

Research and Perspectives
in Endocrine Interactions

J.-P. Bourguignon B. Jégou
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Multi-System Endocrine Disruption

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Research and Perspectives in Endocrine Interactions

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Multi-System Endocrine Disruption

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Preface

In the field of endocrine disruption, the reproductive system has been a priority concern of scientists and environment/public health agencies for several decades, based on observations of fertility impairment in wildlife as well as in humans. In women, intrauterine exposure to diethylstilbestrol (DES) raised the issue of carcinogenicity as early as the 1970s. Although this synthetic compound has been banned for many years, there are still unclear matters of concern such as transgenerational epigenetic effects that provide rationale for monitoring the third generation descending from exposed pregnant women. Another lesson drawn from decades of research is the widened scope of the endpoints of repro-endocrine disruption. In the male, beyond germ cell line differentiation and semen quality, other targets involve testicular descent, epididymis, prostate and external genitalia. The list of potentially disrupting chemicals has also increased dramatically: not only are pesticides and insecticides – including chlordecone and polychlorobiphenyls (PCBs) – involved but also new classes of compounds are emerging, such as analgesics. In addition, chemicals linked to plastic manufacture, like phthalates, have been studied, resulting in convincing evidence of a causal role in the rodent model of testicular dysgenesis syndrome and increasing evidence of association with the occurrence of genital malformations in human males. Although phthalates are banned from some toys and cosmetics, they are still ubiquitous. Among pending questions, the evidence linking early exposure to phthalates and oligospermia/testicular cancer in human males deserves further study. Because phthalates are present in parenteral nutrition or perfusion material, the consequences of prolonged exposure in the critical sensitive period of neonatal life need to be addressed.

More recently, the hypothalamus and the brain have been shown to be possible targets of endocrine disrupting chemicals (EDCs), accounting for neuro-endocrine disruption. In the hypothalamus, the gonadotrophin releasing hormone neurons and afferent neuro-glial system are sensitive to EDCs, leading to disorders of sexual differentiation and maturation. Aromatase and kisspeptin-neurokinin B are likely key mediators of EDC effects. Other endpoints in the CNS possibly include neurogenesis, migration and synaptogenesis in brain cortex and hippocampus. Here, the thyroid hormone system is pivotal, due to its physiological role in early

CNS development. Although they have been banned, PCBs are still among the EDCs involved, due to their persistence in the environment. A still open question is whether stresses other than chemicals (e.g., psychosocial, nutritional, etc.) interact with the sensitivity to deleterious effects of EDCs. For example, does iodine deficiency or exposure to nitrates sensitize the brain to PCB effects?

Another emerging area of endocrine disruption is the central and peripheral control of energy balance. Among other findings, the occurrence of obesity and insulin resistance in adulthood after neonatal exposure to the potent synthetic estrogen DES has opened a new field, possibly substantiating a role for EDCs, including Bisphenol A (BPA), in the epidemics of obesity and type 2 diabetes. BPA accounts for particular epidemiologic and scientific challenges due to its ubiquity and non-linear dose-response curve. The metabo-endocrine endpoints involve adipocytes, pancreatic Beta cells and the intestinal epithelium, where cell proliferation as well as differentiation could be affected by EDCs.

These three areas – repro-endocrine, neuro-endocrine and metabo-endocrine disruption – share common features: ontogenetic disturbances result from particular fetal sensitivity to endocrine disruption with sexually dimorphic responses. Epigenetic mechanisms are likely pivotal.

It has been our privilege, thanks to the Fondation Ipsen, to convene in Paris (May 9, 2011) experts to exchange findings and opinions in the different areas of endocrine disruption summarized above. Questions raised about one system may find answers based on findings in another system, justifying a multi-system perspective in endocrine disruption that appears to be a whole-body burden and a challenge for the whole scientific community, including epidemiologists, toxicologists, geneticists and endocrinologists among others.

The editors wish to express their gratitude to Mrs Astrid de Gérard for the organization of the meeting and Mrs Mary Lynn Gage for her editorial assistance.

Jean-Pierre Bourguignon

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Neuroendocrine Effects of Developmental PCB Exposure, with Particular Reference to Hypothalamic Gene Expression

Rebecca M. Steinberg, Deena M. Walker, Thomas Juenger,
and Andrea C. Gore

Abstract The production and commercial use of novel man-made chemicals over the past century have introduced a variety of industrial compounds into the environment, including polychlorinated biphenyls (PCBs). PCBs alter a multitude of physiological processes in humans and wildlife, and our laboratory and others have been studying the endocrine-disrupting mechanisms by which PCBs specifically affect the hypothalamic control of reproduction. This article will review the literature on PCB effects on reproductive neuroendocrine systems, focusing on effects of exposure during fetal development, a life stage that is particularly susceptible to endocrine disruption. We also provide the example of how the use of a whole genome microarray (Affymetrix rat 2.0) to assay gene expression in the preoptic area (POA; a part of the hypothalamus involved in the control of reproductive physiology and behavior) of female rats fetally exposed to PCBs enabled us to determine whether there was wholesale reprogramming of genes in a manner maintained in adulthood. We also use this method to interrogate the pathways by which PCBs exert these effects, and to ascertain any commonalities of potential dose–response relationships, as endocrine-disrupting chemicals (EDCs) often exert non-traditional, non-monotonic effects on physiological systems. The results show that there are indeed a large number of POA genes and pathways perturbed by prenatal PCB exposure. Although some predicted estrogenic pathways were identified by this method, we also identified other hormonal, neurotransmitter, immune, blood, transcriptional, and intracellular signaling pathways that were affected by the prenatal PCBs. Furthermore, this analysis enabled us to show that the dose–response relationships between exposure and gene expression were almost

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entirely non-monotonic, with U-shaped and inverted U-shaped the most common. The example of PCBs as neuroendocrine disruptors may predict the effects of many other compounds, including anthropogenic chemicals used today such as bisphenol A and phthalates.

Introduction to PCBs and their Endocrine-Disrupting Effects

Polychlorinated biphenyls (PCBs) were in common use worldwide in the mid-twentieth century in industrial manufacturing processes. Their production was halted in the United States in 1978 in response to growing public recognition of their toxic effects on the health of humans and wildlife. However, PCBs are still detected worldwide in soil, air, water and the biomass, due to their dispersal, persistence, and the lack of biological mechanisms to degrade, detoxify or eliminate PCBs from the body burden. Since the time of the initial discoveries of PCBs' overt toxicity, it has become clear that lower-dose, sub-toxic exposures to PCBs can cause endocrine-disrupting effects on reproductive, thyroid, and other endocrine systems (Dickerson and Gore 2007).

Developmental exposures to PCBs, particularly to the fetus or infant, are particularly detrimental due to the vulnerability of rapidly growing and dividing cells. This concept of sensitive or critical developmental periods (Barker et al. 2002; Barker 2003) takes into consideration that the fetus/infant is exposed to the external environment via placental or lactational transfer of natural and man-made compounds from the mother's body. Although potentially subtoxic, the cumulative consequence of these exposures on the fetus, together with the exposed individual's own genetic traits, may predispose that individual to disease later in life. The best example of the "fetal basis of adult disease" in humans come from the study of diethylstilbestrol (DES), a pharmaceutical estrogen given to pregnant women to reduce the risk of miscarriage (Herbst et al. 1971). It was noted that there was increased incidence of adenocarcinoma of the vagina in young women, a population that does not normally have this type of cancer, and it was determined retrospectively that these women had been fetally exposed to DES taken by their pregnant mothers. Thus, a direct link between fetal exposure and adult disease was drawn for the first time in humans. Furthermore, a similar phenotype could be produced in animal models of perinatal DES exposure (McLachlan et al. 1982), a finding that was important both because it enabled mechanistic studies of how fetal DES exposure caused this latent disease and because it showed the conservation of endocrine disrupted traits between animal models and humans.

A growing body of literature now shows that low-dose developmental EDC exposures, including PCBs, may permanently reprogram endocrine and reproductive dysfunctions in adulthood (Dumesic et al. 2007; Steinberg et al. 2007, 2008; Diamanti-Kandarakis et al. 2009). As used here, "reprogramming" refers to how environmental changes early in life may change the capacity of a gene or protein to be expressed later in life, even in the absence of further exposure to the causal

environmental agent. In the case of PCBs, transient fetal or early postnatal exposures in animal models have been linked to reproductive and thyroid disorders in adulthood (reviewed in Dickerson and Gore 2007). In humans, the cause-and-effect relationship between PCBs and disease is less clear, except for cases of toxic poisoning, such as contaminated cooking oil in Japan and Taiwan (Seegal 1996). Epidemiological studies on humans show correlations, albeit not necessarily direct, between PCB body burden and lower IQ, cognitive dysfunctions, other neurobehavioral abnormalities and, more recently, cardiovascular disease (Winneke et al. 1998; Lang et al. 2008). Although the links between developmental exposure and later life dysfunctions are more difficult to prove, there is no doubt that PCBs continue to serve as a valuable model for understanding the effects of both historical (DES) and modern putative EDCs, such as bisphenol A, phthalates, vinclozolin, and many others, due to the similarity of the mechanisms by which EDCs act.

PCB Actions on Neuroendocrine Systems

Much of the published literature on EDCs has focused on their detrimental effects on reproductive systems. It is important to point out that the control of reproduction goes far beyond the gonads and the reproductive tract, the target of most studies in the past. As is the case for several endocrine systems, reproductive function is much more complex: it involves a series of organs and hormones including the brain, and more specifically the hypothalamus at its base, which produces the neuropeptide gonadotropin-releasing hormone (GnRH); the anterior pituitary, and those cells (gonadotropes) that synthesize and release the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH); and the gonads (testis/ovary), which not only produce germ cells (sperm/ova) but also steroid hormones, such as estrogens, androgens, and progestins, and protein hormones, such as inhibin (reviewed in Gore and Crews 2009; Gore 2010). Of paramount importance is that these hormones regulate one another by feed-forward or feedback mechanisms. GnRH release from the hypothalamus stimulates pituitary LH and FSH release, which in turn stimulate gonadal gametogenesis and steroidogenesis. Sex steroid hormones in the circulation feed back to the hypothalamus and pituitary to regulate GnRH and gonadotropin levels in response to the current need of the organism. This concept is crucial to our understanding of EDC effects, because EDCs can act upon the same hormone receptors, enzyme systems, and other targets in the hypothalamus-pituitary that our endogenous hormones utilize. The remainder of this article will discuss evidence for neuroendocrine disruption by PCBs and will focus on the hypothalamic genes that may be reprogrammed by fetal PCB exposures.

Mechanisms and Consequences of Developmental PCB Action in the Hypothalamus

PCBs are grouped into three main categories: coplanar, dioxin-like coplanar, and non-coplanar, with differential actions on various receptor systems (Dickerson et al. 2009). Most research on endocrine disruption has focused on the estrogenic properties of PCBs and their subsequent actions on estrogen receptors in peripheral reproductive tissues (reviewed in Dickerson and Gore 2007). Furthermore, PCBs can act directly upon neuroendocrine tissues, including hypothalamic neurons and cell lines, in which PCBs alter gene and protein expression, including estrogen receptors and the GnRH peptide (Gore et al. 2002; Salama et al. 2003; Dickerson et al. 2009). The cellular/molecular actions of developmental PCBs in vivo are reflected as differences in reproductive physiology and behavior in adulthood (Chung and Clemens 1999; Chung et al. 2001; Steinberg et al. 2007, 2008), showing that there is reprogramming of genes/proteins that underlie these biological processes.

Research in our laboratory has primarily focused on estrogenic PCBs (Petit et al. 1997; Shekhar et al. 1997; Layton et al. 2002; Ptak et al. 2005) such as Aroclor 1221 (A1221), a lightly-chlorinated commercial PCB mixture composed mostly of mono-ortho-substituted and co-planar congeners (Frame 1997). A1221 binds to estrogen receptor alpha and beta, albeit at relatively low affinity compared to endogenous estrogens (Shekhar et al. 1997; Layton et al. 2002). In addition, A1221 or its constituent congeners can also suppress the ability of the P450 aromatase enzyme to convert testosterone to estradiol (Woodhouse and Cooke 2004; Ptak et al. 2006), thereby affecting local estrogen levels in a negative manner. However, A1221 is not purely estrogenic: exposure to A1221 can mimic thyrotoxicosis and alter circulating thyroid hormone levels (Kilic et al. 2005). One report showed that A1221 could inhibit effects of dihydrotestosterone (an androgen) in a prostate cell line (Schrader and Cooke 2003). Clearly more information is needed, as A1221 cannot be easily classified as an endocrine disruptor of a single class of hormone receptors.

Here, we will summarize the literature on the effects of developmental A1221 exposures on adult neuroendocrine function, as well as studies in the hypothalamic GnRH GT1-7 cell line. Research in this arena began with the reports from Clemens' group that perinatal exposure to PCBs caused changes in feminine sexual behavior in rats (Chung and Clemens 1999; Chung et al. 2001; Wang et al. 2002). Based on this research, we began studying the effects of low dose exposure to A1221 given to pregnant rats during late gestation, which is part of the critical period of brain sexual differentiation in the developing fetus. This work has been published and reviewed extensively (Gore 2008, 2010), so we will provide a relatively short discussion, to be followed by a more in-depth presentation of new data on the effects of developmental exposure to A1221 on gene expression in the preoptic area of the hypothalamus.

In vitro studies were performed to examine the effects of A1221 on GnRH gene expression and cell death in immortalized GnRH GT1-7 cell cultures (Gore et al. 2002). When A1221 was given in dose–response experiments, GnRH mRNA levels and GnRH peptide levels were elevated, particularly at intermediate dosages (Gore et al. 2002). Some (but not all) of these effects were blocked by the estrogen receptor antagonist ICI 182,780, suggesting that there were estrogen receptor-mediated properties of A1221 on GnRH function but that other non-estrogenic mechanisms are probably also involved. We followed up on this work by interrogating the mechanism by which PCBs might cause toxicity in this neuroendocrine cell line. Although in our second study we did not specifically test A1221, we found that individual PCB congeners stimulated/inhibited GnRH gene expression depending upon dose and duration, with stimulatory effects caused at lower doses/shorter time points and inhibitory effects at the higher doses/longer time points (Dickerson et al. 2009). We also observed some reversibility of these effects by the estrogen receptor antagonist, supporting our work on A1221 described above. Finally, we found that GT1-7 cell death caused by the PCBs comprised a mix of apoptotic and necrotic mechanisms, again dependent upon dose/duration of the PCB treatment (Dickerson et al. 2009). These studies as a whole support the potential direct effects of PCBs on GnRH neurons, at least in vitro.

In vivo studies have focused on the effects of developmental A1221 on adult reproductive physiology, behavior, and protein expression. In a series of studies, we showed a decrease in the number of cells expressing estrogen receptor beta (ER β) protein in a sub-region of the hypothalamus, the anteroventral periventricular nucleus (AVPV), of the rats treated perinatally with A1221 (Salama et al. 2003). We went on to demonstrate that developmental A1221 exposure impaired paced mating behaviors in the female offspring when tested in early adulthood (Steinberg et al. 2007). In that study, female rats were exposed prenatally to vehicle (DMSO) or one of three doses (0.1, 1, 10 mg/kg) of A1221 on embryonic days 16 and 18, part of the “critical period” of brain sexual differentiation. The paced mating paradigm was used because it enables a focus on female-typical behaviors, as the female rat (not the male) controls the pace of mating, and the female can choose whether (or not) to mate and can also control the timing of her contact with the male. Although not all aspects of mating behaviors were affected, we found the following significant, dose-dependent changes in rats exposed prenatal A1221: the amount of time a female rat took to return to a male rat after he had ejaculated was increased (at the 1 mg/kg dose); an increased number of mating trials were required for the female to mate (at the 1 and 10 mg/kg doses); and a decrease in audible vocalizations (1 mg/kg) were detected (Steinberg et al. 2007). We found it particularly interesting that the 1 mg/kg (intermediate) dosage had the greatest effect upon the behavioral phenotype, whereas the low (0.1 mg/kg) and the high (10 mg/kg) dosages were less consistently effective. This finding will be brought to bear in our gene expression studies discussed later in this article and is relevant to the concept that both endogenous hormones and xenobiotics can exert non-monotonic dose–response effects.

The female siblings of the rats used in the behavioral tests were subjected to other analyses of reproductive physiology, both in the fetally exposed (F1