CORTEXOLONE: BINDING TO GLUCOCORTICOID RECEPTORS IN RAT THYMOCYTES AND MECHANISM OF ITS ANTIGLUCOCORTICOID ACTION

Nurit Kaiser†, Richard J. Milholland, Roger W. Turnell, and Fred Rosen

Department of Experimental Therapeutics
Roswell Park Memorial Institute
New York State Department of Health
Buffalo, New York 14203

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SUMMARY: In rat thymocytes, cortexolone (11-deoxycortisol) competes for binding to glucocorticoid receptors, as identified on sucrose density gradients, and blocks the effect of triamcinolone acetonide (TA) on 2-deoxyglucose uptake. The cellular distribution and sedimentation coefficients of ^{3}H-TA-receptor complexes are dependent on the incubation temperature and ionic strength of the extraction buffer. In contrast, the single receptor complex formed by ^{3}H-cortexolone has a sedimentation coefficient of 3.5S, and is unaffected by changes in temperature and salt concentration.

INTRODUCTION: It is generally accepted that an early event in steroid hormone action involves the binding of the hormone to a specific protein receptor in target tissues (1). An initial step appears to be intracellular binding to a cytoplasmic receptor. The hormone-receptor complex subsequently migrates to the nucleus and binds to acceptor sites on the chromatin (2) and/or to DNA (3).

Certain steroid antagonists have been shown to compete with biologically active hormones for specific binding proteins, and thus to inhibit the physiological action of the hormone (4-9). Apart from competition for sites on the receptor molecule, little is known concerning the interaction and distribution of the steroid antagonist-receptor complex. Cortexolone has been reported to act as an antiglucocorticoid in HeLa cells (10) and rat thymocytes (7), and in the latter case evidence has been obtained that this

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steroid competes with an active glucocorticoid for binding to a specific receptor. In the present study, the binding of ^{3}H-cortexolone to glucocorticoid receptors in rat thymocytes was examined in order to determine the molecular basis for its action.

EXPERIMENTAL PROCEDURES: Suspensions of rat thymocytes were prepared by mincing pooled thymi from 8 to 10 male Carworth Farms rats (75-100 g) in protein-free Roswell Park Memorial Institute 1640 medium using the technique described by J. Rosen et al. (11). For the binding assays, the cells were incubated for 30 minutes in RPMI 1640 medium containing the steroids at 0° or 37°. Specific experimental details are contained in the legend for each figure. Concurrent with the binding studies, the effects of cortexolone alone and in combination with triamcinolone acetonide (TA) on ^{14}C-2-deoxyglucose uptake were determined as previously described (11).

RESULTS AND DISCUSSION: The results shown in Fig. 1a indicate that cortexolone, at 1500-fold higher concentration than TA, is effective in decreasing the binding of ^{3}H-TA to receptors in rat thymocytes by approximately 90%. In thymocytes incubated at 0°, and extracted with either the low or high salt buffer, cortexolone (3×10^{-5} M) was also observed to be an effective competitor (90%) of ^{3}H-TA for specific binding components (data not shown). The cellular uptake of ^{3}H-TA was decreased 20% in the presence of cortexolone. As shown in Fig. 1b, cortexolone did not inhibit the uptake of ^{14}C-2-deoxyglucose into rat thymocytes when used alone but did block the inhibition of 2-deoxyglucose transport caused by TA ($p < 0.01$ in comparison to TA alone). Thus it appears that the hormone-receptor complex is required for biological activity, and that cortexolone forms an inactive receptor complex.

The sedimentation profiles of the TA-receptor complex are shown in Fig. 2. When the cells were extracted with the high salt buffer at 0°, TA was bound in a 4S complex, whereas when low salt buffer was used two peaks of radioactivity at $\sim$3.5S and $\sim$7S were obtained (recoveries of bound steroid on the gradients were $\sim$90%).

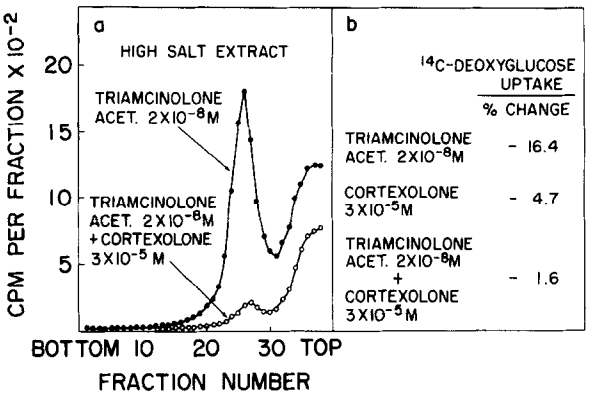

Fig. 1a

Sedimentation of bound ^{3}H-TA on sucrose gradients. Rat thymocytes (4 x 10^{7} cells/ml) were incubated for 30 minutes with 2 x 10^{-8}M ^{3}H-TA (9.5 Ci/mM) with or without 3 x 10^{-5}M cortexolone at 37°. The cells were washed 3 times with RPMI 1640 medium and extracted by homogenization in 1 volume of 0.01 M Tris HCl buffer pH 7.6 containing 0.4 mM Na_2EDTA to which 1/10 volume of 0.11 M Tris HCl buffer pH 7.6 containing 1.65 M KCl was added to give a final buffer concentration of 0.15 M KCl-high salt buffer. 0.5 ml of a 27,000 x g supernatant fraction was layered on 11.5 ml of 5-20% sucrose gradient made in the same buffer used for extraction. 0.5 ml of crystalline bovine serum albumin (4.6S) was run in a separate tube. The gradients were centrifuged at an average force of 201,000 x g in a Beckman SW 41 rotor at 2° for 20 hours. After centrifugation 10 drop fractions were collected from the bottom of the tubes directly into the counting vials and assayed for radioactivity. The migration of the bovine serum albumin was assayed by measuring the absorbance at 280 nm of fractions collected from the gradients.

Fig. 1b

The effect of TA and cortexolone on the uptake of 2-deoxyglucose-(U)-^{14}C (6.3 mCi/mM) into whole cells. Rat thymocytes (4 x 10^{7} cells/ml) were incubated for 3 hours with TA, cortexolone or both. One μCi of 2-deoxyglucose was added to 0.5 ml aliquots of cell suspensions 30 minutes prior to the end of the incubation. Radioactivity was measured in the trichloroacetic acid-soluble fraction. There were 4 samples in each group and the results are expressed as the percentage change of the treated means compared with the control mean.

The total amount of ^{3}H-TA bound to receptors extracted with the low or high salt buffer did not differ significantly. The single receptor complex noted in the high salt extract suggests that there is dissociation of the 7S complex into two subunits, which has been previously postulated for estrogen receptors (12, 13). This is consistent with the observation that when the ^{3}H-TA-receptor complex extracted with low salt at 0° was assayed on a sucrose gradient prepared in a high salt buffer only a 4-5S component was detected.

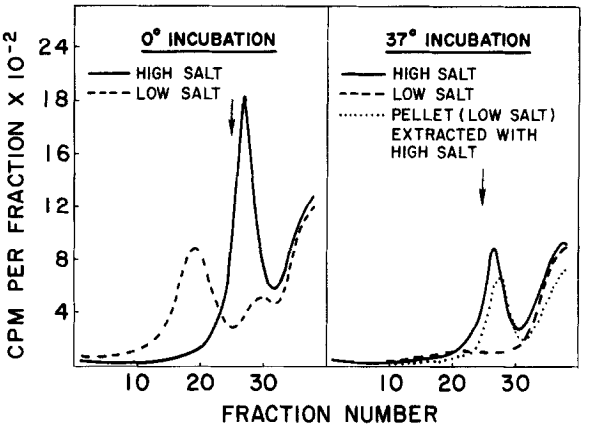

Fig. 2

Sedimentation profiles of ^{3}H-TA-receptor complex on sucrose gradients. Rat thymocytes (4 x 10^{7} cells/ml) were incubated for 30 minutes with 2 x 10^{-8}M ^{3}H-TA (9.5 Ci/mM) at 0° or 37°. After 3 washes the cells were extracted by homogenization in a low salt buffer - 0.02 M Tris HCl buffer pH 7.6 containing 0.4 mM Na_2EDTA, or in a high salt buffer - containing in addition 0.15 M KCl. Sedimentation analysis was performed as described in Fig. 1a. The arrow marks the location of the bovine serum albumin marker.

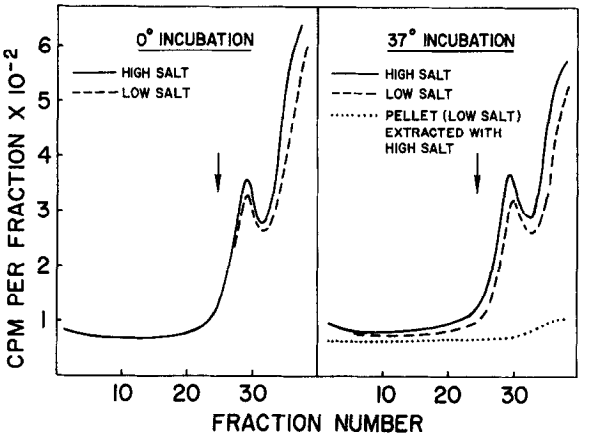

Fig. 3

Sedimentation profiles of ^{3}H-cortexolone-receptor complex on sucrose gradients Cortexolone (35 Ci/mM) was used at a concentration of 3 x 10^{-8}M. See Fig. 2 for details of the experimental procedure.

At 37° the high salt extract contained ^{3}H-TA bound in a 4S complex, whereas under low salt conditions no specific sedimentation coefficient could be determined. However, when the 27,000 x g pellet of the low salt extract was re-extracted with high salt (0.15 M KCl), a complex with a sedimentation coefficient of 3.5-4S was identified. These

data are in agreement with a temperature-dependent translocation of steroid hormone-receptor complexes from the cytoplasm to the nucleus, as observed by others (14).

In contrast to ^{3}H-TA, the cortexolone-receptor complex has a sedimentation coefficient of $\sim$ 3.5S (Fig. 3). This was observed in both low and high salt buffers at 0° and 37° (recoveries of bound steroid on the gradients were $\sim$ 70%). Of special interest is the observation that when the pellet of the low salt extract was re-extracted with two high salt buffers (0.15 M and 0.4 M KCl) there was no ^{3}H-cortexolone complex detected. No specifically bound ^{3}H-cortexolone remained in the pellet after extraction with 0.15 M KCl buffer (15). Since this pellet contained more than 98% of the cellular DNA, it is possible that the cortexolone-receptor complex either failed to get into the nucleus or if it was taken up by the nucleus, it failed to bind to a significant extent in this fraction. Under similar conditions, the amount of ^{3}H-TA-receptor complex extractable with 0.15 M KCl buffer could be increased about 2-fold by using 0.4 M KCl buffer. These results suggest that this complex not only penetrated the nucleus but was quite firmly bound to an intranuclear molecule.

Munck and Wira (7) have suggested that the lack of activity of the cortexolone-receptor complex is a result of a conformational change which allows for its increased affinity to a nuclear acceptor site, but that such an interaction does not mediate a biological response. An alternative explanation is offered by our data. Namely, that the cortexolone-receptor complex has altered physicochemical properties, in comparison to the ^{3}H-TA-receptor complex, and that this modified macromolecule either does not penetrate the nucleus, or has a very low affinity for specific glucocorticoid binding sites in the nucleus. It is not yet known whether inhibitors of estrogens and androgens, which prevent the binding of these hormones to specific receptors (4, 5) in target tissues, act in a manner comparable to that found for cortexolone in the present study.

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REFERENCES:

1. Schering Workshop on Steroid Hormone 'Receptors', Advances in the Biosciences, Vol. 7, G. Raspé, ed. (Pergamon Press, Vieweg, 1971).
2. O'Malley, B.W., Spelsberg, T.C., Schrader, W.T., Chytil, F. and Steggles, A.W., Nature 235, 141 (1972).
3. Yamamoto, K.R., Fed. Proc. 31, 429 (1972).
4. Wyss, R. H., Karszniz, R., Heinrichs, W.L. and Herrmann, W.L., J. Clin. Endocr. Metab. 28, 1824 (1968).
5. Fang, S. and Liao, S., J. Biol. Chem. 246, 16 (1971).
6. Swaneck, G.E., Chu, L.H., and Edelman, I.S., J. Biol. Chem. 245, 5382 (1970).
7. Munck, A. and Wira, C., in Advances in the Biosciences, Vol. 7, G. Raspé, ed. (Pergamon Press, Vieweg, 1971), p. 301.
8. Baxter, J.D. and Tomkins, G.M., in Advances in the Biosciences, Vol. 7, G. Raspé, ed. (Pergamon Press, Vieweg, 1971), p. 331.
9. Samuels, H.H. and Tomkins, G.M., J. Mol. Biol. 52, 57 (1970).
10. Melnykovych, G. and Bishop, C.F., Endocrinology 88, 450 (1971).
11. Rosen, J.M., Fina, J.J., Milholland, R.J. and Rosen, F., J. Biol. Chem. 245, 2074 (1970).
12. Erdos, T., Biochem. Biophys. Res. Commun.32, 338 (1968).
13. Jensen, E.V., Suzuki, T., Numata, M., Smith, S., and DeSombre, E.R., Steroids 13, 417 (1969).
14. Jensen, E.V., Suzuki, T., Kawashima, T., Stumpf, W.E., Jungblut, P.W. and DeSombre, E.R., Proc. Nat. Acad. Sci. USA 59, 632 (1968).
15. Kaiser, N., Milholland, R.J., Turnell, R.W., and Rosen, F., Manuscript in preparation.