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Introduction: neurobiological impact of environmental estrogens

G.C. Panzica [a,*], C. Viglietti-Panzica [a], M.A. Ottinger [b]

[a] *Laboratory of Neuroendocrinology, Rita Levi Montalcini Center for Brain Repair, Department of Anatomy, Pharmacology, and Forensic Medicine, University of Torino, c.so M. D'Azeglio 52, I-10126 Torino, Italy*

[b] *Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742, USA*

Abstract

This paper provides an introduction to a special issue dedicated to the action of environmental estrogens on neural circuits and behavior. The problem of endocrine disrupting chemicals (EDCs), i.e. chemicals that have the capacity to interfere with the endocrine system, has gained increasing attention as it has become clear that these environmental contaminants may be active in humans, as well as in wildlife and domestic animal species. The majority of the early investigations were aimed at the discovery of the toxicological effects of the EDCs, but biomedical observations were among some of the first indications that estrogenic compounds may exert deleterious effects, even some time after exposure. The data derived from women exposed prenatally to diethylstilbesterol provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds. The examination of wild animal populations exposed to industrial chemicals showed that the chemical exposure, though nonlethal, left the individual impaired or even incapable of reproducing.

Among the multiple targets of the action of EDCs, several researches performed in recent years have investigated subtle modifications of the animal behaviors (reproductive, aggressive) that are likely to be related to alterations of specific neural pathways. We have, therefore, focused here on the behavioral studies as one of the more powerful tools to investigate EDCs effects on specific neural circuits.

Keywords: Endocrine disruptors; Industrial chemicals; Xenoestrogens; Central nervous system; Brain circuitries; Behavior

Endocrine disrupting chemicals (EDCs) are compounds that are biologically active and often mimic endogenous hormones, thereby altering hormone modulated responses. EDCs have been shown to disrupt embryonic development, sexual differentiation, reproduction, immune function, behavior, and responses mediated by hormones. The issue of EDCs has gained increasing attention as it has become clear that these environmental contaminants have endocrine activity in humans, as well as in wildlife and domestic animal species. This is a complex issue due to the huge range of chemicals that are suspect and to the variability in the biological target systems affected by these chemicals. Furthermore, there is clearly differential sensitivity to specific EDCs across phyla and even between species in the same phylum.

Due to the high number of these chemicals found at present in the environment, it is important to distinguish the different categories of EDCs, in relation to both their chemical characteristics and the nature of the biological activity identified for that given substance. The reality is that most animal species, humans and other animals alike, are exposed to and accumulate mixtures of these environmental chemicals that then have the capacity to be endocrine active. Furthermore, some of these chemicals, most notably the plant phytoestrogens, may play an important role in the reproductive cycles of small rodents as well as have positive (or negative) effects in other animals including humans.

This is, therefore, an important issue with wide ranging implications. It is critical to understand the consequences of exposure and the parameters that exacerbate deleterious or favorable effects of an EDC. It was the purpose of this special issue of *Brain Research Bulletin* to provide an overview of the effects of EDCs over a range of species and to examine

* Corresponding author. Tel.: +39 011 670 7970; fax: +39 011 670 7732.
E-mail address: giancarlo.panzica@unito.it (G.C. Panzica).

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the range of effects of these environmental chemicals that have estrogenic activity.

1. EDCs: estrogenic compounds and activity on specific target organs or systems

A range of EDC effects has now been documented in a number of animal species, both in laboratory studies and in wild populations [2,6–8,11]. Biomedical observations were among some of the first indications that estrogenic compounds may exert deleterious effects, even some time after exposure. The data derived from women exposed prenatally to diethylstilbesterol [18,29] provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds [19,27]. There was mounting evidence from wild animal populations exposed to industrial chemicals. These data repeatedly showed that the chemical exposure, though nonlethal, left the individual impaired or even incapable of reproducing [15]. As more data became available, it became apparent that a number of chemicals, including industrial products, pesticides, herbicides, and even plant-derived chemicals were endocrine active in a variety of biological systems [14,19,27].

Many of the compounds have been characterized as estrogenic, in that they interact with the estrogen receptor and modulate estrogen-responsive systems [23]. In spite of the majority of environmental chemicals having apparent estrogenic activity, a number of EDCs also have an androgenic or antiandrogenic action, often affecting prostate and other androgen-responsive tissues [2,27,36]. In fact, reported diminished semen quality was an early stimulus for subsequent research [5]. Unfortunately, structure–function relationships are not always predictive of activity and a number of studies have attempted to develop screening techniques [2]. Some of the most effective methods have involved in vitro systems using steroid hormone receptors, and these techniques have contributed a great deal to our understanding of the action of these compounds. Finally, it has been well documented that wildlife generally encounters a mixture of environmental chemicals [11,26]. This is further complicated by other contaminants also present in the environment, including metals [13]. Research is becoming available on these interactions and this potential exacerbation of EDC effects must be considered when determining risk to wild animal populations.

2. Variation in phylogenetic and species-specific sensitivity to EDCs

A great deal of species variation has been documented relative to EDC effects. Some of the variations are due to mechanisms operating during sexual differentiation, exposure venues (e.g. aquatic versus terrestrial), and lifetime reproductive strategies [27]. There have been reports that phytoestrogens may modulate seasonal reproductive cycles in mammals [34]. Therefore, some of the EDCs could facilitate endocrine-related responses. This is in sharp contrast to industrial compounds, pesticides, herbicides, and other environmental contaminants, which appear to have predominantly deleterious effects [7].

Furthermore, it is important to develop animal models appropriate for testing paradigms to ascertain the potential for EDC impact. Single and multigenerational testing paradigms have been developed for assessing impact of selected EDCs in a number of animal species [2,14,17,26]. In birds, the quail provides a model in which the egg can be used for bioassay [3]. These tests take advantage of steroid-responsive morphological measures as well as endocrine, neuroendocrine, and behavioral responses that are sexually differentiated. Such tests are critical for ecological risk assessment for wildlife and for determining acceptable levels of these chemicals in agricultural and other applications.

3. Implications of EDC exposure for animal populations

As mentioned earlier, it is generally the case that wild populations are exposed to a mixture of chemicals as well as other contaminants in their environment. Although field relevant concentrations are generally low, with the exception of industrial or chemical spills, the question of synergistic, additive, or multiplicative actions of these chemicals remains to be elucidated. Research indications are that these environmental chemicals are at least additive in their effects, resulting in impaired reproductive success and morphological abnormalities [9,11,12]. Epidemiology studies are also providing information relative to the association of these environmental events and subsequent impact on wildlife. Databases are collecting the information in a coordinated and understandable form so that it is possible to observe trends and associations in reports at the individual and population levels with chemical analyses in selected regions [32].

The potential consequences of EDC exposure are a critical issue for wild populations. As such, EDCs have emerged as an important problem due to short- and long-term consequences for the individual and potential impact at the population level. Unfortunately, the understanding and assessment of the biological actions of EDCs at the population level has proven difficult for several reasons. First, it is extremely difficult to assess the effects of a myriad of chemicals, especially mixtures of these chemicals that impinge on the animals in the environment. Second, there are many possible mechanisms of action of EDCs leading to biological effects. Third, the timing of exposure to EDCs is a critical factor, as a consequence the effects of a particular EDC will vary over the life cycle of the animal as well as across species and phyla. Often, embryonic exposure to estrogenic EDCs will have life long effects due to action of the estrogenic compounds on sexual differentiation of brain structures. Some compounds target neuroendocrine systems, thereby affecting reproductive

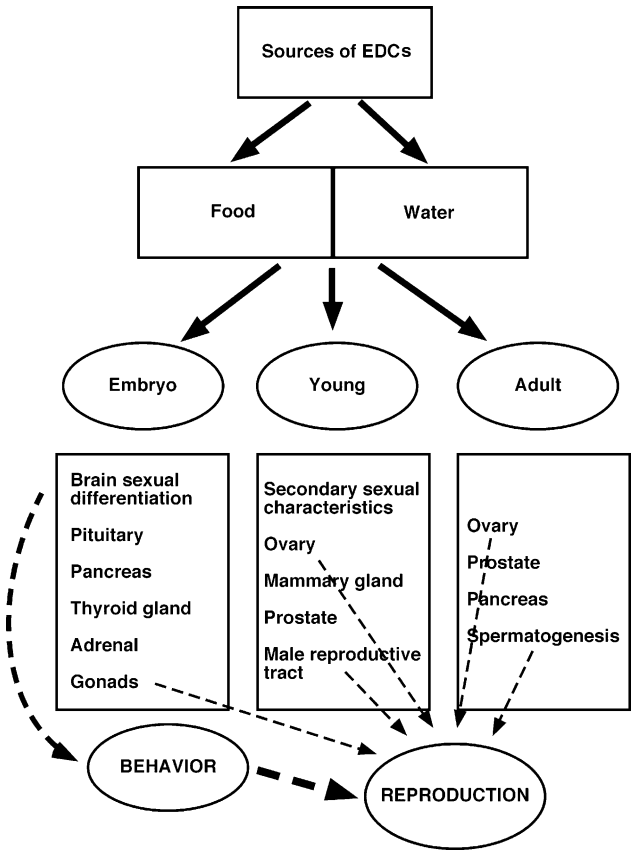

Fig. 1. Diagrammatic summary of effects of environmental estrogens during embryonic, postnatal, and adult life. Actions on estrogen-sensitive neural circuits differentiation during embryonic or early postnatal life may heavily impact adult behavioral activities, that, in turn, control reproduction. During the postnatal and adult life, environmental estrogens act primarily at the level of peripheral organs (gonads, reproductive tracts), interfering with circulating hormonal levels and therefore with the reproduction.

endocrine systems as well as other endocrine systems. Therefore, exposure to the estrogenic chemicals during embryonic development has consequences beyond impaired function of the reproductive axis. This makes the evaluation of short- and long-term effects of EDCs extremely complex to assess.

The disturbance of hormonal systems by EDCs with estrogenic action, particularly during the sensitive periods of organogenesis and sexual differentiation of the brain, can alter the functionality of the reproductive organs and the neurochemistry and organization of the cerebral circuits, and thus, the behavioral responses of the individuals exposed to these substances (Fig. 1). Several researches performed in recent years have investigated subtle modifications of the animal behaviors (reproductive, aggressive) induced by EDCs [38] that are probably related to alterations of specific neural pathways (see [30] for further discussion).

One of the aims of articles collected in this special issue was, therefore, to focus on the behavioral studies as one of the more powerful tools to investigate EDCs effects. Papers within this issue encompass a number of the topics that have emerged as important to our understanding of the actions and consequences of exposure to endocrine-disrupting chemicals.

An interesting area of research is represented by studies on phytoestrogens produced by some plants. The soy isoflavones have gained increasing attention due to the potency of genistein and the other phytoestrogens in soy extracts. The effects of the soy isoflavones at various stages of the life cycle is important because there has been a sharp increase of soy in the diet, as it is a major component of the vegetarian diet and it is a primary protein source in some infant formulas. Furthermore, the concern regarding hormone replacement therapy in women after menopause [37] has stimulated increased use of phytoestrogens for health benefits [20,25]. Research findings and an overview of the field area are presented in two papers by Lephart and co-workers [4,22] that focus on the effects on hypothalamic systems and biological responses, providing a timely review into the mechanisms underlying the physiological and behavioral effects of the soy isoflavones.

The impact of EDCs on mammals has been extensively investigated, especially relative to response by specific target tissues. The liver is often a target for these compounds due to the function of the organ in cleansing the body of contaminants. In the paper by Mussi et al. [24], the authors demonstrate that the mechanism of DDT action on the liver occurs via interaction with the estrogen receptor. Furthermore, their studies show that activation of the estrogen receptor first requires metabolism of the EDC. The timing of exposure to EDCs becomes critical in terms of steroid-dependent behaviors and in the sexual differentiation of endocrine and behavioral components of reproduction. In particular, bisphenol-A was found to diminish conditioned place preferences in female mice prenatally exposed to this EDC or to methoxychlor [21] or impair maternal behavior in female rats when administered during pregnancy or early lactation [10]. Porrini et al. [31] provide further evidence for long-term effects of early administration of bisphenol-A on the socio-sexual behavior of rats. Finally, Razzoli et al. [33] further support the behavioral impairment associated with bisphenol-A exposure and show that this effect occurs also in other mammalian species as the Mongolian gerbils.

The issue of comparative impact of EDCs across phyla is addressed in papers on fish and birds. Alò et al. [1] demonstrate the effects of bisphenol-A in the fish with impact on specific somatostatin receptor subtypes in the diencephalon. Several papers on the Japanese quail suggest the importance of this avian model to study EDCs effects. The paper by Halldin et al. [16] addresses the impact of selected EDCs on reproductive endocrine characteristics that are sexually dimorphic in the adult. Because the quail is a precocial species, reproductive endocrine and behavioral responses are sexually differentiated under the influence of steroid hormones in the last period of embryonic development. Similarly, the paper by Viglietti-Panzica et al. [35] is focused on the effects of diethylstilbestrol on a sexually dimorphic neural circuit (the vasotocin-producing elements of the bed nucleus of the stria terminalis) implicated in the control of male sexual behavior.

It is important to determine if there is a dose-response relationship between EDC and the impairment to the variable under study. In this way, reliable measurement end-points can be validated for detection of EDC activity. The paper by Ottinger et al. [28] provides an overview of some of the issues to be considered in testing birds as well as discusses the multigenerational paradigm and likely measurement endpoints that will be useful for regulatory testing applications.

As discussed above, these contributions provide important information on the action of single EDCs, as well as insights into the neural mechanisms by which these EDCs exert their effects.

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