Testosterone and 5 α -dihydrotestosterone production *in vitro* by rat testicular tissue treated with oestradiol-17 β

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Summary. Decapsulated adult rat testes were assessed for their capacity to produce testosterone and 5 α -dihydrotestosterone when incubated in the presence of oestradiol-17 β for 3 h. Concentrations of 10⁻⁶ and 10⁻⁸ M-oestradiol-17 β had no significant effect on the production of these hormones and did not alter the capacity of the testes to respond to 100 i.u. hCG *in vitro*. It is suggested that oestradiol-17 β does not directly affect acute regulation of testicular steroidogenesis in the adult rat.

Introduction

Prolonged administration of oestrogens to intact adult male animals results in diminished testicular androgen production and cessation of spermatogenesis (Steinberger, 1971), but controversy has developed over whether such effects are secondary to oestrogenic suppression of pituitary gonadotrophins or are due, at least in part, to a direct effect of oestrogens on the testes. Daily administration of oestrogens to intact adult male rats results in decreased plasma and testicular testosterone concentrations, but no discernible change in circulating LH or FSH levels (Danutra *et al.*, 1973; Chowdhury, Tcholakian & Steinberger, 1974; Moger, 1976). Other workers, however, have shown that while plasma testosterone is reduced in oestrogen-treated male rats, such reductions appear to be due to suppression of plasma LH concentrations (Verjans, de Jong, Cooke, van der Molen & Eik-Nes, 1974; de Jong, Uilenbroek & van der Molen, 1975; van Beurden, Mulder, de Jong & van der Molen, 1977). Since many of these experiments employed similar dosages and time courses of administration of oestrogens the reasons for such conflicting results are not apparent. However, Lee, Bird & Clark (1975) reported that oestradiol-17 β appears to increase the metabolic clearance rate of testosterone in male rats and such a change could affect the results of *in-vivo* studies of testicular testosterone production. While the present work was in progress, Bartke, Williams & Dalterio (1977) reported that rat testes incubated *in vitro* in the presence of hCG and 10⁻⁵ M-oestradiol-17 β released testosterone in amounts similar to those produced by testes incubated without oestradiol-17 β , although hCG and 10⁻⁴ M-oestradiol-17 β significantly reduced testosterone production. The present study confirms and extends the experiments of Bartke *et al.* (1977) in that different concentrations of oestradiol were tested and the production of 5 α -dihydrotestosterone was also examined.

Materials and Methods

Experiment 1. Adult male Sprague–Dawley rats (375–400 g body weight) were killed by decapitation between 12:00 and 14:00 h and the testes were rapidly removed, decapsulated and weighed. Alternating right and left testes were transferred to 25 ml Erlenmeyer flasks containing 2 ml Krebs–Ringer–bicarbonate buffer (Umbreit, Burris & Stauffer, 1972; pH 7.4,

supplemented with 2 mg glucose/ml) and 10^{-6} or 10^{-8} M-oestradiol-17 β (final concentration: see Table 1) while the contralateral testis of each animal was incubated without oestradiol-17 β . The tissue was incubated for 3 h at 33°C in a Dubnoff metabolic incubator (144 cycles/min) under a 95% O₂:5% CO₂ atmosphere. After 30, 60, 90 and 180 min, 0.25 ml aliquots of incubation media were withdrawn from each flask, transferred to 25 ml conical glass extraction tubes containing 0.5 ml absolute methanol, and stored at -6°C until analysed for testosterone and 5 α -dihydrotestosterone content. A 0.25 ml volume of the appropriate fresh incubation medium was used immediately to replace the aliquot removed for analysis.

Experiment 2. To investigate the influence of oestradiol-17 β on the response of testes to hCG stimulation *in vitro*, one testis from each rat was incubated (as above) for 1 h in the presence of 10^{-6} M-oestradiol-17 β (experimental incubates) while the contralateral testis was incubated in buffer minus oestradiol-17 β (control incubates). At the end of the 1-h incubation period, 100 i.u. hCG (Antuitrin "S": Parke Davis & Co., Detroit, Michigan, U.S.A.) in 0.1 ml 0.9% (w/v) NaCl were added to the experimental and control flasks, mixed gently by swirling, and incubations continued for an additional 3 h. At the conclusion of the incubations 0.25 ml aliquots of media were withdrawn and stored at -6°C until analysed for steroid content.

Assays. Steroids were extracted from thawed incubate samples with freshly distilled diethyl ether (3 $\times$ 3 volumes); testosterone and 5 α -dihydrotestosterone were separated by celite column chromatography (Nelson, Latham & Finch, 1975). Testosterone was estimated in duplicate samples by a radioimmunoassay procedure (Finch *et al.*, 1977) employing rabbit antibodies to testosterone-3-oxime thyroglobulin (kindly supplied by Dr R. Horton, University of Southern California School of Medicine). Additionally, because the antibodies to testosterone-3-oxime thyroglobulin exhibit significant (>70%) cross-reactivity with 5 α -dihydrotestosterone (Kinouchi, Pages & Horton, 1973), it was possible to utilize these antibodies for construction of standard curves and duplicate estimation of 5 α -dihydrotestosterone in chromatographed incubation media extracts. Recoveries of testosterone and 5 α -dihydrotestosterone after celite column chromatography of incubation media extracts were $64.3 \pm 2.9\%$ (mean $\pm$ s.e.m., $n = 98$) and $32.0 \pm 3.6\%$ ($n = 124$), respectively. Non-specific blanks of the radioimmunoassay methods, based on use of Krebs-Ringer-bicarbonate buffer carried through the entire method, were 6.4 ± 0.7 pg (mean $\pm$ s.d., $n = 6$) and 4.1 ± 1.1 pg ($n = 5$) for testosterone and 5 α -dihydrotestosterone, respectively. The sensitivity of the assay methods, calculated as the method blank plus twice the s.d., was 7.8 pg for testosterone and 6.3 pg for 5 α -dihydrotestosterone. The intra- and inter-assay coefficients of variation were respectively 6.9 and 12.5 for testosterone and 16.3 and 24.3% for 5 α -dihydrotestosterone. Means between experimental and control groups were compared by Student's *t* test for independent means.

Table 1. Testosterone and 5 α -dihydrotestosterone concentrations (ng/2 ml total incubation medium) in incubates of rat testes treated with oestradiol-17 β

Group	No. of testes	Incubation time (min)							
		Testosterone				5 α -Dihydrotestosterone			
		30	60	120	180	30	60	120	180
Control	10	146 $\pm$ 22	164 $\pm$ 22	188 $\pm$ 23	238 $\pm$ 28*	4.9 $\pm$ 0.7	5.4 $\pm$ 1.4	8.6 $\pm$ 2.5	15.6 $\pm$ 2.9*
10^{-8} M oestradiol	10	130 $\pm$ 24	146 $\pm$ 22	165 $\pm$ 24	173 $\pm$ 26	2.7 $\pm$ 1.3	9.1 $\pm$ 5.3	13.0 $\pm$ 5.0	18.9 $\pm$ 4.5*
10^{-6} M oestradiol	10	141 $\pm$ 10	203 $\pm$ 18	209 $\pm$ 14	255 $\pm$ 22*	4.4 $\pm$ 1.5	10.9 $\pm$ 3.5	8.7 $\pm$ 2.0	12.6 $\pm$ 4.0*

Values are mean $\pm$ s.e.m.

* Significantly different from 30 min value of that group, $P < 0.05$.

Results

The results of Exp. 1 are given in Table 1. The increased synthesis of testosterone and 5 α -dihydrotestosterone after 180 min are indicative of net synthesis and secretion of steroids under the incubation conditions selected. Testosterone and 5 α -dihydrotestosterone concentrations in the media from test incubations did not differ ($P > 0.05$) from the corresponding control values at any time. As shown in Table 2, although the presence of oestradiol caused a drop in production of 5 α -dihydrotestosterone by hCG-stimulated testes, the difference was not significant ($P > 0.05$).

Table 2. Testosterone and 5 α -dihydro testosterone concentrations (ng/2 ml total incubation medium) in incubates of rat testes treated with oestradiol-17 β and hCG

Treatment	No. of testes	Testosterone	5 α -Dihydrotestosterone
Control + hCG	5	728 $\pm$ 198	137 $\pm$ 46
Oestradiol (10^{-6} M) + hCG	5	885 $\pm$ 110	75 $\pm$ 22

Values are mean $\pm$ s.e.m.

Discussion

In-vitro incubations of decapsulated rat testes have been utilized extensively as a model for investigations of factors regulating testicular biosynthesis of androgens (Dufau, Catt & Tsuruhara, 1972; Catt & Dufau, 1974) and this system therefore seemed to be particularly well-suited for studies to determine whether oestrogens are capable of exerting direct, acute effects on suppression of testicular androgen production. Endogenous oestradiol-17 β concentration in the rat testis has been reported to be approximately 40 pg/g testis (de Jong, Hey & van der Molen, 1974). The concentrations of 10^{-8} M- and 10^{-6} M-oestradiol-17 β which were used in the present study therefore represent values of approximately 125 and 12 500 times the endogenous concentration. Despite this, and the need for caution in extrapolation of data obtained *in vitro* to the conditions *in vivo*, the failure of the present studies to demonstrate a suppressive effect of oestradiol-17 β on testosterone and 5 α -dihydrotestosterone production by rat testes *in vitro* is consistent with the interpretation that the oestradiol suppression of androgen production *in vivo* is not due to direct inhibition of the testes but to secondary effects such as pituitary inhibition (van Beurden *et al.*, 1977).

The present findings that 10^{-6} M-oestradiol-17 β (approximately 0.5 μ g/incubate) did not inhibit testosterone production by decapsulated rat testes in the presence or absence of hCG are fully consistent with the findings of Bartke *et al.* (1977), but these workers also reported that 300 μ g oestradiol-17 β /incubate resulted in a significant (38%) reduction of testosterone production by decapsulated rat testes. This concentration of oestradiol-17 β is 600 times the largest concentration of oestradiol-17 β used in the present study and about 7.5×10^6 times greater than the endogenous concentration of oestradiol-17 β in rat testes (de Jong *et al.*, 1974). While the present work does not exclude the possibility that concentrations of oestradiol-17 β in excess of 10^{-6} M *in vitro* may have a suppressive effect on testicular steroidogenic enzymes (Samuels, Short & Huseby, 1964), it is questionable whether such concentrations may be achieved in the testes *in vivo* under physiological conditions (de Jong *et al.*, 1974). It is concluded, therefore, that oestradiol-17 β does not appear to participate in the acute regulation of testicular steroidogenesis in the adult rat.

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