Effects of Sex Hormones on Oncogene Expression in the Vagina and on Development of Sexual Dimorphism of the Pelvis and Anococcygeus Muscle in the Mouse

Taisen Iguchi,1 Yugo Fukazawa,1 and Howard A. Bern2

1Department of Biology, Yokohama City University, Yokohama, Japan; 2Department of Integrative Biology and Cancer Research Laboratory, University of California, Berkeley, California

Neonatal treatment of female mice with diethylstilbestrol (DES) is known to induce ovary-independent persistent proliferation and cornification of vaginal epithelium. This irreversibly changed vaginal epithelium persistently expressed higher levels of c-jun and c-fos mRNAs, which was not altered by postpubertal estrogen. Sexual dimorphism was encountered in mouse pelvis and anococcygeus muscle. Postpubertal estrogen changed the shape of the pelvis to the female type and postpubertal androgen changed it to the male type. Neonatal exposure to DES and to the antiestrogen tamoxifen altered the developmental pattern of the pelvis, which contained lower concentrations of calcium and phosphorus than controls. The size of anococcygeus muscle was increased by postpubertal androgen but decreased by postpubertal estrogen. However, neonatal estrogen (DES) exposure permanently enlarged the anococcygeus muscle. Thus, neonatal treatment of mice with estrogen and antiestrogen results in irreversible changes in nonreproductive as well as reproductive structures. — Environ Health Perspect 103(Suppl 7):79-82 (1995)

Key words: developmental effects, diethylstilbestrol, tamoxifen, vagina, oncogene expression, sexual dimorphism, pelvis, anococcygeus muscle, estrogen receptor

Introduction

The study of permanent changes in target organs induced by sex hormones administered during a critical period of development began with experiments on the neonatal mouse treated with estrogens (1-4). The vaginal epithelium of mice treated neonatally with estrogen, including diethylstilbestrol (DES), showed persistent proliferation and cornification, frequently resulting in precancerous and cancerous lesions (5). Perinatally sex hormone-exposed female rodents show lesions in vagina and cervix, uterine metaplasia and tumors, oviductal malformations and tumors, polyovular follicles in the ovary, and mammary gland hyperplasia, dysplasia, and neoplasia (6-12). Perinatal treatment of male mice with estrogenic hormones gives rise to neoplastic changes in coagulating gland, seminal vesicle, and testis (13,14). Nongenital abnormalities have also been reported in mice exposed perinatally to sex hormones and antihormones (12,15-18). This paper describes briefly some genital and nongenital abnormalities found in mice exposed neonatally to the estrogen DES and to the antiestrogen tamoxifen.

Protooncogene Expression in Vagina and Uterus of Mice Exposed Neonatally to DES

Estrogens stimulate DNA synthesis and cell proliferation in the female reproductive tract (19) by initially binding to high-affinity nuclear estrogen receptors that directly regulate transcription of target genes by binding to DNA estrogen-responsive elements (20). Products of the genes activated through the estrogen receptor play a role in amplifying the tissue response. As a primary response to the estrogen receptor complex, c-fos, c-jun and c-myc protooncogenes are expressed in immature and adult rat uterus (21) and mouse vagina (22).

The direct effect of estrogen on the expression of these protooncogenes was examined by Northern blot analysis of vagina and uterus in 50-day-old ovariec-tomized mice exposed neonatally to DES. In ovariectomized, unexposed control mice, the expression of c-jun and c-fos mRNAs in the uterus was stimulated by 17β-estradiol. Within 1 hr after estradiol administration at 50 days, c-jun and c-fos mRNAs increased in concentration, showing a peak 3 hr after estradiol stimulation and decreasing with time thereafter. In the vagina, the concentration of c-jun and c-fos mRNAs increased rapidly, reaching a peak within 1 hr. The expression of c-myc in uterus and vagina was not changed by postpubertal estrogen. Expressions of c-jun and c-fos mRNAs were greater in both the uterus (3- and 6-fold, respectively) and the vagina (18- and 4-fold) of neonatally DES-exposed mice than in control organs. These increased levels of c-jun and c-fos expression were not further altered by postpubertal estradiol and may be related to ovary-independent persistent changes in the genital tract.

Mouse Pelvis: Sexual Dimorphism and Responsiveness to Steroid Hormones

The mouse pelvis is sexually dimorphic (23,24). A pair of innominate bones, the osse coxae, are composed of four separate units: ilium, ischium, pubis, and acetabulum,
which unite at the ventral midline as the pubic symphysis to form the pelvis. The innominate bone is connected dorsomedially with the sacrum by the iliosacral joint. Gardner (23) reported that there is no difference in the shape of the innominate bone in young male and female mice; however, after sexual maturity, the pubic bone in females is thinner than in males. Long-term administration of estrogenic hormones to male mice induces a female-type pelvis with thin pubic bones, indicating that sex hormones play a role in pelvic morphogenesis.

Sexual dimorphism of the innominate bone was found in adult T-strain rats and Chinese hamsters, as well as in mice, by computer-aided morphometric analysis (25). Sexual differences in the pubis and the ischium appeared in mice at 30 and 120 days of age, respectively (24,26). The pubis in female mice was longer and thinner than in males, and the ischium in male mice was shorter and thicker than in females in 14 strains of mice. Serum androgen levels in male mice increased from 30 to 50 days of age (27), suggesting that in male mice the shape of pelvic bones is determined by postpubertally secreted androgens.

The ratio of the width of the ischium to the longitudinal length of the innominate bone in 120-day-old female mice was significantly lower than in 30-day-old females; thus ovarian estrogen secreted postpubertally may participate in the formation of the female pelvic bones. In both male and female newborn mice, estrogen receptors were immunohistochemically detected in mesenchymal cells surrounding pubis and ischium and in the periosteum and osteocytes of pubis and ischium, but not in the innominate bone. A weak reaction for the receptors was also observed in the chondrocytes of pubis and ischium (26).

The innominate bones from male mice castrated on the day of birth and from female mice given daily injections of 20 μg testosterone and 5α-dihydrotestosterone for 5 days starting on the day of birth were examined at 30 days of age. The ratio of the pubis width to innominate length in neonatally androgen-treated females at 30 days was greater than in the age-matched untreated females, whereas this ratio was smaller in neonatally castrated 30-day-old males than in age-matched intact males. In adult testicular-feminized male mice lacking androgen receptors, ischium length and width were significantly smaller than in the wild-type males. Pubis width in testicular-feminized male mice was intermediate between those of wild-type males and females. The ischium in females and castrated males was shorter and thinner than in the males. The pubis in gonadectomized males and females was wider than in intact females and smaller than in intact males. The pubis in intact males and castrated males was shorter than in intact females (26).

In summary, the basic type of ischium is the female type; postnatal endogenous androgen modifies the ischium to the male phenotype, and the pubis phenotype is intermediate between males and females; postnatal endogenous androgen induces the male type, and postpubertal endogenous estrogen induces the female type. These results suggest that the shape of the innominate bone is transformed to the male type under the influence of early postnatal androgen (12,26).

Neonatal tamoxifen treatment caused a long-lasting inhibition of pubic bone calcification; the elastic and cartilaginous nature of the symphysis region continued into adulthood. Neonatally tamoxifen-exposed mice showed hernia of the urinary bladder with or without descent of the caecum through the subpubic space (15,16). Although the mechanism of the bladder hernia is unknown, it may be related to the modified symphysis pubis. Mice treated with tamoxifen for 5 days starting at 0 to 10 days of age had significantly longer pubic ligaments than did the corresponding controls. However, mice treated neonatally with clomiphene and nafaxidine possessed normal pubic bones (16).

In 120-day-old female mice treated neonatally with 100 μg tamoxifen, the total area of the pelvis and the individual areas of the ilium, ischium, and pubis were significantly smaller than in the controls. There was no significant difference in the length of ischium between tamoxifen-treated and control mice of both sexes. However, lengths of ilium and pubis and widths of ilium, pubis, and ischium in tamoxifen-treated male and female mice were significantly smaller than in the respective controls. In contrast, neonatal treatment with 2 μg DES for 5 days from the day of birth did not affect the shape of the pelvis of either sex (24).

Indicators of bone resorption in the endosteal area of the pubic bone were measured on histological sections. The number of active osteoclasts was counted per unit area of bone section. In 15-day-old mice given neonatal injections of tamoxifen, the osteoclastic surface, the number of osteoclasts per unit area, and the number of nuclei per osteoclast were significantly smaller than in the controls. Inhibition of resorption persisted in the junction of pubis and ischium of pelvis transplanted under the kidney capsule after treatment with tamoxifen in vitro (18).

These findings indicate that neonatally injected tamoxifen mainly retards the growth of the ilium and pubis in mice by changing the activities of osteoclasts and osteoblasts and that tamoxifen acts directly on the neonatal mouse pubis as an anti-estrogen to inhibit its ossification.

Neonatally DES-treated female mice showed lower amounts of calcium and phosphorus in pelvis and femur at 12 months of age but not at 2 months of age. The pelvises of 3- to 15-month-old male mice treated neonatally with DES and tamoxifen had lower amounts of calcium and phosphorus than age-matched controls (Figure 1). The femurs of 3- to 15-month-old male mice treated neonatally with tamoxifen, but not with DES, had lower amounts of calcium and phosphorus than age-matched controls (Figure 1). These results indicate that neonatal DES and tamoxifen exposure can result in permanent changes in bone tissue in older male and female mice (17).

![Figure 1. Total amount of calcium per mm length of pelvis (A) and femur (B) in control, DES-, and tamoxifen (Tx)-treated male mice.](image-url)
Mouse Anococcygeus Muscle: Sexual Dimorphism and Responsiveness to Steroid Hormones

The anococcygeus muscle described in rats by Gillespie (28) is a paired, thin sheet of smooth muscle inserting on the rectum, having a tendinous origin largely on sacral vertebrae (28,29). A dense adrenergic innervation is distributed through the muscle along with peptidergic innervation but apparently no cholinergic innervation (30). The physiological role of the muscle, however, has not yet been explained.

The rat levator ani muscle from the perineal muscle complex shows sexual dimorphism and its growth is controlled by testosterone (31). The rat costo-uterine muscle, which provides a skeletal attachment for the longitudinal myometrial layer of the uterine horn, is also responsive to sex steroids (32). The length of the muscle cell increases during pregnancy and after estrogen treatment. However, possible sexual dimorphism and the effect of sex steroids on growth of the anococcygeus muscle have not yet been studied, although it has been reported that the muscle in male rat is bigger than in the female (33). The mouse anococcygeus muscle was examined for sexual dimorphism, responsiveness to neonatal and postnatal sex hormones including DES, and androgen and estrogen receptor expression.

Cross-sections of the anococcygeus muscle of C57BL, ICR and BALB/c mice at 90 days of age showed histology characteristic of smooth muscle. Sexual dimorphism of the muscle was demonstrated in the three mouse strains: the cross-sectional area of the muscle in male mice was significantly larger (1.6 - 3.3 times) than that of females (34). Castration significantly reduced the muscle area in male mice. Implantation of a pellet of testosterone increased the muscle area of castrated males. Ovariectomy at 30 days of age increased the muscle area at 60 days of age, but an implantation of estradiol in ovariecotomized mice further reduced the muscle area. Estrogen also reduced the muscle area in male mice. Both androgen receptors and estrogen receptors were expressed in the muscle cells until 60 days of age in both sexes.

Neonatal exposure to DES significantly reduced the anococcygeus muscle area in 60-day-old male mice, but strikingly increased (3.1 times) the muscle area in age-matched females. These opposite effects of DES on male and female muscles are surprising. Serum androgen levels in neonatally DES-exposed male mice were not different from those in controls (Fukazawa and Iguchi, unpublished data). The decrease in muscle area seen in neonatally DES-exposed male mice at 60 days of age was not evident if mice were castrated at 30 days of age. The muscle area of neonatally DES-exposed female mice was significantly larger than in controls, and ovariectomy at 30 days of age did not alter this, indicating that the increase in the area by neonatal DES exposure had occurred before 30 days of age and was not affected by later ovarian hormone withdrawal. These results suggest that both androgen and estrogen play an important role in induction of sexual dimorphism of anococcygeus muscle: the muscle is under the control of both androgen and estrogen during the pubertal period, and estrogen (DES) has an irreversible stimulatory effect on the muscle in neonatal female mice.

Conclusions

The study of animals treated perinatally with sex hormones and related compounds provides an opportunity to analyze various factors influencing developmental and carcinogenic processes (8-12). Thus, the prenatal and neonatal mouse models continue to indicate possible genetic and nongenital changes in human offspring exposed during development to estrogenic hormones, antihormones, and xenobiotics. In further studies, more attention should be paid to abnormalities in nongenital organs exposed to various estrogenic agents during fetal and early postnatal development in mammals including humans.

REFERENCES

1. Takasugi N, Bern HA, DeOme KB. Persistent vaginal cornification in mice. Science 138:438-439 (1962).
2. Takasugi N. Vaginal cornification in persistent estrous mice. Endocrinology 72:607-619 (1963).
3. Dunn TB, Green AW. Cysts of the epididymis, cancer of the cervix, granulocystic myometritis, and other lesions after estrogen injection in newborn mice. J Natl Cancer Inst 31:425-455 (1963).
4. Takasugi N, Bern HA. Tissue changes in mice with persistent vaginal cornification induced by early postnatal treatment with estrogen. J Natl Cancer Inst 33:855-865 (1964).
5. Takasugi N. Cytological basis for persistent vaginal changes in mice treated neonatally with steroid hormones. Int Rev Cytol 44:193-224 (1976).
6. Forsberg J-G. Developmental mechanism of estrogen-induced irreversible changes in the mouse cervical epithelium. Natl Cancer Inst Monogr 51:41-56 (1979).
7. Mori T, Nagasawa H, Bern HA. Long-term effects of perinatal exposure to hormones on normal and neoplastic mammary growth in rodents—review. J Environ Pathol Toxicol 3:191-206 (1979).
8. Herbst AL, Bern HA, eds. Developmental Effects of Diethylstilbestrol (DES) in Pregnancy. New York: Thieme-Stratton, 1981:203.
9. McLachlan JA, ed. Estrogens in the Environment II: Influences on Development. New York: Elsevier, 1985;435 pp.
10. Mori T, Nagasawa H, eds. Toxicity of Hormones in Perinatal Life. Boca Raton, FL: CRC Press, 1988;184.
11. Bern HA. The fragile fetus. In: Chemically Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection (Colborn T, Clement C, eds). Princeton, NJ: Princeton Scientific Publishing, 1992;9-16.
12. Iguchi T. Cellular effects of early exposure to sex hormones and antihormones. Int Rev Cytol 139:1-57 (1992).
13. McLachlan JA, Newbold RR, Bullock B. Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. Science 190:991-992 (1975).
14. Arai Y, Mori T, Suzuki Y, Bern HA. Long-term effect of perinatal exposure to sex steroids and diethylstilbestrol on the reproductive system of male mammals. Int Rev Cytol 84:235-268 (1983).
15. Iguchi T, Hirokawa M, Takasugi N. Occurrence of genital tract abnormalities and bladder hernia in female mice exposed neonatally to tamoxifen. Toxicology 42:1-11 (1986).
16. Iguchi T, Iriwasa S, Uchima F-DA, Takasugi N. Permanent chondrification in the pelvis and occurrence of hernias in mice treated neonatally with tamoxifen. Reprod Toxicol 2:127-134 (1988).
17. Migliaccio S, Newbold RR, Bullock BC, McLachlan JA, Korach KS. Developmental exposure to estrogens induces persistent changes in skeletal tissue. Endocrinology 130:1756-1758 (1992).
18. Uesugi Y, Sato T, Iguchi T. Morphometric analysis of the pelvis in mice treated neonatally with tamoxifen. Anat Rec 235:126–130 (1993).

19. Galand P, Leroy F, Chretien J. Effect of oestradiol on cell proliferation and histological changes in the uterus and vagina of mice. J Endocrinol 49:243–252 (1971).

20. Yamamoto KR. Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 19:209–252 (1985).

21. Kahn SA, Stancel GM, eds. Protooncogenes and Growth Factors in Steroid Hormone Induced Growth and Differentiation. Boca Raton, FL: CRC Press, 1994; 277 pp.

22. Scrocchi LA, Jones LA. Alteration of proto-oncogene c-fos expression in neonatal estrogenized BALB/c female mice and murine cervicovaginal tumor Lj6195. Endocrinology 129:2251–2253 (1991).

23. Gardner WU. Sexual dimorphism of the pelvis of the mouse, the effect of estrogenic hormones upon the pelvis and upon the development of scrotal hernias. Am J Anat 59:459–483 (1936).

24. Iguchi T, Irisawa S, Fukazawa Y, Uesugi Y, Takasugi N. Morphometric analysis of the development of sexual dimorphism of the mouse pelvis. Anat Rec 224:490–494 (1989).

25. Uesugi Y, Ohta Y, Asashima M, Iguchi T. Comparative study of sexual dimorphism of the innominate bone in rodents and amphibians. Anat Rec 234:432–437 (1992).

26. Uesugi Y, Taguchi O, Nomura T, Iguchi T. Effects of sex steroids on the development of sexual dimorphism in mouse innominate bone. Anat Rec 234:541–548 (1992).

27. Selmanoff MK, Goldman BD, Ginsburg BE. Developmental changes in serum luteinizing hormone, follicle stimulating hormone and androgen levels in males of two inbred mouse strains. Endocrinology 100:122–127 (1977).

28. Gillespie JS. The rat anococcygeus muscle; a new, densely innervated smooth muscle preparation. Br J Pharmacol 43:430 (1971).

29. Larson BA, Gibson A, Bern HA. The effects of urotensins in tetrapods: physiology or pharmacology? In: Neurosecretion and the Biology of Neuropeptides (Kobayashi H, Bern HA, Urano A, eds). Tokyo: Japan Scientific Society Press, 1985; 486–493.

30. Gibson A, Bern HA, Ginsburg M, Botting JH. Neuropeptide-induced contraction and relaxation of the mouse anococcygeus muscle. Proc Natl Acad Sci USA 81:625–629 (1984).

31. Tobin C, Joubert Y. Testosterone-induced development of rat levator ani muscle. Dev Biol 146:131–138 (1991).

32. Guglielmone R, Vercelli A. The costo-uterine muscle of the rat. Anat Embryol 184:337–343 (1991).

33. Gibson A, Gillespie JS. The effect of immunosympathectomy and of 6-hydroxydopamine on the responses of the rat anococcygeus to nerve stimulation and to some drugs. Br J Pharmacol 47:261–267 (1973).

34. Fukazawa Y, Suzuki A, Iguchi T, Takasugi N, Bern HA. Sexual dimorphism and strain difference in mouse anococcygeus muscle. Zool Sci 9:1273 (1992).