OESTROGEN INDUCED LEYDIG CELL REFRACTORINESS TO GONADOTROPHIN STIMULATION

By

J. M. Saez, F. Haour, B. Loras, P. Sanchez and A. M. Cathiard

ABSTRACT

In vivo administration of oestradiol to male rats modifies plasma LH, FSH and testosterone levels, cAMP and testosterone production and DNA synthesis in isolated interstitial cells. Intramuscular injection of oestradiol benzoate (Oe2B) at the dose of 1 or 100 µg/day for 6 days induced a 5- and 10-fold decrease in plasma testosterone, respectively, and a 2-fold decrease in plasma LH and FSH. Plasma testosterone was already significantly decreased 2 h after the Oe2B injection at which point the plasma LH and FSH levels were not yet significantly decreased. In vivo steroidogenic responsiveness to hCG evaluated by plasma and testicular contents was already significantly lower than that of controls 2 h following oestradiol administration. Thereafter response to hCG progressively decreased during the 6 days of 1 or 100 µg oestradiol treatment, reaching 30 and 10 %, respectively, of that of controls on the last day. On the contrary the testicular cAMP content 2 h after hCG injection was significantly higher in oestradiol treated animals than in controls after 24 h. The number of hCG binding sites in isolated Leydig cells decreased to approximately 50 % of that of controls on days 3 to 6 following Oe2B treatment. In vitro testosterone production by isolated interstitial cells, either under basal conditions or under stimulation by hCG or N6O2 dibutyryl adenosine 3'5'-monophosphate (DbcAMP), was lowered as early as 2 h following the injection of Oe2B to the animals. From 1 to 6 days following Oe2B administration, testosterone secretion, in response to both stimuli, was approximately 4 times lower than that of the control animals. Paradoxically, by the second day of oestrogen treatment, basal and hCG induced in vitro cAMP production by interstitial cells was significantly higher than controls despite a significant decrease in the number of binding sites. The incorporation of thymidine into interstitial cells DNA were decreased following 2 days of oestrogen administration. However, the
conversion of pregnenolone to testosterone was unchanged. These inhibitory effects of oestradiol were not overcome by simultaneous administration of hCG.

These results strongly suggest that the rapid inhibitory action of oestradiol on interstitial cell function, steroidogenesis, and DNA synthesis, occurs at the testicular level. Changes observed in gonadotrophin receptor sites or in plasma LH levels may have a long term effect on the steroidogenic refractoriness to hCG. However this refractoriness is primarily related to an abnormality of some step of steroidogenesis beyond cAMP formation.

The inhibitory effects of oestrogenic hormones on testicular function are well known, but explanations of the mechanisms by which such inhibition occurs have been contradictory. Several investigators have suggested that in both men and animals oestrogens produce their testosterone lowering effect through indirect action on the hypothalamic-pituitary axis. This conclusion has been based on the fact that administration of oestrogens induced a concomitant decrease of both plasma testosterone and LH levels (Bartke et al. 1977; Bayns et al. 1974; de Jong et al. 1976; Verjans et al. 1974), and that in vivo administration of LH or hCG\(^1\) overcame the inhibitory effect both on plasma testosterone (Reiter & Kulin 1975; Van Beurden et al. 1977) and on in vitro testicular steroidogenesis (Slaunwhite et al. 1962). Results reported by other group suggest on the contrary that oestrogens produce their inhibitory effects via direct action at testicular levels. Evidence cited is that these steroids produced a decrease in plasma testosterone levels without any significant modification of plasma (Chowdhury et al. 1974; Danutra et al. 1973; Jones et al. 1975; Sholiton et al. 1975; Tcholakian et al. 1974) and that testicular tissue from oestrogen-treated animals (Murota et al. 1966; Oshima et al. 1967; Samuels et al. 1964, 1969; Sholiton et al. 1975) produce less testosterone than that of controls.

In order to clarify these contradictory results we have investigated the effects of in vivo administration of oestradiol on interstitial cells steroidogenesis and DNA synthesis in rats. In addition we have studied the in vivo and in vitro responsiveness of interstitial cells from oestradiol treated animals to stimulation by hCG and dibutyryl cAMP.

**Material and Methods**

**Chemicals**

[\(6-^{3}H\)]Thymidine (27 Ci/mmol) was obtained from C. E. A., Saclay, France, [\(4-^{14}C\)]-pregnenolone (24 mCi/mmol), [\(1,2,6,7-^{3}H\)]testosterone (S. A. 90 Ci/mmol) and carrier free Na\([^{125}I\]) were from Amersham, England. Pregnyl\(\textregistered\) (hCG) was from Organon. Purified hCG was a gift from Serono Laboratories, Italy (13 400 IU/mg), from the NIH (CR 119 hCG) and from Dr. A. Bosch, Oss, Netherlands (15 700 IU/mg). All the other biochemical reagents were obtained from Sigma Chemical Company.
**Animals**

Male Sprague-Dawley rats aged 55 to 60 days were used in all experiments. The rats were housed at 22 ± 2°C on a 12:12 h light-dark cycle. Animals were killed by cervical dislocation between 09.00 and 10.00 h. For each experimental group at least 4 rats were used. hCG (Pregnyl) dissolved in saline and oestradiol benzoate (Oe₂B) dissolved in sesame oil were injected im. All the experiments were repeated at least 2 times to ensure that the qualitative pattern of effects was reproducible.

**Incorporation of radioactive thymidine into DNA**

Rats were given an ip injection of 50 to 100 µCi of [³H]thymidine and killed exactly 2 h later. The tunica albuginea of the excised testes was removed. Interstitial cells and seminiferous tubules were prepared by a modification (Evain et al. 1976) of the method described by Catt & Dufau (1973). Both isolated interstitial cells and seminiferous tubules were submitted to 3 washes, followed each time by centrifugation at 100 × g for 20 min. Then, each pellet was transferred to about 50 ml of 0.1 m Tris-HCl buffer, pH 7.4, containing 6 mM 2-mercaptoethanol and 1 mM EDTA, homogenized and centrifuged at 800 × g for 10 min. The pellet was re-suspended in 50 mM Tris-HCl buffer pH 7.4 and 3 aliquots of 1 ml were separated to determine the specific activity of DNA by the method described elsewhere (Saez et al. 1977a).

**Testicular testosterone and cAMP content**

Testes were removed, decapsulated and immediately immersed in 30 ml of ethanol at −60°C containing [³H]cAMP (≈ 2000 cpm) and [³H]testosterone (≈ 2000 cpm) and kept at this temperature for 1 h. Thereafter the testes were homogenized in the same solvent. After centrifugation at 5000 × g for 10 min the supernatant was saved, and the pellet extracted again with ethanol. The pool of supernatants was evaporated. Half of the extract was chromatographed on a Dowex 1-X2 column (0.6 × 6 cm, 200–400 mesh, Cl⁻ form) using the method described by Mao & Guidotti (1974) and the fraction containing the [³H]cAMP was recovered and evaporated. After acetylation (Harper & Brooker 1975) the cAMP content was measured by radioimmunoassay (Steiner et al. 1972). The testosterone content of the other half was estimated by specific radioimmunoassay after purification in a microcelite column (Forest et al. 1973).

**In vitro studies with isolated interstitial cells**

The interstitial cell suspension were prepared as indicated above. The density of Leydig cells in each suspension was assessed by a differential count of smeared cells stained with May-Grünwald Giemsa. For each of the experiments described below, the number of interstitial cells from different treated groups was similar (± 6 %/s).

a) Testosterone and cAMP production was studied by incubating triplicate aliquots of the cells in MEM medium (Eurobio), pH 7.4, containing 20 mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid), bovine serum albumin (0.1 mg/ml) and 0.1 mM MIX (3-isobutyl-1-methylxanthine) (buffer A). This was done in a Dubnoff shaker for 2 h at 33°C in an atmosphere of O₂:CO₂ (95:5). After incubation, one aliquot

1) The following trivial names and abbreviations are used: testosterone: 17β-hydroxy-4-androsten-3-one; pregnenolone: 3β-hydroxy-5-pregnen-20-one; hCG: human chorionic gonadotrophin; cAMP: adenosine 3',5'-cyclic monophosphate; DbcAMP: N₈O₂ dibutyryl adenosine 3',5'-cyclic monophosphate.
was removed for testosterone determination by direct radioimmunoassay (Forest et al. 1973). The remaining medium was centrifuged in tubes containing 5 volumes of ice-cold ethanol and [3H]cAMP (≥ 2000 cpm). The supernatant was dried by evaporation. The cAMP content was estimated as indicated above.

b) Conversion of pregnenolone into testosterone. About 0.15 and 0.2 μCi of [14C]-pregnenolone dissolved in 50 μl of propanediol were added to the interstitial cells suspended in 2 ml of buffer A. The incubations were carried out as indicated above and were stopped by the addition of 10 ml of ethyl acetate containing 100 μg of non-radioactive testosterone. After shaking for 1 min the ethyl acetate was separated and evaporated to dryness. Testosterone was purified as previously described (Saiez et al. 1977b).

c) The binding of [125I]hCG was measured in interstitial cell particles. Isolated cells were homogenized using a polytron PT 10 homogenizer in 10 mM Tris buffer, pH 7.8, containing 1 mM CaCl₂, 1 mM MgCl₂, 1 mM 1,4-dithioerythritol, and 0.25 M sucrose. The particles were obtained by centrifuging the homogenate at 20,000 x g for 30 min. The pellet was re-suspended in the same buffer and kept at −18°C until used.

hCG was labelled with 125I using the lactoperoxidase method previously described (Haour & Saxena 1974). Specific activity was in the range of 100 μCi/μg. Binding was measured at three different dilutions of interstitial cell particles in the presence of saturating concentrations of [125I]hCG (≥ 5 × 10⁻⁹ M). The incubation was performed in 0.3 ml of 25 mM Tris-HCl buffer, pH 7.4, containing 1 mM CaCl₂, 1 mM MgCl₂ and 20 kalikrein units/ml (Zymophren®, Specia, proteolytic enzyme inhibitor). After incubation at 37°C for 1 h (equilibrium conditions) bound and unbound hormone were separated as previously described (Haour & Saxena 1974; Haour & Saiez 1977). All binding experiments were performed in triplicate. Three additional samples were also run in the presence of a 100-fold excess of unlabelled hCG for estimation of the non-specific binding.

Other methods

Plasma testosterone was estimated by specific radioimmunoassay after purification in a microcelite column (Forest et al. 1973). Rat serum LH and FSH were measured according to the NIAMD directives, except that an immunosorbent as described by Wide (1969) was used. The reagents for radioimmunoassay were kindly provided by the Rat Pituitary Hormone Distribution Program, NIAMD. Standards used were NIAMD rat LH RP 1 and NIAMD rat FSH RP 1. The sensitivity for LH varied from 0.8 to 1 ng/ml and the intra- and inter-assay variations were 7.9 and 15 %, respectively. DNA was determined using the method of Burton (1956).

RESULTS

Effects of Oe₂B administration on plasma testosterone, LH and FSH levels

Plasma testosterone levels decreased significantly ($P < 0.05$) 2 h after the injection of 1 μg of Oe₂B (Fig. 1, upper left panel). By 48 h after administration, testosterone levels were about 5 times lower than those of control animals and they remained so. In contrast, plasma LH and FSH levels 2 h after Oe₂B administration were higher than those of controls, but only the increase of FSH
Effect of *in vivo* administration of oestradiol benzoate (Oe2B) on plasma testosterone (upper panel), LH and FSH (lower panel). Oe2B 1 μg/day (left panel) or 100 μg/day (right panel) were administered for 2 h to 6 days to 57 days old rats. The values are the mean ± sp of those obtained in 4 rats. * Different from control (*P* < 0.05).

was significant (*P* < 0.05) (Fig. 1, lower left panel). After 48 h however, both plasma LH and FSH levels were significantly lower, about one half that of controls, and remained lower during the rest of the experiment. The lowering effect of 100 μg of Oe2B on plasma testosterone is more marked than that of 1 μg, but these differences were less pronounced on plasma gonadotrophins. This discrepancy of the effects of Oe2B on plasma testosterone and gonadotrophin levels suggests that the lowering effect of Oe2B on testosterone levels is not primarily due to a decrease in plasma LH and FSH.

*In vivo and in vitro testicular responsiveness of Oe2B treated rats to hCG*

We have previously shown (*Haour & Saez 1977*) that in rats a peak of plasma testosterone is observed between 1 and 2 h after a single injection of 500 IU of hCG. Two h after administration of 1 μg Oe2B, the *in vivo* steroidogenic responsiveness to hCG of interstitial cells was already significantly decreased (Fig. 2, upper panel, continuous line). Thereafter the responsiveness progressively decreased, and after 6 days of Oe2B treatment it reached 30% of that of controls. The effects of 100 μg of Oe2B are more pronounced.

Steroidogenic refractoriness to hCG following administration of Oe2B was further demonstrated by the fact that, 2 h after the injection of hCG, testicular testosterone content of rats pre-treated with Oe2B was significantly lower than that of control animals (Fig. 2, middle panel). Indeed, testicular testosterone content of Oe2B treated rats which did not receive any hCG (Fig. 2, middle panel, black bars) was significantly lower than that of control as soon as 2 h
after Oe2B injection. In contrast, testicular cAMP content of Oe2B treated rats whether or not they received hCG was similar to that of controls during the first 12 h. Thereafter, the cAMP content after hCG injection was significantly higher in Oe2B treated rats than in control rats (Fig. 2, bottom panel).

Oestradiol treatment at high doses induced a significant decrease in the apparent number of hCG binding sites in interstitial cell particles. Binding sites were 50\% lower than those of controls after 3 days of Oe2B treatment (Fig. 3, upper panel). The effects of 1 µg (data not shown) were less marked than those of 100 µg. These results could suggest that the partial refractoriness of Leydig cells after Oe2B administration could be related to the decrease in hCG binding capacity.

\[\text{Fig. 2.}\]

*In vivo* testicular responsiveness to hCG of oestradiol treated rats. Oe2B 1 µg/day (open bars middle and lower panels) or 100 µg/day (dotted bars middle and lower panels) were administered for 2 h to 6 days. At the time indicated half of the animals (4 rats of each group) were injected with saline, while the other half received 500 IU of hCG. Animals were sacrificed 2 h later. *Upper panel:* Plasma testosterone levels after hCG. Basal values, similar to those of Fig. 1 are not shown. *Middle panel:* Testicular testosterone content of rats who received hCG (whole bar) or saline (bottom black part of each bar). *Lower panel:* Testicular cAMP content of rats who received hCG (whole bar) or saline (bottom black part of each bar). • Significantly different from control \((P<0.05)\).
Effects of in vivo administration of Oe₂B (100 µg/day) on plasma testosterone levels (●), and on the in vitro binding of [¹²⁵I]hCG by interstitial cell particles (▼) (upper panel). on in vitro testosterone (middle panel) and cAMP productions (lower panel) by isolated interstitial cells under several conditions. Four rats were used for each group. The values for testosterone and cAMP productions by interstitial cells are the mean ± s.d. of triplicate determinations of a pool of 8 testes.

This hypothesis was further investigated by studying the in vitro responsiveness of Leydig cells obtained from animals treated in vivo with 100 µg/day of Oe₂B. The results of one representative experiment of the three performed are given in Fig. 3 (middle and lower panels). hCG and DbcAMP had the same effect on cells obtained from untreated control animals. Two h after in vivo Oe₂B administration steroidogenesis of Leydig cells under basal conditions as well as under both hCG or DbcAMP stimulations was significantly lower than that of the controls. Twenty-four h after administration testosterone production was still lower and remained so until the end of that experiment. In contrast, interstitial cell production of cAMP 2 h after administration was
Table 1.

Effects of in vivo administration of Oe₂B, hCG or both on plasma testosterone and specific binding of [¹²⁵I]hCG to interstitial cell particles.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Treatment 2 h before killing</th>
<th>Saline</th>
<th>hCG 500 IU</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma T (ng/ml)</td>
<td>[¹²⁵I]hCG binding: % of control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.8 ± 0.3¹</td>
<td>100 ± 12²</td>
<td>26.2 ± 4.6¹</td>
<td></td>
</tr>
<tr>
<td>Oe₂B</td>
<td>0.2 ± 0.01³</td>
<td>70 ± 6³</td>
<td>7.7 ± 1.8³</td>
<td></td>
</tr>
<tr>
<td>hCG 10 IU + Oe₂B</td>
<td>5.2 ± 0.9³</td>
<td>38 ± 4³</td>
<td>13.2 ± 2.2³</td>
<td></td>
</tr>
<tr>
<td>hCG 500 IU + Oe₂B</td>
<td>2.7 ± 0.5⁴</td>
<td>35 ± 3³</td>
<td>4.7 ± 1.7⁴</td>
<td></td>
</tr>
<tr>
<td>hCG 500 IU + Oe₂B</td>
<td>8.9 ± 1.2³</td>
<td>ND⁶</td>
<td>8.5 ± 1.1³</td>
<td></td>
</tr>
<tr>
<td>hCG 500 IU + Oe₂B</td>
<td>3.5 ± 1.1⁵</td>
<td>ND⁶</td>
<td>3.7 ± 1.9⁵</td>
<td></td>
</tr>
</tbody>
</table>

Adult male rats were pre-treated with oestradiol benzoate (100 µg/day for 2 days) or hCG (single injection) or both. After 2 days, half of the animals received saline and the other four hCG and were killed exactly 2 h later. The binding of [¹²⁵I]hCG was studied only in the interstitial particles prepared from rats only receiving saline.

¹) Mean ± so of 4 rats.
²) Mean ± so of triplicate determinations of a pool of 8 testes.
³) P < 0.01 compared to control group.
⁴) P < 0.01 compared to hCG (10 IU) treated group.
⁵) P < 0.01 compared to hCG (500 IU) treated group.
⁶) Not detectable.

Similar to that of the controls, but thereafter became significantly higher (P < 0.01) under hCG-stimulation. Similar results were obtained in one experiment in which rats received 1 µg/day Oe₂B. However, the inhibitory effect on testosterone production by isolated Leydig cells was less marked (data not shown). These results suggest 1) that oestradiol exerts its inhibitory effects on Leydig cell steroidogenesis beyond cAMP formation; 2) that the effects of oestradiol are not overcome by the acute action of hCG.

Effects of the simultaneous administration of Oe₂B and hCG on Leydig cell function

We have previously shown that in vitro hCG administration induces refractoriness of Leydig cells to gonadotrophin stimulation and a decrease in their hCG binding capacity. These two effects are dose-dependent (Sharpe 1976; Hsueh et al. 1976, 1977; Haour & Saez 1977; Purvis et al. 1977; Saez et al. 1978,
Profile of plasma testosterone after a single injection of 500 IU of hCG in control (upper panel) and Oe2B treated rats (lower panel). All rats of the latter group received Oe2B (100 µg/day) for 6 days, but hCG was injected at different times of oestrogen treatment (i.e. the animals killed 4 days after hCG injection received this hormone on the second day of Oe2B treatment). The values are the mean ± sd of those obtained in 4 rats.

Table 1 shows that oestradiol does not modify the lowering effects of hCG on its own receptors. However, plasma testosterone levels of oestradiol-hCG-treated rats were significantly lower than those of rats treated with hCG alone. These results suggest that the mechanisms by which hCG and oestradiol induce Leydig cells refractoriness are independent.

This hypothesis was further investigated by studying the in vivo pattern of testicular function of Oe2B treated and control animals after a single injection of hCG. In control animals, two main peaks of testosterone at 2 and 96 h after injection were observed (Fig. 4, upper panel). In oestradiol treated rats the peaks were observed at the same time after hCG injection, but the levels were several times lower (Fig. 4, lower panel).
Effects of in vivo administration of hCG or Oe₂B on plasma testosterone levels, on DNA synthesis by interstitial cells and seminiferous tubules and on the conversion of [¹⁴C]pregnenolone to testosterone by interstitial cells.

Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma T ng/ml</th>
<th>[³H]Thymidine incorporation into DNA</th>
<th>Pregnenolone to testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Interstitial cells cpm/µg DNA</td>
<td>Tubules</td>
</tr>
<tr>
<td>Control</td>
<td>1.25 ± 0.22</td>
<td>75 ± 10²</td>
<td>122 ± 12²</td>
</tr>
<tr>
<td>hCG</td>
<td>7.80 ± 0.91</td>
<td>145 ± 12³</td>
<td>132 ± 11</td>
</tr>
<tr>
<td>Oe₂B</td>
<td>0.20 ± 0.05</td>
<td>40 ± 5³</td>
<td>112 ± 10</td>
</tr>
<tr>
<td>hCG + Oe₂B</td>
<td>2.69 ± 0.80</td>
<td>60 ± 11⁴</td>
<td>118 ± 13</td>
</tr>
</tbody>
</table>

Male rats 46 days old were treated with 500 IU hCG (single injection) or oestradiol benzoate (100 µg/day for 2 days) or both. Half of the rats received 100 µCi of [³H]-thymidine and were killed 2 h later. The testes of the rats of each group were pooled and the specific activity of DNA in isolated interstitial cells and tubules was determined. The interstitial cells isolated from the other 6 rats not injected with [³H]thymidine were incubated for 4 h at 33°C with [¹⁴C]pregnenolone (1.3 x 10⁻⁶ M) and its conversion to testosterone determined.

1) Mean ± sd of 6 rats.
2) Mean ± sd of triplicate determination of a pool of 12 testes.
3) P < 0.01 compared to control group.
4) P < 0.01 compared to hCG treated group.

Effects of in vivo Oe₂B administration on in vitro conversion of [¹⁴C]pregnenolone to testosterone by Leydig cells and on Leydig cell DNA synthesis (Table 2)

The percentage of [¹⁴C]pregnenolone converted to testosterone was not modified by in vivo treatment of either Oe₂B (100 µg/day) or hCG. Taking into consideration the number of interstitial cells used (20 to 25 µg of DNA) and duration of incubation (4 h) [¹⁴C]testosterone formed by interstitial cells from control, hCG-, Oe₂B- and hCG + Oe₂B-treated animals can be calculated as 355, 346, 348 and 365 pg/µg DNA/2 h, respectively. These testosterone values are similar to those produced from endogenous precursors by interstitial cells of control animals under maximal in vitro stimulation (Fig. 3, middle panel).

hCG increased by 100 % the incorporation of thymidine into interstitial cells, but did not modify DNA synthesis in seminiferous tubules (Table 2).
On the contrary, 48 h following Oe₂B administration DNA synthesis was significantly decreased in the interstitial cells. When Oe₂B and hCG were injected simultaneously, the steroid blocked the stimulatory effects of the gonadotrophin.

**DISCUSSION**

It is apparent from the present study that oestradiol at the doses used produces a lowering effect on testosterone levels via a direct action at the testicular level, rather than through an indirect action on the hypothalamic-pituitary axis. This conclusion is based on the one hand on the rapid lowering effect of Oe₂B on plasma and testicular testosterone levels at the time when plasma LH and FSH were normal, and on the other hand on the partial *in vivo* and *in vitro* steroidogenic refractoriness of the interstitial cells to hCG stimulation. *Chowdhury et al. (1974)* and *Tcholakian et al. (1974)* have already shown that oestradiol administration produces a significant decrease on testicular and plasma levels of testosterone within 24 h, without any modification of pituitary and/or plasma LH levels. In contrast, the data reported by other investigators suggest that the inhibitory effect of oestrogenic hormones after 7 to 10 days of treatment on testosterone levels acts through the hypothalamic-pituitary axis (*Boyns et al. 1974; de Jong et al. 1976; Reiter & Kulin 1975; Verjans et al. 1974*).

Recently *Van Beurden et al. (1977)* have reported that 1 and 3 h after injection of 0.5 µg oestradiol, testosterone levels in plasma and testis were decreased. These results are similar to ours. In addition, they also found a decrease in plasma LH levels. Possible explanation for this discrepancy includes differences in strains of rats, in the age or in the radioimmunoassay used to determine LH. However, even if oestradiol induced a fast lowering effect on plasma LH levels, its effect on testicular testosterone production should be overcome by exogenous hCG. This seems not to be the case, since 2 h after simultaneous administration of hCG and Oe₂B, testosterone levels in both plasma and testis were lower than in hCG treated rats.

Interstitial cells contain specific oestradiol receptors (*Brinkmann et al. 1972; Mulder et al. 1976*), but the mechanism by which oestrogens inhibit the steroidogenesis is not clear. Our results show that oestradiol induced a decrease in the apparent number of testicular LH/hCG receptors. However, this cannot by itself explain the partial refractoriness of Leydig cells to gonadotrophin stimulation: First, within one day of Oe₂B treatment alone, Leydig cells became less sensitive to hCG stimulation at the time when the number of apparent receptors was more than 75% of that of the control. Second, in these conditions, the hCG stimulation of cAMP production by isolated interstitial cells from oestradiol-treated animals was significantly higher than that of
controls. Third, the partial steroidalogenic refractoriness of Leydig cells from oestradiol-treated rats was similar after both hCG and DbcAMP stimulation. These results, summarized in Figs. 2 and 3 suggest therefore that the main inhibitory effect of oestradiol on Leydig cells steroidalogenesis is localized beyond cAMP formation.

Indeed, in vitro studies have shown that oestrogens are able to decrease the testicular synthesis of testosterone from several precursors. However, the data concerning the effects of oestrogens added to testicular incubations are contradictory. Sholiton et al. (1975) have shown that diethylstilboestrol decreases the conversion of pregnenolone to testosterone and Yanaihara & Troen (1972) have reported that the conversion of dehydroepiandrosterone to testosterone was decreased by oestradiol. However, in both studies the concentration of oestrogens used was very high. Samuels et al. (1964, 1969) have suggested that the inhibition of the microsomal enzyme activities they observed with concentrations of oestrogens higher than 10^{-6} M were probably not specific.

The inhibitory effects of the in vivo administration of oestrogens on the in vitro testicular steroidalogenesis have been reported by several groups. Samuels et al. (1964, 1969) have noted a decrease in the activities of several enzymes (e.g. 17α-hydroxylase, 17-20-lyase and 17β-hydroxysteroid dehydrogenase) after a week or more of in vivo diethylstilboestrol treatment in the mouse. This inhibitory effect was not overcome by the simultaneous administration of hCG. These investigators concluded that the most probable mechanism seems to be an inhibition of the synthesis of these enzymes. A decreased conversion of Δ^{1}-androstenedione to testosterone but not of that of 17α-hydroxyprogesterone to Δ^{1}-androstenedione, has also been observed with human testicular tissues obtained from men treated for several months with diethylstilboestrol (Slaunwhite et al. 1962), but in these cases the inhibition was, however, overcome by in vivo administration of hCG. Moreover recently Kremers et al. (1977) and Rodriguez-Rigan et al. (1977) have reported data which indicate that long-term treatment with high doses of oestrogens reduces testicular 17α-hydroxylase activity.

From our data, the main inhibitory effect observed after 2 days of Oe₂B treatment on Leydig cell steroidalogenesis seems to be localized before the formation of pregnenolone (Table 2). The discrepancy between our results and those of Samuels et al. (1964, 1969), Kremers et al. (1977) and Rodriguez-Rigan et al. (1977) could be due either to the use of different systems (intact cells in our study) or to the duration of oestrogen treatment (2 days versus 4 days or more).

Further evidence for specific inhibitory action of oestrogens on the function of interstitial cells is the decrease in DNA synthesis in those cells, but not in seminiferous tubules. Furthermore, if hCG by itself stimulated specific DNA synthesis in interstitial cells, it was not able to overcome the inhibitory action
of oestradiol on this synthesis. Although the way by which hCG controls DNA synthesis in interstitial cells is unknown, our results suggest that the inhibitory effect of oestrogen on DNA synthesis is gonadotrophin independent.

In an attempt to reconcile our own results with the other data reported so far, three steps could be postulated for the action of oestrogens on testicular steroidogenesis: the first one is a very fast action during the first hours following administration and seems to be localized beyond cAMP formation. The second one could be a decrease in the synthesis of proteins and enzymes involved in testicular steroidogenesis. This diminution would appear after several days of oestradiol treatment. These two inhibiting processes are not overcome by hCG. The third step involves the lowering effect of oestrogens on plasma LH. This effect is observed only after a rather long period of treatment, and is probably the less important of the three effects.

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REFERENCES

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