

Animal Models and Studies of in Utero Endocrine Disruptor Effects

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Abstract

The rate of organ and system development in mammals, including humans, is most rapid during the prenatal period. Perturbations of the endocrine system during this period can have profound effects on later anatomy, physiology, behavior, and the onset of disease. Endocrine-disrupting compounds can cause perturbations during fetal development by mimicking or blocking natural hormones. In experimental studies, compounds that mimic estrogens and those that block androgen action have been shown to have a number of long-term effects. Among these effects are the acceleration of puberty onset, increased incidence of adult cancers such as vaginal and prostate cancers, and alterations in sexually dimorphic anatomy, physiology, and behavior. Laboratory animal models continue to play a crucial role in identifying endocrine disruptors, determining their mode of action, and demonstrating their consequences.

Key Words: animal models; anogenital distance; endocrine disruptors; intrauterine position; prenatal; stress

Introduction

On a rate-of-change basis, the prenatal period is the most active life period that any mammal experiences, including humans. Remarkable changes occur during the time spent in the uterus, approximately 20 days in a mouse or rat and 40 wk in the human. Any perturbations during the prenatal period of development such as trauma, low body mass for time of gestation, or exposure to xenobiotic compounds can significantly affect health and disease later in life (for recent reviews, Chapin et al. 2004; Gillman 2002). Among those xenobiotic compounds are endocrine disruptors—compounds that mimic or block the action of natural hormones. They are naturally produced in some plants, especially soy products, and by humans as industrial chemicals, pesticides, herbicides, fungicides, and drugs. The long-term suite of consequences on the development of reproductive systems following endocrine disruptor exposure is explored herein.

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Background

Large amounts of testosterone are produced by the male fetus during the prenatal period, starting between week 7 and 8 of gestation in humans and from approximately gestation day 12 to postnatal day 5 in male mouse fetuses. This testosterone surge permanently masculinizes the genitalia, the male reproductive tract, and portions of the brain responsible for the expression of male-specific behaviors (Capel 2000; Grumbach and Conte 1998). In female mammals, no similar surge of a steroid hormone such as estrogen occurs (i.e., the development of female characteristics occur in the absence of specific hormone surges during the fetal period). This difference in steroid hormone exposure provides the biological basis for differences between the sexes (Wisemann and Pardue 2001). Fetal development not only results in a newborn with all of the major organs intact, but it also sets the stage for future development and responses to environmental challenges.

How is this process connected to endocrine disruptors? Chemical signals, mostly hormones, play a profound role in initiating and modulating development. Thus, an endocrine disruptor, which mimics or blocks a natural hormone, may interfere with, or at least influence, development. This disruption can result in recognizable phenotypic variation, including serious abnormalities at birth, or it can result in subtle changes that are expressed much later in life.

One of the classic and still most revealing endocrine disrupter effects that has been observed results from exposure to diethylstilbestrol (DES¹). After its discovery in 1938 as a potent estrogen, DES was heavily prescribed from the 1950s through the 1970s to prevent women's miscarriages and for use as a "morning after" pill. It has also been used extensively to speed the growth of poultry and cattle. Not until the early 1970s, when the human fetuses who had been exposed to DES in utero reached puberty, were long-term consequences detected. Young women born to mothers who had taken DES showed an abnormally high incidence of adenocarcinoma of the vagina (Herbst et al. 1971). More than 90% of the exposed women developed benign abnormalities of the reproductive tract such as vaginal adenosis, and approximately 0.1% of exposed women developed a

¹Abbreviations used in this article: CAH, congenital adrenal hyperplasia; DES, diethylstilbestrol; EDCs, endocrine-disrupting compounds; EPA, Environmental Protection Agency; IUP, intrauterine position.

rare form of vaginal cancer (Herbst and Bern 1981; Newbold 1995).

These clinical findings prompted a series of studies using animals to reveal the mechanism of action of DES and to identify additional possible consequences of prenatal exposure to this compound. The mouse proved to be an appropriate model. A similar cancer of the vagina was produced in mice exposed to DES in utero (Newbold and McLachlan 1982). In more recent reviews, Newbold (2001) and Mori and colleagues (2003) describe ongoing progress in understanding the effects of developmental exposure to DES and other endocrine disruptors in rodent model systems and in humans.

Given the numerous known, and many more suspected, harmful effects of prenatal exposure to endocrine disruptors, it is essential that animal models be used to identify potentially harmful chemicals and to determine their modes of action. Continuing basic research on the effects of hormones on development and the expression of species-specific phenotypic traits is essential to provide the base on which these toxicological studies rest. A selection of the animal models used to identify endocrine disruptors and explore their mechanisms of action is described below.

Evidence for an Interaction Between Natural Hormones and Endocrine Disruptors

Sex determination and differentiation provide excellent examples of a set of traits that result from very complex developmental interactions, perhaps including endocrine disruptors. In mammals, the presence of an XX or XY chromosomal complement is only the beginning of a long chain of events that results in an individual's sex identification. For example, a human female with a normal XX chromosomal complement can exhibit a number of masculine traits if hormone production or sensitivity is abnormal during early development (Grumbach and Conte 1998). Pertinent examples of this consequence are girls born with congenital adrenal hyperplasia (CAH¹). CAH results in abnormally high production of an androgen from the fetus's adrenal gland during development. This production results in variable degrees of malformed genitalia that can, if necessary, be surgically corrected. With coworkers, Berenbaum (1998, 2000) has explored the behavioral consequences of CAH. In tests of more than 100 such cases, they found masculinization of toy selection and other sexually dimorphic traits among toddlers born with CAH. The girls with CAH chose toys related to aggression and transportation more frequently than their non-CAH sisters. Wisemann and Pardue (2001) have reviewed endocrine influences on an array of phenotypic traits, including behavior.

Puberty is a sexually dimorphic characteristic in mice, humans, and most mammals, with females attaining puberty earlier than males. The onset of puberty results from changes in the hypothalamic gonadotropin-releasing hor-

mon pulse generator in response to hormones followed by a relatively rapid surge of gonadotropins. Gonadotropins, in turn, induce gonadal hormone release, which results in the adult sexual phenotype (Grumbach and Styne 1998). Because hormones play a primary role in puberty, it is logical to question whether endocrine disruptors influence this complex biological event. To compare results with endocrine disruptors, it is necessary to understand normal prenatal endocrinology in commonly used laboratory rodents, described briefly below.

Influence of Intrauterine Position

A series of studies using rodent models demonstrates the important role of natural variations in hormone concentrations in sexual development. Many animals used as models in biomedical research are polytocous (i.e., they produce litters). In the most common models, the mouse and rat, the uterus is bicornate and the fetuses are lined up like "peas in a pod" in the two halves of the uterus. Because the sequence of fetuses appears to be random in mice and rats, it is possible for a female to be adjacent to one, two, or no males. Male mice produce a relatively large amount of testosterone starting on approximately day 12 of fetal development, and a fraction of this testosterone can passively transfer to adjacent sibling fetuses (vom Saal and Dhar 1992; vom Saal et al. 1990). The action of this transferred testosterone causes a partial masculinization of the adjacent sibling females.

The consequence of such intrauterine position (IUP¹) has been shown to affect a number of sexually dimorphic anatomical, physiological, and behavioral traits (Table 1) (Ryan and Vandenberg 2002; Vandenberg 2003; vom Saal 1984). Thus, the exposure of pregnant females to endocrine disruptors may affect female fetuses differentially as a function of their proximity to a male. The intrauterine position was originally identified by caesarian section (vom Saal and Bronson 1978), and it later became possible to assess the IUP in mice by determining the anogenital distance in day-old or weanling pups. Adjusting the anogenital length for body weight yields an anogenital distance index that reflects the prior IUP with considerable reliability (Vandenberg and Huggett 1995). Thus, for studies in which the prenatal hormone exposure may be important, a noninvasive and very simple measurement of the natural variability of female fetuses to testosterone exposure from their siblings is available for the laboratory mouse. This information could be very valuable in designing experiments because it is possible to assess the masculinity or femininity of individual experimental subjects with little effort and low cost.

With the stage set by a significant amount of basic research on the consequences of several prenatal hormone fluctuations, a test was made of whether an endocrine disruptor with weak estrogenic effects could alter the onset of puberty in mice (Howdeshell et al. 1999). Bisphenol A, a component of many resins and plastics, was delivered orally

Table 1 Effects of intrauterine position on selected phenotypic traits in female mice, except where noted^a

Trait	OM ^b	2M ^b
Morphology		
Anogenital distance	Shorter	Longer
SDN ^p in hypothalamus (rat)	Smaller	Larger
Physiology		
Fetal testosterone concentration	Low	High
Sensitivity to testosterone	Less	More
First estrus (puberty)	Early	Late
Sensitivity to bisphenol-A	More	Less
Behavior		
Mating and impregnation	Earlier	Later
Mounting other females	Less	More
Aggression	Less	More
Home range size in field	Smaller	Larger

^aFor references and a more extensive listing, including other species, see Ryan BC, Vandenberg JG. 2002. Intrauterine position effects. *Neurosci Biobehav Rev* 26:665-678.

^bOM, female fetuses with no males on either side in utero; 2M, female fetuses with a male on both sides in utero; SDN, sexually dimorphic nucleus.

to pregnant mice during gestation. Pups were delivered by caesarian section to determine IUP. The female pups were checked daily for vaginal opening after weaning, and once the vagina had opened, vaginal smears were taken daily to determine first estrus. For female offspring whose mothers were fed bisphenol A, those not flanked by male siblings in utero (OM) females) attained first estrus earlier than females with two adjacent male siblings (2M). Females from the 1M position were intermediate. This development difference indicated an interaction between IUP and bisphenol-A, an estrogen mimic. As expected, there was no effect on age at vaginal openings, which is a good index of puberty in the rat but is not a good measure of puberty onset in the mouse (Cooper et al. 1993).

Frequency and type of play behavior may comprise an interesting phenotype to examine in relation to prenatal exposure to endocrine disruptors. Play is a sexually dimorphic trait, with males showing more frequent and more intense bouts of play than females. This play occurs most intensely during the prepubertal period, when testosterone is at its lowest concentration. It is unique among sexually dimorphic behaviors in that it is organized prenatally by testosterone but is deactivated, rather than activated, when testosterone increases as puberty progresses (Pellis and Pellis 1983, for a review of play behavior in the rat). Recently Hotchkiss and colleagues (2003) showed that exposing rat fetuses to the antiandrogenic endocrine disruptors vinclozolin or flutamide during gestation reduces play behavior in male rats when they are tested immediately before puberty onset. The frequency of play behavior in exposed males was

similar to that seen in females and significantly lower than that of control males. This observation indicates that the organization of the brain resulting in masculine play patterns is altered by antiandrogens.

Influence of Maternal Stress

Because IUP can influence the response of females to an endocrine disruptor, it is possible that other factors that alter the fetal hormone environment can similarly influence experimental results. Maternal stress is an important factor that can alter the fetal development and could interact with exposure to endocrine disruptors. Exposing pregnant mice to intense light and heat increases circulating corticosterone concentrations (Montano et al. 1991). Female rat pups born to similarly stressed dams have delayed vaginal opening and lengthened estrous cycles (Herrenkohl and Politch 1978; Ward 1992). Thus, endocrine-disrupting compounds (EDCs¹) may interact with stressful stimuli.

Several epidemiological studies with humans also reveal the impact of prenatal stress experienced by the mother. Infants born to stressed mothers have an increased incidence of numerous diseases that emerge decades later. Among these diseases are hypertension (Barker et al. 2000; Roseboom et al. 2001), diabetes (Ravelli et al. 1998), and obesity (Ravelli et al. 1976). The “stresses” examined include low body mass for gestation age, trauma, and probable exposure to xenobiotics such as endocrine disruptors. Because these diseases result from altered endocrine experiences during prenatal development, it is possible that endocrine disruptors may induce similar alterations during subsequent development in humans. Research on long-term effects of prenatal endocrine disruption is difficult to conduct on humans because of the long intervals between cause and effect and because of the difficulty in following individuals over most of a life span. Identifying and understanding such long-term effects depend on animal models.

Influence of Dose and Low-Dose Effects

In addition to concerns about how the intrauterine position and stress can be variables that modify responses to EDCs, dose is another important factor that must be considered during the design of experiments using animal models to study EDC effects. Dose is always an important and often a contentious issue in toxicological and other studies of the effects of drugs and chemicals, and recently the accepted linear relation between dose concentration and response has been questioned (Sheehan and vom Saal 1997; Welshons et al. 2003). Some remarkably low doses of endocrine disruptors have been shown to produce measurable effects. An early example of a low-dose effect was that of prostate enlargement in the male mouse following a low dose of DES (0.02 µg/kg/day) delivered to their mothers (vom Saal

et al. 1997). In addition to the finding of anatomical and pathological consequences of low-dose exposure to an endocrine disruptor, a recent report (Palanza et al. 2002) indicates that behavior can also be altered as a result of prenatal low-dose endocrine disruptor exposure. Feeding a low dose of the estrogenic chemical bisphenol A during fetal life (gestation days 14-18) to pregnant mice resulted in the female offsprings' impaired maternal behavior.

Concern over such low dose effects of ECDs led to a workshop hosted by the National Institutes of Environmental Health Sciences and the US Environmental Protection Agency (EPA¹) to explore the validity of the data and to evaluate the consequences of low dose effects (NTP 2003). For this meeting, "low dose effects" were defined as biological changes that occur at environmentally relevant exposure concentrations or at doses that are lower than those typically used in the standard testing paradigm of the US EPA. Although more information is still needed to confirm and understand the impact of low doses of EDCs fully, it is clear that the data available are sufficiently robust to stimulate concern. Welshons and colleagues (2003) have recently published a review of particular low dose effects of EDCs.

Conclusions

The use of animal models remains essential to test the effects of endocrine disruptors and to understand the mechanism whereby they induce developmental anomalies or pathologies. The studies noted above provide examples of how such models are used, and reflect some of the issues that should induce caution in the ongoing design and interpretation of experiments using EDCs. EDCs have many known and even more suspected effects on humans and wildlife species. The well-documented relation between prenatal DES exposure and a specific vaginal cancer is noted above. In addition, correlational evidence suggests that the increase in the incidence of breast cancer and prostate cancer and the decrease in men's sperm counts may relate to increasing levels of xenobiotic compounds in the environment, especially EDCs (Swan et al. 2003). Probable effects on wildlife include hermaphroditism in fishes, demasculinization of reptiles, and feminization of behavior in birds. A more thorough analysis of the possible effects of EDCs on human and wildlife development, health, and disease is available in the book that stimulated much of the work in this field, *Our Stolen Future* (Colborn et al. 1996). More recent book-length analyses are also available in the literature (Damstra et al. 2002; Schettler et al. 1999).

Studies using animal models are necessary to identify EDCs further and to reveal the potential health effects of these compounds on humans. During the course of such work, investigators should consider the possible interactions between the intrauterine position, stress, and the effect of the test compound, as well as the effects of exposure to multiple chemicals during critical periods of development.

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