Pharmacology Biochemistry & Behavior, Vol. 22, pp. 1019–1024, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Neonatal Exposure to DES in BALB/c Male Mice: Effects on Pituitary-Gonadal Function

S. DALTERIO,* A. BARTKE,† R. STEGER† AND D. MAYFIELD*

Departments of Pharmacology and Obstetrics and Gynecology†*
The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284

Received 23 July 1984

DALTERIO, S., A. BARTKE, R. STEGER AND D. MAYFIELD. *Neonatal exposure to DES in BALB/c male mice: Effects on pituitary-gonadal function.* PHARMACOL BIOCHEM BEHAV 22(6) 1019–1024, 1985.—Neonatal male BALB/c mice were injected with diethylstilbestrol (DES), estradiol benzoate (E_2B), testosterone propionate (TP), progesterone or DES, in combination with E_2B, TP or progesterone and examined in adulthood. Body weight was reduced in males exposed to DES, TP or DES + TP, while testicular weight was reduced in animals injected with DES, E_2B, TP, DES + TP or DES + progesterone. Exposure to DES and/or E_2B also produced reproductive tract abnormalities and concomitant progesterone exposure did not further affect this parameter. Concomitant DES did not further alter the reduced plasma luteinizing hormone (LH) levels attributable to neonatal TP or E_2B treatment. Plasma follicle-stimulating hormone (FSH) levels in intact males were increased by DES, DES + progesterone or progesterone alone. Assessment of the feedback effects of exogenous gonadal steroids on pituitary gonadotropin release in castrated adults indicated that injection of 125 μg TP further increased the already elevated post-castration levels of LH and FSH in mice neonatally exposed to progesterone. The increase in testosterone (T) concentration after intratesticular human chorionic gonadotropin (hCG) administration was significantly attenuated in mice neonatally exposed to DES plus E_2B or to progesterone. Basal testicular T levels were significantly elevated in males exposed to DES, alone, or in combination with progesterone. Exposure to DES and TP increased hypothalamic serotonin (5-HT) levels in intact mice, while levels of 5-HT were lower after castration compared to controls. DES + E_2B-treated mice had higher norepinephrine (NE) levels, and E_2B-treated mice also had higher 5-HT levels. These findings indicate that alterations produced as a consequence of neonatal DES exposure include disruptions in pituitary-gonadal feedback and in brain biogenic amine levels, in addition to gross morphological abnormalities in the reproductive tract. Some of the effects of neonatal DES exposure were attenuated by simultaneous treatment with TP, E_2B or progesterone. In addition, treatment with these steroids was also capable of altering pituitary-gonadal feedback. However, gross genital abnormalities were apparent only in males receiving estrogenic preparations. Concomitant exposure to progesterone appeared to be capable of blocking DES-induced morphological effects.

Neonatal exposure Testosterone propionate Progesterone Follicle stimulating hormone
Testicular testosterone concentrations Intratesticular gonadotropin administration Diethylstilbestrol
Estradiol benzoate Luteinizing hormone Pituitary function Castration effects Testicular function

A relationship between human prenatal exposure to diethylstilbestrol (DES), a potent synthetic estrogen, and increased incidence of clear cell vaginal adenocarcinoma and cervical neoplasms appears to be well established [3, 4, 9, 15, 24]. More recently, it has also been reported that young men whose mothers received DES during pregnancy have an increased incidence of epididymal cysts and hypotrophic testes [3,4]. Prenatal DES exposure has also been associated with abnormal spermatogenesis [3,4], and, recently, testicular carcinoma has been identified in DES-exposed men [16]. In laboratory rodents, in contrast to the human, the hormone sensitive phase of the development of the reproductive system and sexual dimorphism of neuroendocrine function occurs in the late prenatal and early postnatal periods [12]. It is well known that exposure to steroid hormones during prenatal or early neonatal periods of development results in long-term alteration in neuroendocrine and reproductive functions in laboratory rodents. Perinatal exposure to either estrogenic or androgenic steroids has been shown to influence gonadotropin release patterns [2, 9, 22] and influence sensitivity of target tissue, such as mammary gland or seminal vesicles [15,16].

In adult male mice exposed to DES neonatally, a significant increase in the incidence of epididymal cysts was observed [11]. In addition, impaired fertility, cryptorchidism, and nodular enlargements of the seminal vesicles or coagulating glands occurred with significantly higher frequency in DES-exposed than in control male mice [22, 23, 25]. However, prenatal DES exposure in the rat resulted in anomalies in female, but not in male, offspring [5].

The present experiments were designed to further characterize the consequences of neonatal DES exposure on the development of the male reproductive system in BALB/c mice, which are particularly susceptible to DES-induced effects. In addition, we determined whether concomitant treatment with testosterone propionate (TP), estradiol ben-

zoate (E_2B) or progesterone influence these effects of early DES exposure. Neonatal androgen treatment has been observed to disrupt pituitary-ovarian function in a manner similar to DES [9], while progesterone has been reported to ameliorate some estrogen-induced effects on sexual differentiation of mammary glands [10,17].

In the present studies we examined the effects of neonatal exposure to DES, E_2B, TP or progesterone, alone or in combination, on the gross and histological appearance of the male reproductive structures, body and testicular weights, plasma levels of testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in intact adult males, as well as hormonal responses to castration and exogenous steroid administration. Testicular responsivity to exogenous gonadotropic stimulation was also examined.

In order to determine the possible influences of neonatal steroid administration on brain neurotransmitters, which are involved in the functional regulation of the hypothalamo-pituitary-gonadal (HPG) axis, we also measured the hypothalamic concentrations of biogenic amines.

METHOD

Animals and Neonatal Treatments

The BALB/c mice were purchased from Timco (Houston, TX) and housed on a 14 hr light:10 hr dark lighting schedule and provided with commercial breeder chow and tap water ad lib. Primiparous female mice were housed in groups of 3 or 4 with a single adult male for one week. Afterwards, the females were checked daily and were isolated as soon as pregnancy became apparent. These animals were examined daily for the birth of litters and within 14 hr of parturition, the pups were injected SC with either DES (5 μg), TP (50 μg), progesterone (100 μg), E_2B (5 μg), or a combination of DES and TP, E_2B or progesterone or 5 μl of sesame oil alone. Some pups were untreated, i.e., not injected or handled, during the 5 days.

Evaluation of Treament Effects

At 55 days of age, a group of males from each treatment were castrated under ether anesthesia, using a midventral incision. Body and testicular weights were recorded. One week post-castration, these animals received a single SC injection of testosterone propionate (TP; 125 μg) or sesame oil and were bled by cardiac puncture under ether anesthesia one hour post-injection. Plasma was stored frozen for the RIA determination of LH and FSH, using the NIAMDD rat FSH kit and Niswender's Anti-ovine LH, as described previously [8]. At two weeks post-castration, these animals, together with the intact males, were sacrificed by decapitation, and trunk blood was collected for RIA determination of LH and FSH. All LH and FSH samples were run in a single assay and the intra-assay coefficient of variation was 2.1% and 1.4%, respectively.

In additional animals from each group, testicular responsiveness to gonadotropin was determined by intratesticular injection of 2.5 mIU human chorionic gonadotropin (hCG) in a 10 μl volume of saline. Thirty min later, testes were removed under ether anesthesia, weighed and homogenized in distilled water (1:9 wgt/v) and stored frozen for radioimmunoassay of testosterone (T), as described in recent publications [6,7].

Statistical Analysis

The Kruskal-Wallis test was used to analyze data on plasma gonadotropin levels, body and testicular weights. Analysis of variance was used to determine the significance of the difference between groups in responsiveness to intratesticular hCG or in brain biogenic amine concentrations.

The oil-injected and untreated males did not differ with respect to any of the parameters tested; therefore, the data from these groups were combined for further statistical analysis and presentation.

Biogenic Amine Determinations

Prior to the amine assay, the brains were partially thawed and the hypothalamus was dissected free. The hypothalamus consisted of a tissue block 2.0 mm deep extending from the rostral margin of the mammillary body to the caudal border of the optic chiasm and laterally to the hypothalamic sulci. The hypothalamic block and the remaining brain tissue were weighed and sonicated in 0.1 N $HClO_4$ containing methyl serotonin (M 5-HT), as a standard for the indoleamine assay, dihydroxybenzylamine (DHBA), as an internal standard for the catecholamine assay, and 1.0 mM sodium metabisulfite.

Indoleamines were separated by high performance liquid chromatography (HPLC) and quantitated by electrochemistry [28,29]. Standards were run concurrently, and serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA)) were calculated by comparison of peak heights with those of the standards. Values were corrected for recovery of the internal standard which averaged 97.3±1.2%. The intra-assay coefficient of variation was 5.6% for 5-HT, and 7.2% for 5-HIAA.

Catecholamines were prepared for chromatography as previously described [25]. Norepinephrine (NE), dopamine (DA) and DHBA were separated by HPLC and quantitated by electrochemistry. The recovery of DHBA averaged 87.5±1.2% and the intra-assay coefficient of variation was 6.1% for NE and 6.7% for DA.

RESULTS

Body and Testes Weights

Neonatal injections of DES, alone or in combination with TP, or progesterone, TP, or E_2B alone resulted in reduced body weights in adulthood. The body weights of animals neonatally injected with progesterone or DES plus E_2B were comparable to controls, which represent combined data from vehicle (sesame oil) and untreated males (Fig. 1, top). Testicular weights were significantly reduced in animals neonatally exposed to DES, DES + TP or TP (Fig. 1, bottom).

In addition, several abnormalities were observed in the reproductive tract, including epididymal cysts in two animals, one neonatally exposed to DES and the other to DES + E_2B; granulomous testicular infiltration in three DES-exposed mice, and a similar lesion in the seminal vesicles of another DES-treated animal. In addition, two males, one neonatally-treated with DES and the other with E_2B, had no discernable testicular tissue and one DES-exposed animal had only one testis. Histological examinations of the tissues appearing abnormal on gross inspection revealed foci of necrosis and mineralization surrounded by a zone of massive neutrophile infiltration. However, there were no morphological changes associated with malignancy. Testicular abnormalities appeared to reflect changes consistent with immunological-type reactions within this tissue. No gross abnormalities were detected in the reproductive systems of control animals.

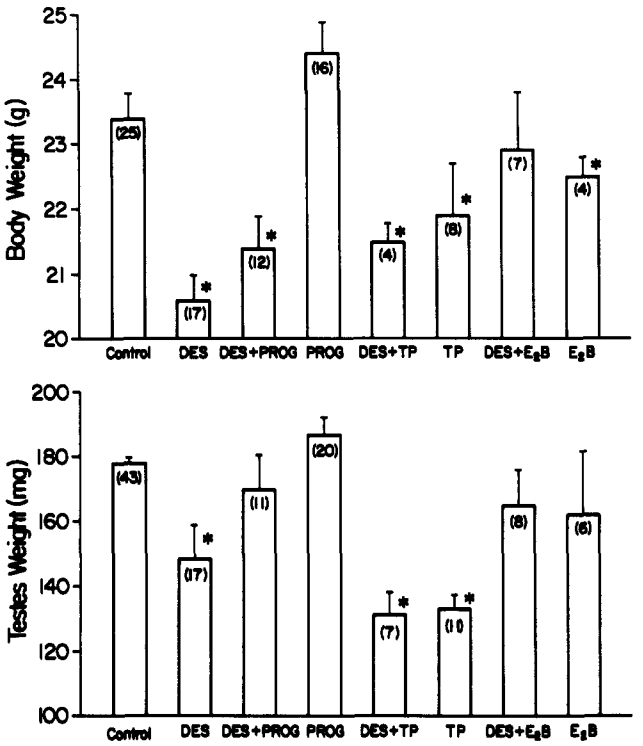

FIG. 1. Effects of neonatal injection (SC) daily for 5 days postpartum with DES (5 μg), alone or in combination with progesterone (100 μg), testosterone propionate (TP; 50 μg), estradiol benzoate (E_2B, 5 μg) or these same doses of progesterone, TP or E_2B alone on body weights (top) and testicular weights (bottom) in adulthood (60–70 days of age). Means±S.E. *Significantly different ($p<0.05$) from controls by analysis of variance and Duncan's multiple range test for pairwise comparisons.

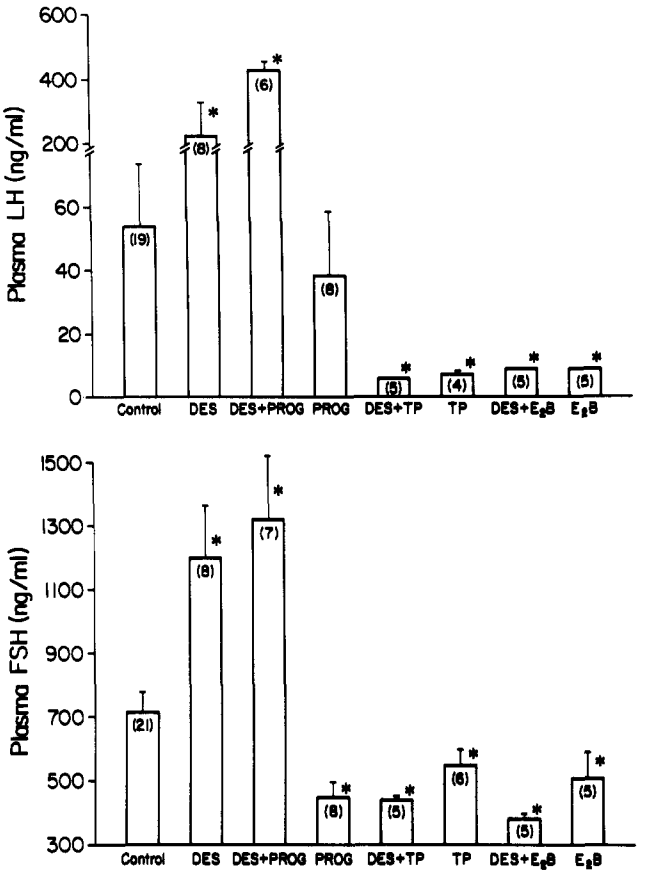

FIG. 2. Plasma levels of LH (top) and FSH (bottom) in adult intact mice neonatally exposed to DES, alone or in combination with progesterone, TP or E_2B, or progesterone, TP or E_2B at doses indicated in the text. Means±S.E. (n). *Significantly different from control ($p<0.05$) by Kruskal-Wallis test.

Plasma LH and FSH

Plasma LH and FSH levels were significantly reduced in adult males which had been neonatally injected with TP or E_2B, alone or in combination with DES (Fig. 2, top). In contrast, LH and FSH concentrations were markedly increased in intact adult males previously treated with DES or DES and progesterone (Fig. 2, bottom). In addition, plasma FSH levels, but not those of LH, were reduced in males neonatally-injected with progesterone alone.

Pituitary Responsiveness to Castration

Post-castration plasma LH levels were lower in mice neonatally-exposed to DES alone, or in combination with TP, E_2B or progesterone, as well as in those treated with E_2B or progesterone alone than those in the controls (Fig. 3). Neonatal TP injection produced post-castration LH levels comparable to those measured in control males.

Post-castration FSH levels appeared to be less affected by neonatal treatments, although FSH concentrations in peripheral plasma were reduced in DES + TP-treated mice, and increased in TP-treated animals, in comparison to values observed in controls (Fig. 3).

Pituitary Gonadotropin Response to TP Injection

Administration of 125 μg TP one week post-castration reduced plasma LH levels in male mice neonatally treated with DES, TP, TP + DES, as well as in control males (Fig. 3), in comparison to values measured in oil-injected castrate males from each of these treatment groups. However, the acute response to TP by castrated male mice neonatally exposed to progesterone consisted of an increase in LH levels compared to those in progesterone-exposed castrates not receiving TP injection.

Plasma FSH levels in castrated animals were largely unchanged 1.5 hr after SC injection of 125 μg TP. Only in males exposed to progesterone alone did injection of TP increase plasma FSH levels (Fig. 3). Insufficient blood samples were obtained from several mice to determine plasma gonadotropin levels; therefore, the treatment groups DES + progesterone, DES + E_2B and E_2B alone are not represented.

Testicular Responsiveness to hCG In Vivo

The T response to intratesticular hCG administration, as measured by the ratio of T concentrations in hCG- versus

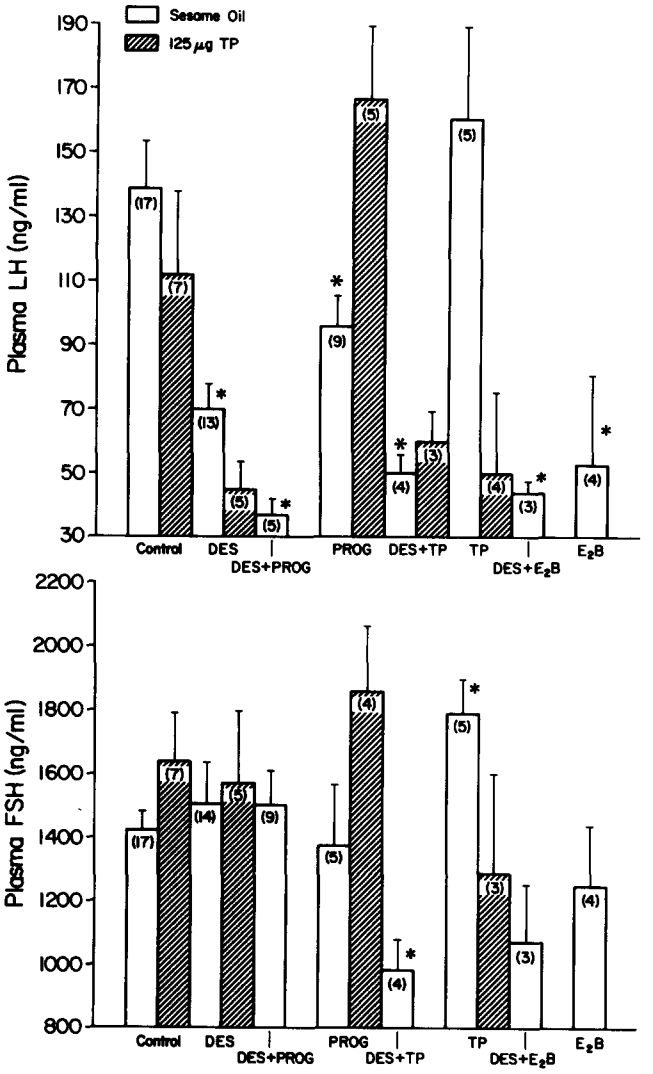

FIG. 3. Plasma LH (top) and FSH (bottom) levels in adult intact mice neonatally exposed to DES, progesterone, TP or E_2B alone or DES in combination with these steroids one week post-castration and 1 hr after SC injection of 125 μg TP. Means±S.E. (n). *Significantly different from control ($p<0.05$) by Kruskal-Wallis test.

saline-injected testes, was significantly reduced by neonatal injection of DES plus progesterone, or DES + E_2B, but was not influenced by the other neonatal steroid treatments (Table 1).

Basal T levels, i.e., concentrations of T, in saline-injected testes were significantly elevated in adult males which had been neonatally exposed to progesterone or progesterone plus DES. Stimulation with hCG resulted in significantly higher levels of T in animals neonatally injected with progesterone, compared to all other groups (Table 1). Animals treated with DES plus E_2B appeared to be unresponsive to hCG, as indicated by significantly lower T concentrations in hCG-injected testes and a reduced T ratio (Table 1).

Biogenic Amines

There was a significant reduction in NE concentrations in hypothalamus from castrated, compared to intact males (Table 2). Levels of 5-HT were increased post-castration in all groups except DES + TP-exposed animals. There were no significant differences between groups in levels of hypothalamic NE post-castration. The levels of NE in hypothalamus were increased in intact DES + EB- and DES + TP-exposed mice, compared to those in intact controls (Table 2).

In adult mice exposed to DES + TP, castration decreased 5-HT levels significantly. In addition, levels of 5-HT in intact males treated with DES + TP were markedly higher than those in intact controls, while castrated DES + TP males had lower 5-HT than castrated controls (Table 2).

Intact EB-treated males had higher levels of 5-HT than those in intact controls. There were no significant effects of castration or neonatal treatments on hypothalamic dopamine or 5-hydroxyindoleacetic acid levels (data not shown).

TABLE 1

BASAL AND HUMAN CHORIONIC GONADOTROPIN (hCG)-STIMULATED TESTOSTERONE (T) LEVELS (ng/ml) AND T RATIOS IN MALE MICE 30 MINUTES AFTER INTRATESTICULAR ADMINISTRATION OF 2.5 mIU hCG (MEANS ± SE)

Treatment	(n)	Basal T (ng/ml)	hCG (ng/ml)	T Ratio
Control†	(16)	53 ± 12	105 ± 18	2.90 ± 0.37
DES	(8)	60 ± 17	165 ± 41	3.03 ± 0.68
DES + Progesterone	(10)	99 ± 11*	132 ± 16	1.37 ± 0.10*
Progesterone	(11)	103 ± 19*	227 ± 32*	2.90 ± 0.60
DES + TP	(4)	64 ± 9	120 ± 12	2.12 ± 0.26
TP	(8)	50 ± 10	118 ± 24	2.84 ± 0.90
DES + E_2B	(5)	83 ± 39	46 ± 18*	1.72 ± 0.53*
E_2B	(4)	75 ± 16	141 ± 41	2.26 ± 0.84

*Significantly different from control ($p<0.05$).

†Oil-treated and untreated animals were not significantly different and were combined for statistical analysis and presentation.

DISCUSSION

The findings of morphological reproductive tract abnormalities reported in the present study are consistent with effects of DES exposure in humans, as well as in laboratory animals [3–5, 33].

Complete testicular atrophy was observed in two mice, one neonatally exposed to E_2B and the other to DES. In addition, another DES-exposed male had only one testis. These findings are consistent with reports on the effects of neonatal 17-β-estradiol treatment in BALB/c mice, although the animals were considerably older (19–27 months) before such anomalies were apparent [18], while the mice in the present study were ~2 months old.

In the female, concomitant exposure to DES and progesterone was reported to alter the timing and severity of associated reproductive tract lesions [17]. Progesterone has anti-estrogenic activity and has been shown to be capable of protecting neonates from disruptive effects of testosterone or estradiol administration [10, 17, 20, 35]. In the present study, concomitant exposure to progesterone appears to block or delay the development of lesions in the reproductive tract of DES-treated males. However, it would appear that these effects of progesterone are not indicative of the ability of progestational agents to block effects of DES on neuroen-

TABLE 2

EFFECT OF NEONATAL STEROIDS AND/OR DES ON HYPOTHALAMIC CONCENTRATIONS OF NOREPINEPHRINE (NE) OR SEROTONIN (5-HT) IN ADULT MALE MICE (MEANS ± SE [n])

Treatment		NE (ng/g)	5-HT (ng/g)
Oil	Intact	2.58 ± 0.12 (25)	2.21 ± 0.09 (28)
	Castrated†	2.20 ± 0.28 (13)	2.78 ± 0.17 (19)*
DES	Intact	2.71 ± 0.22 (26)	2.29 ± 0.13 (12)
	Castrated†	2.46 ± 0.18 (11)	2.52 ± 0.17 (13)
DES + PROG	Intact	2.80 ± 0.23 (5)	2.38 ± 0.34 (6)
	Castrated†	2.76 ± 0.70 (4)	2.69 ± 0.18 (4)
PROG	Intact	2.41 ± 0.31 (7)	2.11 ± 0.14 (7)
	Castrated†	2.18 ± 0.26 (8)	2.76 ± 0.40 (9)*
DES + TP	Intact	3.04 ± 0.10 (3)*	3.20 ± 0.13 (3)
	Castrated†	—	2.38 ± 0.11 (3)
TP	Intact	2.87 ± 0.26 (5)	2.49 ± 0.11 (6)
	Castrated†	2.56 ± 0.58 (4)	2.75 ± 0.32 (6)
DES + EB	Intact	3.33 ± 0.43 (3)*	2.06 ± 0.29 (3)
	Castrated†	2.95 ± 0.48 (3)	2.77 ± 0.32 (4)
EB	Intact	—	—
	Castrated†	2.80 ± 0.39 (4)	2.58 ± 0.48 (3)

*Significantly different from intact controls ($p<0.05$).
†Two-weeks post-castration.

docrine or testicular response parameters. Indeed, plasma LH levels were increased in intact male mice neonatally exposed to either DES or DES + progesterone. In addition, concomitant DES exposure appeared to have no influence on the reductions in plasma LH levels produced by neonatal TP or E_2B treatment. Indeed, the effects of DES on plasma FSH levels in intact males were not influenced by concomitant progesterone treatment and, as with plasma LH levels, concomitant DES exposure added little to the reduction in plasma FSH levels produced by TP or E_2B alone. Interestingly, both progesterone and E_2B appeared capable of attenuating the suppressive effects of DES on body and testicular weights.

The effects of DES and E_2B were usually quite similar. However, plasma levels of LH and FSH were increased in DES-exposed animals, but were markedly reduced in mice receiving E_2B, alone, or in combination with DES. It is possible that there is a qualitative difference between the nonsteroidal estrogen DES and the steroid estrogens, which result in subtle alterations in receptor binding characteristics affecting negative feedback regulation of pituitary function. However, at present the reasons for these differences remain to be elucidated.

Neonatal treatment with DES, particularly in conjunction with steroid administration, significantly attenuated the castration-induced increase in plasma LH levels. Plasma FSH levels appeared to be less affected, although treatment with TP and DES + TP resulted in lower and higher, respectively, levels of FSH than those in the controls.

The changes in plasma gonadotropins in castrated males after a single injection of TP were used to assess pituitary response to steroid feedback. The results indicated that neonatal exposure to progesterone was singularly capable of producing a positive feedback response in males, in terms of both plasma LH and FSH concentrations. However, the mechanism(s) by which progesterone altered the feedback response in castrated mice in the present study is unclear.

The combination of DES with progesterone or E_2B reduced testicular responsiveness *in vivo* to exogenous gonadotropin, while progesterone alone or in combination with DES increased basal testicular T levels. It has been shown that neonatal exposure of rats to high levels of progesterone increases the number of Leydig cells at 1–3 weeks of age [30]. In addition, exposure to DES has been reported to directly inhibit T synthesis by rat testes *in vivo* and *in vitro* [27], and we have previously reported that DES suppresses *in vitro* T production by decapsulated mouse testes [17].

The findings from these experiments suggest that DES exposure, in addition to inducing reproductive tract abnormalities, can affect brain biogenic amines and pituitary-gonadal feedback regulation in males.

ACKNOWLEDGEMENTS

These studies were supported by a grant from Eli Lilly Company and NIH 5 R23-16329 (S.D.) and the Center for Research in Reproductive Biology Grant 5 P30-HD10202.

REFERENCES

1. Bartke, A., K. I. H. Williams and S. Dalterio. Effects of estrogen on testicular testosterone production *in vitro*. *Biol Reprod* **17**: 645–669, 1977.
2. Bern, H. A., L. A. Jones, K. T. Mills, A. Kohrman and T. Mori. Use of the neonatal mouse in studying long-term effects of early exposure to hormones and other agents. *J Toxicol Environ Health* Suppl **1**: 103–116, 1976.
3. Bibbo, M., M. Al-Naqueeb, I. Baccarini, W. Gill, M. Newton, K. M. Sleeper, M. Soner and G. L. Wied. Follow-up study of male and female offspring of DES-treated mothers: A preliminary report. *J Reprod Med* **15**: 29–32, 1975.
4. Bibbo, M., W. G. Gill, F. Azizi, R. Blough, V. S. Fang, R. L. Rosenfield, G. F. B. Schumacher, K. Sleeper, M. G. Sonek and G. L. Wied. Follow-up study of male and female offspring of DES-exposed mothers. *Obstet Gynecol* **49**: 1–8, 1977.
5. Boylan, E. S. Morphological and functional consequences of prenatal exposure to diethylstilbestrol in the rat. *Biol Reprod* **19**: 854–863, 1978.
6. Dalterio, S., A. Bartke, A. Brodie and D. Mayfield. Effects of testosterone, estradiol, aromatase inhibitor, gonadotropin and prolactin on the response of mouse testes to acute gonadotropin stimulation. *J Steroid Biochem* **18**: 391–396, 1983.
7. Dalterio, S., A. Bartke and D. Mayfield. Cannabinoids stimulate and inhibit testosterone production *in vitro* and *in vivo*. *Life Sci* **32**: 605–612, 1983.
8. Dalterio, S., R. Steger, D. Mayfield and A. Bartke. Early cannabinoid exposure influences neuroendocrine and reproductive functions in mice. II: Postnatal Effects. *Pharmacol Biochem Behav* **20**: 115–123, 1984.
9. Davis, M. E. and E. L. Porter. The response of the human fetal reproductive system to the administration of diethylstilbestrol and testosterone propionate during early pregnancy. *Endocrinology* **42**: 370–378, 1948.
10. Dorfman, R. I. The anti-estrogenic and anti-androgenic activities of progesterone in the defense of a normal fetus. *Anat Rec* **157**: 547–558, 1965.

11. Dunn, T. B. and A. W. Green. Cysts of the epididymis, cancer of the cervix, granular cell myoblastoma and other lesions after estrogen injection in newborn mice. *J Natl Cancer Inst* **31:** 425–455, 1963.
12. Gorski, R. A., R. E. Harlan and L. W. Christensen. Perinatal hormonal exposure and the development of neuroendocrine regulatory processes. *J Toxicol Environ Health* **3:** 97–121, 1977.
13. Haney, A. F., R. R. Newbold and J. A. McLachlan. Changes in ovarian morphology and steridogenesis *in vitro* in mice exposed to diethylstilbestrol (DES) *in utero*. *Biol Reprod* **24:** Suppl 11, 130A, 1981.
14. Henderson, B. E., B. Benton, M. Cosgrove, J. Baptista, J. Aldrich, D. Townsend, W. Hart and T. M. Mack. Urogenital tract abnormalities in sons of women treated with diethylstilbestrol. *Pediatrics* **58:** 505–507, 1976.
15. Herbst, A. Summary of the changes in the human female genital tract as a consequence of maternal diethylstilbestrol therapy. *J Toxicol Environ Health Suppl* **1:** 13–20, 1976.
16. Johnson, J. T. and M. Dowie. Revenge of a DES son. *Mother Jones* **40:** 31–40, 1983.
17. Jones, L. A. and H. A. Bern. Long-term effects of neonatal treatment with progesterone, alone and in combination with estrogen, on the mammary gland and reproductive tract of Balb/C_3H mice. *Cancer Res* **37:** 67–75, 1977.
18. Jones, L. A. Long-term effects of neonatal administration of estrogen and progesterone, alone or in combination on male Balb/c and Balb/cF C_3H mice. *Proc Soc Exp Biol Med* **165:** 17–25, 1980.
19. Kalra, P. S. and S. M. McCann. Involvement of catecholamines in feedback mechanisms. *Prog Brain Res* **39:** 185–198, 1973.
20. Kincl, F. A. and M. Maqueo. Prevention by progesterone of steroid-induced sterility in neonatal male and female rats. *Endocrinology* **77:** 859–862, 1965.
21. Ladosky, W., W. M. Kesikowski and I. F. Gaziri. Effect of a single injection of chlorpromazine into infant male rats on subsequent gonadotropin secretion. *J Endocrinol* **48:** 151–156, 1970.
22. McLachlan, J. A. In: *Developmental Effects of Diethylstilbestrol (DES) in Pregnancy*, edited by A. H. Herbst and H. A. Bern. New York: Thieme-Stratton, Inc., 1981.
23. McLachlan, J. A., R. R. Newbold and B. Bullock. Reproductive tract lesions in male mice exposed prenatally to diethylstilbesterol. *Science* **190:** 991–992, 1975.
24. Newbold, R. R., B. C. Bullock and L. A. McLachlan. Exposure to diethylstilbestrol during pregnancy permanently alters the ovary and oviduct. *Biol Reprod* **28:** 735–744, 1983.
25. Nomura, T. and T. Kanzaki. Induction of urogenital anomalies and some tumors in the progeny of mice receiving diethylstilbestrol during pregnancy. *Cancer Res* **37:** 1099–1104, 1977.
26. O'Malley, B. W. and A. R. Means. Receptors and reproductive hormones. In: *Experimental Medicine and Biology*, vol 26, edited by B. W. O'Malley and A. R. Means. New York: Plenum Press, 1973, pp. 73–401.
27. Sholiton, C. J., L. Srivastara and B. B. Taylor. The *in vitro* and *in vivo* effects of diethylstilbestrol on testicular synthesis of testosterone. *Steroids* **26:** 797–806, 1975.
28. Steger, R. W., A. Bartke and B. D. Goldman. Alterations in neuroendocrine function during photoperiod-induced testicular atrophy and recrudescence in the Golden hamster. *Biol Reprod* **26:** 437–444, 1982.
29. Steinlechner, S., R. W. Steger, T. S. King and R. J. Reiter. Diurnal variations in the serotonin content and turnover in the pineal gland of the Syrian hamster. *Neurosci Lett* **35:** 167–171, 1982.
30. Tapanainen, J., G. Pentinnen and I. Huhtaniemi. Effect of progesterone treatment on the development and function of neonatal rat adrenals and testis. *Biol Neonate* **36:** 190–197, 1979.
31. Terakawa, N., R. A. Huseby and L. T. Samuels. Quantitative changes in estrogen receptor produced by chronic DES treatment of two mouse strains differing in susceptibility to Leydig cell tumor induction. *J Steroid Biochem* **16:** 643–652, 1982.
32. Tillson, H. A. and C. A. Lamartiniere. Neonatal exposure to diethylstilbesterol affects the sexual differentiation of male rats. *Neurobehav Toxicol* **1:** 123–128, 1979.
33. Wadsworth, P. F. and R. Heywood. The effects of prenatal exposure of Rhesus monkeys (Macaca mulatta) to diethylstilbestrol. *Toxicol Lett* **2:** 115–118, 1978.
34. Walker, B. Reproductive tract anomalies in mice after prenatal exposure to DES. *Teratology* **21:** 313–321, 1980.
35. Warner, M. R., R. W. Warner and C. W. Clinton. Reproductive tract calculi: Their induction, age incidence, composition and biological effects in Balb/c Crgl mice injected as newborns with estradiol -17β. *Biol Reprod* **20:** 310–322, 1979.
36. Warner, M. R., L. Yau and J. M. Rosen. Long-term effects of perinatal injection of estrogen and progesterone on the morphological and biochemical development of the mammary gland. *Endocrinology* **106:** 823–832, 1980.
37. Ways, S. C. and H. A. Bern. Long-term effects of neonatal treatment with cortisol and/or estrogen in the female Balb/c mouse. *Proc Soc Exp Biol Med* **160:** 94–98, 1979.