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Effects of Diethylstilboestrol on Testicular Function and Luteinizing Hormone Receptors

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Adult male Fisher-344 rats were implanted with DES-filled or empty Silastic capsules. After 14 weeks, capsules were removed and a second group of rats received DES capsules. Seven weeks later, all the rats were sacrificed. DES treatment decreased body, testes and seminal vesicle weights, and removal of the capsules partially restored the weight of these organs. The concentration of testicular LH receptors was increased by DES treatment. Circulating PRL levels were increased and gonadotropin levels were reduced in all animals having received DES at anytime. Plasma testosterone (T) levels were similar in all groups, but testicular T levels were reversibly decreased by DES. Similarly, whereas basal incubation media T levels were unchanged by DES treatment, the steroidogenic response *in vitro* to hCG was abolished by the presence of DES, and removal of the capsules restored this response. It appears that in this animal model DES and PRL exert opposing effects on testicular LH receptor.

Key words: LH receptors, Diethylstilboestrol, Testes, Testosterone, Prolactin, LH, FSH.

Chronic treatment of male Fisher-344 rats with diethylstilboestrol (DES) produces marked pituitary enlargement, lactotroph hyperplasia, hyperprolactinaemia and suppression of gonadotropin release and testicular function (7). When this treatment is discontinued, hyperprolactinaemia, pituitary enlargement and suppression of LH and FSH levels persist, while testicular function recovers (7). Analysis of the effects of hyperprolactinaemia on the hypothalamic-pituitary-testicular axis in this model is complicated by difficulty in separating the effects of prolactin (PRL) from the effects of DES. Both hyperprolactinaemia and oestrogens can produce major changes in male reproductive and endocrine functions (3-6, 17, 19, 36, 40, 44). Furthermore, there is evidence that PRL may mediate some of

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the effects of oestrogenic steroids on the pituitary-testicular axis (45-46). Since both hyperprolactinaemia and oestrogens have the capability of altering testicular LH receptors (4, 9, 20, 21, 24, 28, 30, 38, 39), it was of interest to examine testicular LH receptors and steroidogenic response during DES treatment and after removal of DES-containing Silastic capsules.

Materials and Methods

Male Fisher F-344 rats (< 8 weeks old) were purchased from Harlan Sprague-Dawley, Inc. (F-334/NHsd BR) and maintained in a room with controlled photoperiod (12 h light: 12 h dark) and temperature (22 ± 2 °C). The animals had free access to food and water.

In one group, rats were implanted with either Silastic capsules (length = 5 mm; ID = 1.56 mm; OD = 2.39 mm) containing diethylstilboestrol (DES; ~ 8 − 9 mg) or empty Silastic capsules. Fourteen weeks after implantation, all capsules were removed. At this time a second group of rats received DES-containing Silastic capsules (fig. 1). Seven weeks after this, all animals were sacrificed, and blood and testes were collected. Plasma was stored at −20 °C until assayed for circulating hormone levels. The testes were decapsulated and divided into several fragments which were used to study *in vitro* steroidogenesis, and others were instantly frozen in a solid CO_2/acetone mixture and stored at −70 °C until assayed for testosterone (T) levels and luteinizing hormone (LH) receptors.

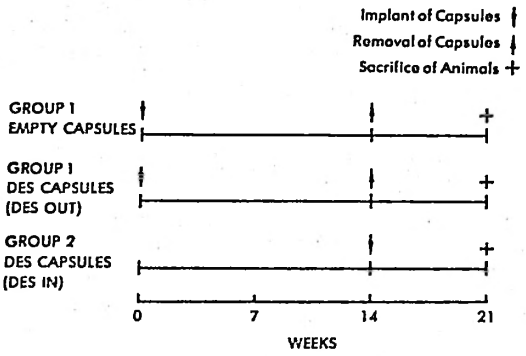

Fig. 1. *Diagram of experimental protocol.*

Testicular LH receptors were measured by radioreceptorassay following procedures previously described (5). The ^{125}I-labelled hCG (CR121, NIH) used had a specific activity of 40 μCi/μg and a maximum binding ability of 30.1 %. The concentration of protein in testicular membrane preparations was determined by a modification of Lowry's method (27), using bovine serum albumin as the standard.

Incubation of testes fragments of similar size, and the subsequent measurement of media T were done following protocols previously described for our laboratory (48). Plasma, LH, FSH and prolactin concentrations were measured by double antibody radioimmunoassays using reagents provided by the National Hormone and Pituitary Program. The reference preparations were LH-RP-2, FSH-RP-2 and PRL-RP-3, respectively. The intra-assay coefficients of variation and average sensitivities of the assays were, respectively, as follows: LH, 6.8 %, .4.0 μg/l; FSH, 2.0 %, 75.0 μg/l; prolactin, 3.0 %, 0.1 μg/l.

The data were assessed by one-way analysis of variance using SPSS-X programs on an IMB mainframe (31-33). All data were tested for normality of distribution by the Kolmogorov-Smirnov test and for homogeneity of variance by Barlett's test. Log or square root transformations were utilized as needed (41, 42).

Results

Treatment with DES-containing Silastic capsules produced the expected significant decreases in body, testes, and seminal vesicle weights. As in our previous studies (7) removal of DES capsules partially re-

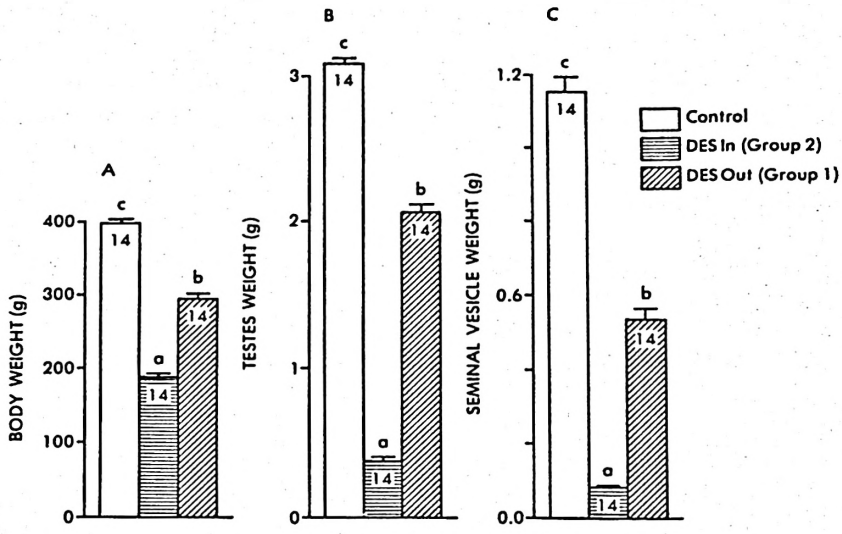

Fig. 2. *Body, Testes and Seminal vesicles weights, as a function of the presence (Group 2) or absence (Group 1) of DES capsules in the rats at the time of sacrifice.*
Values are expressed as mean ± S.E.M. Numbers indicate number of rats per point. Values with a letter in common are not significantly different (Student-Newman-Keuls procedure of the multiple range test).

versed these decreases, so that rats that had DES capsules removed 7 weeks before sacrifice had higher body, testes, and seminal vesicle weights than those measured in animals bearing DES capsules at the time of sacrifice (fig. 2).

Rats with DES capsules did not exhibit significant changes in the concentration of testicular LH receptors when compared to control rats. However, in animals which had the DES capsules removed, the concentration of testicular LH receptors was dramatically increased (fig. 3 a). The total content of testicular LH receptors was reduced in rats with implanted DES capsules, and elevated in those that had the DES capsules removed, compared to control animals (fig. 3 b).

As expected, treatment with DES-containing Silastic capsules dramatically decreased circulating LH and FSH levels (fig. 4 a, b) and increased prolactin levels, and elevation of PRL levels persisted after removal of DES capsules levels (fig. 4 c). Rats which had DES capsules present at

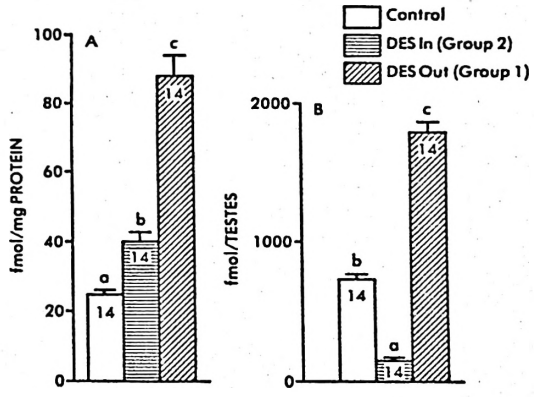

Fig. 3. *Concentration and total content of testicular LH receptors as a function of the presence (Group 2) or absence (Group 1) of DES capsules in the rats at the time of sacrifice.*
Legend as in fig. 2.

the time of sacrifice, had extremely low testicular T levels. Removal of DES capsules 7 weeks before sacrifice, allowed testicular T levels to return to normal (fig. 5 b). Surprisingly, no differences in plas-

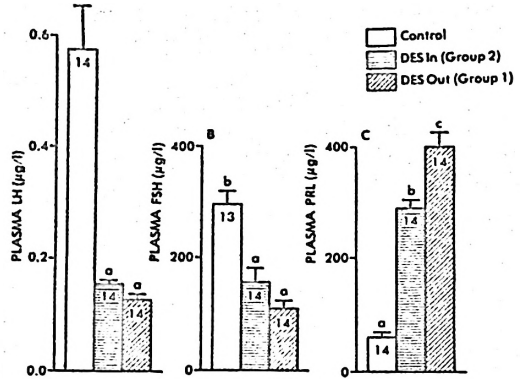

Fig. 4. *Plasma LH, FSH and prolactin concentrations as a function of the presence (Group 2) or absence (Group 1) of DES capsules in the rats at the time of sacrifice.*
Legend as in fig. 2.

ma T levels were observed among the treatment groups (fig. 5 a). Treatment with DES did not cause any differences in basal rates of T accumulation *in vitro*, compared to control animals. Continued presence of DES implants inhibited T response to hCG stimulation, while removal of DES capsules allowed recovery of this steroidogenic response (fig. 5 c, d).

Discussion

In analyzing the effects of hyperprolactinaemia (hyperPRL) it is necessary to consider the characteristics of both the model being used, and the species being studied. The surprising diversity of the effects of hyperPRL among different models for this condition in the rat is summarized in table I. Testicular parameters seem to be affected by hyperPRL as in proportion to the increase in plasma PRL

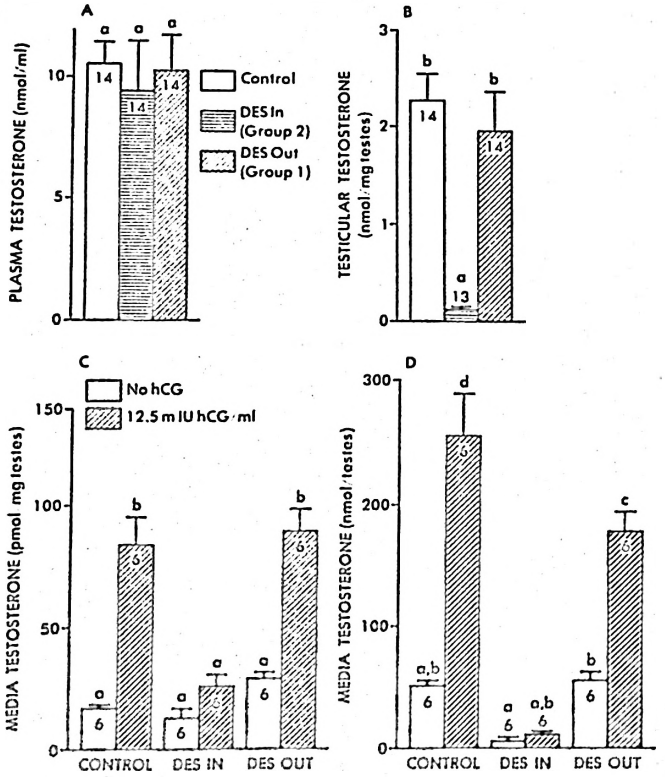

Fig. 5. *Plasma, testicular and incubation media testosterone concentrations as a function of the presence (Group 2) or absence (Group 1) of DES capsules in the rats at time of sacrifice.*
Legend as in fig. 2.

Table I. *Comparisons among different models of hyperprolactinemia in rats**

	DES[a]	SHR[b]	Grafts[c]	MtT-W15[d] Tumours	GH_1B^e Tumours
LH Receptors	Increased	Decreased	Increased	—	—
Testicular Weight	Decreased	Increased	Unchanged or Decreased	Decreased	Decreased
Plasma T	Unchanged	Decreased	Unchanged	Decreased	Decreased
Plasma LH	Decreased	Unchanged	Decreased	Decreased	Increased
Plasma FSH	Decreased	Increased	Decreased	—	Unchanged

* All effects listed in this table are statistically significant.
[a] DES capsules for 14 weeks, then removed 7 weeks before sacrifice; present study.
[b] Spontaneously hypertensive rats (5).
[c] Grafts of pituitaries under the kidney capsule (8, 9, 16).
[d] Transplants of prolactin- and growth hormone-secreting tumours (19).
[e] Transplants of prolactin- and growth hormone-secreting tumours (17).

levels. Thus, testicular LH receptor levels decrease in rats with mild hyperPRL (SHR; 5), whereas in animals with more severe hyperPRL, LH receptor levels are elevated. In rats with transplantable PRL-secreting tumours and extreme elevation of plasma PRL levels, T levels are suppressed (19). Similarly, in SHR rats, plasma T decreases, and in rats with pituitary grafts or DES pretreatment it is unchanged. Testicular weight reflects even more closely the magnitude of hyperPRL. In SHR rats testicular weight increases, in rats with pituitary grafts («medium range» hyperPRL) it is unchanged (8), and in rats pretreated with DES or implanted with PRL-secreting tumours (very severe hyperPRL) testicular weight decreases (17-19). Differences among the effects of hyperPRL in different species are even more pronounced. For example, in Syrian hamsters with pituitary grafts testicular LH receptor levels were elevated, and testicular weight and plasma T levels increased or were unchanged (4). However, in mice with pituitary grafts testicular LH receptor levels decreased and testicular weight was unchanged (25).

Similarly to the effects of hyperPRL, the effects of DES on testicular function vary depending on the model used and species studied (table II). Examination of these data reveals an interesting relationship between the response of LH receptors to oestrogens in a given animal model and that animal's susceptibility to testicular tumourigenesis. Thus, in tumour-resistant rats (Sprague-Dawley) LH receptor concentration decreases after oestrogen exposure, whereas in tumour-susceptible rats (Fischer F-344) it increases (30; and present study). In all strains of mice studied there is an initial increase in LH receptor levels, but in tumour-resistant mice (C3H) receptor concentration subsequently returns to normal, whereas in tumour-susceptible mice (BALB/c) it remains elevated (30). In men, who because of the low incidence of testicular tumours could be considered a tumour-resistant species, LH receptor levels decrease after oestrogen exposure (21). However, oestrogens reduce plasma T levels in both tumour-resistant (Holtzman) and tumour-susceptible rats (Fisher F-344), as well as in the human (7, 21, 24). It seems as if susceptibility to testicular tumourigenesis is inversely correlated to changes in LH receptor concentration. Thus, it is interesting to note that this sus-

Table II. *Comparison of the effects of oestrogen treatment on the Pituitary-testicular axis*.*

	Men[a]	C3H mice[b]	BALB/c mice[b]	Sprague-Dawley Rats[b]	Holtzman Rats[c]	Fisher F-334 Rats with DES[d]	Fisher F-334 Rats after DES[d]
LH Receptor (Concentration)	Decreased	First Increased, then Normal	Increased	Decreased	—	Increased	Increased
(Content)	—	First Increased, then Decreased	Increased	Decreased	Decreased	Decreased	Increased
Testicular Weight	—	Decreased	Normal	Decreased	Decreased	Decreased	Decreased
Plasma LH	Decreased or Normal	—	—	Increased	—	Decreased	Decreased
Plasma FSH	Normal	—	—	—	—	Decreased	Decreased
Plasma PRL	—	—	Increased	Increased	—	Increased	Increased
Plasma T	Decreased	—	—	—	Decreased	Decreased or Normal	Normal
Testicular T	Decreased	—	—	—	Decreased	Decreased	Normal
In Vitro	Decreased	—	—	—	Decreased	Decreased	Normal
Tumour Susceptibility	Low	Low	High	Low	Low		High

* All effects listed in this table are statistically significant.
[a] Prostatic cancer patients (2, 14, 16, 21, 26, 29, 34).
[b] Tumour resistant animals (C3H mice and Sprague-Dawley rats) and Tumour susceptible (BALB/c mice) (13, 22, 30).
[c] Tumour resistant Holtzman rats (24, 34, 35).
[d] Tumour susceptible Fisher F-334 (7, and present study).

ceptibility correlates directly with changes in oestrogen-induced DNA synthesis. Oestrogens increase ADN synthesis independently of hCG, in intact and hypophysectomized tumour sensitive BALB/c mice (23, 43, 47). In contrast, in tumour resistant animals, oestrogens cause either a smaller increase (C3H mice), no increase (Holtzman rats), or a decrease (Sprague-Dawley rats) in DNA synthesis (23, 43, 47). In Sprague-Dawley rats, oestrogens also inhibit the hCG-induced increase in DNA synthesis (37). Therefore, it could be assumed that high susceptibility to testicular tumourigenesis is associated with lower sensitivity to oestrogen-induced decrease in LH receptors, and with higher oestrogen-induced DNA synthesis. In contrast, PRL-induced ADN synthesis (11) could correlate with the hyperPRL-induced increase in testicular LH receptors (7 and present study).

In Leydig cells it is very common to observe that a factor that changes LH receptor levels in one direction, also changes PRL receptor levels in the same direction; therefore, reports that oestrogens also decrease PRL receptors in Sprague-Dawley rats were not surprising (13, 22). However, it should be noted that in genetically hypoprolactinemic IPL rats (12, 18), which appear to have also genetically induced decreases in PRL receptors, oestrogens can induce hyperPRL but cannot reduce the levels of PRL receptors (13). Therefore, it could be suggested that there are three levels of gene-mediated regulation of hormone receptor levels in the Leydig cell. The first (and lowest level) would correspond to PRL, the second to oestrogens, and the third (and highest level) to inherited intrinsic characteristics of the animal's genome.

In the present study, plasma T levels and basal rate of T release *in vitro* were normal in rats exposed to DES. However, in those animals with DES capsules present at the time of sacrifice, testicular T levels were greatly reduced, and the *in vitro* steroidogenic response to hCG was abolished. Removal of DES capsules prior to sacrifice tended to normalize T levels in general. Decreased plasma T levels have been observed in oestrogen-treated men (26) and rats (7, 24, 37). Testicular T levels were reported to be reduced in rats and men treated with oestrogens (21, 24, 26, 37). Basal incubation media T levels also decreased in several species after oestrogen treatment, as was the *in vitro* steroidogenic response to LH/hCG (10, 15, 20, 35, 37, 38). The inhibitory effects of oestrogen on basal and hCG-stimulated T levels are observed as early as 8 hours after *in vivo* administration (1). Furthermore, the inhibition of testicular steroidogenesis by oestrogens appears to involve direct action on several enzymes. *In vitro* incubation of human testes fragments, in the presence of oestrogens, decreased hCG-stimulated T production, and the activities of 3β-hydroxysteriod dehydroyenase, 17α-hydroxylase and C17-20-lyase (14). Testicular cholesterol concentration increases in rats treated with oestrogen (35). Moreover, the effects of oestrogens on testicular steroidogenesis occur faster than those on PRL levels (8 hours vs 2 days) (1, 2). SAIRAM and BERMAN (38, 39) suggested that the effects of oestrogens on testicular function might be a consequence of their being capable of inhibiting gonadotropin binding. Their experiments showed that oestrogens inhibit binding of oLH to receptor preparations in a radioreceptorassay. Therefore, oestrogens could exert their effects on testicular function by regulating gene expression and/or LH receptors.

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Resumen

Ratas machos adultas de la cepa Fisher-344 recibieron cápsulas de Silastic vacías o llenas de dietilestilbestrol (DES), que se les quitan 14 semanas después en que a otro grupo les implantan cápsulas con DES. El tratamiento con DES produce disminución del peso corporal, testicular, y de vesícula seminal, el cual se recupera parcialmente cuando se eliminan las cápsulas. El tratamiento con DES también causa un aumento en la concentración de receptores testiculares a LH, así como en los niveles circulantes de PRL. Los niveles plasmáticos de gonadotropinas disminuyen en los animales tratados. Aunque los niveles circulantes de testosterona no sufren ningún cambio, en el nivel testicular se observa una reducción reversible a causa del DES. De la misma manera, los niveles basales de testosterona en el medio de incubación no se afectan por el tratamiento *in vivo*, la respuesta esteroidogénica a hCG *in vitro* es abolida reversiblemente por DES. Aparentemente, la PRL y el DES tienen, en este modelo, efectos opuestos sobre los niveles de receptores testiculares a LH.

Palabras clave: Receptores LH, Dietilestilbestrol, Testosterona, Prolactina, LH, FSH, Testículos.

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