ACUTE DECREASE IN RNA POLYMERASE ACTIVITY OF RAT THYMUS IN RESPONSE TO CORTISOL INJECTION*

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A significant body of evidence assembled in recent years supports the hypothesis that hormonal augmentation of protein synthetic ability by ribosomal preparations from a number of tissues is reflected in an accelerated rate of RNA synthesis,1−4 including an RNA with rapid turnover, inferred to be messenger RNA.5−12 An early enzymic manifestation of this temporal relationship between increased rates of incorporation of amino acids into ribosomal protein and RNA synthesis has been demonstrated for liver, namely, a rise in RNA polymerase activity. Within 30 min following injection of cortisol into rats, liver nuclear preparations from hormone-treated animals showed an elevation of RNA polymerase activity significantly above that of preparations from untreated controls.13−15 Gorski16 reported that estradiol injection in rats caused an increase in activity of uterine RNA polymerase within 2 hr.

In contrast to the stimulation by cortisol of the ribosomal protein synthetic capacity of liver17, 18 is the inhibitory influence of this hormone on a cell-free amino acid incorporating system prepared from thymus.19, 20 Indeed, liver and thymic tissue taken from rats 3 hr following injection of cortisol showed, respectively, an increase and a decrease in the activities of the ribosomal protein amino acid incorporating systems.19, 20 Exploration of these opposite effects indicated that, for both tissues, the hormonal influence is reflected in alterations at the ribosomal level. The data also suggested that nuclear preparations from thymic tissue of fasted rats injected 3 hr prior to sacrifice with cortisol, as compared with similar preparations from control animals, had a diminished RNA polymerase activity.20 However, no direct evidence was obtained for a deficiency of messenger RNA on ribosomes prepared from cortisol-treated rats. The present paper is a report of studies designed to examine further the response of the RNA polymerase system of rat thymus to cortisol injection.

Materials and Methods.—Male rats of the Sprague-Dawley strain, 6 weeks old, weighing approximately 150 gm, were purchased from Carworth Farms. The animals were kept at least 5 days before use and maintained on Purina chow and water ad libitum.

The following chemicals were obtained from either Sigma Chemical Co., St. Louis, Mo., or Calbiochem, Los Angeles, Calif.: ATP, GTP, UTP, CTP, phosphoenolpyruvate, and pyruvate kinase (suspension in 2.1 M ammonium sulfate (pH 6.0) containing 10 mg/ml). H3-UTP (0.8 c/mmole) and H3-GTP (0.8 c/mmole) were purchased from Schwarz BioResearch, Inc., Orangeburg, N. Y., and cortisol from Steraloids, Inc., New York, N. Y. Other reagents were analytical grade and obtained from commercial sources.

Experimental animals, either fed or fasted overnight with access to water, received intraperitoneally a single dose of cortisol (5 mg per 100 gm body weight). The steroid was administered as a suspension of 15 mg per ml in 0.85% NaCl. Paired treated and control animals were injected with the same volume of saline.

At the desired time after steroid administration, the rats were sacrificed by decapitation. The thymus from two to three control or injected animals were rapidly excised, pooled, and placed in cold homogenization medium (0.25 M sucrose, 0.075 M KCl, 0.01 M MgCl2, 0.035 M Tris-Cl,
TABLE 1
RESPONSE OF RAT THYMIC RNA POLYMERASE TO FASTING AND TO CORTISOL INJECTION

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>Labeled precursor</th>
<th>Average Counts Incorp./mg DNA Control</th>
<th>Injected*</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>H$^3$-GTP</td>
<td>2424(8)</td>
<td>2062(8)</td>
<td>-15</td>
</tr>
<tr>
<td>Fasted</td>
<td>H$^3$-GTP</td>
<td>1491(4)</td>
<td>1128(4)</td>
<td>-25</td>
</tr>
<tr>
<td>Fed</td>
<td>H$^3$-UTP</td>
<td>1037(10)</td>
<td>699(10)</td>
<td>-34</td>
</tr>
<tr>
<td>Fasted</td>
<td>H$^3$-UTP</td>
<td>443(4)</td>
<td>391(4)</td>
<td>-12</td>
</tr>
</tbody>
</table>

* Cortisol (5 mg/100 gm body weight) injected 3 hr prior to sacrifice of rats.
† Numbers in parentheses are number of individual experiments, each representing two pooled thymis.

pH 7.8). The tissue was homogenized with 10 ml of the same medium, using a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 700 $\times$ g for 5 min in an International refrigerated centrifuge. The supernatant fluid was discarded, the precipitate washed twice with the above medium, and the final pellet resuspended in a known amount of the medium.

The incubation medium contained 0.25 ml of nuclear suspension (equivalent to 1.5 mg DNA), 1 $\mu$ mole each of ATP, GTP, CTP, UTP, 5 $\mu$ moles phosphoenol pyruvate 10 $\mu$ g of pyruvate kinase, 50 $\mu$ moles Tris-Cl, 16 $\mu$ moles MgCl$_2$, and 6 $\mu$ moles of 2-mercaptoethanol, in 0.5 ml. When H$^3$-UTP (1 $\mu$) was employed, the unlabeled UTP was omitted from the system, and when H$^3$-GTP (1 $\mu$) was used, the nonradioactive GTP was omitted. Incubation was carried out for 10 min at 37°.

At the end of the incubation, 3 ml of 5% perchloric acid were added, and the precipitate separated by centrifuging. The precipitate was washed twice with 5% cold perchloric acid and then extracted with 1 ml of 5% perchloric acid at 90° for 15 min. The tubes were chilled and centrifuged.

DNA was determined in a 0.1-ml aliquot of the supernatant solution by the diphenylamine reaction. Aliquots (0.1 ml) of the supernatant solution were placed in counting vials containing 15 ml of Bray’s solution and neutralized immediately with an equivalent amount of KOH. The samples were counted in a Packard liquid scintillation counter. The counts obtained at 0 min incubation were subtracted from those obtained after incubation. Satisfactory linear relationship was found between the rate of the polymerase reaction and enzymic (nuclei) concentration.

Results.—Table 1 presents data for the RNA polymerase activity of thymic preparations from control and from cortisol-injected fed and fasted rats, utilizing either H$^3$-GTP or H$^3$-UTP in the enzymic assay. RNA polymerase activity appears to be higher in nuclei prepared from the thymi of fed, as compared with fasted, rats. Injection of cortisol 3 hr prior to sacrifice of the animals results in a significant decrease in RNA polymerase activity in the thymic nuclei. The lower enzymic level seen in the thymus of fasted as compared with fed rats may be a reflection of the augmented endogenous secretion of adrenal steroids and their effects on lymphoid tissue consequent to fasting. In view of this, fed animals have been employed in all studies except, as noted, in Table 1.

The data in Table 2 indicate that although maximum RNA polymerase activity is evident when all four nucleoside triphosphates are present in the assay system, demonstrable incorporation of either H$^3$-GTP or H$^3$-UTP is seen in the absence of any one of the triphosphates.

Animals sacrificed at varying periods following cortisol injection provide data suggesting that the hormonal influence on thymic RNA polymerase activity is an acute one (Table 3). Within 30 min after steroid administration, this enzymic activity has declined significantly in the assays utilizing H$^3$-UTP.

Discussion.—The data presented in this paper establish that the level of activity of thymic RNA polymerase, like that of liver, is influenced by adrenal cortical steroids. However, whereas elevated concentrations of adrenal steroids lead to
increased activity of liver RNA polymerase, this enzymic activity diminishes in the thymus of rats injected with a potent adrenal cortical steroid, namely, cortisol. Indeed, in the same animal receiving this steroid, the temporal rise in liver RNA polymerase activity is similar to the decrease in activity of this enzyme in thymus. Moreover, these enzymatic alterations in liver and thymus are evident, under the experimental conditions used, within 30 min following cortisol administration. The data from this time study are in good relative agreement with *in vitro* studies of the incorporation of H\(^3\)-uridine into thymocyte RNA.\(^{25}\) Thymocytes obtained from rats injected with cortisol 40 min prior to sacrifice showed a 23 per cent decrease, as compared with cells from control rats, in their capacity to incorporate H\(^3\)-uridine *in vitro* into RNA.\(^{25}\)

The lower level of RNA polymerase activity in thymic tissue of fasted as compared with fed rats may be an additional reflection of the influence of adrenal steroids on this enzyme in thymus, since fasting augments endogenous levels of these hormones.\(^{23, 24}\) As is the case for the RNA polymerase activity of other mammalian tissues, e.g., rat liver,\(^{26}\) brain,\(^{27}\) and chick embryo,\(^{28}\) deletion of one of the nucleoside triphosphates from the incubation medium reduced the incorporation of labeled precursor into RNA. However, thymic nuclei showed somewhat less measurable
dependence on the complete nucleoside triphosphate system than reported for RNA polymerase measurements in other mammalian tissues. It is also of interest that the influence of cortisol on thymic RNA polymerase activity is still apparent in assay systems lacking one of the triphosphates.

Gabourel and Fox have described a marked decrease in the total RNA of thymic tissue of rats injected 6 hr prior to sacrifice with cortisol (1.5 mg/100 gm body weight). A single injection of cortisol acetate in rats or the prolonged administration of cortisol to mice has been reported to inhibit the in vivo incorporation of a labeled precursor into thymic RNA. In this laboratory, a significant inhibition was observed in the in vitro incorporation of added H-uridine into thymocyte RNA either when the cells were obtained from rats injected prior to sacrifice with cortisol or when the steroid was added directly in vitro, in concentrations of $10^{-4}$ to $10^{-6} M$ to the thymocytes. While these latter studies were in progress, Kidson reported that an inhibition of incorporation of H-uridine into RNA was evident within a few minutes after addition in vitro of cortisol hemisuccinate to lymphocyte suspensions prepared from rabbit mesenteric lymph nodes. These results, coupled with the early acute effects of cortisol injection on the levels of thymic and liver RNA polymerase activity, suggest RNA metabolism as an early locus of adrenal steroid action, perhaps directly at the polymerase level in both tissues. However, in view of the opposite directions in which RNA polymerase activity is affected at the same time in the two tissues by steroid, it is difficult to visualize an identical mode of action of the hormone on this enzyme system in liver and in thymus.

The data presently available do not permit the exclusion of some earlier biochemical reaction, preceding alterations in RNA synthesis, as a basis for the initial site of steroid hormone action in liver and in thymus. Such an earlier event might be exerted at the level of DNA required as participant in the RNA polymerase catalyzed reaction. Support for this suggestion has been derived primarily from evidence of increased formation of rapidly labeled RNA consequent to hormonal stimulation. Dahmus and Bonner have recently reported that administration of cortisol caused a 30 per cent increase in the activity of rat liver chromatin template for RNA synthesis.

Another possible variable influencing the data reported might be one of alterations in intranuclear nucleoside triphosphate pool size as a consequence of cortisol administration. This possibility was examined by addition of a five- to tenfold concentration of unlabeled GTP or UTP in the RNA polymerase assay. However, this addition did not alter the acute decrease in thymic RNA polymerase activity in response to cortisol administration, as described in this communication.

Summary.—RNA polymerase activity in thymic nuclei obtained from rats injected 3 hr prior to sacrifice with cortisol is significantly lower than that observed in similar preparations from control rats. Alterations in activity of thymic RNA polymerase activity resulting from hormone injection are discernible as early as 30 min following steroid treatment.

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The discovery by Hoogsteen\textsuperscript{1,2} that methylated derivatives of adenine and thymine (both carrying the substituent at their glycosidic nitrogen) co-crystallize as a hydrogen-bonded complex, whose configuration is, however, different from that which Watson and Crick observed in the nucleic acids, stimulated a large number of investigations on the co-crystallization of purine and pyrimidine bases. Among the most outstanding results in this field are (1) the observations that while the derivatives of adenine and thymine co-crystallize in at least two different complexes,\textsuperscript{3,4} both different from that of Watson and Crick, the derivatives of guanine...