4 - 70 EFFECTS OF ESTRADIOL AND PROGESTERONE ON THE ACTIVITIES OF TYROSINE AMINOTRANSFERASE AND ORNITHINE DECARBOXYLASE AND THE LEVELS OF PROTEIN IN THE LIVER AND MUSCLES OF RUMINANTS

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Ornithine decarboxylase activity is enhanced in the liver after treatment with somatotropic hormone (4). Enhanced ornithine decarboxylase (OD) activity can be related to inducing growth by an enhanced synthesis of putrescine, a product of ornithine decarboxylation and of the spermidine formed from putrescine by the addition of propylamine from the decarboxylation of S-adenosyl methionine (7). The application of insulin, cortisole, glucagon and thyroxin in adrenalectomized mice stimulates OD activity (6, 7). Mallette and Exton (4) demonstrated in vitro insulin to enhance ornithine decarboxylase activity by acting directly in the liver with or without the presence of amino acids in the perfusion medium.
Estrogenic hormones are pronounced anabolitics in ruminants. The objective of the present study was to test the possible direct action of estradiol and progesterone on the enzymatic activity of ornithine decarboxylase and tyrosine aminotransferase and on the quantitative changes of protein in liver and muscles.

Material and Methods

The investigations were made using 2 year old castrated ewes. The liver was perfused in situ by a recycling medium of 3000 ml by the method described by Mortimore (2,3). The medium consisted of heparinized sheep blood diluted 1:1 with Krebs' bicarbonate buffer and containing, per 100 ml, 0,67 g of hydrolysed caseine (Sigma), 250 mg of glucose, 50 000 IU penicilline, and 107 mg of streptomycine sulfate. The hormones were added after 10 min from the beginning in concentrations of 100 and 300 µg/ml for estradiol, and 10 and 20 µg/ml for progesterone. Before the start, a piece of liver was removed for a control analysis from L. intermedius hepati, at 1 hour of infusion, from l. dexter hepati, and at 3 hours, from l. sinister hepati, after which the animals were killed. The pieces of liver were put immediately into an ice-cold buffer of 0,05 M sodium phosphate, and disodium EDTA, 0,001 M, of a pH of 7,2. The remaining liver tissue was homogenized in 4 volumes of phosphate-EDTA buffer in a glass homogenizer. The homogenate was centrifuged at 10 000 x g for 20 min at 0,5°C and the supernatant was taken for enzymatic analyses.
Ornithine decarboxylase was determined by the method of Russell and Synder modified by Mallette and Exton (5). Its activity was expressed in cp in the CO_2 released per minute by the enzyme present in 1 g of fresh liver tissue. Tyrosine aminotransferase (TAT) was determined by Rossen's method modified by Mallette and Exton (4). Its activity was expressed in mmol p-hydroxy-phenyl-pyruvate produced by 1 g of fresh liver tissue/hour. Protein in liver and the M. long. dorsi muscle was determined after Kjeldal.
The effects of estradiol and progesterone on the activities of ornithine decarboxylase and tyrosine aminotransferase and the levels of liver and muscle protein were tested in an experiment with 3 groups each of 6 castrated ewes: group I, control; group II, experimental, treated with 50 mg of progesterone; and group III, experimental, treated with 60 mg of estradiol after a preliminary premedication for 5 days using 20 mg of progesterone per day. On day 2 of the treatment, pieces of liver were removed by laparotomy, and of the M. long. dorsi muscle, by biopsy, and they were tested by the methods described above. The results were processed by Barov's method of variation statistics.

Results and Discussion

It is obvious from the results shown in Table 1, that estradiol tested in its higher dose (300 µg/ml) caused a statistically demonstrated increase in TAT and OD registered already at one hour after the infusion ($p<0,01$). The lower estradiol dose resulted also in an increase in the activities of the two enzymes which was statistically demonstrated only at 3 hours from the beginning of infusion.
The lower progesterone dose (10 µg/ml) did not cause demonstrated changes in TAT and OD activities. The higher dose (20 µg/ml) stimulated reliably TAT activity throughout the whole investigation period, while in OD, a statistically non-demonstrated trend towards increase was observed.
The results shown in Table 2 on the effects of estradiol and progesterone on TAT and OD activities in the organisms of ewes after a single administration, point to a dy-

Table 1. Changes in the activities of ornithine decarboxylase and tyrosine aminotransferase in perfused ewe liver

Hormones	N	Tyrosine aminotransferase mmol/g dry matter^{-1} x h^{-1}			Ornithine decarboxylase mmol/g dry matter^{-1} x h^{-1}		
		Control	After 1 h	After 3 h	Control	After 1 h	After 3 h
Estradiol 100 μg/ml	8	41±1,3	44±1,5	49±1,2	44±1,8	46±1,5	78±1,4
300 μg/ml	8	42±1,8	54±1,3	89±2,1	84±1,6	98±2,1	120±3,2
Progesterone 10 mg/ml	6	40,2±1,1	41±2,1	44±1,2	46±1,4	48±2,1	49±1,4
20 mg/ml	6	42±1,3	58±1,8	71±2,1	42±1,5	46±1,8	46±1,4

Table 2. Changes in the activities of tyrosine aminotransferase and ornithine decarboxylase and in the level of liver and muscle protein after a single treatment with progesterone and estradiol in ewes

Groups, hormone and dose	N	Tyrosine aminotransferase, mmol/g dry matter^{-1} x h^{-1}		Ornithine decarboxylase, mmol/g dry matter x h^{-1}		Liver protein, g%		Muscle protein, g%	
		Control	After 2 days	Control	After 2 days	Control	After 2 days	Control	After 2 days
Group I, control	6	52,3±1,2	57,5±1,2	58,3±1,2	52,1±1,1	17,3±0,6	16,1±0,4	17,8±0,4	15,3±0,6
Group II, experimental 50 mg progesterone	6	53,3±1,1	56,5±1,3	57,2±1,0	54,3±1,2	17,1±0,5	16,9±0,5	16,9±0,5	17,2±0,4
Group III, experimental, 50 mg estradiol	6	51,1±1,0	65,6±1,2	57,5±1,1	92,6±1,6	16,9±0,4	19,5±0,3	17,1±0,4	18,2±0,5

namics of estradiol and progesterone-induced changes in the enzymes close to the one found on testing them in situ.

Surgical intervention causes a demonstrated increase in TAT activity and reduction in OD sctivity with a non-demonstrated trend towards a reduction in liver and muscle protein levels, which changes can be explained by the stress reaction of the organism (Krustev, 1). On this background, it is impossible to differentiate the single treatment with progesterone tested in the dose of 50 mg in which nearly the same reaction is found as in the control group. The situation is the cpposite after a single treatment with estradiol in the dose of 60 mg. In this case, a reliable increase is registered in TAT and OD activity and in liver and muscle protein level ($p<0,05$).

The results described demonstrate ornithine decarboxylase activity to increase during treatment with estradiol after a preliminary premedication with progesterone. This increase is accompanied by an activation of tyrosine aminotransferase and a rise in the level of protein in liver and muscles. This reveals part of the intimate mechanism of the anabolitic action of estrogenic hormones in ruminants found by Krustev (1) and some others.

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