Effects of Early Exposure to Xenoestrogens on the Prostate Gland

Project Scope

Past studies on rodents indicates that brief exposure to natural estrogens during early development results in permanent alterations of the prostate (imprinting), including differentiation defects, altered gene expression and squamous metaplasia, dysplasia, hyperplasia, and adenoma formation with aging. Synthetic environmental contaminants with estrogenic properties might likewise affect the prostate gland, even at low doses. Hence, the present project tested the hypothesis that exposure to environmentally relevant doses of certain xenoestrogens during prostate developmental will permanently imprint the prostate gland and promote dysplasia or tumor formation with aging.

The specific objectives of this research were to:

- Test the hypothesis that brief exposure to environmentally relevant doses of estrogenic chemicals during prostate development will produce abnormal growth patterns and predispose to prostate cancer; and
- Investigate the molecular mechanisms by which xenoestrogens affect the prostate.

Project Results and Implications

The first set of experiments focused on determining the dose-dependence of neonatal treatment with synthetic estrogens in Sprague-Dawley (SD) rats. Animals were given subcutaneous injections of either nonylphenol (NP) or bisphenol A (BPA) at doses ranging from 0.1 µg/kg to 100 mg/kg. The results provided equivocal evidence that neonatal exposure to low doses of NP might advance puberty in male rats, since preputial gland separation occurred significantly earlier and ventral prostate and testis weights were increased in 35 day-old animals. At day 90, values were not different from control or high-dose animals, however. Furthermore, NP exposure super-masculinized several liver enzymes, which are known to be hormonally regulated and can be imprinted in the male rat. A repeat experiments failed to verify the results none of the affects of low-dose NP exposure could be duplicated nor was there any difference in epididymal sperm counts between control NP-exposed animals. No effect of BPA exposure on prostate development was observed at any dose. The investigators attributed the failure to replicate the NP findings to possible differences in test agent purity of within-strain variability in sensitivity to NP exposure.

Grant Title and Principal Investigator

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Key Findings

- Neonatal exposure to low doses of nonylphenol (NP) may advance puberty in male rats, and appeared to increase the expression of liver enzymes known to be under androgenic control in SD rats, but these results could not be duplicated.
- Dose-response study of estradiol benzoate (EB) showed that neonatal exposure to estrogens results in an inverted U-shaped dose-response curve, with low doses transiently increasing prostate weights at day 35 and high doses decreasing weights compared to controls. At 90 days, increased organ weights are seen at lower doses than at 35 days.
- Hepatic 2α- and 16 α-testosterone hydroxylase activities were significantly increased in low-dosed EB animals, suggesting that increased prostate sizes in the low-dose groups were due to advancement of puberty.
- Fisher 344 rats appear to be more sensitive to estrogen treatment than the traditionally used Sprague-Dawley strain, suggesting that the former strain may be the better model for testing environmental endocrine disruption.

Publications include 2 peer reviewed articles

Project Period: January 1998 to January 2001

Relevance to ORD's Multi-Year Research Plan

This project contributes to all three ORD Multi-Year Plan long-term goals (LTGs): (1) providing a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors, (2) determining the extent of the impact of endocrine disruptors on humans, wildlife, and the environment; and (3) supporting EPA's screening and testing program. Specifically, the results help determine critical biological factors during development that result in toxicities later in life; understand the mechanisms that operate in the low end of the dose-response curve; determine the extent to which exposure to endocrine disruptors contribute to the onset or increase in the severity of diseases; and key risk assessment issues (e.g., non-linear dose response, sensitivities of different animal strains).

During the second year of the grant, a dose-response experiment was performed with estradiol benzoate (EB) in which SD rats were treated at postnatal days 1, 3, and 5 by subcutaneous injections of EB (0.015 µg/kg to 15.0 mg/kg). Neonatal low-dose treatment resulted in significant increases in dorsal prostate weights on day 35, whereas high doses reduced prostate weights significantly. This non-monotonic, or inverted-U, dose-response was not observed in 90-day old animals, where only high-dose EB significantly reduced prostate weights. Preputial gland separation was not significantly early in any treatment group, although animals treated with the highest doses did not reach PGS at all. Hepatic 2α- and 16α-testosterone hydroxylase activities were significantly increased in low-dosed animals on day 35, suggesting that the effect in low dose animals was due to an advancement of puberty (Figure1). The dose-response for liver enzyme activities was also non-monotonic.

An inverted U-shaped dose response curve also was seen in both the testes and epididymis, where low EB doses caused increases in weights while high doses of estrogens resulted in reduced organ weights. At 90 days, the stimulatory effect of EB exposure on testicular and epididymal weights was seen at lower doses than the effect at 35 days. These effects were correlated with circulating testosterone levels, suggesting alterations in endocrine control mechanisms.

Since there had been reports of differential sensitivity of various rat and mouse strains to estrogenic substances, the EB dose-response experiment was repeated in Fisher 344 rats. On day 90, there was no significant difference observed in prostate lobe weights, except for significant reductions at the highest treatment concentrations. The investigators judged the Fisher rats to be somewhat more sensitive to the high-dose effects of EB than the SD rats.

Investigators concluded that neonatal exposures to certain estrogens results in non-linear dose-response relationships for reproductive developmental effects, with significant effect seen at concentrations below previously reported no-effect levels. Low doses of natural estrogens administered perinatally were found to advance the onset of the pubertal phase in male rats, thus resulting in temporarily increased reproductive organ weights, circulating testosterone levels, and liver enzyme activities. The Fisher 344 rat appeared to be more sensitive to estrogen-induced organ weight depression, suggesting that the Fisher 344 rat may be a better model for testing environmental endocrine disruption.

Investigators

Gail S. Prins - University of Illinois at Chicago

For More Information

Prins Research Lab Home Page:
http://www.uic.edu/labs/prins/index.htm
NCER Project Abstract and Reports:
http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/170/report/0

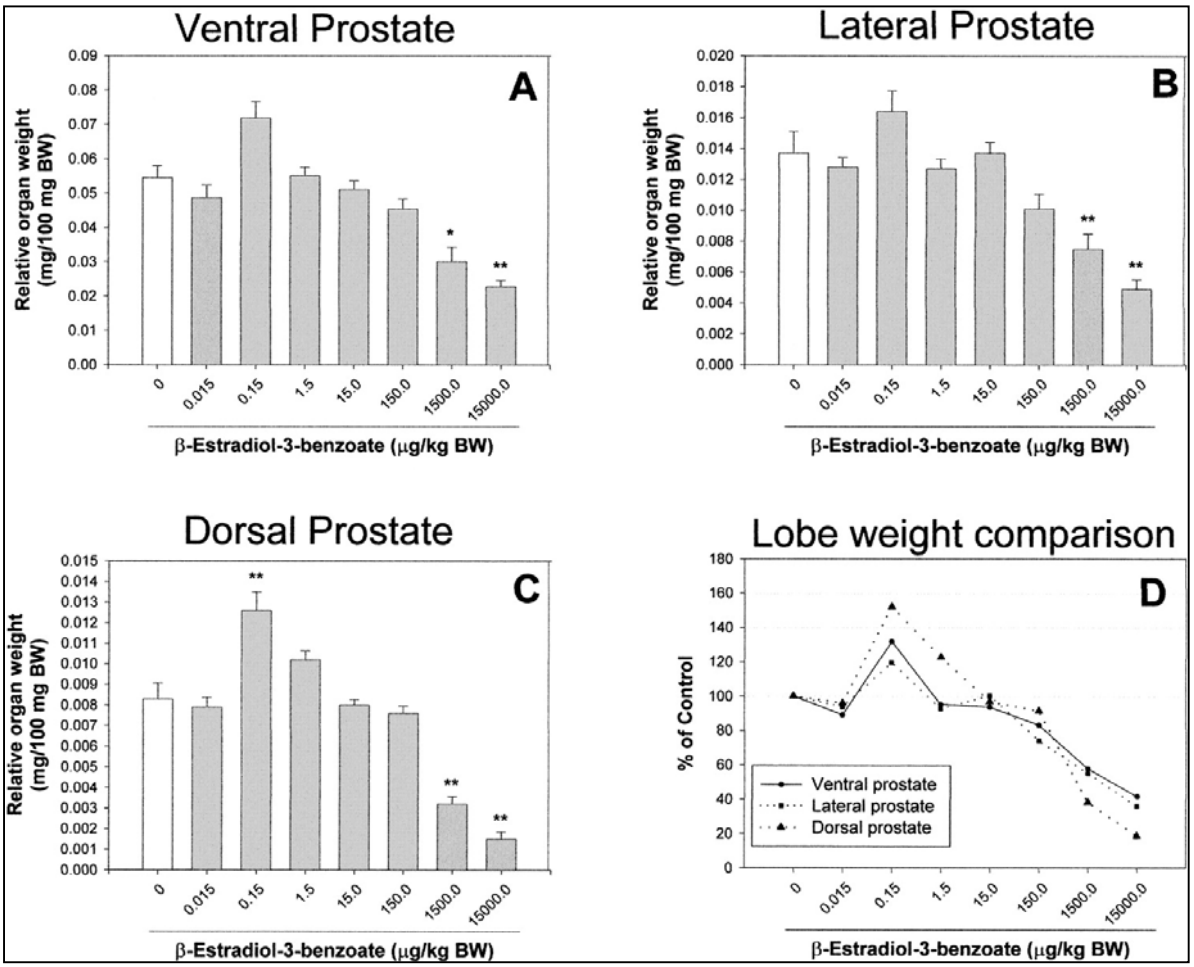

Figure. 1. Effects of neonatal EB administration on relative prostate weights (mg/100 mg BW) of SD rats on post-natal day 35. A) Although not significant, relative ventral prostate weights were increased in animals treated with a low concentration of EB (0.15 μg/kg BW). The opposite high-dose effect was significant at 1500 and 15 000 μg/kg BW. B) The response curve for relative lateral prostate weights shows a similar nonsignificant increase at low-dose EB and significant decrease at high-dose EB as that for the ventral lobe. C) In the dorsal lobe, the relative organ weight increase at the low dose was significant, as were the decreases in lobe sizes at the high doses. D) The direct comparison of all three prostate lobe responses (shown as percentage of control values) indicates that the dorsal lobe reacted most sensitively to the treatment and that the entire prostatic complex responded in a dose-dependent fashion, resembling an inverted U-shaped response curve. All values are mean ± SEM. •, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$. (Putz O, Schwartz CB, Kim S, LeBlanc, GA, Cooper, RL, Prins, GS. (2001). Neonatal Low- and High-Dose Exposure to Estradiol Benzoate in the Male Rat: I. Effects on the Prostate Gland. *Biology of Reproduction* **65**: 1496-1505.
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