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Effects of Estrogen Treatment on the Responses of cAMP and Sex Steroids to hCG by the Testes of Patients with Prostatic Cancer

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ABSTRACT

The effects of diethylstilbestrol diphosphate (DESDP) on testicular steroidogenesis were investigated in patients undergoing orchiectomy for prostatic carcinoma.

In non-DESDP treated patients the concentration of cAMP in the spermatic venous blood was 17.8 ± 3.1 (SE) pmoles/ml, which increased to 343.3 ± 18.6 (SE) pmoles/ml 30 min after hCG (1,000 IU) injection into the testis. T increased gradually from the pretreated level of 26.5 ± 7.2 (SE) μg/dl at 50 min. A slight increase in DHT was observed after hCG injection. Ten days' injections of DESDP significantly reduced the responses of T and DHT to hCG and 20 days' injections decreased T and DHT responses as well as cAMP response to hCG.

These findings indicate that DESDP can directly suppress the hCG stimulated steroidogenesis and cAMP production in human testis *in vivo*. The inhibitory effects of DESDP on Leydig cell function are probably on T biosynthesis as well as on cAMP formation.

The use of estrogens to depress human testicular androgen production in patients with prostatic carcinoma is well established[3]. The suppression of testicular function by exogenous estrogen administration has generally been thought to be mediated by the hypothalamus and/or the anterior pituitary. Recent findings suggest that estrogen may also be important in the physiological control of testicular endocrine function[7,9].

Leydig cells in the rat testis contain an estrogen receptor, which may mediate some of the inhibitory effects of estrogen on testicular steroidogenesis[1,6].

The present study was therefore designed to determine the influence of a synthetic estrogen [diethylstilbestrol diphosphate (DESDP)] on human testicular steroid secretion *in vivo* and on pituitary gonadotropin secretion in patients with advanced prostatic carcinoma. The aim of this study was to obtain additional knowledge about the site of inhibition of estrogens on human testicular steroid production, to further evaluate the role of cAMP in this process, and to test the efficiency of regimens used to suppress testicular androgen production.

MATERIALS AND METHODS

Patients and study protocols

Eighteen patients with prostatic carcinoma who required orchiectomy were selected for this study. The mean age of these patients were 74 years. None of the patients had any previous endocrinological disease, nor had they received any endocrine therapy except that described in this report.

The first group of 8 cases had never received any form of hormonal treatment, the second

group of 5 cases had been treated with DESDP (500 mg iv daily for 10 days) and third group of 5 cases treated with the same dose of DESDP for 20 days prior to orchiectomy.

The orchiectomies were performed under local anesthesia. At that time, left spermatic vein was separrated and canulated with polyethylene tube. Blood samples from the left spermatic vein were collected first, and then 1,000 IU of hCG in 2 ml of physiological saline was directly injected into the left testis. Spermatic venous blood was collected 5, 10, 15, 30, 40 and 50 min after the hCG injection. Peripheral venous blood samples were draw before and after the treatment. These blood samples were collected into EDTA coated tubes and then centrifuged and plasma were frozen at −20°C until assayed.

Hormone assays

Assays of LH and FSH were done using double antibody RIAs (kits purchased from Daiich Radioisotope Institute, Tokyo, Japan). Plasma cAMP concentration was determined by RIA method without chromatography, which had been described previously[2,15]. Testosterone (T), 5 α—dihydrotestosterone (DHT) and androstenedione were extracted from 50—200 μl of plasma samples twice with 5 ml of ethylether by shaking for 1 min. The combined ethylether phase was evaporated to dryness under a stream of air. The extract was dissolved in a small quantity of ethanol, applied to thin layer chromotography plates (pre-coated silica gel G plates, Art 5721, E, Merck Japan Ltd.) and developed in a solvent system of chlorofrom/ethyl acetate/petroleum ether (50/45/5, vol/vol/vol). T, DHT and androstenedione standards were run as markers at each edgs and were detected by an UV light or Allen's reagent. The area of silica corresponding to T, DHT and androstenedione was scrapped with razor blads and then extracted with methanol. Aliquots of methanol extracts were pipetted into 10×75 mm glass tubes for T, DHT and androstenedione determinations. These steroid concentrations were measured by RIAs[10,19]. The intra- and interassay coefficients of variation for these assays were, respectively: LH, 10% and 12%; FSH, 9% and 11%; T, 9% and 13%; DHT, 11% and 15%: androstenedione, 9% and 12%. All assays for a given hormone in each individual were performed in the same assay run. The RIA dose-response curves were analyzed by an iterative least squares method for logistic curve fitting developed by Rodbard and Hutt[12]. The significance of differences between results for different groups was calculated using a two-tailed Student's t-test.

RESULTS

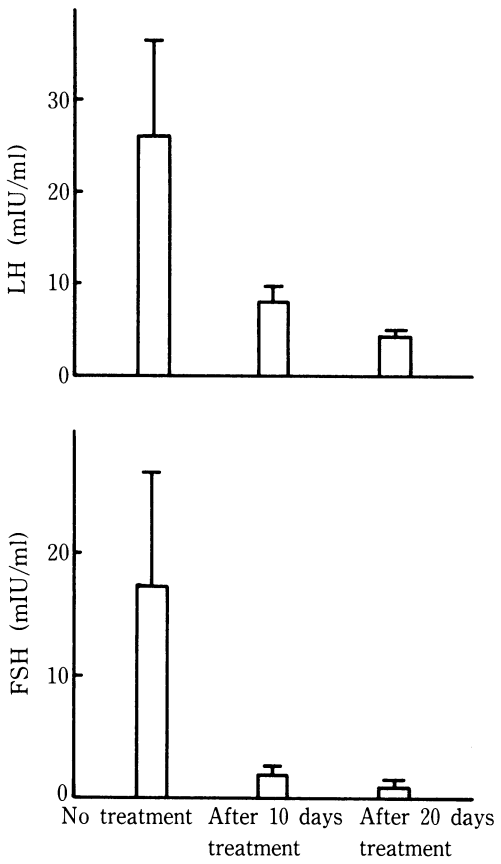

Fig. 1. Effect of DESDP on LH (upper panel) and FSH (lower panel) levels in patient with prostatic cancer

Effect of DESDP therapy on LH and FSH levels in the peripheral venous blood

Concomitant changes in peripheral plasma LH and FSH after DESDP treatments are depicted in Fig. 1. The mean concentrations of LH and FSH were 26.3 mIU/ml and 17.6 mIU/ml in patients who had not been treated with DESDP, which were significantly suppressed to the levels of 7.3 mIU/ml and 2.1 mIU/ml in pa-

tients treated for 10 days and further suppressed to the levels of 4.8 mIU/ml and 1.4 mIU/ml in patients treated for 20 days. The concentration of FSH was relatively more suppressed than that of LH during DESDP treatment.

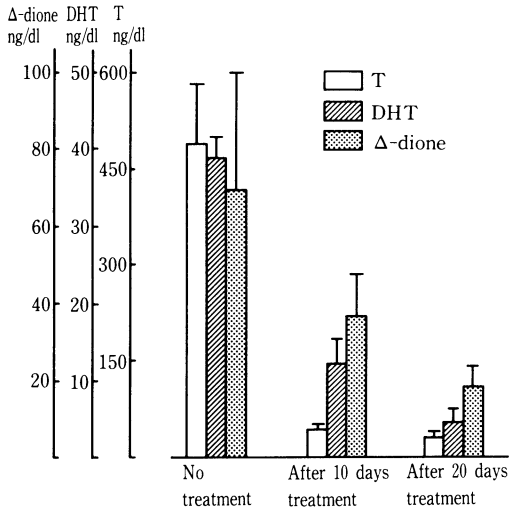

Fig. 2. Effect of DESDP on the blood levels of androstenedione (Δ-dione), DHT and T in patients with prostatic cancer.

Effect of DESDP therapy on T, DHT and androstenedione levels in the peripheral venous blood

The mean concentrations of T, DHT and androstenedione were 490 ng/dl, 40 ng/dl and 70 ng/dl in patients who had not been treated with DESDP. A significant decrease in these steroids was seen in patients treated for 10 days. A further decrease in T, DHT and androstenedione down to 28 ng/dl, 4 ng/dl and 18 ng/dl were observed in patients treated for 20 days. Compared to DHT and androstenedione, a decrease in T was large in patients treated with DESDP (Fig. 2).

Effect of DESDP on hCG-stimulated testicular cAMP production

To study the influence of DESDP on hCG-stimulated cAMP production, we measured the concentrations of cAMP in the spermatic venous blood after the injection of hCG into the testes. The cAMP concentration in the spermatic vein increased sharply in patients who had not been

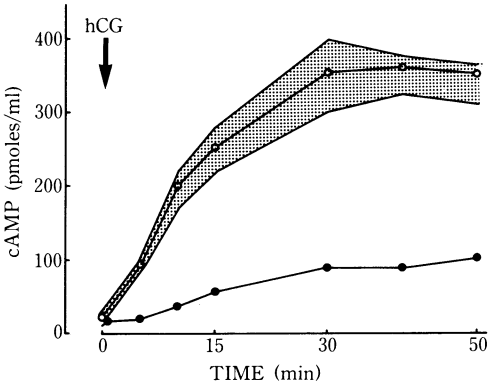

Fig. 3. Concentrations of cAMP in the spermatic venous blood after a single injection of 1000 IU hCG. Mean values from 10 days treatment: 0, mean values from 20 days treatment: ●. Shaded area represents the mean ± SE in patients who have not been treated with DESDP.

treated with DESDP, reached a level of 340 pmoles/ml 30 min after hCG injection, which was 20-fold higher than the initial level. The maximal response of cAMP to hCG in patients treated for 10 days did not differ from that of patients who had not been treated with DESDP. However, the responsiveness to increasing concentrations of cAMP after hCG in patients treated for 20 days was reduced by more than 70% of that observed in patients who had not been treated with DESDP (Fig. 3).

Effect of DESDP on hCG-stimulated testicular T production and on spermatic venous DHT

The mean concentrations of T and DHT were 27.2 μg/dl and 133 ng/dl in patients who had not been treated with DESDP. T gradually increased and reached a level of 50 μg/dl 50 min after the hCG injection. The pattern of the changes in spermatic DHT was similar to that of T, however, no significant increase was demonstrated after hCG injection in these patient. On the other hand, a significant decrease in the concentrations of both T and DHT were observed in patients treated for 10 days. Although T response to hCG still remained, the level of T 50 min after hCG decreased down to 4 μg/dl. A further significant decrease of T and DHT were observed in patients treated for 20 days and hCG administration caused no increase in spermatic T concentration (Fig. 4).

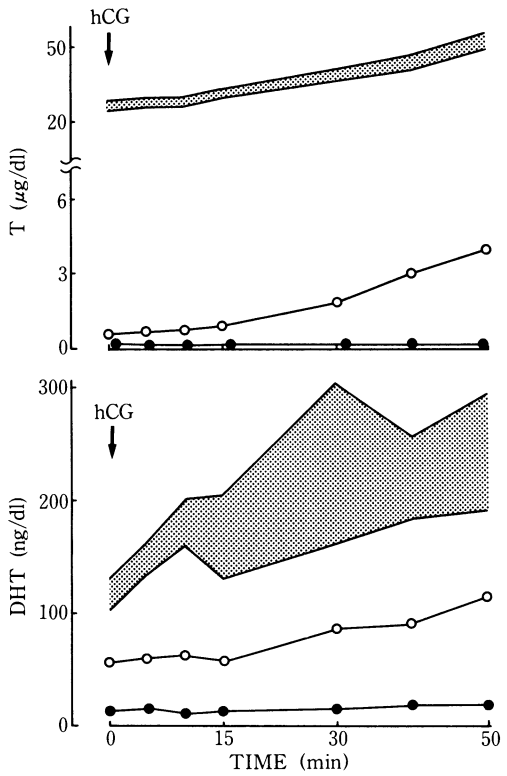

Fig. 4. Concentrations of T (upper) and DHT (lower panel) in the spermatic venous blood after a single injection of 1000 IU hCG. Mean values from 10 days treatment: 0, mean values from 20 days treatment: ●. Shaded area represents the mean ± SE in patients who have not been treated with DESDP.

DISCUSSION

Several studies have examined the effects of estrogen on gonadotropin secretion in men. Large doses of estrogen decrease the levels of both plasma FSH and LH[16,18]. In our study, a decrease in the concentration of plasma LH was noted in patients treated for 10 days and this effect was more pronounced in patients treated for 20 days. Although the pattern of plasma FSH was similar to that of LH, DESDP has a greater suppressive effect on FSH than on LH. These findings may be similar to those of Sawin et al[17] who demonstrated that chronic administration of ethynil estradol to normal adult men caused a rapid decrease in FSH levels whereas the LH levels were raised above control levels. Our findings indicate that DESDP alone may preferentially inhibits FSH secretion, while it did not do so in our subjects.

Estrogen treatment has been shown to inhibit numerous testicular enzymes depending on the estrogen used, its dosage, and experimental conditions. Our finding of decreased peripheral concentrations of androstenedione, T and DHT in association with decreased peripheral concentration of LH suggests that DESDP exercise its main effect by the testes by means of pituitary suppression of LH, which thereby decreases T synthesis. On the other hand, the decrease in the concentration of T was statistically significant in patients treated for 10 days whereas the fall in DHT concentration was relatively smaller than that of T, as well as androstenedione. A similar observation was also made by Leinonen et al[8] who showed that the depletion of testicular T, DHT and androstenedione was occurred in patients with prostatic cancer who had also been treated with DESDP, suggesting that a synthetic estrogen, DESDP was very potent in depressing testicular steroid production. However, the present deta do not justify any definite conclusions about the human testicular enzymes which are inhibited *in vivo* DESDP administration.

In order to investigate the possibility that DESDP might have a direct effect on T synthesis in addition to effects via its negative feedback action on LH secretion, we examined the changes in testicular response to hCG by spermatic venous cAMP, T and DHT determinations, which had been previously established[20]. The spermatic venous cAMP in non-DESDP treated patients continued to increase until 30 min after hCG injection, which is about 20 times higher than that of pretreated level. Although the pattern of cAMP response to hCG in patients treated for 10 days was similar to that of the patients who had not been treated with DESDP, a significant decreased T concentration after hCG was observed whereas the T response to hCG still remained. These results suggest that estrogen may be acting at a step distal to receptor-mediated cAMP production in the Leydig cells. A possible direct action of estrogen upon steroidogenic enzymes had been suggested because of these enzymes have been shown to be inhibited by estrogens in testes of normal rats and cryptochid mice *in vivo* and *in vitro*[11,14].

Finally, it is of note that chronic admistration of DESDP caused a decreased receptor-mediated cAMP production, as judged by the testes of patients who had also been treated with DESDP for 20 days. Estrogen treatment of intact[13], hypophysectomized-FSH maintained[4] or hypophysectomized-hCG and FSH maintained rats[5] reduces the number of LH receptors in the testis. However, this reduction may not account for the reduced response to LH as LH-stimulated cAMP production is either unchanged[4] or increased[13]. These results indicate that the major block to LH-stimulated T production in estrogen-treated animals is at a point beyond the production of cAMP. Our results are different to these results in cAMP response to hCG, which indicate that there may be differences in hormonal regulation of human and rat testes due to *in vivo* or *in vitro* conditions.

In conclusion, our results suggest that there is a third mechanism of DESDP action on human testicular steroidogenesis, in addition to the known suppression of gonadotropin secretion and testicular enzymes involved in androgen biosynthesis. This mechanism is the suppression of the testicular cAMP formation in response to hCG stimulation. However, additional studies are needed to confirm a causal relationship between the estrogen-induced decrease in cAMP formation being not mediated via gonadotropin suppression and androgen production.

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