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PROGESTERONE RECEPTOR IN THE CHICK THYMUS

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Summary: Specific, high affinity binding sites for progesterone and promegestone /R-5020/ have been shown to be present in the chick thymus measured by experiments with intact cells or under cell-free conditions. In isolated thymocytes most of the receptor-R-5020 complex is bound to the nucleus. Dissociation constants were determined in thymic cytosol by Scatchard plot analysis and were found to be 3.1 and 2.6 nM for progesterone and R-5020, respectively. On the basis of competition assays the binding sites seemed to be specific for progesterone and R-5020. Glucocorticoids bind only slightly and only at high concentrations. By gel-filtration experiments the thymic R-5020 binding site was shown to be a macromolecule. In vivo treatment of chicks with progesterone or R-5020 caused a significant increase in thymidine kinase activity of the thymus.

INTRODUCTION

Progesterone has been reported to have antiglucocorticoid action in a number of steroid sensitive systems including lymphoid cells /1,2/. On the other hand, some progesterone analogs possess glucocorticoid-like activity in vivo since they can cause thymus involution or induction of tyrosine aminotransferase in the rat /3/. Furthermore, R-5020 , a potent synthetic progestational agent was shown to be equipotent with cortisol in inhibiting PHA-stimulation of human peripheral lymphocytes /4/.

Abbreviations used are: R-5020, dimethyl-19-nor-pregna--4,9-diene-3,20 dione, 17α ,21 [17α -methyl ^{3}H] ; TA, triamcinolone acetonide; Dex, dexamethasone; PHA, phytohemagglutinin; EDTA, ethylenediamine tetraacetate; DDT, dithiothreitol; BSA, bovine serum albumin; TK, thymidine kinase.

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Steroid hormones are believed to exert their biological effects via the receptor system. One obvious possibility for antiglucocorticoids to act is by competing with the glucocorticoids for binding to their receptors and thus inhibiting hormone action. This hypothesis was supported recently /5,6/. However, the glucocorticoid-like effects of progestational agents can not be explained using this concept. The mechanism of action of these agents might be the following: progestational agents bind to glucocorticoid receptor so that the complex formed can be translocated to the nucleus and increase the rate of specific mRNA synthesis. This possibility would mean that glucocorticoid receptors are not absolutely specific for glucocorticoids. Another alternative is that progesterone or progestational agents have specific receptors in the given cells. The aim of the present study was to investigate the latter possibility in the chick thymus.

MATERIALS AND METHODS

Chemicals: [1,2,4-^{3}H]-triamcinolone acetonide /TA, 21 Ci/mmole/, [1,2-^{3}H]-dexamethasone /Dex, 26 Ci/mmole/, [1,2,6,7-^{3}H]-progesterone /110 Ci/mmole/ were purchased from Amersham, [17α-methyl ^{3}H]-R-5020 /promegestone, 87 Ci/mmole/ from New England Nuclear. The following compounds were purchased: non-labeled steroids from G. Richter Ltd. /Hungary/ and Merck Sharp and Dohme /N.J./, Norit A, Triton X-100 and dithiothreitol from Serva, bovine serum albumin from Sigma, Sephacryl S-200 /superfine/ from Pharmacia. All other chemicals were from REANAL /Hungary/.

Methods: Determination of steroid binding in cell-free system was performed as described elsewhere /7/. In brief, cytosol was prepared in 10 mM Tris-HCl, 1.5 mM EDTA, 2 mM dithiothreitol and incubated for 6 h at 0^o with various concentrations of the labeled steroids in the presence or absence of 0.5-5 μM non-labeled steroid. Free steroid was

removed by charcoal /Norit A, activated/.Binding capacity and dissociation constants were obtained from Scatchard analysis of the data /8/. Determination of steroid binding in whole cells and nuclear translocation of the hormone-receptor complex was performed as described previously /9/. Competition experiments: Determination of steroid binding in the cytosol /at 0° for 6 h/ or in whole cells /at 37°C for 40 min/ was performed in the absence or presence of the competing unlabeled steroids.

Sephacryl S-200 chromatography was performed as described elsewhere /7/. A 1.5x20 cm column was loaded with 1.4 ml cytosol labeled with [^{3}H]-R-5020 and washed successively with 10 mM Tris, 1.5 mM EDTA, 2 mM dithiothreitol, 300 mM KCl. Fractions /1.1 ml/ were collected and assayed for radioactivity and protein content. The column was calibrated with dextran blue, BSA, cytochrom c and [^{32}P]-Na-phosphate. Protein was determined by the Coomassie brilliant blue method /10/.

The effect of in vivo treatment with progesterone or R-5020 was monitored by measuring thymidine kinase /TK/ activity in the thymus 48 h after injecting the chicks with 0.5 mg/100 g b.w. of the steroid. TK activity was assayed according to Bresnick /11/. In vitro treatment of isolated thymus cells was performed by incubating the cells ± 1 µM progesterone or R-5020 at 37° for 20 h. Then cells were washed, sonicated and TK activity assayed in the cytosol.

RESULTS

Progesterone and R-5020 binding sites were found by incubating these steroids with chick thymus cytosol at 0-4°C for 6 h[1]. The high affinity binding sites were saturable /Fig. 1/ and could be displaced by the addition of excess unlabeled progesterone or R-5020. Scatchard analysis of the data revealed a single class of binding sites for both steroids with a K_d 3.1 nM for progesterone

1. This time was sufficient to reach equilibrium even at the lowest steroid concentrations.

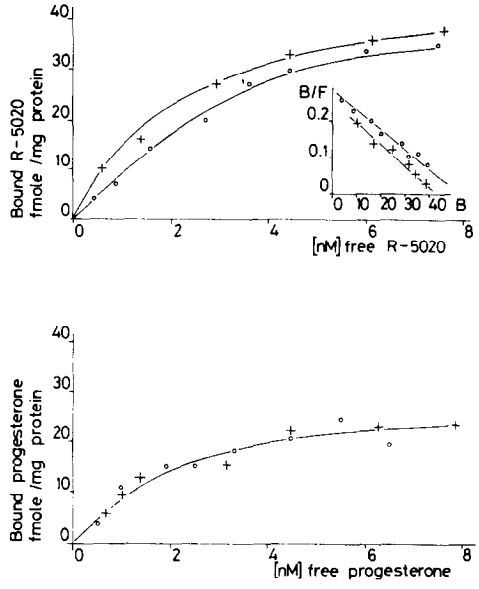

Fig. 1. Specific binding of $[^3H]$-R-5020 and $[^3H]$-progesterone to chick thymus cytosol. Cytosol was incubated for 6 h at 0-4°C with various concentrations of $[^3H]$-steroids in the presence /o——o/ or absence /x——x/ of 0.1 μM dexamethasone. The binding data were analysed according to Scatchard.

and 2.6 nM for R-5020. Since it is known that both progesterone and R-5020 have high affinity for glucocorticoid receptor in some mammalian lymphoid tissues /5/ and breast cancer cells /4/ the same experiments were performed after incubating the cytosol for 1 h at 0°C with saturating concentration /0.1 μM/ of Dex. Analysis of the data resulted in similar K_d-s /Fig. 1/. Specific binding of $[^3H]$-R-5020 was 96 % of that of the control cytosol /incubated without Dex/.

In order to establish the specificity of this binding, competition experiments were performed with various steroids. It can be seen in Fig 2 that progesterone and R-5020 were

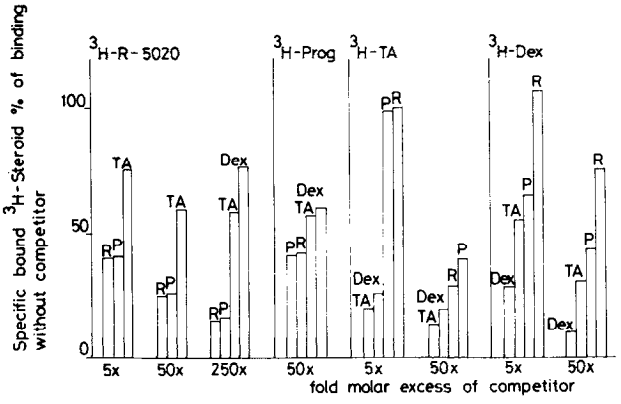

Fig. 2. The binding of [^{3}H] -steroids to chick thymus cytosol; competition by unlabeled R-5020 /R/, progesterone /P/, triamcinolone acetonide /TA/, and dexamethasone /Dex/, in 5, 50 and 250 fold excess. The concentration of the [^{3}H]-steroids was 20 nM. **Specific binding of [^{3}H] -steroids in the absence of unlabeled steroids was taken as 100 %.**

equipotent in competing for [^{3}H] -R-5020 binding sites. TA competed less and Dex only very poorly even at high concentrations. Dex competed stronger for [^{3}H] -progesterone binding than for [^{3}H]- R-5020 binding sites. Similar results were obtained from cross-competition experiments. Unlabeled R-5020 competed better for [^{3}H]-TA binding than for [^{3}H]-Dex binding /the latter was almost negligible/.

The nature of [^{3}H]-R-5020 interaction with the chick thymus cytosol was also studied by gel filtration experiments. Cytosol was incubated with 50 nM [^{3}H]-R-5020 in the presence or absence of 0.1 μM unlabeled Dex and then chromatographed on a Sephacryl S-200 column. Protein bound radioactivity eluted in a single peak at the void volume. The size of this peak decreased only very slightly when excess of unlabeled Dex was present during the incubation /Fig 3/. This

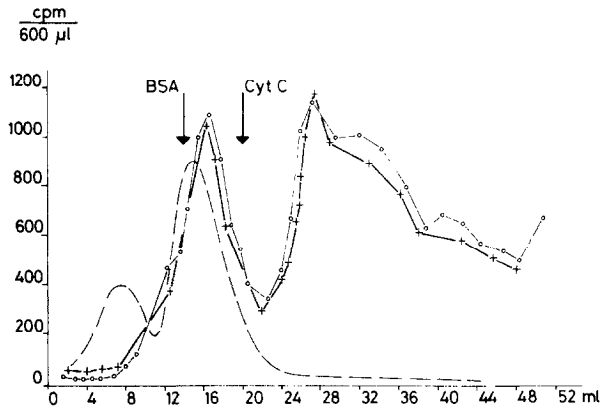

Fig. 3. Sephacryl S-200 chromatography of the chick thymus cytosol receptor-R-5020 complex. The cytosol was equilibrated with [^{3}H] -R-5020 in the presence /x——x/ or in the absence /o——o/ of 1 µM unlabeled dexamethasone and chromatographed as described in Methods. 600 µl of each fraction was transferred into scintillation vial. Optical density at 280 nm is shown by dashed line.

peak was completely eliminated when 5µM unlabeled R-5020 was present during the incubation /not shown/.

In further experiments [^{3}H] -R-5020 binding of isolated thymic lymphocytes was studied at 37°C. The isolated cells specifically bound R-5020 in all experiments performed. This binding seemed to be specific for R-5020 since only non-labeled R-5020 could compete effectively /29 % of the total radioactivity remained bound when cells were incubated with 250 fold excess of cold R-5020/. 5µM Dex given together with [^{3}H] -R-5020 competed very slightly /85 % of the total radioactivity remained bound/. In order to determine if R-5020-receptor complex can be translocated to the nucleus specific binding in cell fractions were measured after incubating the cells with [^{3}H] -R-5020. 76 % of the total

specific binding was found in the nuclear fraction. The ratio of the nuclear transfered receptor-hormone complexes did not change when cells were pre-incubated with 50 nM unlabeled Dex at 37°C for 30 min. /nuclear transfer = 72 %/.

In vivo treatment of chicks with progesterone or R-5020 caused a significant increase in TK activity of the thymus. TK activities were 1.52 ± 0.3; 3.96 ± 0.5 and 2.34 ± 0.4 U/mg protein in the control, progesterone and R-5020 treated chicks, respectively. In vitro experiments resulted in a similar increase in TK activity of isolated thymocytes following treatment with 1 μM progesterone or R-5020. TK activity was 340 and 170 % of control value for progesterone and R-5020, respectively.

DISCUSSION

Our results suggest the existence of specific progesterone and R-5020 binding macromolecules in chick thymus. Since isolated thymic lymphocytes bind R-5020 with the same high affinity as cytosol does, it seems inpossible that only epithelal cells are target for these sex steroids as it was found for the bursa Fabricius /12/. In this respect the chick thymocytes seem to be different from either rat thymus cells or human peripheral lymphocytes where others failed to demonstrate high affinity binding sites for sex steroids /5, 13/.

The progesterone- and R-5020 binding sites found in our experiments differ from the glucocorticoid receptor of chick thymocytes since incubation of the cells or the cytosol with concentrations of Dex saturating glucocorticoid receptors decreased the amount of bound progesterone or

R-5020 only very slightly. Chromatographic experiments confirmed this statement /Fig 3/.

The results of competition experiments suggest that there are at least two specific binding sites in the chick thymus: one of them binds progesterone and R-5020 with high affinity /and glucocorticoids with very low affinity/ and the other, the glucocorticoid receptor, which also can bind progesterone and R-5020 with lower affinity.

Although it is plausible to assume that these binding sites are functional receptors the following alternatives may arise. Some enzyme involved in progesterone metabolism or an intracellular "transcortin-like" protein may behave like "receptor". However, the fact that in cells incubated with the labeled hormone at 37°C most of the specific bound radioactivity could be measured in the nuclear fraction makes these possibilities unlikely.

The fact that treatment with progesterone or R-5020 caused an increase in TK activity of the thymus prove that these steroids have biological activity on the chick thymus, which is manifested through the receptors described here.

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