Effects of Cortisol–Protein Complexes on RNA Polymerase Activity in Isolated Pig Liver and Thymus Nuclei[1] (36586)

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(Introduced by E. V. Morse)

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Physiological actions of glucocorticoids have been studied over the past 40 years, but a precise definition of their action is still being pursued. The liver has been recognized for many years as a target organ for the anabolic actions of glucocorticoids (12) and the thymus as a target organ for their catabolic actions (10). Stimulatory actions of glucocorticoids on hepatic gluconeogenesis (14) and inhibitory actions on extrahepatic tissues (10) are well documented.

An attractive hypothesis concerning action sites of these steroids is that they have direct effects on nucleic acid metabolism and protein synthesis. The ability of corticosteroids to increase RNA and protein synthesis in the liver (14) and decrease RNA and protein synthesis in the thymus has been reported by numerous investigators (3, 10).

Very soon after treatment with a labeled glucocorticoid, specific binding of the hormone occurs in the cytoplasm of liver (7, 9) and thymus cells (10). These interactions are of great interest and may be related to physiological actions of glucocorticoids. We have found ^{3}H-cortisol bound to cytoplasmic proteins in the liver and thymus of pigs 45 min after injection (4). Molecular-sieve chromatography of the liver supernatant fraction produced 2 peaks of radioactivity associated with the high molecular weight fractions. In the thymus supernatant only one peak of radioactivity was found associated with the high molecular weight fractions. Recent experiments in this lab have shown that similar binding occurs *in vitro*.

The work reported here tested the possibility that cytosol steroid–protein complexes from the liver and thymus could alter RNA polymerase (nucleoside triphosphate:RNA nucleotidyltransferase EC 2.7.7.6) activity in homologous nuclei. These experiments seemed appropriate since it is well known that cortisol *in vivo* alters RNA polymerase activity in liver (1, 2) and thymus (11).

Materials and Methods. Preparation of nuclei. Young Yorkshire-Chester white female pigs weighing 4–6 kg were killed by electrocution and exsanguinated with heparinized saline. The liver and thymus were removed and placed in cold 0.24 *M* sucrose, 4 m*M* ethylenediaminetetraacetate (EDTA), in 10 m*M* potassium phosphate buffer, pH 7.0 (SMEP). Each tissue was passed through a tissue press (Harvard Apparatus Co., Inc.) in order to remove the connective tissue and then homogenized in 4 vol of SMEP buffer using a Potter-Elvehjem homogenizer. This was filtered through several layers of cheesecloth to remove any remaining connective tissue or intact cells. The filtrate was centrifuged at 800*g* for 10 min in order to obtain a nuclear pellet. The pellet was washed twice with 5 vol of SMEP buffer and saved for the RNA polymerase assay by resuspending in sucrose–KCl–$MgCl_2$–Tris buffer (0.25 *M* sucrose, 0.25 *M* KCl, 0.01 *M* $MgCl_2$ in 0.05 *M* Tris–HCl, pH 7.5). Each milliliter contained nuclei from 1 g of tissue, about 2.0 mg DNA. Duplicate samples were saved for DNA determination (8).

Preparation of cytosol steroid–protein complexes. Samples of the liver and thymus were

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homogenized in Krebs–Ringer–phosphate buffer (pH 7.4). A cytosol fraction was obtained by first centrifuging at 800*g* and the resulting supernatant was centrifuged at 105, 000*g*. The pH of 4 ml of the cytosol was adjusted to pH 8 with NaOH and incubated (37°) for 30 min with a final concentration of 10^{-7} *M* ^{3}H-cortisol (14 μCi/μg cortisol). A 2 ml aliquot was layered on a column of Sephadex G-100 and eluted with 20 m*M* phosphate buffer. Five milliliter fractions were collected and assayed for protein and radioactivity. Four milliliters of the effluent from tubes containing protein and the highest amount of radioactivity were concentrated by ultrafiltration using Centriflo membrane ultrafilters (Amicon Corporation, Lexington, MA). Each tube was centrifuged for 10 min at 1100*g*. One milliliter of the concentrated steroid–protein complexes was incubated with nuclei.

Incubation of nuclei with free cortisol and steroid–protein complexes. Duplicate 1 ml samples of nuclei, prepared as indicated above, were incubated at 37° with (a) 1.0 ml of buffer, (b) 1.0 ml of buffer containing 10^{-5} *M* cortisol and, (c) 1.0 ml steroid–protein complex with 10^{-5} *M* cortisol. After incubation for 10 min all reactions were stopped by placing the tubes in an ice bath to chill and then centrifuged at 1500*g* for 5 min. Hypotonic lysis of nuclei was accomplished by resuspending the pellets in 5.0 ml cold 0.05 Tris–HCl buffer (pH 7.9) and allowed to stand for 20 min at 2°. The mixture was then centrifuged at 10,000*g* for 10 min. The resulting "nuclear sediment" was then thoroughly homogenized in 0.3 ml of 0.065 *M* Tris–HCl buffer (pH 7.9) and used for the determination of RNA polymerase activity.

RNA polymerase activity in nuclei. The RNA polymerase assay system contained 50 μmoles Tris–HCl (pH 8.0), 10 μmoles $MgCl_2$, 1.0 μmole of ATP, 0.25 μmoles of GTP and CTP, 0.1 μCi UTP-2-^{14}C (sp act 50 mCi/mmole) and 200 μl of "nuclear sediment." The incubation period was either 0 or 10 min at 37°. All reactions were stopped by adding 3.0 ml cold 5% trichloroacetic acid (TCA) and centrifuged at 10, 000*g* for 10 min. The pellet was washed twice with 3.0 ml of TCA and once with 3 ml of water. The pellet was resuspended in 1.0 ml of water, transferred to a liquid scintillation vial and the radioactivity was counted using a Beckman DPM-100 liquid scintillation counter. Net counts were obtained by subtracting the cpm in the 0 min incubation from each sample. The results are expressed as cpm of UTP-2-^{14}C incorporated into RNA per milligram of DNA. Data were analyzed by a two-way analysis of variance in order to remove variation due to different runs. The treatment means were compared by Newman-Keuls sequential range test.

Results. The effects of cortisol, cytosol proteins and steroid–protein complexes on RNA polymerase activity when incubated with homologous nuclei are shown in Table I. Two different steroid–protein complexes from the liver were separated by gel filtration and incubated with nuclei. The elution volume for the first steroid–protein peak (P_1) was about 50 ml and for the second peak (P_2) it was about 80 ml.

Incubation of isolated pig liver nuclei with free cortisol had no significant effect on RNA polymerase activity when compared to those nuclei incubated with buffer alone. The steroid–protein complexes from P_1 significantly increased RNA polymerase activity when compared to that observed for buffer ($p < .01$) as well as that observed for buffer plus free cortisol ($p < .01$). Nuclei incubated with the steroid–protein complexes from P_2 significantly increased RNA polymerase activity when compared to that observed for buffer ($p < .01$), however there was no significant increase over that observed for buffer plus cortisol (Table I).

In order to determine if the protein without the associated steroid had any effect upon RNA polymerase activity, pigs were adrenalectomized in order to remove endogenous steroids. The liver cytosol proteins were obtained and incubated in the RNA polymerase assay system. It was found that the isolated proteins of both peaks, P_1 and P_2, without bound steroid had no effect on RNA pollymerase activity (Table I).

The effects of cortisol and steroid–protein complexes, isolated from the thymus, on

TABLE I. Effects of Free Cortisol, Cytosol Proteins and Cytosol Steroid–Protein Complexes on RNA Polymerase Activity in Homologous Pig Cell Nuclei.[a]

Nuclei	Buffer	Buffer and cortisol	Cytosol steroid–protein complexes		Free cytosol protein[b]	
			P_1	P_2	P_1	P_2
Liver[c]	540 ± 92	591 ± 116	810 ± 114[e]	737 ± 120[f]	684 ± 185	533 ± 141
Thymus[d]	616 ± 106	435 ± 111[f]	398 ± 97[f]		—	—

[a] Results are expressed as cpm ^{14}C-UTP Incorporated into RNA per milligram DNA.
[b] Free cytosol protein was isolated from adrenalectomized pigs. P_1 and P_2 represent the two groups of liver cytosol proteins which bind cortisol.
[c] Results are the average of 7 determinations ±SE.
[d] Results are the average of 5 determinations ±SE.
[e] $p < .01$ value compared to buffer and buffer + free cortisol.
[f] $p < .01$ value compared to buffer.

RNA polymerase activity of thymus nuclei are also shown in Table I. Only one peak of radioactivity was associated with the protein fraction of thymus cytosol when separated by gel filtration. Analaysis of the data indicated that both free cortisol and the steroid–protein complexes significantly decreased RNA polymerase in homologous nuclei. The effect of the steroid–protein complex was no different than that observed for free cortisol.

Discussion. The observations that the cytosol steroid–protein complexes enhanced liver RNA polymerase activity indicates that an association of cortisol with cytosol proteins may be an early event in the action of cortisol (Table I). Since neither free cortisol (Table I), nor cytosol proteins without the bound steroid altered RNA polymerase activity, these data support the thesis that association of cortisol with proteins is necessary for activity in the liver. These results agree with those reported by Beato *et al.* (2). They reported that cytosol macromolecules enhanced the ability of cortisol to stimulate *in vitro* RNA polymerase activity in isolated rat liver nuclei. The effects *in vitro* are similar to *in vivo* effects of cortisol since several investigators have shown that *in vivo* injections of cortisol increased RNA polymerase activity and protein synthesis in the liver (2, 6, 13).

The results of these experiments indicate that a cytosol steroid–protein complex in the thymus significantly decreased RNA polymerase activity in thymus nuclei when compared to buffer, but the difference was no greater than that observed for free cortisol (Table I). This indicates that a cytosol steroid–protein complex may not be necessary for cortisol action in thymus nuclei. The observation that free cortisol as well as steroid–protein complexes decreased RNA polymerase activity in isolated nuclei is consistent with *in vivo* effects of cortisol. Brunkhorst (5); Munck and Brinck-Johnsen (10); Nakagawa and White (11), and Young (15) have all shown that cortisol decreased RNA polymerase activity and protein synthesis in the thymus.

It is possible that the thymus cytosol steroid–protein complexes used in this experiment represent nonspecific binding (10) and may not be related to specific physiological effects of cortisol.

We conclude that association of cortisol with liver cytosol proteins is an early action of cortisol in the liver. The steroid–protein complex is then capable of nuclear changes which alter RNA synthesis. Cortisol also associates with cytosol proteins in the thymus, but this association does not appear to be necessary for cortisol actions in the thymus.

Summary. Cortisol is known to alter RNA polymerase activity in liver and thymus. It also binds to cytosol proteins in several tissues. These experiments tested the possibility that cytosol steroid–protein complexes could alter the RNA polymerase activity of homologous nuclei.

Steroid–protein complexes were prepared

by incubating liver and thymus minces with 10^{-7} *M* ^{3}H-cortisol at 37°. The partially purified steroid–protein complexes were obtained by fractionation of the cytosol on Sephadex G-100. Steroid–protein complexes were incubated with intact homologous nuclei and a nuclear sediment was prepared by hypotonic lysis of nuclei and centrifugation. RNA polymerase activity was determined in the nuclear sediment.

It was found that a liver cytosol steroid–protein complex significantly ($p <$ .01) increased RNA polymerase activity when compared to free cortisol or buffer. Cytosol proteins from adrenalectomized pigs had no effect on nuclear RNA polymerase. A steroid–protein complex in the thymus significantly decreased RNA polymerase activity in thymus nuclei when compared to buffer, but the difference was no greater than that observed for free cortisol. This indicates that an association of cortisol with liver cytosol proteins may be an early event in the action of cortisol.

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1. Beato, M., Homki, J., and Sekeris, C. E., Exp. Cell Res. **55**, 107 (1969).
2. Beato, M., Homoki, J., Doenecke, D., and Sekeris, C. E., Experentia **26**, 1074 (1970).
3. Bethell, J., Feigelson, H., and Feigelson, P., Biochim. Biophys. Acta **104**, 92 (1965).
4. Bottoms, G. D., Stith, R. D., and Burger, R. Q., Proc. Soc. Exp. Biol. Med. **132**, 1133 (1969).
5. Brunkhorst, W. K., Biochem. Biophys. Res. Commun. **35**, 880 (1969).
6. Dukes, P. P., Sekeris, C. E., and Schmid, W., Biochim. Biophys. Acta **123**, 126 (1966).
7. Fiala, E. S., and Litwack, G., Biochim. Biophys. Acta **124**, 260 (1966).
8. Kirby, K. S., Biochem. J. **66**, 495 (1957).
9. Litwack, G., Fiala, E. S., and Filosa, R. J., Biochim. Biophys. Acta **111**, 569 (1965).
10. Munck, A., and Brinck-Johnsen, T., J. Biol. Chem. **243**, 5556 (1968).
11. Nakagawa, S., and White, A., Endocrinology **81**, 861 (1967).
12. Silber, R. H., and Porter, C. C., Endocrinology **52**, 518 (1953).
13. Stackhouse, H. L., Chetsanga, C. J., and Ian, C. H., Biochim. Biophys. Acta **155**, 159 (1968).
14. Weber, G. A., Shivastava, S., and Singhal, R. L., J. Biol. Chem. **240**, 750 (1965).
15. Young, D. A., J. Biol. Chem. **245**, 2747 (1970).

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