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Biochemical aspects of the interaction of androgens with Sertoli cells

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Summary. Cultured rat Sertoli cells have been shown to contain the components of an androgen-responsive system. A macromolecule distinguishable from androgen binding protein and exhibiting a K_d of 6.3 nM for ^{3}H-testosterone has been found in Sertoli cell cytosol. Effectiveness of unlabeled steroids in competing for ^{3}H-testosterone binding sites was found to be testosterone = dihydrotestosterone $>$ R1881 $>$ progesterone = cyproterone acetate $>$ 5 α-androstane-3 α, 17 β-diol $>$ androsterone $>$ estradiol. After labeling the cells in culture with ^{3}H-testosterone, macromolecular bound steroid could be extracted from the nuclear fraction with 0.4 M KCl and chromatographed on Sephadex G-200. Rechromatography of the bound fraction revealed negligible dissociation of the complex. Specific binding to the nuclear fraction was saturable with an apparent Kd of 2.2 nM. Chromatin prepared from Sertoli cell nuclei bound 3-4 times as much cytoplasmic androgen-receptor complex as did thymus or liver chromatin. The binding was optimal at 0.1 M NaCl and exhibited two binding components when the assay was performed in the presence of a constant total protein concentration. The high affinity component exhibited a K_d of 8 pM and 6 fmoles of sites/mg DNA. These data indicate that the Sertoli cell has the capacity to respond to androgens in a manner similar to that described for other steroid target cells.

Introduction.

The recent expansion of biochemical knowledge concerning hormone action in the testis has supported the concept that the Sertoli cell mediates hormonal effects on spermatogenesis (see Steinberger E. (1975), Steinberger E. *et al.* (1977) and Hansson *et al.* (1975) for reviews). Thus Sertoli cells bind FSH and respond to this hormone with elevated cAMP levels and secretion of an androphilic protein called androgen binding protein or ABP (Steinberger E. *et al.*, 1977). That the Sertoli cell can also respond to androgen (and hence indirectly to LH) was suggested by the fact that under certain circumstances production of ABP can also be regulated by androgens (Elkington *et al.*, 1975 ; Means *et al.*, 1976 ; Louis and Fritz, 1977). Furthermore, testicular androgen receptor-like macromolecules which persisted following hypophysectomy and germ cell depletion have been described (Hansson *et al.*, 1974 ; Mulder *et al.*, 1974).

This presentation summarizes our evidence that Sertoli cells are target cells

for androgen action. We show that macromolecules with the properties of androgen receptors can be found in the cytoplasmic fraction from cultured Sertoli cells prior to incubation with steroid and in the nuclear fraction after incubation with steroid. We also show that cultured Sertoli cells contain chromatin acceptor sites for androgen-receptor complexes.

Materials and methods.

Preparation of androgen receptor. — Sertoli cells were isolated from 28-30 d animals and cultured for 2-3 d as described by Steinberger A. *et al.* (1975). The cytoplasmic form of the receptor was prepared as described elsewhere except that the precipitate obtained at 40 p. 100 saturation with $(NH_4)_2SO_4$ was collected, redissolved and desalted on a Sephadex G-25 column (Sanborn *et al.*, 1977). The void volume eluted from this column was used as cytoplasmic receptor. Androgen-receptor complex for use in the chromatin acceptor assay was prepared from the testes of 14-21 d hypophysectomized rats 60 d of age of surgery by a similar procedure (Tsai *et al.*, 1977).

To obtain the nuclear form of the receptor, cells were washed and then incubated with ^{3}H-testosterone (1, 2, 6, 7,-^{3}H, 85 Ci/mmole, New England Nuclear) for 30 min at 37 °C, and then treated by a procedure involving homogenization, centrifugation and subsequent washing of the pellets once with 0.3 p. 100 Triton X-100 and twice with buffer (Sanborn *et al.*, 1977). The pellets were extracted with 0.4 M KCl, 1 mM EDTA, 0.01 M Tris HCl pH 7.4 and/or ether. The resulting pellets were used to determine DNA and extracts were counted as described elsewhere (Sanborn *et al.*, 1977).

Chromatin acceptor assay. — Nuclei from thymus and liver of 30-31 d old rats were isolated by procedures described by Tsai *et al.* (1977). Sertoli cell nuclei were obtained from cultured cells by lysing the cells in 5 mM $MgCl_2$, collecting the nuclei by centrifugation through 0.25 M sucrose, washing the nuclei with 0.3 p. 100 Triton X-100 and purifying the nuclei by centrifugation through a discontinuous gradient composed of 2.2, 1.8, and 1.6 M sucrose in buffer (Tsai *et al.*, 1977). Chromatin was prepared as described elsewhere (Tsai *et al.*, 1977), using 0.08 M NaCl-0.02 M EDTA pH 6.4 to lyse the nuclei and washes of 0.3 M NaCl and 1.5 mM NaCl-0.15 mM Na citrate pH 7.0. Sertoli cell chromatin preparations were subjected to an additional centrifugation through 1.7 M sucrose.

Initial studies involved incubation of chromatin preparations (100-200 μg DNA) with ^{3}H-testosterone-receptor complex at 26 °C for 1 hr in 0.15 M NaCl, 0.5 mM EDTA, 5 mM Tris HCl pH 7.4. More recent studies employed optimized incubation conditions (see figure legends). After the desired interval, cold 0.15 M NaCl-10 mM $MgCl_2$ was added and the suspensions were centrifuged (15 000 $\times$ g, 20 min). The precipitated chromatin was washed twice by a similar procedure and the radioactivity was extracted with ether, evaporated, and counted (Tsai *et al.*, 1977).

Results and discussion.

The cytoplasmic form of the androgen receptor. — Cytosol fractions from cultured Sertoli cells were labeled with ^{3}H-testosterone either prior to the ultracentrifugation

step or subsequent to chromatography, depending on the purpose intended. Yields using either procedure were approximately equivalent, indicating the relative stability of the receptor during the separation procedures. The androgen-receptor complex was clearly distinguishable from ABP by electrophoresis on 0.5 p. 100 agarose/3.3 p. 100 polyacrylamide gels (Sanborn *et al.*, 1977). ABP exhibited an Rf of 0.79 while the receptor complex exhibited an Rf of 0.43. This behavior is consistent with the behavior of receptor complexes isolated from whole testes (Hansson *et al.*, 1974). Furthermore, incubation of the labeled complexes with excess unlabeled steroid for 30 min at 0 °C prior to electrophoresis abolished binding in the ABP area but did not affect that in the receptor area, consistent with the shorter $t_{\frac{1}{2}}$ of dissociation of the ABP complex (Sanborn *et al.*, 1975).

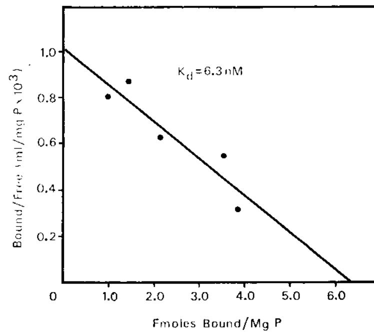

FIG. 1. — *Scatchard plot of the specific binding of* ^{3}H-*testosterone to cytosol prepared from cultured Sertoli cells.* Aliquots of cytosol were incubated with 0.5-9 nM ^{3}H-testosterone or labeled steroid plus 100-fold molar excess of unlabeled steroid at 0° for 18 hr. Samples were applied to 0.5 × 7 cm Sephadex G-25 columns, aliquots were collected and counted, and the counts eluting in the void volume were calculated. Specific binding was considered to be the difference between binding of the labeled steroid in the absence and in the presence of unlabeled steroid.

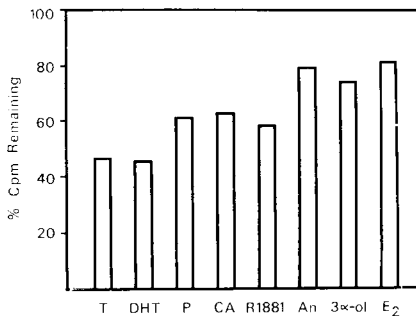

FIG. 2. — *The ability of 100-fold molar excess of unlabeled steroids to compete with* ^{3}H-*testosterone for binding sites in Sertoli cell cytosol.* ^{3}H-testosterone (9 nM) and unlabeled steroids were mixed prior to the addition of cytosol. After 18 hr at 0°, bound steroid was estimated as described for Figure 1. T : testosterone, DHT : 17 β-hydroxy-5 α-androstan-3-one ; P : progesterone ; CA : 1, 2 α-methylene-6-chloro-4,6-pregnadien-17 α-ol-3, 20-dione-17-acetate ; RI881 : 17 β-hydroxy-17 α-methyl-estra-4, 9, 11-trien-3-one ; An : 3 α-hydroxy-5 α-androstan-17-one ; 3 α-ol : 5 α-androstane-3 α, 17 β-diol ; E_2 : 17 β-estradiol.

Figure 1 shows that binding of androgen to the cytosol fraction from Sertoli cells was saturable with a K_d in the nM range. The concentration of binding sites was usually in the range of 10 fmoles/mg protein. The binding sites showed specificity for androgens, with the greatest competition exhibited by testosterone and dihydrotestosterone, but progesterone also competed with ^{3}H-testosterone for binding sites (fig. 2). This order of specificity is similar to that reported elsewhere for binding at the nuclear sites in Sertoli cells (Sanborn *et al.*, 1977). The interaction of progestogens with androgen receptors in other target tissues has been described at some length by Bardin *et al.* (1975).

The nuclear form of the androgen receptor. — Accumulation of label into the nuclear fraction from cultured Sertoli cells was temperature-and time-dependent (Sanborn *et al.*, 1977). Binding was also saturable with an apparent K_d of 2.2 nM for both 0.4 M KCl extractable and total specific binding with 0.14 and 0.30 pmoles of binding sites/mg DNA, respectively (Sanborn *et al.*, 1977). In general, about 30 p. 100 of the radioactivity was extractable with 0.4 M KCl in 1 hr ; larger amounts could be extracted by incubation for an additional 16 hr. The material extracted with 0.4 M KCl eluted from a Sephadex-200 column in the void volume (~ 33 p. 100) and as unbound steroid (67 p. 100) (fig. 3). The proportion of label in the bound fraction could be increased by prior extraction of the nuclei with 0.1 M KCl, but the total amount of bound label remained essentially constant (data not shown). Rechromatography of the material eluting in the void volume did not result in further dissociation of the complex (fig. 3).

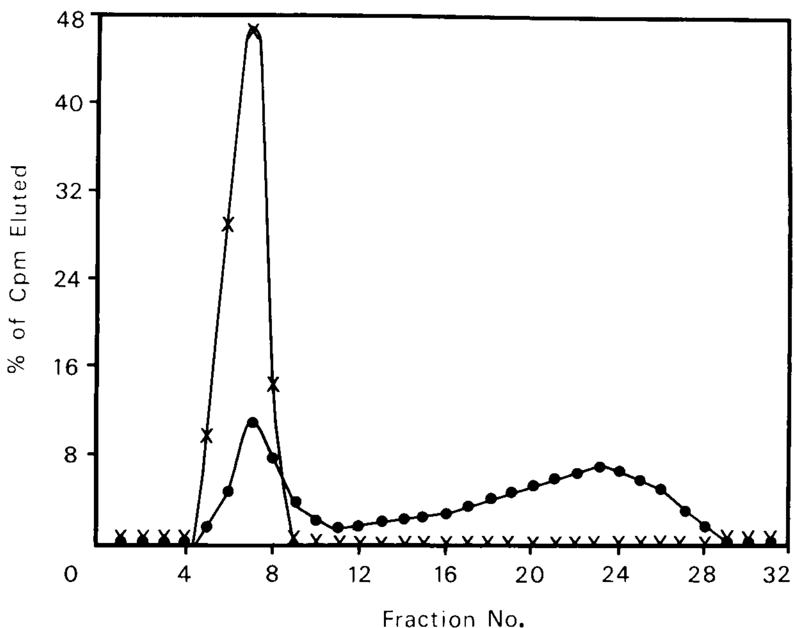

FIG. 3. — *The elution pattern on Sephadex* G-200 *of a* 0.4 M KCl *extract* (1 hr, 4 °C) *of the Sertoli cell nuclear pellet after exposure of the cells to* ^{3}H-*testosterone.* ●—●—●, initial extract; X-X, an aliquot from the void volume (fractions 5-8) rechromatographed on the same column after standing 4 hr at 0 °C.

Chromatin acceptor sites for androgen-receptor complexes. — Chromatin acceptor sites for androgen-receptor complexes have been found in preparations from rat testis by Klyzsejko-Stefanowicz *et al.* (1976) but the cell types involved were not delineated. While attempting to purify Sertoli cell nuclei in preparation for chromatin isolation, we noted that the nuclei obtained from cultured cells did not sediment through 2.2 M sucrose. The Sertoli cell nuclei sedimented at the 1.6-1.8 M and 1.8-2.2 M sucrose interfaces of a discontinuous sucrose gradient while the residual germ cell nuclei sedimented through 2.2 M sucrose at 120 000 × g. The use of Sertoli cells from 2 day cultures combined with this gradient separation yielded a preparation containing 95 p. 100 recognizable Sertoli cell nuclei (Tsai *et al.*, 1977). The chromatin preparations isolated from purified nuclei of rat liver, thymus and cultured Sertoli cells exhibited acid-soluble protein/DNA ratios of 1.0, 0.98 and 1.2, respectively. Using a fixed amount of labeled receptor complex with 200 μg chromatin DNA, Sertoli cell chromatin bound 3-4 times as much ^{3}H-T receptor complex as did the chromatin from thymus or liver (Tsai *et al.*, 1977). Using a fixed amount of chromatin and increasing amounts of labeled cytosol complex, a biphasic Scatchard plot of binding was observed at 0.15 M NaCl, 26 °C. Subsequent experiments showed the optimal temperature and time for incubation to be 15 °C for 1 hr (Tsai *et al.*, manuscript in preparation). Figure 4 shows that the optimal salt concentration using androgen-receptor complex which had been precipitated with $(NH_4)_2SO_4$ was 0.1 M NaCl.

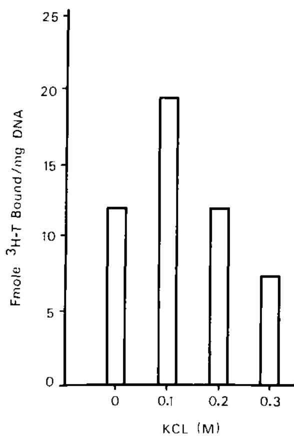

FIG. 4. — *Variation in the amount of* ^{3}*H-testosterone-androgen receptor complex bound to Sertoli cell chromatin* (200 *μg DNA*) after incubating for 1 hr at 15 °C in the presence of the NaCl concentration indicated, 0.5 mM EDTA and 5 mM Tris-HCl pH 7.4. Bound complex was estimated as described in Methods.

A binding analysis was subsequently performed on data obtained after incubation of receptor complex with a fixed amount of Sertoli cell chromatin. Total protein concentration was kept constant by addition of heat-inactivated cytosol processed in a manner identical to that used for the fractionation of the receptor complex. Figure 5 shows that under these conditions, the Scatchard plot was still biphasic. Resolution of the binding components yielded a K_d of ~ 8 pM for the high affinity component

with 6 fmoles of binding sites/mg DNA. These data indicate that binding sites of limited capacity and high affinity are present in the chromatin obtained from cultured Sertoli cells.

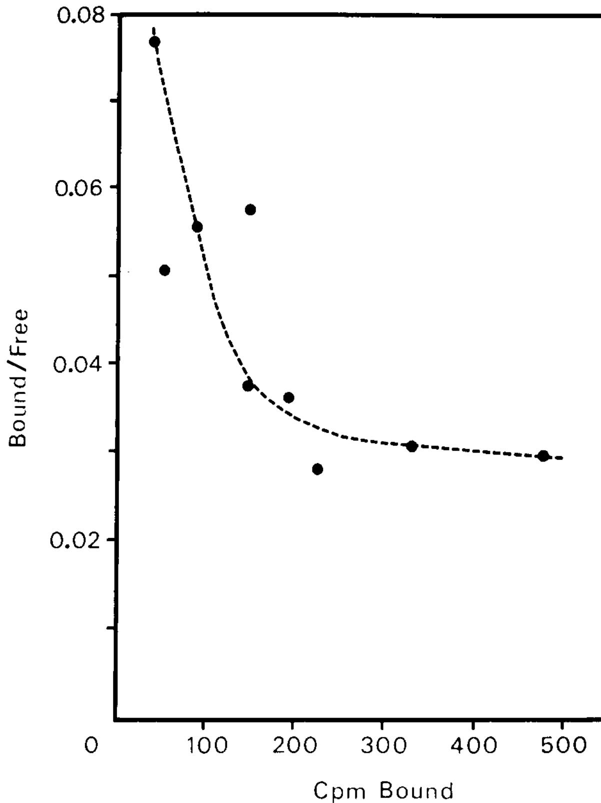

FIG. 5. — *Scatchard analysis of the binding of* ^{3}*H-testosterone-androgen receptor complex* (3-195 *pM*) *to Sertoli cell chromatin* (100 *μg DNA*) *after incubation at* 15 °*C for* 1 *hr in the presence of* 0.10 *M NaCl.* The total protein concentration was kept at 4.5 mgP/ml by the addition of heat -inactived cytosol concentrated in a manner identical to that used for active cytosol. The specific activity of ^{3}H-testosterone was 8.5×10^4 cpm/pmole.

Conclusions.

The Sertoli cell has been demonstrated to have the capacity to respond to androgen in a manner similar to that described for other steroid hormone target tissues. (Baulieu, *et al.*, 1975 ; Buller and O'Malley, 1976). It possesses a cytoplasmic form of androgen receptor which is distinguishable from ABP. After incubation of cells with labeled hormones, a nuclear form of receptor can be isolated. Finally, the chromatin from cultured Sertoli cells possesses a limited number of high affinity acceptor sites for cytoplasmic androgen-receptor complex. *In vivo* (Elkington *et al.*, 1975 ; Means *et al.*, 1976) and *in vitro* (Louis *et al.*, 1977) evidence suggests that the production of

ABP by Sertoli cells is regulated in part by androgens. Together these data provide strong evidence that Sertoli cells are primary target cells for androgen action in the testis.

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Résumé. Les cellules de Sertoli de rat en culture contiennent les éléments d'un système sensible aux androgènes. Le cytosol des cellules de Sertoli contient une macromolécule différente de l'ABP et présentant un K_d de 6,3 nM pour la ^{3}H-testostérone. L'efficacité compétitive des stéroïdes froids sur les sites de liaison avec la ^{3}H-testostérone est dans l'ordre : testostérone = dihydrotestostérone > R1881 > progestérone = acétate de cyprotérone > 5 α-androstane-3 α, 17 β-diol > androstérone > estradiol. Après marquage des cellules en culture par la ^{3}H-testostérone, le stéroïde lié à la macromolécule peut être extrait de la fraction nucléaire par du KCl 0,4 M et une chromatographie sur Séphadex G. 200. Une seconde chromatographie de la fraction liée n'entraîne qu'une dissociation négligeable du complexe. La liaison spécifique à la fraction nucléaire est saturable, avec un K_d apparent de 2,2 nM. La chromatine préparée à partir des noyaux des cellules de Sertoli a un pouvoir de liaison avec le complexe cytoplasmique liant les androgènes 3 à 4 fois supérieur à la chromatine du foie ou du thymus. La liaison est optimale dans du NaCl 0,1 M et on met en évidence deux composants quand l'expérience est réalisée en présence d'une concentration protéique totale constante. Le composant à forte affinité se définit par un K_d de 8 pM et une concentration de 6 fmoles de sites/mg ADN. Ces résultats montrent que la cellule de Sertoli a la capacité de répondre aux androgènes de la même manière que celle décrite pour les autres cellules cibles des stéroïdes.

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