INFLUENCE OF ESTRADIOL ADMINISTRATION ON PROTEIN SYNTHESIS IN HOMOGENATES OF HEN OVIDUCT*

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Recent studies in this laboratory on protein synthesis in slices of seminal vesicle have demonstrated that the administration of testosterone to immature rats results in the enhancement of a specific step in protein synthesis, the peptide bonding of transfer RNA-amino acids to form microsomal ribonucleoprotein (Wilson, 1962). However, the formation of peptide bonds in protein biosynthesis is a complex reaction, requiring, in addition to the transfer RNA-amino acids and the ribosome acceptor, the cofactor GTP, a soluble transfer enzyme, magnesium, and, in some preparations, sulfhydryl compounds (Nathans and Lipmann, 1961). In order, therefore, to localize further the mechanism of this hormonal action, the influence of estradiol administration on protein synthesis has been studied in cell-free preparations of immature hen oviduct. The hen oviduct was chosen for this study because of the demonstration by Brandt and Nalbandov (1956) that protein synthesis in this tissue is regulated by both androgens and estrogens.

EXPERIMENTAL PROCEDURE

Ten-week old, White Leghorn pullets were given, intramuscularly, 5 mg per day of estradiol for two days. Normal control and estradiol-treated chickens were decapitated, and the oviducts were quickly removed and placed in ice-cold buffer. Several oviducts were pooled for each experiment; three to one homoge-

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nates were prepared in a Dounce homogenizer using either the sucrose-electrolyte buffer as described by Zamecnik and Keller (1954) or the tris(hydroxymethyl)-aminomethane-electrolyte buffer described by Niremberg and Matthaei (1961). The homogenates were centrifuged at 10,000 X G at 0° for 10 minutes. The 10,000 X G supernatant was then either incubated directly or fractionated at 104,000 X G into soluble and microsomal fractions. The incubations were performed at 37° in a Dubnoff metabolic shaker after gassing with 95% oxygen-5% carbon dioxide. The methods for the purification of the protein and the assay for C^{14} have been previously described (Wilson, 1962). In some experiments microsomal RNA, prepared by the method of Nomura, Hall and Spiegelman (1960) from estradiol-treated oviduct was added to the incubation mixtures.

RESULTS

The results of fractionation of oviduct homogenates from estradiol-treated and control pullets are demonstrated in Table I. In the absence of an ATP generating system (creatine phosphate and creatine phosphate-ATP transphosphorylase) protein synthesis was negligible in all preparations. Upon the addition of the ATP generating system to the 10,000 X G supernatant a ten-fold enhancement of the incorporation of L-valine-U-C^{14} into protein was demonstrable in the estradiol-treated preparations as compared with the control values.

The 10,000 X G supernatant was then separated into microsomal and soluble fractions. The recombination of estradiol-treated microsomes and soluble fraction and of control microsomes and soluble fraction gave similar results to the unfractionated system. While the recombination of control microsomes and estradiol-treated soluble fraction gave no enhancement of protein synthesis over the control system, the system of estradiol-treated microsomes and control soluble fraction resulted in as great an enhancement of protein synthesis as was seen in the complete estradiol-treated system, demonstrating that the site of enhancement of protein synthesis in this tissue by estradiol administration is secondary to an effect on the microsome itself.

The results of studies which further characterize the estradiol-sensitive protein synthesizing system of hen oviduct are shown in Table II. In this

TABLE 1.

FRACTIONATION OF THE PROTEIN SYNTHESIZING SYSTEM OF OVIDUCT FROM ESTRADIOL-TREATED HENS

Homogenate	System				ATP Generating System	L-Valine-U-C^{14} Incorporated Into Protein
						cpm/mg
10,000 X G Supernatant	Estradiol-treated					31
	Estradiol-treated				+	463
	Control					8
	Control				+	40
Fractionated Homogenate	Microsomes		Soluble Fraction			
	Estradiol Treated	Control	Estradiol Treated	Control		
	+		+			38
	+		+		+	363
		+		+		13
		+		+	+	45
	+			+		9
	+			+	+	359
		+	+			4
		+	+		+	36

The incubation system contained L-valine-U-C^{14} (1.06×10^6 cpm), creatine phosphate (2.5×10^{-2} M), creatine phosphate-ATP transphosphorylase (30 γ), sucrose (.35 M), $KHCO_3$ (3.5×10^{-2} M), KCl (2.0×10^{-2} M), $MgCl_2$ (1×10^{-2} M), KH_2PO_4, pH 7.8 (1×10^{-2} M), and, as indicated, oviduct fractions containing the following amounts of protein to make a final volume of 0.8 ml: unfractionated homogenate (estradiol-treated, 3 mg; control, 2 mg), microsomes (estradiol-treated, 0.6 mg; control 0.4 mg, and soluble fraction (estradiol-treated, 2.4 mg; control 1.7 mg). Incubated at 37° for 45 minutes.

TABLE II.

CHARACTERIZATION OF THE ESTRADIOL-SENSITIVE PROTEIN SYNTHESIZING SYSTEM OF HEN OVIDUCT

System	L-valine-U-C^{14} Incorporated into Protein by Oviduct Microsomes from: Estradiol-treated Hens	Control Hens
	cpm/mg	cpm/mg
Complete System	137	12
- phosphoenolpyruvate, pyruvic kinase	0	0
- microsomes	0	13
- soluble fraction	0	0
+ chloramphenicol (0.05 μm)	135	13
+ puromycin (0.05 μm)	5	0
+ microsomal RNA (4.1 mg)	205	21
+ estradiol (1γ)	126	21

The complete system consisted of 0.5 ml estradiol-treated soluble fraction (1.9 mg protein and 0.02 mg RNA) plus either control microsomes (0.12 mg protein and 0.032 mg RNA) or estradiol-treated microsomes (0.09 mg protein and 0.023 mg RNA) and L-valine-U-C^{14} (1.06×10^6 cpm), phosphoenolpyruvate (5×10^{-3} M), pyruvic kinase (15 μg), tris(hydroxymethyl)aminomethane, pH 7.8 (1×10^{-2} M), magnesium acetate (1×10^{-2} M), KCl (6×10^{-2} M), and mercaptoethanol (6×10^{-3} M) to make a final volume of 0.8 ml. Incubated at 37° for 45 minutes.

experiment, soluble fraction from estradiol-treated hens was added either to control microsomes or microsomes from estradiol-treated hens. Protein synthesis in this homogenate clearly required the presence of treated microsomes, an ATP generating system, and the soluble fraction. In addition, although the synthesis of protein was markedly inhibited by puromycin, the addition of similar concentrations of chloramphenicol had no effect. Furthermore, the addition of microsomal RNA from estradiol-treated oviduct or of estradiol itself to the control microsomes did not result in significant enhancement of protein synthesis. In other experiments the addition of GTP also had no effect on protein synthesis in either the treated or control preparation.

DISCUSSION

These studies clearly demonstrate that the acceleration of protein synthesis in hen oviduct by the administration of estradiol is the result of an effect on the microsome itself and exclude the possibility that the enhancement of peptide bonding by this treatment is secondary to effects on either transfer enzyme or to soluble cofactors of the reaction. Together with the previous demonstration in slices of rat seminal vesicle that testosterone administration influences this same step in protein synthesis (Wilson, 1962) these results suggest that the influence of steroid hormones on protein synthesis in general may be mediated at this reaction. The inability of estradiol to accelerate this reaction when added directly to untreated homogenates suggests that this effect is not the result of a direct hormonal action.

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