INTRACELLULAR BINDING OF CORTICOSTERONE IN THYMUS TISSUE[1]

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Received May 13, 1969

Summary

The intracellular distribution of radioactivity was determined in rat thymus cells 15, 45 and 90 minutes after the animals had received intraperitoneal injections of corticosterone-1,2-H^3. Cytoplasmic and nuclear fractions were isolated and the nuclei were fractionated to yield various components of the chromatin structure. Radioactivity of cytoplasmic fractions decreased rapidly from an initial high level at 15 minutes. The concentration of radioactivity associated with histones and with material solubilized by DNAse increased during the time interval studied. This observation suggests a possible specific receptor site for glucocorticoids in thymocyte nuclei.

It has been proposed that the primary action of steroid hormones is mediated by interaction with a specific receptor molecule in the cells of target tissues. Binding of glucocorticoids by subcellular fractions of thymus in in vitro systems has been demonstrated by De Venuto (1) and by Brunkhorst (2). Munck and Brinck-Johnsen (3) have presented evidence, obtained from an in vitro study, for a non-specific rapidly dissociable binding of glucocorticoids and a much smaller slowly dissociating fraction which they propose consists of molecules responsible for glucocorticoid activity. The report does not suggest a location of the receptor site but does demonstrate that the interaction is dependent on cellular ATP levels.

The present report describes preliminary results of intracellular distribution of corticosterone in rat thymus 15, 45 and 90 minutes after administration of hormone.

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[1]The investigation was supported by Grant GB-6849 from the National Science Foundation.

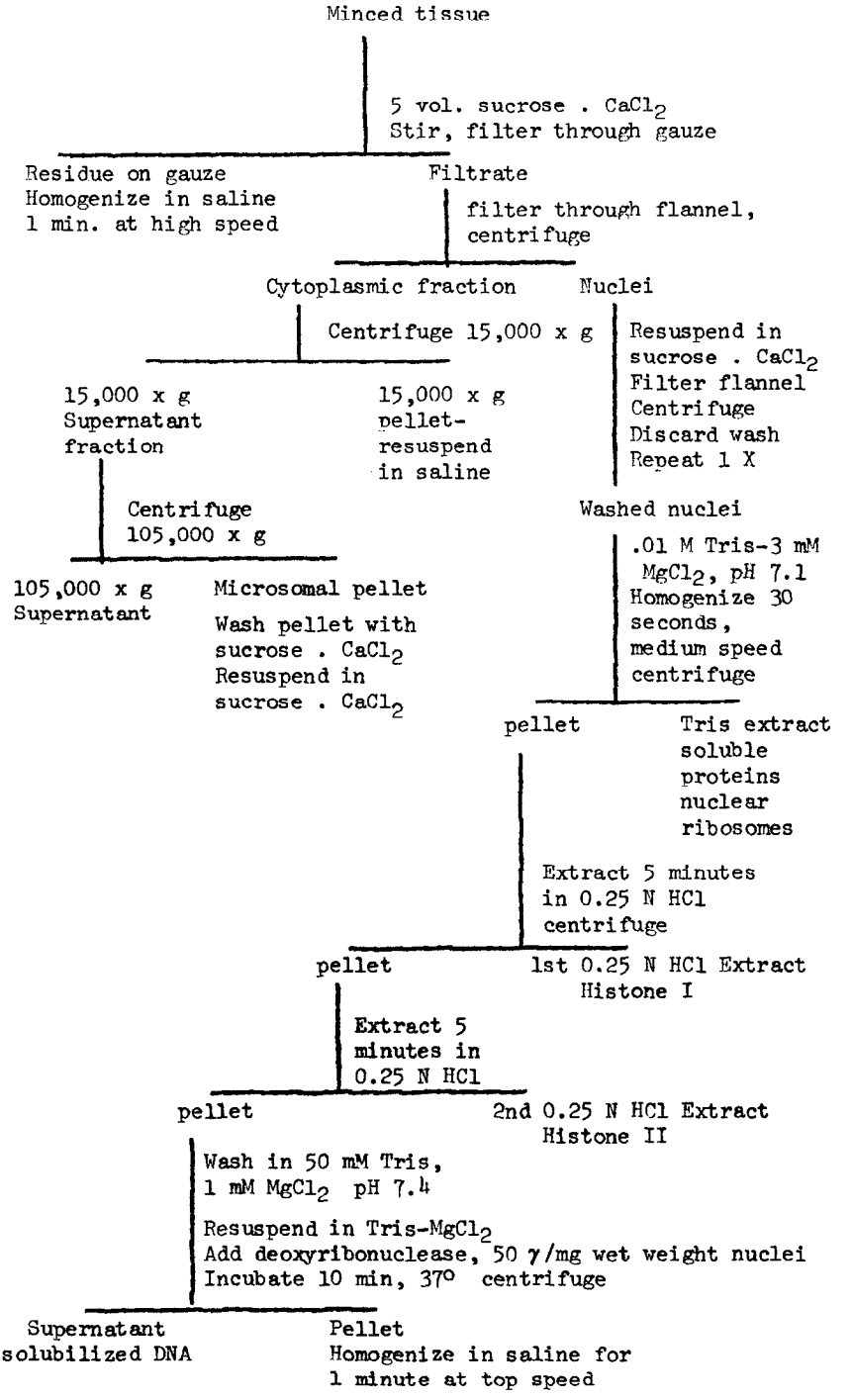

Fig. 1. Procedure followed for fractionation of thymus tissue.

Methods

Male Sprague-Dawley rats, 40-45 days old, were adrenalectomized and maintained on Purina rat chow and 0.9% saline for 5 days. 1,2-H^3-corticosterone, specific activity 57.2 C/mole, was obtained from New England Nuclear Corporation. The labeled steroid was taken to dryness and transferred in ethanol to a saline solution containing unlabeled corticosterone. One ml of saline, containing 33 μc and 1γ hormone was injected intraperitoneally into each rat.

At 15, 45 and 90 minutes after injection of the corticosterone ten animals at each period were decapitated and the thymuses removed and chilled immediately. All subsequent operations were carried out at 4°.

Thymuses were cleaned, minced and forced through a Harvard tissue press. The procedure used for fractionation of the tissue is outlined in Figure 1. Amounts of radioactivity in the fractions were determined as follows: (A) 0.5 ml aliquots of serum, whole nuclei, gauze residue homogenate and the DNAse insoluble residue were placed in scintillation vials along with 0.5 or 1.0 ml of 1 N NaOH. The samples were heated at 75°C for 1 hour, and neutralized with Biosolve #2 solubilizer (Beckman Instruments Co.) before 10 ml of scintillation fluid (prepared from Liquifluor, New England Nuclear) were added to each vial. (B) 0.5 ml aliquots of all other fractions were solubilized in 1.0 ml of Biosolve #3 solubilizer and 10 ml of scintillation fluid were added to each vial. Radioactivity of the samples was determined by counting in a liquid-scintillation spectrometer. Counting efficiency was determined by adding internal standard to the vials after the initial counts were taken. Radioactivity in the whole nuclear fraction and the fraction solubilized by DNAse is expressed as dpm/mg DNA positive material. The method of Webb (4) was used to determine the amount of DNA. Activities of other fractions are expressed as dpm/mg protein, the method of Lowry *et al.* (5) being used to measure the protein.

Results and Discussion

Recoveries of radioactivity from all the cellular subfractions indi-

cated that 0.1% of the injected corticosterone was present in thymus. The data for the distribution of radioactivity in the cell fractions and the changes in concentration with increasing intervals of time after injection of corticosterone are given in Table I.

Table I

Intracellular Distribution of Radioactivity following Injection of Corticosterone-1,2-H^3 into Rats

Cytoplasmic	15 MIN	45 MIN	90 MIN
Total cytoplasm	5553 ± 1281	2325 ± 813	1013 ± 85
15,000 x g supernatant	5915 ± 1825	2487 ± 998	1035 ± 169
105,000 x g supernatant	8263 ± 1693	3090 ± 865	1447 ± 107
15,000 x g sediment	644 ± 171	286 ± 49	94 ± 14
Microsomes	441 ± 245	225 ± 137	111 ± 41
Nuclear			
Intact nuclei	295 ± 39	247 ± 8	135 ± 18
Tris extract	419 ± 124	512 ± 170	438 ± 120
Histone I	67 ± 25	60 ± 16	49 ± 17
Histone II	209 ± 71	224 ± 71	247 ± 111
DNAse soluble	70 ± 30	81 ± 36	128 ± 52

Radioactivity in intact nuclei and in DNAse soluble fractions is expressed as dpm/mg DNA positive material. Activities of other fractions are expressed as dpm/mg protein

Values given are means ± S.E.

See Fig. 1 for explanation of fractions

The gauze residue fraction represents cytoplasmic and nuclear components of reticular tissue (6). It was calculated that 40% of the radioactivity in the entire thymus was present in this fraction. No attempt has been made at this time to obtain subfractions of this material.

The highest concentration of radioactivity was present in the 105,000 x g supernatant of cytoplasm. It is possible that this represents non-

specific binding of the hormone or its metabolites since the concentration decreased rapidly from its initial high levels. Fifteen minutes after administration of hormone particulate fractions of cytoplasm have high specific activities relative to some of the nuclear fractions. However the loss of radioactivity with time from microsomes and the 15,000 x g sediment, which contains mitochondria, occurs at a rate very similar to that of the soluble cytoplasmic fraction. This distribution of radioactivity is similar to that reported by Litwack, Sears and Diamondstone (7) for cytoplasmic fractions of liver.

The most significant observations were made with subfractions of thymocyte nuclei. Calculated curves of best fit for the data are drawn in Figure 2 for histone I, histone II and the material solubilized by DNAse. The activity of histone I decreases slowly from a maximum at 15 minutes as shown by the negative slope of the curve. On the other hand the observations indicate that histone II and the DNAse fraction accumulate radioactivity over the time interval studied. The variations from experiment to experiment is such, however, for these fractions that one is not permit-

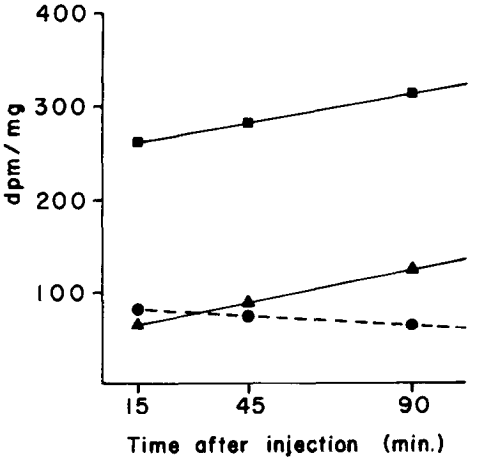

Fig. 2. Concentration of radioactivity in nuclear fractions of rat thymocytes after following injection of corticosterone 1,2-H^3 ●- - ● Histone I; ■——■ Histone II; ▲——▲ DNAse soluble fraction. Histones dpm/mg protein, DNAse dpm/mg DNA.

ted to say that the slopes differ significantly from zero. It should be noted that the specific activities of histone II are always approximately four times those of histone I. The histones in Fraction II are assumed to be more tightly bound to DNA since they were not solubilized during the first extraction with HCl. This higher specific activity in the histones more tightly bound to DNA, and the concentration of radioactivity with time in this fraction and in the DNAse fraction (which is 90% DNA by analysis) suggests that these components of chromatin could contain a specific receptor for the hormone.

From their experimental data Munck and Brinck-Johnsen have determined m, the saturation concentration of cortisol in the specific fraction, as 4×10^{-8} moles per liter of cells. Each thymus cell contained 5,000 specifically bound molecules at saturation. Using the data reported in this paper, it was calculated that thymus cells contained approximately 3,000 molecules corticosterone per cell and nuclei before fractionation contained approximately 1,000 molecules per nuclei. This value seems in rather good agreement with that of Munck and Brinck-Johnsen when one considers that equilibrium conditions are disrupted during fractionation procedures and saturation of all sites is probably not maintained.

It is difficult to relate the radioactivity found in the Tris extract to the discussion of chromatin as the possible receptor site. The specific activities of this fraction are higher than for other nuclear fractions, with the concentration reaching maximum at 45 minutes. Jensen and coworkers have reported binding of estradiol to a specific fraction in nucleoplasm of uterine cells (8).

Attempts are being made to identify the radioactive material present in thymus. Thymus tissue itself does not contain enzymes involved in metabolism of glucorticoids in liver (9). However, it is possible metabolites present in blood are present in thymus. It has been reported that thymus tissue contains 11β-OH dehydrogenase so that 11-dehydrocorticosterone is

a possible metabolite also. Munck and Brinck-Johnsen (3) report that most of the steroid in their specific fraction is in the form of cortisol, the hormone added to incubation medium. If the chromatin is the site of binding of this specific fraction then the radioactivity associated with it should be corticosterone. However, positive identification must be made by extracting the radioactive material and identifying it by chromatographic procedures.

Acknowledgment

The author thanks Mr. Lawrence Sikora for his capable technical assistance.

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