Influence of Neonatal Diethylstilbestrol Treatment on Prolactin Receptor Levels in the Mouse Male Reproductive System (43093)

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Abstract. **Neonatal exposure to the synthetic estrogen, diethylstilbestrol, is known to affect the structure of the male reproductive system; thus, changes may also occur in the levels of hormone receptors. Prolactin receptor levels from the reproductive systems of male BALB/c mice exposed neonatally to diethylstilbestrol were analyzed. Neonatal exposure to diethylstilbestrol caused significant decreases (i) in prolactin receptor levels in the seminal vesicle, ductus deferens, and anterior and ventral prostates and (ii) in tissue weight and protein content in reproductive organs other than the ventral prostate.** [P.S.E.B.M. 1990, Vol 194]

The influence of steroid hormones during differentiation and development of male mouse sex accessory gland is well documented (1–3). Perinatal exposure to exogenous estrogens at critical periods causes alterations in the normal development of the reproductive system in male rodents. These permanent structural changes include hyperplastic and/or metaplastic changes in the epithelium of the seminal vesicle (SV), anterior prostate (AP; coagulating gland), ventral prostate (VP), and epididymis (4–13).

We have shown (14) that neonatal diethylstilbestrol (DES) exposure caused significant decreases in cytosolic androgen and cytosolic and nuclear estrogen receptor levels in the anterior prostate and cytosolic estrogen receptor levels in the ventral prostate. A significant increase was seen in the cytosolic estrogen receptor levels in the seminal vesicle (14, 15). Inasmuch as blood prolactin (PRL) levels may be modified by neonatal DES treatment (see Discussion), PRL receptor levels may also be altered, especially because PRL receptors are regulated by PRL itself (16, 17). In this study, we have examined the levels of PRL receptors in the AP, VP, SV, ductus deferens (DD), testis, and liver of neonatally DES-treated male mice to determine whether early DES exposure results in significant changes.

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Materials and Methods

Mice. A total of 480 male BALB/cCrgl mice were given daily subcutaneous injections of either 1 μg of DES (lot no. 29C-0318; Sigma) in 0.02 ml of sesame oil (Hain Pure Food, Los Angeles, CA) or sesame oil alone for the first 5 days of life beginning within 18 hr after birth. Litters were adjusted to four to six pups with the addition or removal or newborn females. All mice were weaned at 1 month of age. At 2 months of age, the mice were killed, and AP, VP, SV, DD, testis, and liver from a total of 60 mice were separately collected and pooled in glass containers submerged in ice. At the end of the collection of tissues, they were frozen in liquid nitrogen until membrane preparation.

Membrane Preparation. Membranes were prepared according to the method of Haro *et al.* (18) as summarized here. Tissues were weighed and homogenized in 4 volumes (w/v) of buffer using a Polytron PT-10 at a setting of 5 for three periods of 10 sec each, with cooling intervals of 10 sec with the flask in ice during the entire homogenization. The homogenization buffer was composed of 200 m*M* glycine-NaOH, 150 m*M* NaCl, 50 m*M* EDTA, 50 m*M* EGTA, 300 m*M* sucrose at pH 9.0, 1 m*M* phenylmethylsulfonyl fluoride, and 100 kallikrein inhibitor units of Trasylol/ml of buffer. Homogenates were then centrifuged in a fixed angle rotor at 9500*g* for 20 min at 4°C. The resulting pellet

was resuspended with a Teflon-glass homogenizer in 100 μl of 25 m*M* Tris-HCl/10 m*M* $MgCl_2$ at pH 7.6. An aliquot (10 μl) was taken to determine protein content (19), and the remaining preparation was stored at −80°C until assayed.

Hormone. Ovine prolactin (oPRL, NIH-oPRL-18) was a gift from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases.

Iodination. Ovine prolactin was iodinated by a lactoperoxidase method previously described (20) with some modifications. Ten microliters of oPRL in 10 μl of 0.05 *M* sodium phosphate buffer (pH 7.5), 10 μg of lactoperoxidase in 10 μl of 0.05 *M* sodium phosphate buffer (pH 7.5), and 10 μl of 1:50,000 dilution of 30% H_2O_2 in distilled, deionized water were added to 1 mCi of carrier-free Na ^{125}I (10 μl). After 1 min, 10 more μl of diluted 30% H_2O_2 were added. This procedure was repeated twice. One minute after the final addition of H_2O_2, the reaction mixture was diluted with 300 μl of 0.05 *M* sodium phosphate (pH 7.5). The diluted mixture was immediately applied in a 1- × 50-cm Sephadex G-75 column previously equilibrated with 25 m*M* Tris-HCl/10 m*M* $MgCl_2$, 0.1% bovine serum albumin, and 0.1% sodium azide (pH 7.6). Sequential 2-ml fractions were collected in 13- × 100-mm glass tubes and the radioactivity was monitored in a gamma counter. The specific activity of ^{125}I-labeled oPRL was 64 μCi/μg.

Prolactin Receptor Determination. Specific binding was estimated by incubating 150 μg of membrane protein in 50 μl of buffer (100 m*M* Tris, 500 m*M* sodium acetate, 10 m*M* $MgCl_2$ containing 0.1% bovine serum albumin) at pH 7.6, with 25,000 cpm of ^{125}I-labeled oPRL in 50 μl of buffer in the presence or absence of excess unlabeled oPRL (1 μg in 50 μl of buffer). The final volume was adjusted to 250 μl with buffer. The mixture was incubated for 16 hr at room temperature (21). The incubation was terminated by the addition of 1.5 ml ice-cold buffer. Free and bound ^{125}I-labeled oPRL were separated by centrifugation at 2000*g* for 30 min. The supernatant was decanted, and the tubes containing the membrane pellet were dried and counted in a gamma counter. Results were expressed as

$$\% \text{ specific binding } (SB) = (\text{total binding } (TB) - \text{nonspecific binding } (N))/\text{total counts } (TC) \times 100,$$

where *TB* is the radioactivity bound in the absence of unlabeled hormone, *N* is the radioactivity bound in the presence of a large excess of unlabeled hormone, and *TC* is the amount of ^{125}I-labeled oPRL added to the binding reaction. In each experiment, total binding and nonspecific binding were determined in triplicate. In the competition studies using oPRL as both labeled and unlabeled ligand, binding constants were derived using the LIGAND computer program (22). The LIGAND software uses a statistically valid, appropriately weighted least squares curve-fitting algorithm and gives objective measurement of goodness of fit.

Statistics. Data were analyzed by one-way analysis of variance. All statistical analyses were performed using CRISP version 3.03 (Crunch, Oakland, CA) for the IBM personal computer.

Results

Prolactin Receptor Levels. The effects of neonatal DES treatment on prolactin receptors (PRL-R) are shown in Table I. In the SV, DD, and AP there was a significant decrease in PRL-R compared with control when expressed on a membrane protein basis. There were no significant modifications seen in PRL-R levels in the VP, testis, or liver. However, when the data were compared on a tissue weight basis, which is more indicative of the cellular content of receptors since there were decreases in protein content in DES-treated reproductive tissues (see below), the PRL-R level was drastically decreased (by 75% to 50%), not only in SV, DD, and AP but also was decreased in the VP. Scatchard analysis (Table II) of PRL binding in the different tissues indicates no appreciable difference in the dissociation constant between DES-exposed and control tissues.

Protein Content. Table III shows the effect of neonatal DES treatment in the protein content and tissue weight. There was a significant decrease in protein content in SV, AP, VP, and DD and in tissue weight in SV, AP, and DD when compared with controls.

Discussion

Prolactin is increasingly recognized as a synergist in growth of the prostate and possibly other sex accessories (23). The present study indicates a significant decrease in PRL-R levels in the DES-exposed SV, VP, AP, and DD. This effect appears to be specific for these tissues since no effect was observed on the testis or liver of the same animals. Scatchard analysis of PRL binding in the different tissues indicates no changes in the dissociation constant between DES-exposed and control tissue, thus indicating that the number of binding sites but not the affinity is decreased in DES-exposed tissue. Differential effects may be related to a variation in sensitivity of the neonatal prostatic lobes and SV to estrogen. We have shown recently that the same treatment results also in decreased levels of estrogen and androgen receptors in AP and VP but not in SV (14). Our results now suggest that SV, VP, AP, and DD may be less sensitive to PRL following DES treatment. Indeed, recent experiments from our laboratory indicate that isolated mammary epithelial cells of DES-exposed mice may be less sensitive to PRL (24). The significant decrease in protein levels seen in SV, VP, AP, and DD confirms earlier reports on protein levels from similarly

Table I. Prolactin Receptor Content in Reproductive Organs and Liver

	Liver	Testis	Seminal vesicle	Anterior prostate	Ventral prostate	Ductus deferens
n	4[a](60)[b]	4(60)	4(60)	4(60)	4(60)	4(60)
	% Specific binding/300 g membrane protein (mean ± SE)					
Control	4.83 ± 0.24	10.53 ± 0.44	8.97 ± 1.03	3.02 ± 1.77	2.35 ± 0.03	11.35 ± 0.87
DES-treated	6.87 ± 1.05	9.33 ± 0.40	4.37 ± 0.63[c]	1.37 ± 0.03[c]	2.58 ± 0.13	4.38 ± 0.31[c]
	Fmol/g tissue (mean ± SE)					
Control	71.45 ± 9.12	34.58 ± 2.37	21.86 ± 1.23	11.80 ± 0.96	11.61 ± 0.18	31.61 ± 2.19
DES-treated	89.29 ± 14.77	39.15 ± 2.18	5.50 ± 0.26[c]	4.94 ± 0.04[c]	6.88 ± 0.38[c]	15.31 ± 1.15[c]

[a] Number of experiments.
[b] Total number of animals used for tissue pools in parentheses.
[c] Significantly different from controls ($P < 0.05$).

Table II. Scatchard Analysis of Prolactin Receptor in Reproductive Organs and Liver

	Liver	Testis	Seminal vesicle	Anterior prostate	Ventral prostate	Ductus deferens
Control	3.6[a]	5.4	9.3	2.3	4.2	4.4
DES-treated	5.2	7.6	5.5	3.2	4.2	5.4

[a] Dissociation constant ($K_d \times 10^{-10}$ *M*).

treated mice (14). Immunocytochemical localization of the mPRL receptor could be useful to see in which compartment(s) levels are altered.

There is some controversy as to whether neonatal treatment of mice with DES or other estrogens results in increased circulating PRL levels and in increased prolactin synthesis and/or release in adult life. The picture is confounded by the use of different mouse strains, the presence or absence of expressed mammary tumor virus, and the neonatal dosage of hormone employed. Thus, the data of Nagasawa *et al.* (25) on BALB/c mice include some evidence of increased blood levels; pituitaries from estrogen-treated mice showed a significantly greater number of prolactin cells (26). Kalland *et al.* (27) found no increased PRL levels in their mouse stock but did see increased sensitivity of the pituitary prolactin cells to estrogen stimulation as a result of neonatal DES treatment. Huseby and Thurlow (28) found evidence for hyperprolactinemia in C3H × BALB/c and BALB/c mice exposed prenatally to "low-dose" DES. Lopez *et al.* (29) found that high doses (2.5 μg daily) of DES given neonatally to C3H/mammary tumor virus-positive mice decreased circulatory levels of PRL as well as PRL synthesis by pituitaries incubated *in vitro* (release was the same in the DES and control pituitaries). The decreased PRL levels at high DES dose levels are consistent with the minimal mammary development seen in BALB/c mice so exposed (Mills and Bern, unpublished data) and also consistent with the present results indicating decreased PRL receptor levels in the male reproductive tissue. PRL receptors are regulated by PRL itself (16, 17). Thus, decreased circulating levels of PRL could result in decreases sensitivity to PRL in male sex accessories. Neonatal exposure of mice to estrogen, as well as to androgen (30), appears to exert both direct effects on the male reproductive tract and indirect effects via the hypothalamo-hypophysio-gonadal axis.

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Table III. Protein Content and Weight of Reproductive Organs and Liver

	Liver	Testis	Seminal vesicle	Anterior prostate	Ventral prostate	Ductus deferens
n	60[a]	60	60	60	60	60
	Mg membrane protein/g of tissue (mean ± SE)					
Control	30.14 ± 7.15[b]	6.70 ± 0.41	5.15 ± 0.55	9.60 ± 0.79	9.83 ± 0.09	9.31 ± 0.33
DES	26.02 ± 0.50	8.23 ± 0.42	3.14 ± 0.07[b]	6.20 ± 0.59[b]	5.12 ± 0.21[b]	5.61 ± 0.37[b]
	g Tissue/animal (mean ± SE)					
Control	1.19 ± 0.12	0.196 ± 0.016	0.093 ± 0.008	0.032 ± 0.003	0.011 ± 0.001	0.09 ± 0.01
DES	1.13 ± 0.07[b]	0.155 ± 0.003	0.034 ± 0.002[b]	0.013 ± 0.001[b]	0.012 ± 0.001	0.03 ± 0.01[b]

[a] Number of measurements.
[b] Significantly different from controls ($P < 0.05$).

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