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Endocrine Disruptors and Hypothalamic Sexual Differentiation

Contents

Abstract

The so-called "endocrine disruptors" have been described as compounds which interfere with the estrogen action in their receptors and may exert a crucial role in the development of the reproductive tract and in the brain sexual differentiation. Thus, conducts and/or exposure to these drugs in the perinatal period that apparently do not endanger the neonate may cause side effects. During embrionary development, the gonads, through discharge of a small quantity of reproductive hormones, will guarantee the phenotype of male or female at birth, as well as actuate in specific areas sexual differentiation of the central nervous system. Several experimental models have shown an interference of drugs acting as endocrine disruptors in hypothalamic sexual differentiation. Thus, reproductive function is impaired by exposure to estrogen in the perinatal life of rats and the mechanisms involved in this effect are distinct for males and females. Perinatal exposure to drugs which may be considered endocrine disrupters may induce an incomplete masculinization and defeminization of the central nervous system. Alterations in these processes, if present, generally are perceived only at puberty or adult reproductive life. These later alterations may include anomalies in the process of fertility or in sexual behavior.

Key words: endocrine disruptors, brain sexual differentiation, reproductive function, estrogen, fertility, sexual behavior.

Invited Mini-review

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Introduction

The importance of hormones in a variety of reproductive processes is common knowledge; these processes include development, puberty, behavior, gametogenesis, and integrated sexual function. The ability of foreign compounds to affect the functioning of various endocrine systems is currently thought responsible for a variety of effects (Chapin *et al.*, 1996). Warnings about the risks of exposure to chemicals that are capable of acting as endocrine disruptors have been in evidence in the international scientific literature. Conducts and/or exposure to some drugs in the perinatal period that apparently do not endanger the neonate may lead to later side effects (Carlos *et al.*, 1996; Arena & Pereira, 2002; Gerardin & Pereira, 2002; Pereira *et al.*, 2003[a,b,c]). Thus, their impact upon health and ecosystems is debated. The process of hypothalamic sexual differentiation and the long-term effects of these drugs on reproductive physiology and sexual behavior will be reviewed, with emphasis on recent studies in our laboratory.

Endocrine Disruptors

It has been described that compounds which interfere with the estrogen action in their receptors may exert a crucial role in the development of the reproductive tract and in the brain sexual differentiation. An endocrine disruptor may be defined as an exogenous agent that interferes with synthesis, storage/release, transport, metabolism, binding, action or elimination of natural blood-borne hormones responsible for the regulation of homeostasis and the regulation of developmental processes (Kavlock *et al.*, 1996).

The endocrine system consists of a number of central-nervous-system pituitary-target-organ feedback pathways involved in the regulation of a multitude of bodily functions and the maintenance of homeostasis. As such, there are several target-organ sites at which an environmental agent could disrupt endocrine function (Cooper & Kavlock, 1997). Over the past 2 decades, there has been great concern that the incidence of congenital disorders of male sexual differentiation is increasing, which has led to the suspicion that environmental chemicals are detrimental to normal male genital development in utero (Sultan *et al.*, 2001). In this sense, several reports indicate an increase in the prevalence rates of hypospadias, cryptorchidism, and micropenis (Toppari & Skakkebaek, 1998). Thus, the ubiquitous presence of endocrine disruptors in the environment and the increased incidence of neonatal genital malformation support the hypothesis that disturbed male sexual differentiation may, in some cases, be caused by increased exposure to environmental xenoestrogens and/or antiandrogens (Sultan *et al.*, 2001).

Concern over the possibility that the hormonal system may be disrupted by chemicals in the environment plus the intrinsic complexity of evaluating chemicals for estrogenic activities confirm the need for rigorous attention to experimental design and criteria for assessing estrogenic activity. Thus, many efforts have been made to develop assays for detecting endocrine disruptors. Among them, the uterotrophic assay is known

to be efficient for detecting endocrine disruptors, especially estrogenic compounds (Odum *et al.*, 1997; Kang *et al.*, 2000; Andrade *et al.*, 2002). However, caution is recommended when assays are used to evaluate chemicals for potential therapeutic use. Product safety bioassays conducted with animals selected for fecundity may greatly underestimate disruption of male reproductive development by estradiol and environmental estrogenic compounds (Spearow *et al.*, 1999; Yamada *et al.*, 2001). Thus, concern over the potential hazard posed to humans and wildlife by exposure to environmental endocrine-disrupting chemicals has led to several calls to all substances for the compilation of lists of endocrine disrupting chemicals (Ashby *et al.*, 1997).

Endocrine disruptors are becoming a problem of serious concern in terms of public and environmental health. In addition, exposure to chemicals that act as endocrine disruptors during the perinatal period has a long-term effect on reproductive physiology by interfering in processes necessary to perpetuate the different species (Pereira *et al.*, 2003[b]).

Hypothalamic Sexual Differentiation

In birds and mammals, the sex is determined by two distinct processes: sexual determination and sexual differentiation. Sexual differentiation has far-reaching consequences throughout the life of the organism, in terms not only of reproductive activity but also a wide variety of other physiological processes that function differently in adult males and females (Bardin & Catterall, 1981; Wilson *et al.*, 1981). Sexual differentiation is the result of complex mechanisms involving developmental genetics and endocrinology (Hiort & Holterhus, 2000). During embrionary development, the gonads, through release of a small quantity of reproductive hormones, will guarantee the phenotype of male or female at birth, as well as act in specific areas to permit the sexual differentiation of the central nervous system. In rats, the critical period for sexual differentiation begins in the last phase of gestation and continues through the first week of postnatal life. If the action of these hormones is prevented, sexual differentiation of the central nervous system is impaired (MacLusky & Naftolin, 1981). Neuroendocrine events during the first hours after birth may set a permanent mark on the sexual development that may not be offset by testicular secretions later in life (Matuszczyk *et al.*, 1990).

Thus, in mammals, sexual differentiation begins with the genetic determination of the gonads, which, once completed, will determine the sex of the brain (McCarthy *et al.*, 1997). Before sexual differentiation, the hypothalamus is organized as female type, so that in males it needs to be defeminized and masculinized to guarantee a normal reproductive function. This process depends on an abrupt discharge of testicular testosterone that occurs during the perinatal period in the newborn male. In male rats the concentration of serum testosterone increases by almost 400% between 0 hour *in utero* and 2 hours after birth while in human infant boys testosterone at birth increases dramatically during the first 12 h (Corbier *et al.*, 1992). In this sense, exposure to testosterone

or its metabolites during this period is critical for the masculinization and defeminization of sexual behavior, the establishment of gonadotropin secretion patterns, and also for various morphological indices. In the absence of testosterone or its metabolites, sexually dimorphic structures and functions are feminized (Rhees *et al.*, 1997).

Data are also consistent with the hypothesis that androgen-induced defeminization of feminine behavioral and neuroendocrine responses to estrogen may involve selective reductions in the estrogen sensitivity of critical components of the neural circuitry regulating these responses, mediated in part through a reduction in estrogen receptor biosynthesis (MacLusky *et al.*, 1997). However, it is not androgen *per se* that is responsible for masculinizing the brain (Roselli & Klosterman, 1998); it is necessary to have the conversion of androgen to estrogen. The conversion of testosterone to estradiol *via* cytochrome *P*450 aromatase is an important step in the sexual differentiation processes. This enzyme is increased in the preoptic-hypothalamic area during the perinatal period. Thus, conversion of androgen to estrogen in specific brain areas depends on aromatase cytochrome P450; and there is evidence for the utilization of alternative promoter(s) in man and rodents in driving aromatase gene expression in the brain (Lephart, 1996). It is suggested that neonatal sex hormones influence the sensitivity of the hypothalamic-pituitary-adrenal axis to sex hormones in adulthood and, thus, that they have organizational effects in addition to activational effects on hypothalamic-pituitary-adrenal function (McCornick *et al.*, 1998). Thus, the development and differentiation of the brain involve a complex series of events which begin during gestation and continue, at least in rodents, in the early postnatal period (Negri-Cesi *et al.*, 2001).

Long-term Effect of Manipulation and/or Endocrine Disruptors on the Reproductive Physiology and Sexual Behavior

In the course of their differentiation, certain cells of the brain express genes for steroid hormone receptors, which enable them to respond to hormones that regulate particular aspects of brain development, as well as activate behavioral and neuroendocrine functions in adult life. Manipulation of steroid hormones in the perinatal period may result in sexually dimorphic neuroendocrine events, such as the regulation of gonadotropin secretion. In this sense, several experimental models in development in our laboratory have shown an interference of drugs in the hypothalamic sexual differentiation.

Kacsóh *et al.* (1986) showed that early lactation milk is necessary for a normal masculinization of the hypothalamus-pituitary axis in rats. In addition, it was proposed that the intake of early lactation milk during the neonatal period is important to the later sexual development of rats and that GnRH is somehow involved in this effect (Carlos *et al.*, 1996).

Sexual differentiation of the hypothalamus of male and female rats involves complex phenomena and an important participation of estrogen, as well as androgens (Dohler, 1991). Thus, reproductive function is impaired by exposure to estrogen in the

perinatal life of rats and the mechanisms involved in this effect are distinct for males and females (Pereira *et al.*, 1997). In order to obtain more information about the participation of estrogen during the period of brain sexual differentiation, male rats were treated with clomiphene in the neonatal phase. The estrogen antagonist activity of clomiphene during this phase had a long-term effect on the reproductive physiology and sexual behavior of these male rats as shown by a significant reduction in the frequency of mounts. When these adult male rats were castrated and received estrogen, sixty percent presented female sexual behavior (Pereira *et al.*, 2003[b]). On the other hand, the effects of aromatase inhibitor during the perinatal period of brain sexual differentiation also impaired the reproductive performance and sexual behavior of male rats. There was a decrease in the number of spermatozoa found in the testes and in the daily sperm production. Only fifty percent of these males were capable of presenting male sexual behavior, while twenty five percent of the males did not present male sexual behavior, showing female sexual behavior when castrated and pretreated with estrogen (Gerardin *et al.*, 2002). These results, plus data from Dohler (1991), demonstrated that the differentiation of the male rat hypothalamus is not exclusively estrogen dependent and that, during differentiation of the brain, estrogen is supportive of the primary actions of androgens.

It has been also reported that prenatal stressors such as immobilization, electric foot shocks (Velazquez-Moctezuma *et al.*, 1993), cold and ether anesthesia (Matuszczyk *et al.*, 1990), or perinatal exposure to picrotoxin (Silva *et al.*, 1998; Teodorov *et al.*, 2002) may induce changes in the adult sexual behavior of the offspring. Thus, reproductive function may be impaired by exposure to stress in the perinatal life that can compromise the success of mating and species perpetuation. Pereira *et al.* (unpublished data) showed that prenatal stress exposure in rats induced enduring neurochemical alteration in a region-specific manner that may be related to sexual behavior damage previously observed. Probably, the activation of the serotoninergic system may be responsible for the reduction in copulation efficiency, as observed by the increase in latency for the first mount and intromission, which are involved with motivational aspects of male sexual behavior.

Exposure of male rats to ethyl ether during the critical period of male brain sexual differentiation probably delayed or reduced the testosterone peak, necessary to the processes of masculinization and defeminization of the hypothalamus, endangering the later spermatozoa production as well as the sexual behavior. The decreased fertility plus the appearance of homosexual behavior when these male rats were castrated and pretreated with exogenous estrogen suggest endocrine disruption through an incomplete masculinization and defeminization of the central nervous system (Arena & Pereira, 2002).

Stress may be part of normal life, so that, to a certain extent, some stressful situation such as physical exercise and various emotional states usually may be considered healthy. However, manipulation of the hypothalamic-pituitary-adrenal axis either by stress or by the administration of pituitary/adrenal stress hormones during the last third of pregnancy may influence the process of brain sexual differentiation and have a long-term effect on the

reproductive physiology of male rats (Ward, 1972; Anderson *et al.*, 1986). These changes appear to be dependent upon stress-induced hormonal changes in the pregnant mother and/or the fetus, such as altered levels of corticosterone (Ward & Weisz, 1984).

The maintenance of physiological levels of corticosteroids in perinatal life is of fundamental importance to support the later contractile response pattern of the seminal vesicle to the mediator acetylcholine in the adult phase, which may be crucial to the reproductive process. In addition, exposure to hydrocortisone during this critical period of brain sexual differentiation has a long-term effect of decreasing the testosterone production in adult life of male rats (Pereira *et al.*, 2003[c]). Exposure of male rats to hydrocortisone in the later stages of pregnancy also may have a later effect on fertility and sexual behavior. Thus, males exposed to hydrocortisone during the prenatal period were able to mate with normal females, which became pregnant but exhibited an increased number of post-implantation losses. In spite of this, these treated males exhibited decreased male sexual behavior and the appearance of female sexual behavior after these male rats were castrated and pretreated with exogenous estrogen. All these alterations may be a consequence of an incomplete masculinization and defeminization of the central nervous system induced by the high plasma levels of corticosterone in perinatal life (Pereira *et al.*, 2003[a]).

On the basis of these considerations, all these results suggest that manipulation and/or perinatal exposure to drugs which may be considered endocrine disrupters induce an incomplete masculinization and defeminization of the animal central nervous system. Alterations in these processes, if present, generally are perceived only at puberty or adult reproductive life. These later alterations may include anomalies in the process of fertility and in sexual behavior.

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