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TO THE MECHANISM OF THE REGULATORY ACTION OF ECDISTEN AND NEROBOL ON THE PROCESSES OF PROTEIN SYNTHESIS IN THE BODY OF HIGHER ANIMALS

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Annotation. A comparative study of the regulatory effect of the preparation of ecdisten (created based on the phytoecdysteroid ecdysterone) and nerobol (a synthetic anabolic preparation) on protein biosynthesis was carried out in mammals (mice) in in vivo and in vitro experiments. We have defined that the activation of protein-synthesizing processes by ecdisten in the organism of higher animals is not associated with its effect on the synthesis of mRNA, but is only a reflection of the acceleration of translational processes. The corresponding action of Nerobol is directed primarily at transcription processes with subsequent generalized stimulation of the synthesis of protein macromolecules at the cytoplasmic level due to an increase in the number and activity of polyribosomes.
Keywords: phytoecdysteroids, ecdisten, nerobol, higher animals, protein biosynthesis.

Among the most active agents, currently used to stimulate protein-synthesizing processes in the body are steroid anabolic preparations (methyandrostenediol, retabolil, phenobolin, etc.) [11]. Along with this, in a number of cases, to enhance protein-anabolic processes began to be used, although less active, but nevertheless, quite effective ecdysteroid-containing agents (ecdisten, ecsumid, serpisten, etc.) [13,16,17,18,23]. While the former are synthetic derivatives of the male sex hormone testosterone, the latter are based on phytoecdysteroids that are widespread in nature. The fact of the creation based on phytoecdysteroids of medicines and biologically active food supplements used in clinical and sports – medical practice, it seems at least surprising. For a long time it was believed that for most phytoecdysteroids, which are close or identical in structure to zooecdysteroids - hormones of moulting and metamorphosis of insects [24]. Only the effect on protein-synthesizing processes in their body is characteristic and is associated primarily with the stimulation of the synthesis of certain protein-enzymes at the level transcription, contributing to the sclerotization of the cuticle of the larvae in the process of transformation into pupae [15,6,21].

Higher animals are far from insects in evolutionary terms. They are not capable of endogenous production of ecdysteroids and their metabolic processes (including protein

synthesizing ones) are largely subject only to the regulatory influence of their own highly specialized hormonal system. Therefore, the mechanism of the regulatory effect of phytoecdysteroids on the protein synthesis system in mammals, which ultimately leads to a general anabolic effect, it is not fully understood.

The works carried out in this regard are largely debatable and do not bring complete clarity to the issue under consideration [7,8,23]. The aim of this research was to define in the most general terms the principles of the regulatory effect on protein biosynthesis in the body of higher animals of ecdysteroid-containing preparations (for example, ecdisten) in comparison with steroid anabolic preparations (for example, nerobol).

Materials and methods:

The effects of ecdisten and nerobol were analyzed on the processes of protein biosynthesis in the body of higher animals, leading to a general anabolic effect by changes in the activity of the protein synthesizing system of liver cells in mice (males, 18–20 g). When conducting experiments in vivo, ecdisten, created based on a natural ecdysteroid – ecdysterone (20-hydroxyecdysone), isolated from *Rhaponticum carthamoides* (Willd.) Iljin and *Ajuga turkestanica* (Rgl.) Brig [13, 17] and the reference preparation of Nerobol [11] was administered to animals orally at doses of 5.0 and 10.0 mg / kg, respectively, 4 hours before the start of the experiment. In experiments with actinomycin, D-antibiotic was used 30 minutes before the administration of the tested drugs at a dose of 2 mg / kg. 10 minutes before decapitation of mice, a mixture of 14C-amino acids (leucine and valine) was injected intraperitoneal, 10 μCy per mouse, after which the liver was removed and placed in liquid nitrogen.

The incorporation of ^{14}C-amino acids into the total proteins of the liver tissue homogenate and into the complete polypeptide chains of polyribosomes, as well as the radioactivity of the acid-soluble fraction, was determined as described in literatures [1,2]. The synthesis time of the middle polypeptide chain was calculated as described in the method [9]. In experiments in vitro, all operations for the isolation of subcellular liver components were carried out in a cold room at a temperature of 2-4 °C, the necessary solutions were cooled to 0° C on ice, and they were prepared in bidistilled water. Polyribosomes and cell sap from the liver of mice were isolated according to the method [25] with some additions [12]. The concentration of polyribosomes and cell sap was determined according to literatures [20, 4]. The functional activity of polyribosomes was studied in a cell-free protein synthesis system as described in [22]; their sedimentation analysis was performed in a linear gradient (10-50%) of sucrose density. The gradient was applied with 5 mg of polisome in a volume of 0.5 ml and centrifuged at 26,000 rpm in an SW-30 rotor on a VAC-601 preparative ultracentrifuge for 120 min. After that, the gradients were distributed into test tubes by 20 drops, 2.0 ml of water was added, and spectrophotometric was taken at 260 nm [2]. The sedimentation constants of the polyribosome fractions were calculated using the 80 S marker. 80 S ribosomes were obtained by treating liver polyribosomes of normal animals with ribonuclease. For this purpose, pancreatic ribonuclease from Reanal was added to a suspension of polyribosomes (5 mg in 1 ml of solution) containing 0.0035 M $MgCl_2$, 0.055 M KCl, 0.03 M Tris-HCl buffer (pH 7.6) to a final concentration of 2 μg / ml, incubated for 5 min at 37 ° C and cooled in ice water, and then applied to the gradient. They were centrifuged in parallel with experimental samples. The ratio of “heavy” and “light” liver polyribosomes was calculated conditionally, taking for “heavy” polyribosomes fractions with constant sedimentation greater than 105 S. The radioactivity of the samples was measured on a

Mark II scintillation counter ("Nuclear Chicago", USA). The results were processed statistically by using the Student's t-test.

Results and discussion

Conducted experiments have shown that in mice after administration of both ecdisten and nerobol, the activity of protein biosynthesis in the liver increases markedly (in the latter case, this process was more pronounced). Calculation of the radioactivity of the total proteins of the liver homogenate showed that the inclusion of ^{14}C-amino acids in them in experimental mice receiving ecdisten was 75.5% higher, and in those receiving Nerobol, it was 97.8% higher than in the control (Fig. 1).

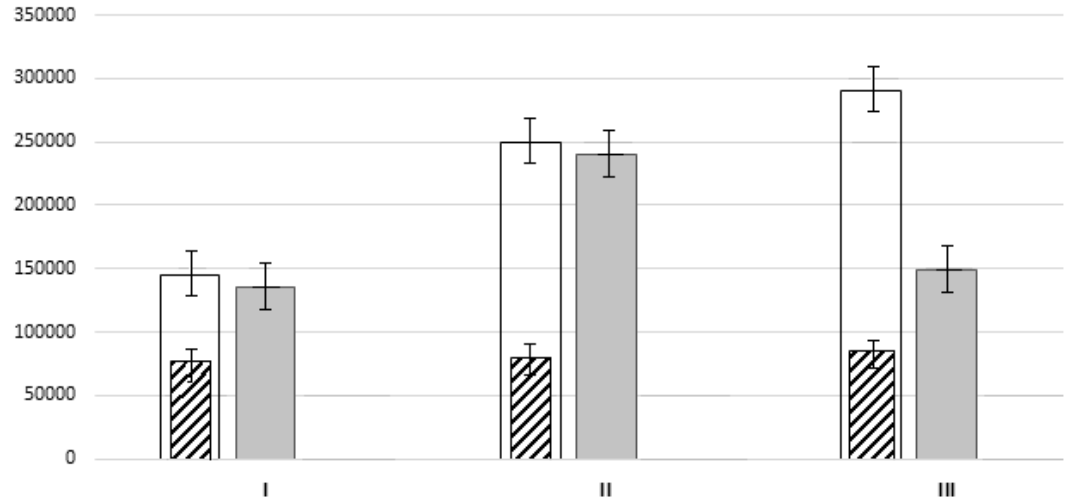

Fig. 1 Radioactivity (imp / min / g liver) of the total proteins of the homogenate (light bars - normal animals, dark bars - pretreated with actinomycin D) and acid-soluble fraction (shaded bars) of the liver of mice in control (I) and after administration of ecdisten to animals (II) and nerobol (III). Each group consisted of 6-8 animals.

The concentration of labeled amino acids in the intracellular fund in control animals, as well as in those treated with ecdisten and nerobol, it is the practically same (the radioactivity of the acid-soluble fraction did not change significantly in any of the experimental variants). The time of synthesis of the polypeptide chain, calculated based on data from a specially set series of experiments (Table 1), decreases. All this suggests that the more pronounced stimulation of protein synthesis, noted by us under the action of both steroids in the mammalian body, is associated with an increase in the absolute rate of synthesis of protein macromolecules.

Table 1

Influence of ecdisten and nerobol on the incorporation of labeled amino acids into mouse liver peptides (M ± m, n = 6-8)

Experimental conditions	Radioactivity of polypeptides, imp / min / g liver			Polypeptide synthesis time, sec
	Total	Completed	Growing (share,%)	
Control	98385 ±1449	92383 ± 1251	5942 (0.0604)	80.1
Ecdisten	160284 ±1672*	153100 ± 1286*	7184 (0.0448)	57.6
Nerobol	188300 ± 1520*,[1]	182520 ± 2452*,[1]	5780 (0.0307)	39.7

Note: the time, elapsed from the moment of labeling to the moment of immersion of the liver in liquid nitrogen, which had to be taken into account when determining the synthesis time of the middle polypeptide chain according to the method of [9], in each case it was determined using a stopwatch. In our conditions, in the control it was 663 sec, in animals, receiving ecdisten and nerobol – 643 sec and 647 sec, respectively.

* - Differences compared to control are significant; [1] - the differences between the two experimental groups are significant (p <0/05).

This was also indicated by the data obtained when studying the effect of ecdisten and Nerobol on the functional activity of liver polyribosomes, when conducting kinetic studies of the inclusion of ^{14}C-amino acids into peptides in a cell-free protein synthesis system. That is, when studying the activity of liver polyribosomes in control and experimental animals depending on time incubation of the protein synthesizing system. Figure 2 shows that the norm of the liver polyribosome includes ^{14}C-amino acids in peptides mainly during the first 20 minutes (84% of the total radioactivity of ^{14}C-amino acids).

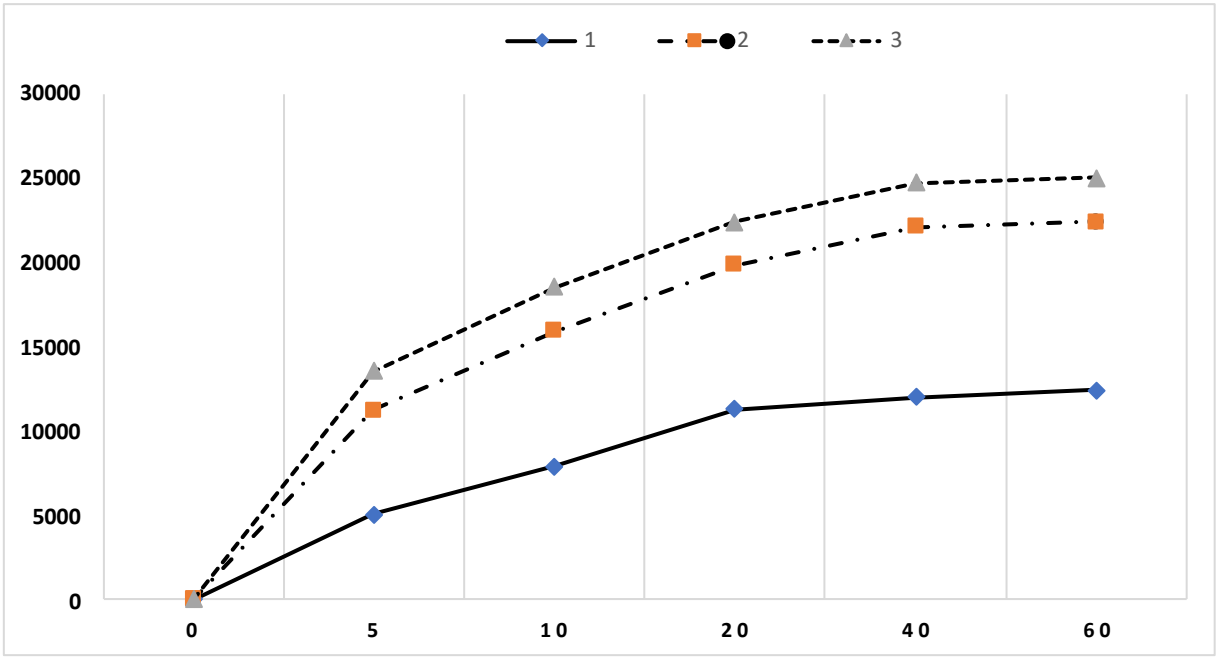

Fig. 2. The kinetics of the inclusion of ^{14}C - amino acids by polyribosomes of the liver in the cell-free system: 1. control; 2. ecdisten; 3. Nerobol. Y-axis - radioactivity (imp / min / mg polyribosomes)

By the 40th minute, the incorporation of labeled amino acids almost ceases and the kinetic curve reaches a plateau. In animals treated with ecdisten and nerobol, the kinetics of ^{14}C-amino acid incorporation completely repeats the kinetics, observed in the study of normal polyribosomes with the only difference that the processes of protein synthesis in this case proceed more intensively. These experiments show that the time of effective functioning of polyribosomes in relation to the control does not change. Consequently, the observed increase in the incorporation of ^{14}C-amino acids into peptides by polyribosomes under the action of both

steroids at the same time of their functioning once again indicates an increase in the rate of assembly of protein molecules. However, the fundamental difference between ecdisten and nerobol was that this process, when administered to mice with ecdisten, it is not associated with the incorporation of new genes and the induction of mRNA synthesis. Since the preliminary administration of actinomycin D, which selectively blocks the synthesis of DNA-dependent RNA in animal cells [10], is not eliminated the revealed effect of stimulation of protein synthesis by it (Fig. 1). Upon preliminary administration of actinomycin D to animals, the protein-anabolic effect of Nerobol was almost completely inhibited (Fig. 1).

The differences, revealed in these experiments in the mechanism of action of the compounds from the two classes of steroids under consideration in the mammalian organism, we have found their continuation in the clarification of the question of the localization of their action on the individual components of the protein synthesizing system. It was occurred, when conducting cross experiments with polyribosomes and cell sap from control and experimental animals, as well as in the analysis of sedimentation profiles of ribosomal structures from their liver in a sucrose density gradient of ecdisten Effect, in contrast to Nerobol, it depends mainly on changes in the polyribosome apparatus of liver cells (Table 2). Its effect at the level of cell sap factors was not manifested practically, while the stimulating effect of Nerobol on the protein-synthesizing system of hepatocytes was realized at both levels.

Table 2

Results of cross experiments with fractions of control and experimental protein synthesizing systems from the liver of mice treated with ecdisten and nerobol in the in vitro system (M ± m, n = 9)

Polyribosomal fraction	Cell juice	Incorporation of ^{14}C - amino acids into protein, ipm/min/mg polyribosomes
Control (from the liver of intact animals)	I. “Control”	12212 ± 374
	II. “Ecdisten”	13101 ± 451
	III. “Nerobol”	14200 ± 492*
“Ecdisten”	IV. “Control”	23133 ±301*
	V. “Ecdisten”	24090 ± 326*
“Nerobol”	VI. “Control”	25561 ± 131*
	VII. “Nerobol”	31600 ±498*,**

Note. * -Reliable in relation to I; ** - significant between VII and VI ($p < 0.05$).

Firstly, this was seen with a combination of polyribosomes from the liver of control animals and cell sap from the liver of experimental animals (a significant activation of the reconstructed cell-free protein-synthesizing system was observed only if nerobol was tested: the incorporation of ^{14}C-amino acids into peptides significantly increased by 16.3%). Secondly, the addition of the corresponding cell juice to the polyribosomes, isolated from the liver of mice, treated with Nerobol made an additional noticeable “contribution” to the activation of protein synthesis (the radioactivity of the samples increased by 23.6%). In a series of experiments, we also found that an increase in the functional activity of polyribosomes in animal liver cells after administration of ecdisten is not accompanied by significant changes in their distribution in the sucrose density gradient. The ratio between the translating and non-translating material in the experimental and control animals remained within the same values. Under the action of Nerobol,

this ratio significantly increased due to an increase in the proportion of polyribosomes (i.e., heavy component> 105S) and a decrease in the content of dimers and monomers (Table 3).

Table 3

Content and ratio of "heavy" and "light" components in polyribosomal material from the liver of mice treated with ecdisten and nerobol, %

Experimental conditions	Material		Ratio
	"heavy" (>105S)	"light" (80-105S)	
Intact animals	79,9	20,1	3,9
Ecdisten	79,4	20,6	3,8
Nerobol	88,2	11,8	7,5

The received data indicate that the enhancement of protein biosynthesis in mammals (protein-anabolic effect) under the influence of ecdisten is not determined by its effect on the pathways of genetic information transmission, as in insects [14]. The activation of protein synthesis in this case serves only as a reflection of the acceleration of translational processes, apparently due to the conjugate stimulation of translation initiation and elongation. As a result, the revealed by us an increase in the absolute rate of protein synthesis under the influence of ecdisten is not accompanied by a change in the polyribosomal profile. At the same time, the regulation of protein biosynthesis in mammals by Nerobol primarily depends on its activating effect on transcription processes, enhancement of the synthesis of ribonucleic acids and, above all, mRNA. As a result, the proportion of translating ribosomes increases and, accordingly, their functional activity in the cell-free system of protein synthesis increases. However, if the effect of Nerobol was determined only by enhanced synthesis of mRNA, then with an increase in the number of protein molecules, the absolute rate of their biosynthesis should not change. Having received exactly opposite results in this aspect, it is safe to say that Nerobol, providing actinomycin D-dependent stimulation of protein synthesis, significantly accelerates the translation processes through the factors of cell juice. It is possible that transport RNA can take a significant part in this. Thus, using the example of the preparation of ecdisten (substance - ecdysterone), it was shown that these steroids are fundamentally different in the mechanism of stimulation of protein synthesis processes in mammals and from their inherent effect in the body of arthropods [14,15] and from steroid anabolic preparations - synthetic analogs of male sex hormones. The action of ecdysteroids (phytoecdysteroids) in this case is largely similar to the action of other plant substances; in particular, compounds isolated from ginseng and *Eleuthero coccus* [5] and does not appear any specificity. Their effect in mammals is associated with the impact on the most general mechanisms of metabolic processes, which can lead to an increase in the functional activity of cell polyribosomes. As a result, the synthesis of proteins characteristic of a given organism is activated, and only against the background of their genetically determined induction. This material explains in our opinion, the fact that with the introduction of compounds from the class of ecdysteroids (phytoecdysteroids), or preparations created on their basis, higher animals observe (in contrast to steranabols) a harmonious course of anabolic reactions under conditions of a whole organism [16]. So there adaptogenic action [19], not accompanied by any specific hormonal effects and toxic effects during their long-term use [3].

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