FUNCTION OF SEXUAL GLANDS AND MECHANISM OF SEX DIFFERENTIATION

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ABSTRACT — Prior to any investigation of toxicant effects on sexual development it is necessary to have a complete understanding of the relevant physiology of reproductive development. Beginning at conception, development of males and females diverge to form the respective reproductive systems. From the prenatal period to the interval following puberty, radical changes take place in the hypothalamo-pituitary-gonadal axis of males and females. The complexity of each of these systems and their development is mirrored in the many possibilities for the means by which chemicals may produce adverse effects. For example, a chemical that affects hormone synthesis may, if administered at the proper time, affect hypothalamic development. As a consequence, pubertal development may not occur normally. In this chapter, we have outlined the basics of reproductive development and provided examples of adverse effects by endocrine disrupting chemicals (EDCs) on such development.

KEY WORDS: Reproductive development, Hypothalamus, Ovary, Testis, Abnormal development, Puberty, Endocrine disruption

INTRODUCTION

In order to investigate and comprehend toxicologic effects on development, the male reproductive system, and the female reproductive system, it is necessary to have an understanding of the process by which prenatal differentiation and pubertal maturation lead to functional reproductive systems. The development of the anatomical and physiological features of male and female reproductive systems can be divided into several stages representing a time line of development. Appropriate prenatal differentiation of ovarian and testicular tissue is critical to postnatal and peripubertal changes that ultimately lead to adult reproductive functioning and fertility following puberty. In adulthood, gametogenesis and steroidogenesis in both sexes are regulated by the hypothalamic-pituitary-gonadal-axis (HPG) which itself must undergo maturation (Johnson and Everitt, 1980). Proper development of each portion of the HPG axis is essential. For example, in female mammals, the hypothalamus develops the potential to participate in reproductive cyclicity early in life. However, manifestation of such cyclicity does not occur until puberty (Kaplan et al., 1976).

Each stage of reproductive development can also be thought of as a window of vulnerability to toxicant exposures. Exposure to chemicals during prenatal or perinatal development, for example, can impact not only the developmental changes that occur during those intervals but also may have far-reaching consequences with respect to potentially impaired fertility in adulthood (Dwivedi and Iannaccone, 1998). Because each class of toxicants can have varied mechanisms of action of action, each of these classes of chemicals may perturb the development of the reproductive system in a specific way. Examples of such mechanisms include...

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structures are evident by the 4th or 5th week of human development of the diencephalon. Both of these rudimentary areas are formed from a ventral projection of the oral ectoderm termed Rathke’s pouch, the anterior pituitary is formed prenatally as an evagination of the oral ectoderm termed Rathke’s pouch, and the posterior lobe is formed from a ventral projection of the diencephalon. Both of these rudimentary structures are evident by the 4th or 5th week of human fetal life. Autocrine and paracrine activities involving adjacent ectoderm and neural tissue mediate the early differentiation of these areas (Siler-Khodr, 1999).

Also early in fetal life, the hypothalamic-pituitary system undergoes differentiation toward a capability for endocrine function. Gonadotropic hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH) are produced in the human anterior pituitary as early as five weeks of gestation. Gonadotropin releasing hormone (GnRH), as well as other releasing hormones, are present in the developing hypothalamus around the same time. The action of these releasing hormones on the anterior pituitary can occur only after the formation of the portal vessels through which the hypothalamic-releasing hormones are transported to the pituitary (Siler-Khodr, 1999).

As the gonads begin to produce hormones in the course of fetal development, interactions and reciprocal development take place between the gonads of each sex and the hypothalamic-pituitary axis. Although Leydig cells differentiate in males by the 7th to 8th week of human gestation and begin producing androgens under regulation by placental human chorionic gonadotropin (hCG), the earliest peak of testosterone occurs after 12 weeks (Marty et al., 2003). In the female fetus, estrogen is present by 10 to 14 weeks, but the hormone does not peak until 20 weeks. This peak in estrogen production follows peaks in hypothalamic GnRH and pituitary gonadotropin concentrations. Subsequently, a negative feedback of estrogen leads to suppression of the hypothalamus and pituitary, and lowered levels of pituitary gonadotropin synthesis are seen toward the end of gestation (Kaplan et al., 1976). There is a perinatal “programming” of the hypothalamus of the female that promotes the development of the capability of the organ to respond to gradually rising estrogen levels. This capability permits the triggering of the surge of LH that is a requisite for ovulation, but these events will not occur until after puberty and full maturation of the system (Kaplan et al., 1976). In males, neonatal exposure of the hypothalamus to androgen is needed for sexual differentiation of the LH release mechanism, allowing LH secretion to be modified by either androgen or estrogen (Kaplan et al., 1976).

Sex determination and development

Sex determination consists of the regulation of the development of the gonadal sex. Sex differentiation encompasses the events subsequent to gonadal organogenesis. These processes are regulated by at least 70 different genes that are located on the sex chromosomes and autosomes. These genes act via a variety of mechanisms involving gonadal steroids, peptide hormones, and tissue receptors (George and Wilson, 1994).

In early embryo-fetal development, primordial gonadal tissue is bipotential, having the capacity to become an ovary or testis. Bilateral urogenital ridges arise from the coelomic epithelium and underlying mesenchyme. Gonadal ridges are colonized by germ cells thus producing the bipotential gonad primordium. In mammals, gonadal sex is determined by genetic sex. The presence of a Y chromosome is associated with the development of testes. This genetic switch that triggers the indifferent gonad to develop into a testis has been identified in humans as the SRY gene (Harley and Goodfellow, 1994). It is the influence of factors produced by the genetic male that directs the development of the gonad toward the male phenotype. In the absence of these factors, the development of the female phenotype occurs. The ability of the primitive male and female reproductive tissue (Wolffian and Mullarian ducts) to differentiate into their respective functional reproductive tracts depends on the appropriate develop-
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Development of the gonads and secretion of the respective male and female hormones (Teixeira and Lee, 1999).

**Genetic regulation**

The molecular mechanisms by which SRY triggers testis development are unknown. In addition to the SRY gene, a number of genes having a critical role in male or female sex determination have been identified (Roberts *et al.*, 1999). WT1 (Wilms Tumor), a transcriptional regulator, appears to act on embryonic metanephric blastema tissue early in urogenital development. SF-1 (steroidogenic factor-1), a nuclear receptor involved in transcriptional regulation, is expressed in both male and female tissues where it is required for synthesis of testosterone and estrogen. In Sertoli cells, this gene regulates the anti-mullerian hormone gene (Parker *et al.*, 1999). WT1 and SF-1 appear to play important roles in the differentiation of the genital ridge from the intermediate mesoderm. DAX-1 is a gene coding for an “anti-testis” factor and is found in the primate gonadal ridge several days before the peak expression of SRY. SRY and DAX-1 appear to act antagonistically in gonadal dysgenesis (Parker *et al.*, 1999). The human SOX-9 gene transcripts are present in the gonadal ridge of both male and female embryos, and the expression of the gene is increased as the indifferent gonad develops toward a testis and is decreased in the course of ovarian development (Wizemann and Pardue, 2001). Two genes, DMRT-1 and DMRT-2, localized on autosomal chromosome 9, also appear to play a role in human sex determination. WNT-1 contributes to the regulation of steroidogenesis in the fetal gonad (Uusitalo *et al.*, 1999). WNT-4 is down-regulated in the fetal testis leading to testosterone synthesis in individuals carrying the Y chromosome. Expression of this gene in the fetal ovary appears to inhibit gonadal androgen biosynthesis.

**Ovary**

In the absence of testis determining genes, including SRY, the gonadal primordium develops as an ovary (Table 1; Parrott and Skinner, 1999). Complete development of an ovary requires the initiation of meiosis, enclosure of the germ cells into follicles, and the differentiation of steroid producing and interstitial cells. Evidence suggests that primordial germ cells (PGCs) reside in the epiblast of the inner cell mass of the blastocyst (Byskov and Hoyer, 1994). PGCs migrate from the extra-embryonic site to the embryonic mesoderm of the primitive streak. Migration continues further to the visceral endoderm of the yolk sac and to the developing hindgut from whence the PGCs migrate up through the dorsal mesentery and reach the gonad (McKay *et al.*, 1953; Copp *et al.*, 1986). Somatic cell lineages also populate the gonad after which morphologic sex differentiation occurs. About the same time, weeks 7-8, germ cells of the ovary enter the first meiotic prophase. Little or no steroids are produced until follicles are formed, around week 12. During the migration of PGCs, the germ cells increase in

<table>
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<td>Months 2.5-7</td>
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(Reprinted with permission from Parrott and Skinner, 1999.)
number from ~100 to ~5000. After migration is complete, oogonia continue to proliferate, as the ovary differentiates, until human embryonic month 7 when the number reaches 6 to 7 million (Byskov and Hoyer, 1994). Gonads are first identified as ovaries when oogonia enter meiotic prophase, an event beginning around weeks 7-8 in human fetuses. Oogonia are then called oocytes (Parrott and Skinner, 1999). At this time the Wnt gene may suppress the differentiation of steroidogenic cells in the fetal ovary, as seen in the mouse (Parrott and Skinner, 1999). Meiosis occurs in developing oocytes until all are arrested in late prophase of the first meiotic division, and further division is delayed until around the time of ovulation in the adult. This is in contrast to the male in which meiosis begins at puberty and proceeds without delay (Teixeira and Lee, 1999). Oocytes having undergone meiotic arrest become encased in presumptive granulosa cells, and oocytes in these primordial follicles can remain arrested in meiosis I for up to 50 years in the human ovary. The number of germ cells in the ovary is at a lifetime maximum in the fetal ovary, and their number decreases throughout life. Primordial follicles represent the pool from which all developing follicles will emerge (Byskov and Hoyer, 1994). All follicles that begin development in embryo-fetal or prepubertal ovaries will degenerate before maturing. However, in pubertal and adult ovaries, some follicles will continue development and successfully ovulate and some will not (Parrot and Skinner, 1999). As follicles develop, cells resembling steroid producing cells can also be found in the ovary, and cells with ultrastructural characteristics of steroid producing cells are present after the 20th week in the human fetus. In the absence of the Mullerian Inhibiting Substance (MIS) and testosterone, which induce male directed differentiation, the Mullerian duct in the female grows and gives rise to the oviduct, uterus, and upper part of the vagina. In addition, the Wolffian duct degenerates. If, however, a genotypic female is exposed to sufficient testosterone or other androgens at an appropriate time in fetal development, her phenotype may be masculinized. The ovary itself plays no role in the differentiation of the female genital tract (Wizemann and Pardue, 2001).

**Testis**

By the end of the bipotential stage of gonadal development, the primordial germ cells have migrated to the gonadal ridge, and germ cells are continuing to proliferate. The coelomic epithelium is separated by a lamina from the germ cells where sex cords develop, and the tunica albuginea is formed from mesenchymal tissue (Byskov and Hoyer, 1994). Testicular development is induced by factors encoded on the Y chromosome including the SRY gene. Presumptive Sertoli cells become associated with the germ cells in testicular cords. When the male germ cells become encased in the testicular cords, they are then called spermatogonia. These spermatogonia divide mitotically in parallel with morphologic differentiation before entering a period of mitotic arrest. Spermatogonia are not released from mitotic arrest nor enter meiosis until puberty occurs (Teixeira and Lee, 1999).

Leydig cells develop shortly after gonadal sex differentiation, when the testis is identifiable, at which time steroid production begins. The concentration of human gonadotrophic hormone (hCG) peaks prior to Leydig cell differentiation. Testicular concentrations of testosterone peak between 12 and 14 weeks then decline until a second peak occurs around 28 to 32 weeks which is itself followed by a decline in hormone levels by term (Siler-Khodr, 1999). There is evidence that endocrine feedback systems, involving FSH, inhibin, LH and testosterone, develop and are functional in the fetus (Marty et al., 2003). Also, fetal testicular androgens are believed to engage in the process of priming the brain in the male direction at early developmental stages. Factors that interfere with these developing feedback axes have the potential to lead to abnormal development of the hypothalamic-pituitary-testicular axis, impaired sexual maturation, and reduced fertility in adulthood (Marty et al., 2003). Testosterone actively induces differentiation of the Wolffian duct into the epididymis, vas deferens, and seminal vesicle. This differentiation of the Wolffian duct is dependent on testosterone itself, via the androgen receptor (AR) in target tissues. By contrast, the conversion of testosterone to dihydrotestosterone (DHT) by 5-alpha-reductase in target tissues is essential for the DHT-dependent differentiation of the urogenital sinus and genital tubercles. Mullerian Inhibitory Substance (MIS), produced by the Sertoli cells, promotes regression of the Mullerian ducts. If the testis of a genotypic male fails to secrete testosterone, masculinization will not take place and the phenotype will be feminized (Siler-Khodr, 1999). The Sertoli cell also secretes inhibin, nurtures the germ cells, expresses stem cell factor, synthesizes an androgenic binding protein, and temporarily prevents meiosis until puberty.
Reproductive development.

POSTNATAL DEVELOPMENT

Because reproductive development is actually very much a continuum, it is difficult to subdivide in any precise way the period of development that begins just after birth and ends during the pre-pubertal period. In this section, we will start with perinatal development, the period immediately following birth, and end with the pre-pubertal period. The subsequent section will discuss the prepubertal to pubertal interval. The period between birth and puberty has also been divided by some scientists into the infantile, juvenile, and prepubertal intervals.

Perinatal - infancy period

Progression to puberty can be described as a time of neuroendocrine functional development. A hypothalamic neuronal network that is responsible for the pulsatile discharge of GnRH into the hypophysial portal system and thus regulates gonadotropin secretion has been termed the hypothalamic GnRH pulse generator. In human fetal development, the principal stimulus to the fetal gonadotropes is provided by an intermittent pattern of GnRH discharge by the hypothalamus. Fetal concentrations of the gonadotropins reach adult levels in the second trimester of gestation, suggesting that the GnRH pulse generator is fully functional by that time (Klein, 1999). Gonadotropins fall somewhat during the last trimester under negative feedback regulation by placental steroids, and the activity of the pulse generator is diminished. At parturition this inhibition is lost, GnRH pulse generator activity is once again active during early infancy, and gonadotropin levels rise. As neonatal gonads become able to respond to stimulation by gonadotropins, ovarian follicle maturation occurs in the female infant, and adult levels of serum estradiol (in the female) or testosterone (in the male) may be found during the early infantile period. During this time, gender-related differences in gonadotropin secretion are evident, based in gender-specific differences in GnRH pulsatility which, in turn, have been attributed to the effects of gonadal androgens on the developing male fetal brain (Klein, 1999). In the male perinatal hypothalamus, the GnRH pulse generator operates at a frequency typical of that in the adult male, and increased secretion of LH and FSH stimulates the Leydig cells of the neonatal testis to secrete testosterone. Later in infancy, the pattern of GnRH pulsatility shifts, producing a hypogonadotropic state leading to gonadal quiescence. The GnRH pulse generator is said to be “brought into check,” a condition lasting until the prepubertal phase (Plant, 1994). In the early postnatal testis, immature Sertoli cells are the most common cell type and are accompanied by limited numbers of germ cells. There is a biphasic increase in Leydig cell number in parallel with changes in testosterone secretion. In infant boys, there is an increase in total germ cell number with a peak in early infancy followed by a decrease in older boys prior to puberty. Spermatogonia increase in number 6-fold between birth and 10 years of age (Marty et al., 2003).

In the infantile female, the GnRH pulse generator does not operate at the adult frequency, and a pattern of gonadotropin secretion different from that in the male is seen. Circulating FSH concentrations are sustained at elevated adult levels for several years, whereas circulating LH exhibits a developmental pattern similar to that observed in the infantile boy, with a rise to low adult levels during early infancy. Until about 12 to 24 months of age, the ovaries respond to the increased FSH by secreting estradiol which reach levels resembling those of adulthood. The secretion of estradiol then decreases, with its lowest point at about 6 years of age. During this time there is a decrease in the pulsatile release of GnRH which may be based in both gonadal and central nervous system restraints (Klein, 1999).

Pre-pubertal phase

From about 2 years of age until the onset of puberty, there is a “prepubertal hiatus” during which an arrested hypothalamic-pituitary system maintains FSH and LH secretion at low levels. Prepubertal girls may experience episodes of non-ovulatory follicular activity, but circulating estradiol remains low. In boys, the Leydig cells and seminiferous tubules are relatively inactive, and serum testosterone levels remain low as well. The mechanism for the pre-pubertal attenuation of GnRH pulsatility and decreased stimulation of gonadotrophs by the hypothalamus in humans is not fully understood. Work in prepubertal monkeys suggests that the restraint on the GnRH pulse generator is imposed by an inhibitory neuronal input originating upstream from the GnRH-secreting neurons (Plant, 1994). A possible mediator of such a signal inhibitory to GnRH is gamma amino butyric acid (GABA), a major inhibitory neurotransmitter in the brain (Fig. 1). In support of this hypothesis is the finding that, in the rat, hypothalamic release of GABA declines with the onset of puberty and that the interruption of GABA synthesis or action elicits GnRH release during prepubertal development (Scacchi et al., 1998; Roth et al., 1997).
PUBERTY

Timing of the onset of puberty is not dependent on the maturation of the pituitary or the gonads, as they are capable of response at all ages. Rather, the events of puberty originate with the ability of the hypothalamus to generate GnRH in a pulsatile rhythm with specific frequency and amplitude (Plant, 1994). In the same way that the mechanism by which the GnRH pulse generator is held in check during the prepubertal hiatus is as yet understood, the neurologic or other event that leads to the removal of the pre-pubertal brake on the GnRH pulse generator is still under investigation (Klein, 1999). The critical event is presumably timed by specific developmental cues, but none of the cues thus far investigated, such as the attainment of a particular state of somatic maturation, has been shown unequivocally to be the mechanism by which the GnRH pulse generator is reinstated. Two processes potentially involved include 1) a desensitization of the gonadostat of the hypothalamus to negative feedback by gonadal steroids and 2) a decrease in intrinsic central inhibition of GnRH secretion (Fig. 1).

Hypothalamic-pituitary axis

Consequences of the reawakening of the GnRH pulse generator include an increase in the responsiveness of the pituitary gonadotrophs to GnRH stimulation and an increase in the synthesis of LH and FSH. These events are mediated at least in part by an induction of cell surface peptide hormone receptors and an increase in the expression of the genes that encode the GnRH receptor, the gonadotropin subunits, and paracrine factors (such as activin and follistatin) that are involved in gonadotropin release. In male humans, the pubertal reawakening of the hypothalamic-pituitary-Leydig cell axis is characterized by nocturnal elevations of both LH and testosterone secretion (Plant,
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1994). The pubertal activation of the hypothalamic-pituitary axis in the female is also characterized by nocturnal elevations in LH secretion which, as in the male, are sleep related and taken to reflect an increase in the amplitude of GnRH pulses. In girls, as puberty progresses the diurnal variation becomes less pronounced. At full maturation in girls, LH and FSH pulse amplitude and frequency vary with the phases of the menstrual cycle, under modulation by ovarian steroids (Boyar, 1978).

As gonadotropin activity is increased in the course of puberty, gonadotropic hormones affect the gonads leading to higher levels of steroid secretion and to gamete maturation. In the female, spontaneous pre-ovulatory gonadotropin surges are first observed at the time of puberty. These surges are elicited by the positive feedback action of estradiol on LH and FSH release and the development of the ability of the hypothalamic-pituitary axis to respond to such feedback (Plant, 1994). Estradiol enhances the pituitary’s midcycle release of LH and, along with inhibin, temporarly inhibits FSH release. For estrogen to exert the positive feedback that leads to the LH-surge, the pituitary must be exposed to a permissive level of estradiol for an adequate period of time.

Female

The size of the ovaries increases from birth through puberty. Although the number of germ cells reached its maximum during the prenatal interval, the number and size of antral follicles as well as the volume of medullary stroma are increased between birth and puberty (Klein, 1999). Growth and atresia of ovarian follicles occur during the prepubertal phase, but preovulatory (Graafian) follicles are not found until puberty. In the female, estradiol concentrations increase at initial stages of puberty, at 8 - 10 years of age in the human. Initial clinical signs of puberty such as the initiation of breast enlargement and the development of external genitalia are then observed and are considered markers of pubertal onset (Beckman and Feuston, 2003). Amplification of follicular growth and steroidogenesis continues as early puberty progresses, and further development of the secondary sexual characteristics and menarche occur. Menarche, the onset of menstrual cyclicity, is an overt sign of pubertal progression, and the first ovulation occurs about 6 months after the first cycle in human females. A regular recurrence of ovulatory menstrual cycles is not established for several more years. Gonadotrophs acquire the capacity to respond to the stimulatory action of estradiol on LH secretion after menarche. Resumption of meiosis in oocytes begins at the time of ovulation at which time meiosis is once again arrested until fertilization of the oocyte at which time meiosis is completed (Beckman and Feuston, 2003).

Male

In the human male, there is an initiation at puberty of a dramatic increase in testicular size. Although data are sparse, it is probable that the development of Leydig cells and a rise in intratesticular androgen concentration precede both the acceleration in testicular growth and the rise in circulating androgen levels (Marty et al., 2003). Daytime levels of plasma testosterone increase progressively before reaching values typical of adulthood by 14 or 15 years of age (Klein, 1999). Initial activation of testicular testosterone secretion during early puberty occurs nocturnally. Further maturation of the testis occurs under the regulation of LH, FSH, and local growth factors and cytokines. Changes in Sertoli and Leydig cell function are necessary for the initiation of spermatogenesis. Primordial germ cells that have remained in a state of mitotic arrest since fetal development undergo spermatogonial division. Also at the onset of puberty, a lumen in the seminiferous tubules is formed and tight junctions between the Sertoli cells that separate the adluminal surface from the interstitial space are established, creating a blood-testis barrier. In primates, the acceleration in growth of the testis during early puberty results largely from an increase in diameter and compacted length of the seminiferous tubules that is associated with the appearance of definitive Sertoli cells and with a proliferation of spermatocytes (Plant, 1994). Spermatogenesis is considered to become established between the 12th and 16th year of human life. The initiation and maintenance of spermatogenesis requires the hormonal action of FSH and testosterone and is dependent on the supportive and paracrine actions of the Sertoli cells and on androgen production by the Leydig cells. In the mature testis, spermatogenesis occurs in cycles with well-defined stages of gametogenesis along the seminiferous tubules. Spermatogonia divide several times before entering a meiotic phase to become preleptotene spermatocytes. Following sequestration for variable periods of time, the preleptotene spermatocyte then completes meiosis and undergoes another mitotic division to yield the haploid spermatid which then differentiates to the mature spermatozoa (Plant, 1994).
TOXICOLOGIC EFFECTS

Evidence is accumulating that environmental chemicals can affect normal endocrine function in animals. Consequently, there is an evolving concern regarding the potential effects of these endocrine disrupting chemicals (EDCs) on reproduction and developmental events including the development of the reproductive organs. It is commonly believed that considerable homology exists in the endocrinology of vertebrates. Hence, toxicants that alter endocrine function in one species are likely to produce adverse effects in another. That said, there are some significant differences between species in endocrine function that warrant consideration in the question of homology and interspecies extrapolation. Although hormones, hormone synthesis, and their receptors are highly conserved, the role of specific hormones in reproductive function and development can vary greatly. In addition, significant differences in metabolism of EDCs can result in marked inter-species differences in responses to these chemicals. It is important to consider these factors when interpreting results from rodent studies. This section will examine animal research on the effects of EDCs on the development of the reproductive system, from the prenatal to the pubertal period, focusing on classes of chemicals with known modes of action.

Androgen receptor (AR) mediated effects

One of the means by which toxicants can alter reproductive development and function is via interaction with the androgen receptor (AR) in either an agonistic or antagonistic manner. For example, vinclozolin is a dicarboximide fungicide with AR antagonistic activity. Vinclozolin metabolites M1 and M2 competitively inhibit the binding of androgens to the mammalian AR (Kelce et al., 1997). The chemical has been shown to alter gene expression in vivo in an antiandrogenic manner and to inhibit growth of androgen-dependent tissues in the castrate-immature-testosterone-treated male rat, a further demonstration of its antiandrogenic action (Gray et al., 1994). With respect to reproductive development, a range of antiandrogen-mediated teratogenic effects were seen in male offspring following late gestational exposure to vinclozolin, including a female-like anogenital distance (AGD), retained nipples, cleft phallus with hypospadias, suprainguinal ectopic testes, vaginal pouch, epididymal granulomas, and small or absent accessory sex glands at birth and delays in preputial separation at puberty (Gray et al., 1994, 1999a).

Several other toxic substances that have been shown to display AR-antagonistic activity include the DDT metabolite DDE, the methoxychlor metabolite HPTE, the organophosphate fenitrothion, and the dicarboximide fungicide procymidone (Kelce et al., 1995; Gaido et al., 1999; Ostby et al., 1999). Linuron is a urea-based herbicide that displays weak affinity for the AR, but the effects induced in male offspring indicate that it may alter mammalian sex differentiation via more than one mechanism of action (Lambright et al., 2000).

Estrogen receptor (ER) mediated effects

The ability of certain pesticides to act as estrogens and induce a uterotrophic response has been known for over 30 years (Bitman et al., 1968). Many estrogenic chemicals, often termed xenoestrogens, have been identified including methoxychlor, chlordcone, octylphenol, nonylphenol, bisphenol A and B, phytoestrogens (genistein), ethynyl estradiol, and fungal mycotoxins (zearalenone). The estrogenic action of these chemicals is mediated by the binding of the chemical or metabolite(s) to the estrogen receptor followed by the sequelae of hormone action, in the case of agonists, or the blockade of hormone action, as in estrogen antagonistic chemicals.

Methoxychlor is a pesticide that is metabolized to several more active compounds, including HPTE, which displays both estrogen receptor (ER) agonist activity and AR antagonist activity (Waters et al., 2001; Bulger et al., 1978). Treatment of adult male rats with methoxychlor at very high doses alters fertility by inhibition of spermatogenesis. If methoxychlor is administered to male rats at weaning, the chemical will induce a delay in puberty and a reduction in accessory gland weights (Gray et al., 1999b). In a study in which methoxychlor was given to female rats for the week before and the week after birth, with the pups then directly dosed with methoxychlor from postnatal day 7, puberty was delayed in males and females, fertility was reduced in males, and estrous cyclicity was altered in females (Chapin et al., 1997). In contrast to the delay of puberty by estrogenic compounds in the male rat, peripubertal estrogen administration accelerates the onset of the pubertal process in the female. Methoxychlor administration beginning at weaning was found to advance vaginal opening and increase the length of estrous cycles (Gray et al., 1989). Xenoestrogens nonylphenol and octylphenol, through gavage but not dietary administration, also accelerated vaginal...
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Inhibitors of steroid hormone synthesis or metabolism

The enzyme 5-alpha-reductase is responsible for the conversion of testosterone to DHT, a more potent AR agonist. DHT acts specifically to masculinize the external genitalia of the male. Finasteride is a 5-alpha-reductase inhibitor used clinically to treat androgen-dependent prostate cancer. Following oral exposure of rats on days 6 to 20 of gestation, AGD was reduced and hypospadias were produced in offspring. Wolfian differentiation was not affected by inhibition of DHT, demonstrating its testosterone dependency, but seminal vesicle growth was impaired (Imperato-McGinley et al., 1992).

Phthalates are a broad class of chemicals used as plasticizers in manufacturing processes, and developmental effects of several phthalates are mediated by alterations in testosterone-synthesizing ability of the fetal testes (Parks et al., 2000). Male rat pups exposed during sexual differentiation to DBP or DEHP exhibit malformations in androgen-dependent tissues, apparently by a non-receptor mediated mechanism (Gray et al., 2000). In a DBP multigenerational study, marked effects on the fertility of rats in the F1 generation were seen, with a 50% decrease in sperm count. These F1 animals showed numerous male reproductive tract malformations at the highest dose level tested (Wine et al., 1997). When pregnant and lactating animals were exposed and their offspring examined, a high incidence of epididymal malformations and decreased sperm count were found, as well as delays in preputial separation and decreases in AGD of the male pups (Mylchreest et al., 1998). Evidence supporting the proposal that these effects are not mediated via AR antagonism include data showing that maternal DEHP and DBP treatments induced dramatic reductions of fetal testosterone synthesis and androgen levels, and altered Leydig cell morphology and function were evident (Parks et al., 2000; Mylchreest et al., 1998).

AhR agonists: TCDD

Male rats exposed in utero to a single dose of TCDD on day 15 of gestation displayed reduced fertility, delayed puberty, and altered reproductive organ weights at maturity (Gray et al., 1997). Treated progeny displayed transient reductions in ventral prostate and seminal vesicle weights, and epididymal sperm reserves and glans penis size were permanently reduced. Female offspring from this treatment regimen showed a delay in puberty and, in some cases, a persistent vaginal thread. Although fertility rates were normal, time to pregnancy was delayed (Gray et al., 1997). F1 female hamster offspring exposed in utero to TCDD also displayed external urogenital malformations with most females having complete clefting of the phallus (Wolf et al., 1999). Altered neurological development is another outcome associated with prenatal exposure to TCDD in experimental animals. Maternal exposure of male rats led to demasculinization and feminization of sexual behavior (Mably et al., 1992).

CONCLUSIONS

The development of reproductive competence begins with bipotential gonads and a hypothalamic-pituitary axis that is undifferentiated. As the gonads respond to genetic signals and become specifically male or female, reciprocal development of the hypothalamus leads to hypothalamic-pituitary axes that are programmed in the male or female direction by the end of gestation. Whereas ovarian gametogenesis is arrested in meiosis, intra-testicular development of spermatogenesis is arrested prior to the initiation of meiosis. There is an interval of quiescence of both hypothalamic and gonadal function during the juvenile period between infancy and puberty. At puberty there is a reawakening of the GnRH pulse generator which stimulates pituitary and gonadal activities in a sex specific manner. The female pattern of gonadotropin secretion develops in a manner that permits the cyclic pattern of reproductive function and, ultimately, ovulation. At the same time, development of the hypothalamic-pituitary-gonadal axis in boys leads to a capacity for sustained steroidogenesis and spermatogenesis that is typical of testicular function. Endocrine disrupting chemicals have the capability to disrupt the development of normal reproductive function in male and female animals. This disruption can occur via exposures during prenatal, perinatal, and peripubertal intervals, and long term adverse consequences relating to the development of fertility can occur in males and females. Key modes of action of EDCs on the developing gonad that have been identified include estrogen and androgen receptor antagonism, estrogen and Ah receptor agonism, and inhibition of gonadal hormone synthesis. Based on homologies of reproduction physiology between humans and the rodents on which most EDC studies are done, it is possible that the chemicals could affect human reproduction and development. However, due to insufficient data, an unequivocal state-
ment to that effect cannot be made.

REFERENCES


Gray, L.E. Jr., Ostby, J.S., Furr, J., Price, M., Veeramachaneni, D.N. and Parks, L. (2000): Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters


