ($p<0.001$) 24 and 48 hours after hormone administration. At the next time intervals studied (96 and 120 hours), its concentrations remained increased. Norepinephrine showed a significant increase 24 hours after administration of the hormone. At subsequent intervals, its levels did not differ from those before hormonal stimulation. An increase in the levels of plasma dopamine and norepinephrine after administration of the extrapituitary hormone PMSG was found in our previous report (Pástorová and Várady 1992). An increase in the catecholamine levels was significant 48 hours after administration of serum gonadotropin during ovulation.

The increase in dopamine and norepinephrine observed in the plasma of sheep 24 and 48 hours after administration of FSH is considered to be related to the increase of oestrogens in the plasma at the given time (Schiewe *et al.* 1991, Arendarčík *et al.* 1990). This suggestion is supported by findings of Miyake *et al.* (1987), who recorded changes in the levels of catecholamines in the plasma of women after direct administration of oestrogens. At the same time they observed a decrease in plasma LH and FSH levels with a subsequent pronounced increase in LH levels. In our experiment a superovulatory response to FSH was 3.28 ± 0.21 versus 1.20 ± 0.23 in sheep with non-stimulated synchronized control ovulations. The administration of 18 mg FSH induced a significant increase in the ovary volume ($p<0.01$) and the number of prominent follicles ($p<0.001$). The effect of 24 mg FSH on the ovarian apparatus of sheep was less marked (Eliáš *et al.* 1992). The effect of FSH on plasma catecholamine levels was not dose dependent and their increase was pronounced especially in the period of ovulation.

We suggest that our findings could contribute to a better understanding of the effect of superovulatory preparations on the peripheral adrenergic system affecting the levels of plasma catecholamines.

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Reprint Requests

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