Long-Term Effects of Neonatal Hormonal Treatments on Plasma Prolactin Levels in Female BALB/cfC3H and BALB/c Mice

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ABSTRACT

In view of morphological indications that the effect of neonatal sex steroid exposure on mammary gland development and tumorigenesis in mice may be a consequence of sustained prolactin secretion, homologous radioimmunoassay was used to measure plasma prolactin levels in variously treated mice at several ages. Female BALB/cfC3H/Crgl and BALB/cCrgl mice received daily s.c. injections of 5 μg diethylstilbestrol, 20 μg diethylstilbestrol, 20 μg 17β-estradiol, 20 μg testosterone, or 20 μg ovine prolactin for the first 5 days of postnatal life. In mice killed at about 2, 7, and 15 months of age, plasma prolactin levels of BALB/cfC3H females treated neonatally with diethylstilbestrol, 17β-estradiol, or prolactin were comparable with those of the controls at proestrus/estrus and usually significantly higher than the levels in those at metestrus/diestrus. Prolactin levels in mice treated with testosterone were usually significantly higher than those in the other treated groups and in all control groups. In BALB/c females at about 7 and 15 months, most steroid-treated groups showed prolactin levels greater than control values at metestrus/diestrus but comparable with those at proestrus/estrus. However, testosterone treatment resulted in levels greater than those at proestrus/estrus. The importance of prolactin in mammary tumorigenesis in mice has been established by the administration of prolactin or prolactin suppressors, by pituitary grafting, and by hypothalamic lesions (2, 3, 14, 17, 21, 22). Neonatal treatment of mice with E₂ or TE leads to increased mammary tumor incidence and to an earlier age of tumor onset in MTV-expressed BALB/cfC3H female mice (1, 5, 10). Neonatal injections of OPRL did not have this effect (10). The frequent occurrence of dilated ducts and lobules in mice exposed neonatally to sex steroids, especially in those animals developing tumors (5, 10), led to this investigation of plasma prolactin levels at several ages in female mice of the same strain with and without expressed MTV and treated neonatally with DES, E₂, TE, or OPRL.

INTRODUCTION

The importance of prolactin in mammary tumorigenesis in mice has been established by the administration of prolactin or prolactin suppressors, by pituitary grafting, and by hypothalamic lesions (2, 3, 14, 17, 21, 22). Neonatal treatment of mice with E₂ or TE leads to increased mammary tumor incidence and to an earlier age of tumor onset.

MATERIALS AND METHODS

Female BALB/cfC3H/Crgl (MTV-expressed) and BALB/c-Crgl (MTV-unexpressed) mice received daily s.c. injections of 5 μg DES, 20 μg DES, 20 μg E₂, 20 μg TE, or 20 μg OPRL for the first 5 days of postnatal life. DES and steroids were injected in 0.02 ml sesame oil and OPRL in 0.9% NaCl solution. Control mice were given the vehicle only (control-oil or control-0.9% NaCl solution).

When the mice were about 2 [2.1 ± 0.0 (S.E.)], 7 (7.0 ± 0.0), and 15 (14.8 ± 0.2) months of age, blood was collected from the vena cava under light ether anesthesia with a heparinized syringe in the afternoon (1:00 to 5:00 p.m.). Plasma was frozen and kept at −20° for the assay of prolactin by homologous radioimmunoassay (18). Plasma prolactin levels in the controls were separated into 2 categories according to the stage of ES (PE/ES or ME/DE) as judged by the histological conditions of the vagina at autopsy. Mice were killed after bleeding, and their mammary glands and genital tracts were removed for histological observation. The mammary glands were fixed in 10% formalin and stained as wholemounts with iron-hematoxylin. Vaginae, uteri, and ovaries were fixed in Bouin’s fluid, sectioned in paraffin at 7 μm, and stained with hematoxylin and eosin.

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RESULTS

Body Weight. Body weight increased with age, and no differences were observed at autopsy following neonatal treatments in either substrain.

Plasma Prolactin Levels. All groups treated neonatally with steroids or DES showed vaginal cornification at the
time of autopsy; OPRL-treated and control mice were in various stages of the estrous cycle. In the OPRL-treated groups, there was no significant difference in plasma prolactin levels between PE/ES and ME/DE, except at 2 months of age in the BALB/cfC3H substrain. Therefore, the plasma prolactin levels in the OPRL-treated groups were combined regardless of stage of ES.

The results from female BALB/cfC3H mice are presented in Chart 1A. Prolactin levels of control mice were significantly higher at PE/ES than they were at ME/DE for all ages examined (\(p < 0.05\)). At all ages prolactin levels of DES- and \(E_2\)-treated mice did not differ significantly from those of PE/ES controls, except for DES (20 \(\mu g\))-treated mice at 2 months, in which the value was lower; however, the levels were higher than those in ME/DE controls (\(p < 0.05\)), except for those given \(E_2\) (at 7 months) and 20 \(\mu g\) DES (at 2 months). TE-treated mice had prolactin levels significantly greater than those of the controls at both PE/ES and ME/DE at all ages (\(p < 0.05\)). Furthermore, levels in TE-treated mice were significantly greater than those in all other treatment groups at 2 and 7 months of age and in OPRL-treated mice at 15 months of age (\(p < 0.05\)). Prolactin levels of OPRL-treated mice were comparable with those of PE/ES control mice at all ages and were significantly higher than those of ME/DE control mice at 2 and 15 months (\(p < 0.05\)).

The results from BALB/c female mice are presented in Chart 1B. Prolactin levels of control mice were significantly greater at PE/ES than they were at ME/DE at all ages (\(p < 0.05\)), with the exception of the 2-month-old groups, where both control values were inexplicably high with large variations. At 2 months all steroid- and DES-treated groups had lower levels than did both PE/ES and ME/DE controls (\(p < 0.05\)), except for TE-treated mice, in which the values were not different from those of ME/DE controls. At 7 and 15 months, levels in TE-treated mice were significantly higher than they were in PE/ES controls (\(p < 0.01\)), and all other steroid and DES treatments resulted in levels comparable with PE/ES control values. \(E_2\) and TE-treated mice had higher levels than did ME/DE controls at these ages (\(p < 0.05\)). OPRL-treated mice had lower levels than did PE/ES controls (\(p < 0.05\)), which were comparable with ME/DE controls at 2 months, but levels were comparable with those of PE/ES controls and higher than those of ME/DE controls at both 7 and 15 months (\(p < 0.05\)).

In both substrains prolactin levels were generally higher at 7 and 15 months of age than they were at 2 months in experimental groups and in the BALB/cfC3H controls.

**Mammary Glands.** The results were essentially consistent with previous reports (1, 5, 10). Table 1 shows the incidence of mammary duct dilation and hyperplastic nodules in the several groups used, as judged from wholemounts of the mammary apparatus. As was reported earlier (10), TE is particularly effective in inducing duct dilation, lobuloalveo-
lar development, and nodules. Even in BALB/c mice with MTV unexpressed, there was a high incidence of dysplasia, which was often nodule-like (cf. Ref. 6).

**Ovaries.** In both substrains the majority of ovaries in the control and OPRL-treated mice contained both follicles and corpora lutea at various stages of development. However, the ovaries of mice receiving steroid hormones or DES neonatally consisted only of follicles and lacked corpora lutea at all ages examined.

**Uterus and Vagina.** The response of the uterus and vagina to neonatal treatment with hormones was essentially similar to the results of previous workers (19). The vaginal epithelium was cornified and hyperplastic and showed downgrowths in mice of both substrains receiving steroid hormones or DES. The control and OPRL-treated mice showed various stages of the estrous cycle and no hyperplastic vaginal downgrowths.

Stratification was observed in the uterine epithelium of some mice treated with steroids or DES neonatally but not in the control or OPRL-treated mice.

**DISCUSSION**

In BALB/cfC3H mice there is a tendency for significantly greater prolactin values to result from neonatal DES and steroid treatments in almost all cases if comparison is made with control mice at ME/DE, where values are lower than they are at PE/ES. Inasmuch as the mice treated neonatally with DES or sex steroids are noncycling, the important difference between them and the controls lies in the occurrence of continuously high prolactin levels, at least comparable with those of control mice in ES. Beyond this, notably high values occur as a consequence of neonatal TE treatment, consonant with observed changes in the mammary gland seen here and elsewhere (10) and also with mammary tumor incidence in 1 series of experiments reported earlier (10). In BALB/c mice at 7 and 15 months of age, this increase again prevails.

In general, although the data are not consistently clear-cut, inspection of Chart 1 reveals a tendency toward increased prolactin levels in mice neonatally exposed to steroid hormones or DES. As found in previous studies, the ovaries of steroid- or DES-treated mice consisted only of anovulatory follicles, whereas ovaries of OPRL-treated and control mice showed both follicles and corpora lutea. It is well established in rats that neonatal hormone treatment induces a permanent alteration in the hypothalamopituitary-ovarian axis, i.e., release of gonadotropin (presumably largely follicle-stimulating hormone) from the anterior pituitary at a constant rate and consequent continuous secretion of estrogen, a potent stimulator of pituitary prolactin secretion, by the anovulatory ovarian follicles (11-13, 20). Cytological studies show increased numbers of prolactin cells in adult female mice and rats (16) treated neonatally with estrogen.

Thus, the data suggest that pituitary secretion of prolactin may be stimulated constantly in mice treated neonatally with sex steroids or DES. Since prolactin is a principal factor in the induction of mammary tumors in mice (2, 16, 22), increased mammary development, including preneoplastic and neoplastic changes, in mice treated with steroid hormones or DES can be ascribed at least in part to the higher levels of circulating prolactin in these mice.

Whereas neonatally OPRL-treated female BALB/cfC3H...
mice showed plasma prolactin levels comparable with those of E2-treated mice, preneoplastic and neoplastic mammary development was not enhanced in these mice (Ref. 10 and results herein). Since the ovaries of OPRL-treated mice contained corpora lutea, any alteration of the hypothalamo-pituitary-ovarian system by neonatal treatment with prolactin is less severe than it is after neonatal steroid or DES treatment. This suggests that the hormonal milieu of OPRL-treated mice is more like that of the controls. Higher levels of prolactin alone (as appear to occur in OPRL-treated mice) do not result in increased mammary dysplasia; it is probable that both continuous estrogen secretion and higher prolactin levels are associated with the enhanced mammary abnormalities.

Neonatal exposure of castrated male and female rats to estrogen favors increased prolactin levels and increased sensitivity to prolactin release by estrogen in the adult, whereas androgen does not have these effects (7, 8). In neonatally androgenized intact rats, the plasma and pituitary prolactin levels are higher (although not always significantly) than they are in age-matched control rats (4, 9). This is in agreement with our present data, in which neonatal TE appears to be a major determinant of prolactin secretion (cf. Ref. 15). Neonatal administration of 5β-dihydrotestosterone, a supposedly biologically inactive steroid, also stimulates pituitary prolactin secretion and induces an ovarian anovulatory syndrome associated with enhanced mammary gland and tumor development in a manner similar to that seen with biologically active sex steroids and DES (23).

Pituitary prolactin secretion was stimulated by neonatal DES and steroid hormone treatment in MTV-unexpressed female BALB/c mice in a fashion similar to that seen in BALB/cF344 females. Although some dysplasias were encountered, mammary tumors did not occur in the glands of this substrain (Ref. 10 and results herein). These findings support the view that the presence of MTV is essential for the occurrence of mammary tumorigenesis in neonatally steroid- or DES-treated mice, as it is in normal mice (10).

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