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Endocrine disruptors: from Wingspread to environmental developmental biology[☆]

Caroline M. Markey, Beverly S. Rubin, Ana M. Soto, Carlos Sonnenschein*

Department of Anatomy and Cellular Biology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111-1800, USA

Abstract

The production and release of synthetic chemicals into the environment has been a hallmark of the “Second Industrial Revolution” and the “Green Revolution.” Soon after the inception of these chemicals, anecdotal evidence began to emerge linking environmental contamination of rivers and lakes with a variety of developmental and reproductive abnormalities in wildlife species. The accumulation of evidence suggesting that these synthetic chemicals were detrimental to wildlife, and potentially humans, as a result of their hormonal activity, led to the proposal of the endocrine disruptor hypothesis at the 1991 Wingspread Conference. Since that time, experimental and epidemiological data have shown that exposure of the developing fetus or neonate to environmentally-relevant concentrations of certain synthetic chemicals causes morphological, biochemical, physiological and behavioral anomalies in both vertebrate and invertebrate species. The ubiquitous use, and subsequent human exposure, of one particular chemical, the estrogen mimic bisphenol A (BPA), is the subject of this present review. We have highlighted this chemical since it provides an arresting model of how chemical exposure impacts developmental processes involved in the morphogenesis of tissues and organs, including those of the male and female reproductive systems, the mammary glands and the brain. © 2003 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The anecdotal association between chemical contamination of the natural environment and the onset of disease, reproductive failure and death of wildlife species began to take form as early as 1947 in the US [67]. At that time, both amateur and professional ornithologists began to observe a sharp decline in east coast populations of the American bald eagle, a phenomenon that was associated with changes in courtship behavior and the maternal care of chicks. Since then, a growing number of cases came to highlight that the contamination of lakes and rivers with sewage from local manufacturing industries and chemical plants and also from agricultural run-off was the cause of developmental and reproductive problems in certain aquatic species, and by virtue of their higher position on the food chain, avian, reptilian and mammalian species. For example, in the 1960s, the mink industry around the Great Lakes region in the US suffered economic hardship due to reproductive failure of these

animals, a repercussion of its heavy reliance on local fish that were later found to be contaminated with, among other chemicals, polychlorinated biphenyls (PCBs). In this same decade, a high incidence of tumors was observed in fish that resided within Californian waters receiving sewage from local industries. In the 1970s, the herring gull population of Lake Ontario in Canada was observed to suffer from developmental deformities such as club feet, missing eyes, twisted bills and the presence of adult feathers in chicks instead of down, phenomena that were attributed to dioxin exposure. In the 1980s, alligators in Lake Apopka, Florida were observed to exhibit micro-phallus and other reproductive-related disorders, a consequence of a local industrial accident and, more recently, agricultural run-off of chemicals including 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT).

2. The road toward Wingspread

Thus it became vividly clear that certain industrial and agricultural chemicals could cause developmental and reproductive anomalies in wildlife, a phenomenon that was foreshadowed by Carson in her 1962 book “Silent Spring” [12], and one that evoked fears of similar effects in humans. As a result, the agricultural and industrial use of DDT and

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* Corresponding author. Tel.: +1-617-636-2451; fax: +1-617-636-3971. E-mail address: carlos.sonnenschein@tufts.edu (C. Sonnenschein).

PCBs was banned in 1973 and 1977, respectively, within the US. Nonetheless, new and different cases of wildlife disturbances continued to emerge over the next few decades, due to bio-persistence of the banned chemicals (DDT has a half-life of 57 years) and the production of a plethora of new chemicals. Motivated by these findings, Dr. Theo Colborn, currently of the World Wildlife Fund, convened the 1991 Wingspread Conference in Racine, Wisconsin. This scientific meeting heralded the notion that these chemicals might be inducing their observed effects as a result of their hormonally active nature. The conferees addressed the issue that “a large number of man-made chemicals that have been released into the environment . . . have the potential to disrupt the endocrine system of animals, including humans,” and made the observation that some of the effects documented in the genital tracts of wildlife were comparable to those seen in the daughters and sons of women who had been exposed during pregnancy to the synthetic estrogen diethylstilbestrol (DES). Administered to pregnant women between the years 1948 and 1971 as an anti-abortion therapy in the US, Europe and Australia, fetal exposure to this potent estrogen induced a rare cancer, clear cell adenocarcinoma of the vagina, and other severe reproductive disorders that became apparent after puberty; in some instances these malformations manifested when these women tried to become pregnant [28,43]. The conference participants recognized that the human DES syndrome was an extreme expression of the plasticity of the fetus in response to environmental cues, and further, it provided a template for the potential effects that other hormonally active chemicals could have on human health.

3. The complexity of endocrine disruptors

Environmental “endocrine disruptors” have been defined by the US Environmental Protection Agency as “exogenous agents that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior.” A subset of these chemicals have been identified largely on the basis of a battery of *in vitro* and *in vivo* assays that define estrogen and androgen agonists and antagonists, since their chemical structure alone is not a reliable indicator of their hormonal activity. The most notable of the estrogenic bioassays are the (a) estrogen receptor (ER) competitive binding assay [20], (b) yeast-based reporter gene assay [24], (c) E-SCREEN assay [61], and (d) mouse uterotrophic assay [51]. These assays measure the ability of a suspected hormonally active chemical to (i) competitively bind the mouse uterine ER, (ii) transcriptionally and translationally activate a reporter gene construct via an estrogen response element, (iii) induce proliferation of estrogen-responsive human MCF-7 cells, and (iv) induce increased uterine wet weight in the prepubertal or adult ovariectomized mouse, respectively. While

these methods have certainly provided insight into the hormonal nature of both synthetic and natural chemicals, they are not equivalent [20,56]. The ER binding, reporter gene and E-SCREEN assays are consistent in detecting estrogenic activity, albeit with different sensitivity [2,20]. The uterotrophic assay, although quite insensitive, is able to detect pro-estrogens, that is, chemicals that do not bind to the ER until they undergo metabolism. Nonetheless, the E-SCREEN assay, for example, has been the one that revealed the estrogenic nature of most of the estrogenic, environmental chemicals described so far [62]. Environmental chemicals with estrogenic activity include: (a) insecticides: DDT, methoxychlor, kepone, dieldrin, toxaphene, endosulfan, lindane; (b) anti-oxidants: alkylphenols, butyl-hydroxy-anisole; (c) plasticizers and monomers: phthalates, bisphenol A (BPA); (d) disinfectants: phenyl-phenol; (e) other chemicals such as PCBs, sunscreens, and fire retardants.

Endocrine disrupting chemicals are believed to exert their effect by (1) mimicking normal, endogenous hormones such as estrogens and androgens; (2) antagonizing endogenous hormones; (3) altering the pattern of synthesis and metabolism of endogenous hormones; and (4) modifying hormone receptor levels. Natural, endogenous estrogens are involved in the development and maintenance of the female reproductive tract and secondary sexual characteristics, and regulation of the menstrual cycle, pregnancy and lactation. At the cellular level, these endogenous hormones mediate cell proliferation, and also the synthesis and secretion of cell-specific proteins in reproductive tissues such as the ovary, oviduct, uterus, vagina, hypothalamus, pituitary and mammary gland. These effects are mediated, for the most part, by ER α and ER β in the female, although the expression of these same receptors in the male reproductive tract and in non-reproductive organs such as the thyroid gland, cardiovascular system and bone indicates the vast reach of these synthetic estrogenic chemicals [14].

Although the environmental chemicals are usually less potent than endogenous hormones, such as estradiol, it is now clear that they act *additively* with them. This explains how low, seemingly insignificant levels of xenoestrogens, such as the “weak” BPA, may have an impact when added to the already significant levels of endogenous steroidal hormones [57]. Further, several endocrine disruptors have unequivocally been shown to induce non-monotonic dose–response curves of biological effects; that is, lower doses induce a more profound effect than higher doses (“low dose effect”). This phenomenon, which has been known for a long time regarding sex steroid action, was first described for endocrine disrupting agents in the mouse prostate. Fetal exposure to increasing doses of estradiol and DES [71], and the “weak” xenoestrogen BPA [45], was shown to induce increased prostate weight that persisted into adulthood. Further, exposure of the prepubertal mouse to a range of BPA doses induces a U-shaped curve for the endpoints of age of vaginal opening and uterine weight, two parameters

that are the hallmarks of estrogen action, such that a more apparent effect is observed in the lower and higher doses relative to the medium range doses [1]. Endogenous hormones are known to act on hormone-sensitive endpoints in this manner, as evidenced by the observation that prostate cells undergo increased cell proliferation in response to low doses of androgen but decreased cell proliferation in response to high doses of androgens [59]. It has been postulated that this phenomenon is due to the operation of different and discrete pathways at low and high doses [25]; in other instances, low dose effects have been observed to occur due to down-regulation of the receptor by the ligand.

4. Endocrine disruptors within a developmental context

The deleterious effects of endocrine disrupting chemicals vary according to the age at which an organism is exposed. The developing organism is critically sensitive to both endogenous and exogenous hormones, a phenomenon that led Bern to coin the phrase the “fragile fetus” [6]. The “critical window” of exposure differs depending upon the time at which specific developmental events occur in particular tissues or organs. For example, clear cell adenocarcinoma of the vagina was observed in daughters of pregnant women exposed in utero to DES, but only if exposure occurred before the 13th week of gestation. It is clear that the developing fetus and neonate are profoundly sensitive to hormonally active chemicals as demonstrated by the observation that mammary gland dysgenesis occurs in the adult mouse following fetal exposure to concentrations of BPA that are 4000-fold lower than those required to induce an uterotrophic effect in the prepubertal animal [1,39]. In 1998 the DDT metabolite DDE, and other chemicals such as PCBs and phytoestrogens, were shown to be the most frequently recovered contaminants in amniotic fluid of women aged 35 years and over in the US [23]. This finding is of great concern because it occurred in spite of a restriction in the use of the parental chemical DDT since 1973. To understand the impact of environmental chemical exposure on human health and disease, we must consider exposure within a developmental context. The study of BPA provides one pertinent model by which to address this issue, since the chemical is present ubiquitously in our environment, has high potential for fetal exposure, and the literature provides multiple examples of developmental effects.

5. Fetal and neonatal exposure to BPA

Although it had been known since 1943 that BPA was estrogenic [17], this chemical was serendipitously discovered to leach from polycarbonate plastics in concentrations that were sufficient to up-regulate the expression of progesterone

receptor and induce cell proliferation in estrogen-target, serum-sensitive MCF-7 cells through binding to the ER [35]. BPA (4,4'-isopropylidenediphenol) is a diphenyl compound that contains two hydroxyl groups in the “para” position making it remarkably similar to the synthetic estrogen DES. Used in the manufacture of polycarbonate plastics and epoxy resins, BPA is present in a multitude of products including the interior coating of food cans, wine storage vats, water carboys, milk containers, food storage vessels, baby formula bottles, water pipes, dental materials, automotive lenses, optical lenses, protective coatings, adhesives, protective window glazing, compact disks, thermal paper, paper coatings, and as a developer in dyes. Halogenated derivatives of BPA, tetrabromobisphenol A (TBBPA) and tetrachlorobisphenol A (TCBPA), are widely used as flame retardants for building materials, paints, synthetic textiles, and plastic products including epoxy resin electronic circuit boards and other electronic equipment [40]. Studies have shown that incomplete polymerization of these products during manufacture, and/or depolymerization due to increased temperatures (induced either intentionally for sterilization/heating purposes or unintentionally during storage in warehouses) causes BPA and its derivatives to leach into foods (4–23 µg per can; 7–380 µg/kg) [10], beverages (7–58 µg/g) [7], infant formula from plastic bottles and cups (7–58 µg/g) [7] and saliva (90–913 µg in saliva collected in a 1 h period following application of dental sealant) [47]. BPA has been found in air and dust samples from certain residential and commercial environments in Massachusetts [53] and in leachates from a waste water treatment plant and river water in Japan [4]. Similarly, TBBPA and its dimethylated derivatives have been found in river sediment in Osaka, Japan (0.5–140 µg/kg dry weight), in sites downstream from a plastics production facility in sewage sludge samples in Sweden (270 µg/g dry weight TBBPA; 1500 ng/g dry weight as the dimethyl derivative) [55], and in air samples in electronic recycling plants [58].

The ubiquitous use of BPA provides great potential for exposure of both the developing fetus, indirectly through maternal exposure, and the neonate, directly through ingestion of tinned food, infant formula or maternal milk [73]. In rodents, BPA has been shown to readily traverse the placenta [65], and to bind α -fetoprotein with negligible affinity relative to estradiol; this results in its enhanced bioavailability during neonatal development [42]. Further, it is present in the mouse fetus and amniotic fluid during maternal exposure in higher concentrations than that of maternal blood [74]. These data, and those revealing the presence of 4–200 pg TBBPA and TCBPA/g of human plasma lipids [68] and 1 ng BPA/g umbilical cord fat/serum from Japanese women/neonates [44], indicate that the developing human fetus is readily exposed to this ubiquitous chemical. Further, they suggest that the pharmacokinetics and pharmacodynamics of BPA may exacerbate the impact of this chemical on the developing fetus and neonate [74].

6. BPA: a model of chemically-induced developmental disruption

Numerous studies have described the effects of BPA exposure in rodents, although more recent data has described effects in some aquatic species. We will review mostly those studies that outline low, presumably environmentally-relevant exposures during fetal or neonatal development, but will also include a few examples of high exposures when a paucity of the former data exists for various organs/systems.

6.1. Early developmental effects of BPA

The time course of development for various tissue and organ systems spans different periods of gestational, neonatal and adult life, the length of which depends upon the specific system of interest. Since the hormonal milieu of estrogen-sensitive systems, such as the reproductive tract and mammary glands, is of critical importance during fetal development, the addition of hormonally active agents like BPA during this time have been shown to exert a profound effect on how these tissues respond to the genetic or hormonal cues that shape their structure and function later in life. Morphogenesis of the mouse Mullerian ducts, which ultimately develop into the female reproductive tract, starts during embryogenesis and progresses through a series of distinct morphological and physiological events, such as vaginal opening, first estrus, and ultimately the onset of regular estrous cycles in adulthood. These events are influenced initially by maternal estrogens that traverse the placenta, and later by the gradually increasing levels of prepubertal estrogens that ultimately lead to a functional hypothalamic–pituitary–ovarian axis at puberty.

Prenatal exposure to low doses of BPA alters the timing of these morphological events and, once established, the functionality of the female reproductive system. Exposure of pregnant mice to 20 µg/kg BPA from gestational days 11 through 17 has been shown to induce both vaginal opening and first vaginal estrus at a significantly earlier age in female offspring [29]. A similar phenomenon has been observed following the same period of exposure of pregnant mice to 2.4 µg/kg BPA, which specifically reduced the number of days between vaginal opening and first vaginal estrus in female offspring situated in utero between two females (0M) but not in those situated between two males (2M) [30]. This developmental precociousness of the pups was associated, not surprisingly, with an increased body weight at the time of weaning (day 22), whereby the weight of those females and males situated between two females (0M) increased by 22%, 1M females increased by 9%, and 2M females remained unaffected [30]. The developmental events that mediated these BPA-induced changes during neonatal and prepubertal growth appear to have significant consequences in adulthood. Exposure of pregnant rats from gestational day 6 through lactation to 100 µg/l BPA in drinking water was shown to significantly increase body weight in both

female and male offspring from the time of birth to 110 days and disrupt the regularity of estrous cyclicity in females aged 4 and 6 months [52]. Female offspring born to mothers that were exposed to 25 and 250 µg/kg BPA during pregnancy exhibited a state of persistent estrous/metestrus at 3 months of age [38].

Developmental studies in male rodents have shown that prenatal exposure to BPA increases ano-genital distance in the neonate [26] and affects various testicular parameters suggesting that this chemical advances testicular/spermatogenic development. Exposure of rats from postnatal days 2 to 12 to 500 µg per rat advanced seminiferous tubule formation and increased Sertoli cell nuclear volume per testis by postnatal day 18, and spermatocyte nuclear volume per unit Sertoli cell nuclear volume by puberty [3]. While interesting, this study examined the effects of BPA doses that may not necessarily reflect environmentally-relevant exposures. However, studies in fish have shown that exposure to 10 µg/l BPA is able to induce feminization of testes (ovo-testis) in Japanese medaka [41]; these concentrations are comparable to those found in waste water discharged from sewage treatment plants. Further, injection of BPA (200 µg/g egg) into the yolk of quail and chicken eggs during early incubation caused Mullerian duct malformation in female quail embryos and ovo-testis in the male chicken embryos [5]. The same treatment with TBBPA (45 µg/g egg) caused high embryo mortality in both species, while a higher dose of BPA (67 and 200 µg/g egg) induced mortality in chickens only.

6.2. Male reproductive tract and gonads

The establishment of a functional reproductive system in the female and male rodent is a consequence of genetic, epigenetic and hormonal cues. Although it has not been determined fully how BPA interferes with any one of these factors, multiple studies have established that fetal and neonatal exposure to this chemical impacts the ultimate morphology and function of the reproductive tract and gonads. One of the earliest studies to show that BPA can alter developmental pathways resulting in permanent changes described how adult prostate weight was increased by 30 and 35% in male offspring born from mice exposed to 2 and 20 µg/kg BPA, respectively, from gestational days 11 to 17 [45]. Findings that in utero exposure to BPA increased androgen receptor binding activity of subsequently cultured prostate tissues [26], and that in vitro exposure to BPA-induced enlargement of the prostate through the presence of ER in the stroma [30] suggests some mechanisms by which this chemical impacts organ size. Observed alterations in the fibroblastic to smooth muscle cell ratio of the peritubular stroma [49] and an increase in prostatic glandular buds [69] in rats further reflect how fetal exposure to BPA affects prostate histoarchitecture.

Other male reproductive parameters are affected by prenatal and neonatal exposure to environmentally-relevant doses of BPA. Exposure of pregnant mice to 2 and 20 µg/kg BPA

from gestational days 11 to 17 has been shown to permanently increase the size of the preputial gland, reduce the size of the epididymis, and decrease daily sperm count by 20% (20 $\mu\text{g}/\text{kg}$ group) in male offspring [70]. Further, exposure of prosobranch snails to 1 and 100 $\mu\text{g}/\text{l}$ BPA throughout their complete life-cycle has been shown to cause decreased penis and prostate length, and reduced sperm storage within the vesicula seminalis [46]. An interesting phenomenon has been observed regarding species and strain-specific responsiveness to estrogen, and this perhaps may also be pertinent to xenoestrogens such as BPA [63]; different strains of both inbred and outbred mice exhibit different degrees of spermatogenic changes following exposure to increasing doses of estradiol. In some strains, such as CD-1 mice, no effects were seen at all.

6.3. Female reproductive tract and gonads

The developmental changes that were described earlier in the female reproductive tract as a result of fetal exposure to low doses of BPA have been shown to affect adults. Three-month-old female offspring born to mothers that were exposed to 25 and 250 $\mu\text{g}/\text{kg}$ BPA while pregnant show a decrease in uterine weight [38], a change that is associated with increased DNA synthesis within the glandular epithelium, and a striking increase in the expression of both ER α and PR within the luminal epithelium (Markey et al., unpublished). The consequence of these changes may be to alter the responsiveness of the uterus to endogenous hormones imposed under different physiological conditions, such as pregnancy, or predispose the tissue to disease and carcinogenesis. Indeed, transgenic mouse models in which ER expression is up-regulated by 25% develop uterine adenocarcinoma at a far earlier stage in adult life relative to the wild type following neonatal exposure to DES [13]. Alterations in the relationship between cell proliferation and apoptosis may also predispose the uterus to carcinogenesis.

While humans may be considered the primary targets of BPA exposure by contaminated foods and drinks, and dental materials, recent evidence has revealed that invertebrate species are also exposed through the contamination of the natural environment. Freshwater snails exposed to concentrations of BPA similar to those found in rivers and lakes generate “super-female” phenotypes, that is, they exhibit additional reproductive organs [46]. Female mice have also been shown to develop an ovary-independent stratification of the vaginal epithelium in response to high BPA doses (150 μg per pup BPA during postnatal days 1–5) [64] and a down-regulation of vaginal ER α specifically during estrus following maternal exposure to 0.1 and 50 mg/kg BPA [54]. Ovarian changes have also been observed in mice exposed to BPA during development. The phenomenon of polyovular follicles was shown to be induced in female mice exposed daily to 150 μg BPA per pup from postnatal days 1 to 5 [64]. Further, much lower doses of BPA (25 and 250 $\mu\text{g}/\text{kg}$) administered to pregnant mice from gestational days 9 to

20 have been shown to result in an increased number of female offspring exhibiting blood-filled ovarian bursa by 6 months of age [38]. All of these changes suggest that fetal and neonatal exposure to BPA may impact fertility, age of reproductive senescence, and onset of disease later in life. It is interesting to note that serum BPA has been found in higher concentrations in men and in women with polycystic ovary syndrome than in normal women; this may be due to differences in androgen-related metabolism of BPA [66].

6.4. Mammary gland

The development of the mammary gland represents an interesting model to evaluate the effect of endocrine disruptors because this organ undergoes dramatic morphological and functional changes throughout the lifetime of an animal. The epithelial primordium of the mouse mammary gland initiates its development at gestational day 10 to ultimately establish a ductal tree of distinct epithelial and stromal tissue compartments before postnatal day 10. The tissue is relatively quiescent until puberty ensues, at which time the epithelial ductal tree migrates into the stroma, led by a front of large terminal end buds that are very active sites of both DNA synthesis and apoptosis, in response to increasing systemic levels of estrogens. The gland fills out the fat pad and eventually establishes a network of ducts, terminal ends, terminal ducts, and a few alveolar buds. This morphology remains relatively quiescent, once again, other than minor fluctuations with each estrous cycle, until pregnancy when the entire epithelial compartment undergoes dramatic proliferation resulting in a plethora of alveolar buds and lobulo-alveolar units in preparation for lactation. Once the period of lactation is over, the mammary gland undergoes rapid involution, a process associated with widespread apoptosis, to return to its pre-pregnancy state [15,27].

Exposure of the developing mouse fetus to BPA (25 and 250 $\mu\text{g}/\text{kg}$ BPA to the pregnant mother) has been shown to impact certain aspects of this developmental process. By postnatal day 10, the relationship between the epithelium and stroma in terms of DNA synthesis becomes altered, a phenomenon that is also observed at puberty (day 30) and at 6 months of age. At this latter time point, these virgin mice exhibit mammary glands that resemble those of pregnant mice. This is reflected by a significant increase in the percentage of ducts, terminal end buds, terminal ducts and alveolar buds [39]. These BPA-induced changes in mammary gland development are consistent with the notion that prenatal exposure to estrogens may predispose the tissue to cancer. Indeed, the persistence of epithelial structures, such as the terminal end buds and terminal ducts, has been associated with increased carcinogenesis in both rodents and humans [50].

Estrogen exposure throughout a woman's life is a major risk factor for the development of breast cancer, as demonstrated by the increased risk associated with early age of menarche, late age of menopause, and obesity in

the postmenopausal period (and thus increased aromatase activity and estrogen production). The positive correlation between increased intrauterine levels of estrogens (a phenomenon observed in twin births) and breast cancer in daughters born from such pregnancies also supports this link [9,18,72]. The aforementioned studies describing how fetal exposure to low doses of BPA induces modulations in cell proliferation and apoptosis, and the timing of developmental events, fits the notion that this chemical can predispose the mammary gland to carcinogenesis. Indeed, various epidemiological case–control and cohort North American and European studies have revealed a positive correlation between blood serum levels of other endocrine disruptors, such as dieldrin, DDT and PCBs, in women and breast cancer incidence [31,32]. Controversy abounds on this interpretation of the data mainly because none of these chemicals can be construed to be a marker of a total xenoestrogen exposure [60]. However, it is worth noting that women who were exposed therapeutically to DES while pregnant between the years 1948 and 1971 now show a higher incidence of breast cancer [11]. The multitude of environmental chemicals to which we are all exposed involuntarily, in addition to medically used hormones (hormonal contraceptives or hormone replacement therapy) may be a cumulative cause in the increase of breast cancer incidence that has been observed during the last 50 years.

All of the morphological and functional changes that have been described earlier as a result of in utero and neonatal exposure to BPA suggest a mechanism of action that involves both alterations in the way developmental genes, such as the *Hox* or *Wnt* genes, direct tissue patterning, as well as alterations in the hypothalamic–pituitary–gonadal (H–P–G) axis. Fetal exposure to DES has been shown to down-regulate the expression of *Wnt7a* [34] and several *Hox* genes [8,37] in the mouse Mullerian duct and uterus. The uterus phenotype observed in mice carrying null mutations of these genes is similar to that resulting from fetal exposure to DES, suggesting that the DES effect is due to the down-regulation of these genes [34]. Our laboratory is currently investigating this hypothesis in the BPA-exposed mouse mammary gland.

6.5. Hypothalamic–pituitary–gonadal (H–P–G) axis function

Evidence of alterations in H–P–G axis function is probably best exemplified by the disturbances in estrous cyclicity observed in female rats and mice exposed to BPA during development [38,52]. Data from several studies reveal alterations at the hypothalamic and/or pituitary level in BPA-exposed animals. Exposure of pregnant rats to BPA in their drinking water (100 µg/l) from gestational day 6 through lactation results in altered response to long-term ovariectomy in female offspring manifested as decreased plasma levels of LH [52]. The exposure of male rats to far higher doses of BPA, namely 500 µg per rat, from postnatal

days 2 to 12 results in increased levels of plasma FSH by day 25 [3]. Further, both male and female rats injected with 100 and 500 µg BPA per rat from postnatal days 1 to 5 show a progressive increase in serum prolactin levels that reaches three-fold that of the controls by postnatal day 30 [33]. This latter finding is relevant to the aforementioned changes observed in the BPA-exposed mammary gland; this mammary phenotype strongly suggested a state of hyperprolactinemia based upon the striking increase in alveolar development.

Prenatal and neonatal exposure to BPA can alter the expression of sex steroid receptors in the hypothalamus and pituitary. The study described above in which increased prolactin levels were observed following neonatal exposure to 100 and 500 µg BPA per rat during postnatal days 1–5, also documented a concomitant increase in the expression of ER α and ER β mRNA in the anterior pituitary of male, but not female, rats at postnatal day 30. In addition, female rats in this study exhibited an increase in ER α mRNA in the medial basal hypothalamus at this same time point [33]. Changes in the expression of sex steroid receptors in the pituitary as well as in brain regions important for LH surge induction reveal how developmental exposure to BPA might impact steroidogenesis and estrous cyclicity.

6.6. Evidence of direct effects of BPA on the developing brain

Emerging data suggests that the developing brain may be a primary target for BPA action. A few studies in rats have now documented measurable changes in the brain of offspring exposed to BPA during development. As mentioned earlier, daily injections of BPA into rat pups on postnatal days 1–5 increased the levels of ER α mRNA measured in the mediobasal hypothalamus of the females on postnatal day 30 providing conclusive evidence of a direct effect of neonatal BPA exposure on the brain [33]. Exposure of rats to 40 or 400 µg/kg BPA during pregnancy and lactation resulted in measurable effects on one of the five known somatostatin receptor subtypes, *sst2*, in the brains of the offspring [19]. Variations of low affinity and high affinity *sst2* binding parameters were documented in hypothalamic and limbic structures in BPA-exposed offspring when examined on postnatal days 10 and 23 providing additional evidence for a direct central effect of early BPA exposure. In yet another study, when examined in adulthood, offspring born to rats treated with BPA (1.5 mg/kg per day) throughout pregnancy and lactation, exhibited measurable alterations in the volume and the number of cells in the locus coeruleus, a major noradrenergic nucleus of the brainstem [36].

Perhaps the most striking evidence of BPA action on the developing brain is provided by the emerging data from behavioral studies in rodents. The first reports of developmental effects of BPA on behavior in adulthood were provided by the studies of Farabolini et al. in rats [22]. Female rats

were exposed to a low dose of BPA (40 $\mu\text{g}/\text{kg}$ per day) beginning 10 days before mating and continuing until weaning of the pups, or they were exposed to a 10-fold higher daily dose (400 $\mu\text{g}/\text{kg}$ per day) from day 14 of gestation through postnatal day 6. Both treatment regimens revealed similar findings in behavioral tests. An overall reduction in motivation to explore was observed in all BPA-exposed offspring. Moreover, depressed motor activity was detected in BPA-exposed females and reduced anxiety-like behaviors were observed in BPA-exposed male offspring. In the studies of Kubo et al. mentioned earlier [36], the significant sex differences in activity levels, exploratory behaviors and avoidance memory noted in control offspring were obliterated in offspring born to mothers treated with 1.5 mg/kg per day BPA throughout pregnancy and lactation. Recent studies have also documented effects of perinatal exposure of BPA on sociosexual behavior in male and female rats [21]. The behaviors observed suggested that female offspring born to mothers exposed to 40 $\mu\text{g}/\text{kg}$ per day BPA during pregnancy and lactation exhibited a potentiation of female typical behavior and male offspring exhibited a reduction in male typical behavior. The examination of play behaviors in young rats (postnatal days 35, 45 and 55) born to females exposed to 40 or 400 $\mu\text{g}/\text{kg}$ per day BPA in the regimens described above, revealed differences in male and female BPA-exposed offspring [16]. Two components of play behavior were masculinized in female offspring born to BPA-exposed females and one component of male play behavior was intensified in BPA-exposed males. The recent work of Palanza et al. [48] indicates effects of developmental exposure to BPA on maternal behavior in mice. BPA exposure in this study was limited to 10 $\mu\text{g}/\text{kg}$ per day administered to pregnant females on days 14–18 of gestation. In adulthood, the females exposed to BPA during gestation spent less time nursing their pups and more time out of the nest compared to females born to control mothers. The demonstration of measurable effects on behavior in adult mice exposed to as little as 10 $\mu\text{g}/\text{kg}$ per day BPA for 5 days during gestation suggests that the developing brain may be a particularly sensitive target for BPA exposure. Additional studies are needed to further characterize the effects of BPA on other behavioral endpoints and to delineate the critical time periods of BPA exposure for the different behavioral parameters assessed.

7. Conclusions

The data collected so far in the field of environmental toxicology is sufficient to raise concerns among scientists and public officials about the potential deleterious impact of endocrine disrupting chemicals on human development. It has always been controversial to extrapolate from data that is obtained from laboratory animal experiments in which the dose and schedule of chemical exposure does not always represent conditions that humans face in their

habitat during a lifetime. However, it would be derelict to ignore the increasing evidence coming from these experimental studies, the naturally occurring malformations and behavioral problems found in chemically-exposed wildlife, and the increasing incidence of comparable malformations and behavioral problems in human populations that are exposed to the same chemicals during different developmental stages. All of this evidence should be sufficient to encourage us to apply the precautionary principle and thus ban or substitute those chemicals that are likely to be harmful to the normal development of humans and wildlife.

One impediment to achieving the aforementioned goal is the general belief that environmental endocrine disruptors, particularly those that are steroid hormone agonists and antagonists, are less potent than their natural counterparts, that is, endogenous sex steroids, and therefore do not have the capacity to cause health-threatening effects. However, it has been shown convincingly that endocrine disrupting chemicals, acting through the same receptor pathway as endogenous sex steroids, act additively. For example, xenoestrogens that have been deemed of low potency relative to estradiol are able to shift the dose–response curve for estradiol to the left. Moreover, endogenous hormones, and their environmental mimics, show non-monotonic dose–response curves resulting in different effects at low and high doses. Finally, it is irrefutable that the susceptibility and sensitivity of the organism varies depending on the developmental stage at the time of exposure. While environmentally-relevant doses of xenoestrogens are insufficient to evoke an uterotrophic effect in prepubescent animals, they are certainly capable of inducing dramatic morphological, biochemical, physiological and behavioral changes in laboratory animals exposed in utero or in ovo.

The contamination of our environment with endocrine disrupting chemicals is providing evidence that mammalian development is far more plastic than previously thought, and that hormones such as maternal estrogens, previously considered irrelevant to early mammalian development, produce subtle but measurable effects in the behavior of these animals. The findings that exposure to BPA during morphogenesis of estrogen-target tissues and organs results in changes that become fully manifested during adult life have provided important information towards our understanding of how BPA (and potentially other estrogen mimics) exposure effects male and female reproduction in mammals. This ongoing research has practical implications in the realization that wildlife and humans are being affected by environmental exposure to hormonally active chemicals at levels previously considered to be irrelevant. From a theoretical perspective, we are learning to reconsider the prevalent view that development is simply an unfolding of a genetically determined program, and therefore to incorporate an understanding of the ecological regulation of development (phenotype plasticity). With these new insights, the emerging field of environmental endocrine disruption will

provide an understanding of the mechanisms that underlie the development of hormone-target organs.

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