Retinol binding protein in rat testicular cells

J. Huggenvik and M. D. Griswold

Program in Biochemistry, Washington State University, Pullman, Washington 99164, U.S.A.

Summary. Cellular retinol-binding protein (CRBP) was identified in the cytosols of cultured Sertoli cells and peritubular cells from the testes of 20-day-old rats. CRBP was not detected in spermatids or spermatocytes obtained from the testes of 60-day-old rats. Cultured Sertoli cells and peritubular cells contained up to a 5-fold enrichment of CRBP/mg protein compared to whole testis homogenates. FSH- or FSH + testosterone-treated cultures of Sertoli cells showed a 60% increase in the specific activity of CRBP when compared to untreated cultures.

Introduction

Vitamin A is required for normal male reproductive function (Thomson, Howell & Pitt, 1964). In vitamin A-deficient rats, spermatogenesis is arrested and can be alleviated by the administration of retinol (Moore, 1967). It has been proposed that the action of vitamin A is mediated by a cellular binding protein in target tissue (Bashor, Toft & Chytil, 1973) which then may alter gene expression and protein glycosylation (Chytil & Ong, 1978; Adamo, DeLuca, Silverman-Jones & Yuspa, 1979). In homogenates of whole rat testes, a cellular retinol-binding protein (CRBP) and a cellular retinoic acid-binding protein (CRABP) have been reported (Bashor et al., 1973; Ong & Chytil, 1978) and retinol has been shown to affect the synthesis of an androgen-binding secreted by rat Sertoli cells in culture (Karl & Griswold, 1980).

In this study the different cell types of the seminiferous tubule were screened for the presence of CRBP and the amount of CRBP in cultured Sertoli cells and peritubular cells was determined. In addition the effect of FSH and testosterone on the amount of CRBP in cultured Sertoli cells was examined.

Materials and Methods

Male Sprague–Dawley rats were obtained from the Laboratory Animal Resource Center at Washington State University. [3H]Retinol (sp. act. 4.5 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, Massachusetts). All other chemicals were from Sigma (St Louis, Missouri), Beckman (Anaheim, California), and Amersham Co. (Chicago, Illinois).

Cell culture

Cultures of Sertoli cells from 20-day-old rats were prepared as described by Dorrington & Fritz (1975) with the modifications of Wilson & Griswold (1979). The Sertoli cells were maintained in culture for 6 days before use. After 2 days in culture the medium was changed and FSH (0.1 µg NIH-S12/ml) and testosterone (0.1 µg/ml) were added to some cultures.

Testicular peritubular cells were obtained from the supernatant after collagenase digestion of the tubules (Wilson & Griswold, 1979). The peritubular cells were plated in tissue culture flasks.

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in Ham’s F-12 culture medium (Gibco, New York) containing 10% calf serum and subsequently subcultured once before use. Germinal cells were isolated from 60-day-old rat testes as described by Dorrington & Fritz (1973).

**Cytosol preparation and incubation**

The medium was aspirated from the cultures of Sertoli cells and peritubular cells and the cells were gently removed from the culture plates with a rubber policeman. The cells were suspended in Hanks’ balanced salt solution (HBSS) and were centrifuged for 5 min at 1400 g at 0°C. The germinal cells were suspended directly into HBSS and centrifuged. The pellet was transferred to a glass teflon homogenizer with a 1 ml 50 mM Tris–HCl buffer, pH 7.5, and homogenized for 3–5 min at 4°C. The homogenate was then centrifuged at 47 000 g for 30 min at 4°C. The supernatant (cytosol) was used in the incubation studies.

[3H]Retinol (40 pmol) was added in 5 μl ethanol to 0.3–0.6 ml aliquots of cytosol. The volume of the cytosol added was varied to adjust for differences in protein concentration. All incubation mixtures were made up to 0.6 ml with buffer. The mixtures were incubated at 4°C for 12–16 h. In binding competition studies 8 nmol unlabelled retinol or unlabelled retinoic acid were added in 5 μl 95% ethanol before the incubation; ethanol alone was added to the control mixtures.

**Sucrose gradient centrifugation**

The binding protein was analysed on a linear 5–20% sucrose gradient in 50 mM Tris–HCl, pH 7.5, as previously described by Bashor et al. (1973). The polyallomer tubes (Beckman) were centrifuged 18–24 h at 189 000 g at 4°C.

Fractions (0.5 ml) were collected from the gradient. Samples of each fraction (50 μl) were added to 5 ml scintillation cocktail (ACS, Amersham Co.) and the radioactivity was determined in a Beckman LSC scintillation counter at 45% counting efficiency. The protein content was determined by the colorimetric method described by Bradford (1976).

**Results**

Sucrose gradient centrifugation of the Sertoli cell cytosol after incubation with [3H]retinol showed a peak of radioactivity sedimenting at 2S (Text-fig. 1). When Sertoli cell cytosol was incubated with [3H]retinol in the presence of a 200-fold excess of unlabelled retinol, the radioactive binding peak was eliminated (Text-fig. 1). Unlabelled retinoic acid in a 200-fold excess failed to compete with [3H]retinol for binding to CRBP.

Cultured rat testicular peritubular cells also exhibited a 2S peak of bound radioactive retinol (Text-fig. 2). CRBP in peritubular cell cytosol has the same ligand specificity for retinol as in Sertoli cells (data not shown). The peritubular cells appeared by light microscopy to be fibroblasts or myoid cells and there were no discernible Sertoli cells (Tung, Dorrington & Fritz, 1975).

The germinal cell preparations consisted primarily of spermatids and spermatocytes which were at the leptotene stage of prophase or more advanced stages. Less than 5% of the germinal cells were spermatogonia or preleptotene spermatocytes (Dorrington & Fritz, 1973) and little or no CRBP was detected in the cytosol fraction (Text-fig. 3).

The linear binding of [3H]retinol to CRBP in the range of 1–10 pmol is demonstrated in Text-fig. 4 and a summary of cytosol binding data is presented in Table 1.

FSH or testosterone treatment of the Sertoli cell cultures affected the amount of detectable CRBP (Text-fig. 5). The cytosol from the hormone-treated cells bound 100% more [3H]retinol
Text-fig. 1. Sucrose gradient centrifugation of rat Sertoli cell cytosol after incubation with 40 pmol [³H]retinol (●) in the presence or absence of a 200-fold excess of unlabelled retinol (■) or retinoic acid (▲). Samples were incubated for 14 h then centrifuged for 24 h at 189 000 g. ---, Total protein/100 µl.

Text-fig. 2. Sucrose gradient centrifugation of peritubular cell cytosol from the testes of 20-day-old rats after incubation with 40 pmol [³H]retinol (●). Samples were incubated for 14 h then centrifuged for 24 h at 189 000 g. ■, Total protein/100 µl.
Text-fig. 3. Sucrose gradient centrifugation of germinal cell cytosol from the testes of two 60-day-old rats after incubation with 40 pmol [3H]retinol (●). Samples were incubated for 16 h then centrifuged for 24 h at 189 000 g. ■. Total protein/100 μl.

Text-fig. 4. The linear relationship between the amount of CRBP and the volume of rat Sertoli cell cytosol.

Table 1. Cellular retinol-binding protein in rat testicular cells

<table>
<thead>
<tr>
<th>Age of rat</th>
<th>Tissue</th>
<th>pmol CRBP/mg protein</th>
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<tbody>
<tr>
<td>20 days</td>
<td>Sertoli cells</td>
<td>13·3 ± 3·5 (9)</td>
</tr>
<tr>
<td>20 days</td>
<td>Testicular peritubular cells</td>
<td>20·1 ± 3·4 (4)</td>
</tr>
<tr>
<td>20 days</td>
<td>Testis homogenate</td>
<td>3·7 ± 0·4 (3)</td>
</tr>
<tr>
<td>Adult</td>
<td>Testis homogenate</td>
<td>3·9 ± 0·8 (3)</td>
</tr>
</tbody>
</table>

Values are mean ± s.d. for the no. of determinations in parentheses. All assays were done so that the binding of [3H]retinol was 3–7 pmol/assay.
Text-fig. 5. Sucrose gradient centrifugation of rat Sertoli cell cytosol after incubation with 40 pmol [3H]retinol and with FSH (○), FSH and testosterone (△), or without any hormone added to the culture medium (○). Samples were incubated for 14 h, then centrifuged for 24 h at 189 000 g.

Discussion

Sertoli cells and testicular peritubular cells contained a cellular retinol-binding protein (CRBP) which demonstrated saturable and specific binding to retinol. We did not estimate the binding constant for the CRBP because Ong & Chytil (1978) reported a value of 1.6 × 10⁻⁸ M; since our experiments were done in 7 × 10⁻⁸ M-retinol the $K_d$ in our system was probably similar. Spermatids and spermatocytes have no detectable CRBP activity and the action of retinol or the maintenance of spermatogenesis may therefore be confined to the non-germinal elements of the testis. However, we have not assayed for CRBP in enriched populations of spermatogonia. In addition, this assertion is based on the premise that retinol has biological actions only on cells which contain a CRBP.

The specific activity of cytosol from isolated Sertoli cells and peritubular cells is 3–5 times greater than in cytosol obtained from whole testis homogenates, again suggesting that the germinal cell mass does not contribute to the total CRBP. The specific activity of the CRBP from the cultured cells is greater than has been reported for other tissue (Ong & Chytil, 1975).
Ong & Chytil (1975) reported that the testis cytosol bound 3-1 pmol [3H]retinol/mg protein. We obtained similar values of 3-7 and 3-9 pmol for homogenates of testis from 20-day-old and adult rats, respectively. The cultured Sertoli cells and fibroblasts bound up to 20 pmol/mg protein and represent nearly a 5-fold enrichment of the binding activity.

It is possible that the action of FSH on CRBP synthesis is specific, although the general synthesis of proteins in Sertoli cells which was measured by the incorporation of radioactive amino acids has been reported to show a 2-fold stimulation in the presence of FSH (Wilson & Griswold, 1979). The stimulation of the synthesis of CRBP probably therefore reflects the stimulation of a number of actively synthesized proteins. The addition of testosterone has no significant effect on protein synthesis or the amount of CRBP activity.

In vitamin A-deficient rats, Rich & de Kretser (1977) have reported that the production of androgen-binding protein (ABP) is greatly decreased and they suggested that this was a result of impaired Sertoli cell secretory function since ABP and several other glycoproteins are actively secreted by Sertoli cells. It has also been shown that the secretion of these proteins in Sertoli cell cultures can be stimulated by the synergistic actions of FSH, insulin and retinol (Karl & Griswold, 1980). As shown in Text-fig. 5, FSH can stimulate the amount of CRBP in cultured Sertoli cells. Perhaps the stimulation by one hormone of the binding protein for another biologically active agent is part of the mechanism of this synergism.

It has been proposed that retinol can act in the nucleus in a manner similar to steroid hormones and it has been demonstrated that retinol can affect protein glycosylation (Chytil & Ong, 1978; Adamo et al., 1979). Phosphorylated retinol has been shown to incorporate mannose and other sugars (Adamo et al., 1979). Retinol may be involved in the transfer of these sugars to glycoconjugates. Experiments with vitamin A-deficient hamsters showed a 79% decrease in mannose incorporation into liver glycoproteins (DeLuca, Bhat, Sasak & Adamo, 1979). The results of our experiments do not support any particular theory concerning the mode of action of vitamin A but the action of retinol on testicular cells in vivo and in culture is consistent with a mode of action which affects protein glycosylation.

References


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