ACUTE EFFECT OF A SINGLE IN VIVO INJECTION OF CORTISOL ON IN VITRO AMINO ACID INCORPORATING ACTIVITY OF RAT LIVER AND THYMIC PREPARATIONS*

A. Peña, B. Dvorkin and Abraham White

Department of Biochemistry
Albert Einstein College of Medicine
Yeshiva University
New York 61, N.Y.

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INTRODUCTION

The classical involution of lymphoid tissue seen following injection of certain adrenal cortical steroids in mice, rats, and rabbits, and the concomitant lymphopenia were described in detail approximately twenty years ago (Dougherty and White, 1944, 1945). These alterations included a marked inhibition of mitosis in lymphoid structures with an accompanying dissolution of lymphocytes in thymus, lymph nodes and spleen. Somewhat later, the in vivo antianabolic action of these steroid hormones was documented (Hoberman, 1950).

The stimulating effect of glycogenic adrenal cortical steroids on protein synthesis by the liver (White and Dougherty, 1947, Roberts, 1952) and on the ribonucleic acid content of liver (Lowe et al., 1958) has also been described as has evidence that these hormonal agents augment synthesis of several liver enzymes,

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e.g., arginase (Fraenkel Conrat et al., 1943) tryptophan pyrrolase (Knox et al., 1956), glutamic-alanine transaminase (Beaton et al., 1957) and glutamic-tyrosine transaminase (Lin and Knox, 1957). In addition, several laboratories have reported a stimulating effect of adrenal cortical steroid injection on the amino acid incorporating systems of cell free preparations from livers of hormone treated rats (Korner, 1960, Hultin et al., 1961, Leon et al., 1962). The data suggest that the locus of this adrenal cortical steroid effect is the ribosomes. These findings are in harmony with the in vivo results of Feigelson and associates (1962) revealing an early stimulatory influence of injected cortisone acetate on the incorporation of glycine into the proteins and purines of rat liver. More recently, Feigelson (1964) has reported that simultaneously with the above stimulatory action of injected cortisone acetate in adrenalectomized rats on glycine-2-C^{14} incorporation in vivo into liver acid soluble adenine, RNA and protein, there is also a depression of these metabolic processes in thymus and in spleen.

Our interests have also centered on the established observations that an injected hormone, e.g., cortisone or cortisol, may affect similar processes in two different organs in the same animal in opposite directions. Administration of certain adrenal cortical steroids appears to augment synthesis of specific liver constituents while simultaneously causing marked involution of the thymus. Experiments were therefore initiated to ascertain whether these gross changes in tissue composition and size were reflected in demonstrable alterations at a subcellular level and to assess the enzymic basis of such changes.

This paper records preliminary observations on the early effect of a single injection of cortisol in the rat on the incorporation of leucine-C^{14} into the total protein of cell free preparations of liver and thymic tissue. Three hours following an intraperitoneal injection of cortisol (5 mg/100 g body weight) into male rats

weighing 200 to 300 g, liver and thymic tissue broken cell preparations from these animals showed a marked increase and decrease, respectively, in the amount of radioactivity incorporated into the total proteins, as compared with similar tissue preparations from control rats not receiving steroid.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain were purchased from either the Holtzman Co., or Carworth Farms. Animals 7 to 12 weeks of age and weighing 200 to 300 g were used generally not prior to one week following arrival in the laboratory, where they were maintained on Purina Chow and water ad libitum. In view of the responsiveness of liver and thymus to adrenal cortical secretion (see below), it is of some importance to avoid stimuli that cause release of endogenous adrenal cortical steroids.

The following chemicals were obtained from Sigma Chemical Co., St. Louis, Mo.: ATP[1], and GTP, as disodium salts; PEP as trisodium salt; pyruvate kinase (suspension in ammonium sulfate containing 10 gm/ml). Uniformly labeled leucine-C^{14} with a specific activity of 240 mc/mmole was purchased from New England Nuclear Corp. Cortisol was kindly supplied in crystalline form by Dr. Elmer Alpert of Merck, Sharp and Dohme Co.

Animals were fasted overnight prior to use, with access to water. The following morning, experimental rats were given a single, intraperitoneal injection of cortisol (5 mg/100 g body weight). The steroid was given as a microcrystalline suspension in 0.85% NaCl containing 15 mg cortisol/ml. Control animals received an intraperitoneal injection of 0.85% NaCl equal in volume to that used in the experimental rats.

Three hours following steroid administration, the rats were sacrificed by

1. The following abbreviations are used: ATP, adenosine triphosphate; GTP, guanosine triphosphate; PEP, phosphoenolpyruvate; TCA, trichloroacetic acid.

decapitation. The thymus and liver were rapidly excised, blotted carefully, weighed on a torsion balance, and placed in an ice cold medium used for homogenization (Leon et al., 1962). This medium consisted of 0.25 M sucrose, 0.075 M KCl, 0.01 M $MgCl_2$ and 0.035 M Tris buffer, pH 7.8. The tissues were minced with scissors, washed several times with the above medium, and then

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Protein determinations were done using the biuret reagent and reading at 540 mμ on precipitates obtained after addition of 2 vols of 10% TCA and two washings with 95% ethanol.

TABLE I

Influence of Cortisol Injection On Leucine-C^{14} Incorporation into Total Proteins of Thymic and Liver Cell-Free Preparations*

	Control	Cortisol-Injected	t**	P
Liver	459 ± 35	709 ± 49	4.16	<0.01
Thymus	279 ± 29	171 ± 17	3.18	<0.01

*Values in table are means ± standard errors of means, and are expressed as counts/min/mg total protein. Each mean represents eight individual experiments.

**"t" values calculated from the formula for treatment of two small uncorrelated samples (Fisher, 1958).

Each incubation mixture contained, per 1.0 ml: 0.3 ml. of 105,000 x g cell supernatant, 0.35 ml of the resuspended microsomes, 2 μmoles ATP, 2 μmoles GTP, 10 μmoles PEP, 20 μg pyruvate kinase, 0.04 μmoles leucine-C^{14} equivalent to 1 μc. After 30 min. incubation at 37° C in a Dubnoff shaker, the reaction was stopped by addition of 1 ml of 20 percent TCA containing 0.1 M leucine, followed by 8 ml of 10 percent TCA. The precipitate was washed twice with 10% TCA, then extracted for 15 min. with the same TCA solution at 90° C, centrifuged, washed again with 10% TCA, then extracted once with ethanol, once with ethanol-ether (1:1) and once with ether. The dry residue was plated, dried in an oven at 150° for 30 min., and counted using a Geiger-Müller tube with a thin window, and a Nuclear-Chicago automatic sample changer and scaler.

homogenized with 2.5 volumes of the same medium in a Potter-Elvehjem homogenizer with a Teflon pestle.

The homogenate was centrifuged at 10,000 x g for 30 min. in the international PR-2 Centrifuge (head #296), and cellular debris, nuclei and mitochondria discarded. The supernatant was then centrifuged at 105,000 x g for 60 min. in the Spinco Model L preparative centrifuge (rotor #40). The cell sap was decanted. The microsomal pellet was rinsed and resuspended in approximately 6.0 ml of medium for each 5 g of original liver, or in 3.0 ml of medium for each 5 g of original thymus. The protocol of the in vitro experiments is indicated in Table I.

RESULTS

The results of several experiments are summarized in Table I. It is evident that both liver and thymic tissue taken from rats three hours following a single injection of cortisol show a response to the hormone, but in opposite ways with regard to hormonal influence on cell free amino acid incorporating systems prepared from the two tissues. The stimulating and inhibiting effects, respectively, on liver and thymic preparations are evident from the data. No significant change in thymic weight was evident at the time of sacrifice of the animals. Preliminary histologic studies also indicate no significant influence on thymus morphology of this dose of cortisol given three hours prior to removal of the tissue.

From initial studies designed to localize the hormonal effect, the following observations seem pertinent. The stimulatory effect of cortisol in liver resides in the microsomal fraction. The inhibitory influence of the thymic preparations is evident both in the 105,000 x g supernatant solutions and in the sedimented microsomal fractions of homogenates of thymus from cortisol-injected rats. Moreover, such high speed supernatant solutions show a greater inhibitory effect than does the control thymic cell sap on the leucine incorporating activity of liver

microsomal preparations from either uninjected or cortisol injected rats.

Experiments are in progress to examine the locus of the observed cortisol influence in thymic tissue as well as to assess further the previous suggestions of the nature of adrenal cortical steroid action in liver protein synthesis.

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