Endocrine System and Endocrine Disrupting Chemicals (EDCs)

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Abstract: Recently, much has been focused on the potential for a wide range of chemicals to interact with the disruption of the endocrine system in humans and animals. Some of those chemicals have been implicated as endocrine disrupting chemicals (EDCs). We represent this review that the current endocrine systems including of molecular biology and effects of EDCs are discussed, as examples pituitary and mammary gland. Alterations to the endocrine environment caused by EDCs may occur through interference with the synthesis, secretion, transport, binding, action, or elimination of natural hormones responsible for vital homeostasis, differentiation, development, reproduction, and/or behavior. It is important consideration for assessing the adverse effects of EDCs to have an understanding of normal endocrine systems, subsequently, dose, duration of exposure, age, sex, situation, and frequency are estimated. (J Toxicol Pathol 2001; 14: 59–64)

Key words: endocrine disrupting chemicals, pituitary, mammary gland, molecular biology, hormonal environment

Introduction

Endocrine disrupting chemicals (EDCs) have been the issues of the interest in not only the scientific aspect but also the general community. Recent reports have suggested that background levels of EDCs including agricultural products, industrial chemicals, and other environmental pollutants, may contribute to an increase in the incidence of cancer in mammary gland, testis and prostate, a decrease in sperm counts, and developmental abnormality in humans^{1–4}. In addition, a decrease in reproductive success and developmental defects also have been reported in wildlife^{1,2,5}. The hypothesis that environmental estrogen, such as potent estrogenic action, contributes to these changes has gained considerable attention with the report of wolff *et al.*⁶.

Hormones act via classical endocrine pathways: they are secreted by glands, transported by the circulatory system, and act on the target cells at several peripheral sites via specific receptors located on the plasma membrane. The regulation of hormonal secretion is complex and involves modulators that exert (stimulatory) or negative (inhibitory) feedback control^{7,8}. In addition, many hormone receptors including subtypes or isoforms have been also identified. Thus, we deal with changes in the endocrine system, not like

the other organ systems, it should be re-emphasized that the endocrine system is highly interacting and this is summarized as hypothalamus-pituitary-gonad axis^{7,8}.

While, the development of current knowledge of the cellular and molecular biology is rapidly progression, especially the nuclear receptors including the estrogen receptor (ER) are ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction^{9–14}. Their primary function is to mediate the transcriptional response in target cells to hormones such as the sex steroids, adrenal steroids, vitamin D₃, and thyroid hormones, in addition to a variety of metabolic ligands^{11,12}. The estrogenic actions of EDCs are mediated through binding specifically to nuclear ER, ligand-activated regulatory proteins that act as dimmers on specific target genes containing defined DNA sequences called estrogen response elements (ERE)^{11,12,14}. In the pituitary gland of most species described, lactotrophs and gonadotrophs exhibit the highest level of ER α with lower and more varied levels of localization to the other cell types^{11,15,16}. The upstream regulatory sequences of the rat prolactin (PRL) gene have been found to process an ERE that binds ER α and functions synergistically with the pituitary-specific factor (Pit-1), to promote expression^{11,16–20}. In addition, even in the same peripheral tissues, paracrine and autocrine systems have been identified²⁰.

Now, novel experimental approaches to the study of selected EDCs both *in vitro* and *in vivo* have been established according to priority^{21–23}. The risk assessment of EDCs has been introduced in many other articles.

This review deals with the endocrine system, especially

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Table 1. Effect of Endocrine Disrupting Chemicals

Actions	Chemicals
Estrogenic action	DDT and metabolites (DDE), Chlordane, Endosulfan, Bisphenol-A, Nonylphenol, Alkyl-phenol, Benzylbutylphthalate, Phytoestrogens (Isoflavonoid, Lignans) <i>etc.</i>
Anti-androgenic action	DDE, Vinclozolin, Flutamide, Permethrin <i>etc.</i>
Anti-thyroid hormone action	Polychlorinated biphenyls (PCBs), TCDD <i>etc.</i>

the pituitary and mammary gland, with special emphasis on the pathol-physiologic reaction to various EDCs. We suggest that the complex hormonal environment should be taken into account in experimental studies, such as effect for other hormones and/or factors to significant process of physiological phenomena.

Endocrine Disrupting Chemicals (EDCs)

The EDCs have a broad range of effects including: 1) estrogenic action, 2) anti-androgenic action, and 3) anti-thyroid hormone action *etc.* (Table 1)^{1, 4, 5, 24}. The estrogenic effect is the most common, and not easily distinguish from the activity of endogenous estrogen in adult human and animals²⁵. Consequently, the estrogenic action of EDCs tends to hide inclusive physiological adaptation as vital homeostasis. Recent reported that exogenous chemicals may bind to a receptor, for example the estrogen receptor, and mimic or block the actions of its natural hormone²⁵.

The adverse effects of estrogen in laboratory animals are summarized in Table 2²⁶. From the table, it is clear that estrogen is associated with adverse effects in the genital tract (testis, prostate, seminal vesicle, uterus, ovary, and mammary gland). In fact, the effects on the female and male genital systems and mammary gland have been investigated extensively. It is also apparent that exogenous estrogens influence not only endogenous estrogen but also other hormones in the rat, especially PRL, and seem to be acting in synergy. Therefore, toxicological and biological scientists would need to judge for change of single or synergy action using rat experiments.

Pituitary Gland and Transcription Factor

It is known that tumors in the human anterior pituitary gland are classified into those of growth hormone (GH)-, PRL-, adrenocorticotrophic hormone (ACTH)-, thyroid stimulating hormone (TSH)-, and gonadotrophin (LH, FSH)-secreting adenomas as well as non-functioning adenomas. Experimental models of pituitary adenomas have included estrogen-induced PRL-secreting adenomas and thyroidectomy-induced TSH-secreting adenomas. In addition, genetic engineering has produced functional pituitary adenomas including GH-secreting tumors in human growth hormone-releasing hormone (GHRH) transgenic mice^{27, 28}. The pituitary tumors in these

Table 2. Overview of the Effect of Estrogen on Various Organs

Tissues/Organs	Findings
Pituitary	Hyperplasia of PRL-secreting cells
Blood	Hyperprolactinemia
Testis	Hypospermatogenesis Leydig cell hyperplasia
Prostate, Seminal vesicle	Squamous metaplasia
Ovary	Atrophy
Uterus	Endometrial hyperplasia
Mammary gland	Development
Adrenals	Cortical cell hyperplasia
Bone	Osteosclerosis

transgenic animals are positive for GH, GHRH, PRL, TSH, and also GHRH-receptor. Tumorigenic effects of GHRH through an autocrine and/or paracrine mechanism have been proposed^{27, 28}.

Various transcription factors for hormone production have been identified, and synergy between transcription factors and other receptors was reported¹¹⁻¹⁴. One example is pituitary specific transcription factor (Pit-1), which belongs to the POU-domain family, Pit-1 proteins comprise 291 amino acids. Most nuclear binding proteins are 31 and 33 KDa in size. Pit-1 was found in the nucleus of rat pituitary cells and shown to enhance the transcription of GH, PRL, and TSH β ²⁹⁻³¹. In rodents, Pit-1 mRNA was detected in all types of pituitary cells. However, Pit-1 protein was limited to three cell types; somatotrophs, lactotrophs, and thyrotrophs³². Similarly, in humans, point mutations in the Pit-1 gene in patients deficient in GH-PRL-TSH have been reported³³. The co-expression of Pit-1 mRNA with GH, PRL, and TSH β in pituitary adenomas was detected by Sanno *et al.* using a combination of *in situ* hybridization and immunohistochemistry³⁴. Therefore, it is suggested that the functional expression of these hormones in the adenoma cells resulted from the DNA binding of Pit-1 protein.

Synergistic factors for Pit-1 in the functional differentiation of pituitary cell types include GHRH receptor (GHRHR), estrogen receptor (ER), thyroxine receptor (TR), retinoic acid receptor (RAR), and retinoid X receptor (RXR) (Fig. 1). In PRL-secreting adenomas, Pit-1 mRNA and ER nuclear protein coexist in the same tumor cells which suggests action of Pit-1 and ER¹⁹ (Fig. 2). As mentioned above, other hormone receptors coexist in the same tumor cells, in GH-secreting adenomas and TSH β -secreting

Fig. 1. The synergy of Pit-1 and other factors in GH, PRL, and TSH β expression. GHRHR; Growth hormone releasing hormone receptor, TR; Thyroxine receptor, RAR; Retinoic acid receptor, ER; Estrogen receptor, RXR; Retinoid X receptor.

Fig. 2. Double staining shows the presence of Pit-1 mRNA (cytoplasm-blue) and ER (nuclei-brown) in the same cells of PRL-secreting adenoma.

adenomas, respectively. Therefore, the synergy of Pit-1 and ER promote transcription into PRL, that of Pit-1 and GHRHR, TR and RAR promotes transcription into GH, and that of Pit-1 and RXR promotes transcription into TSH β in human pituitary adenomas, respectively^{16–20} (Fig. 1). However, it has not been elucidated whether EDCs interact with transcription factors or not. Transcription factors may be also important to the endocrine environment in pituitary gland for preventing exogenous estrogenic effects.

Caution in the Assessment of Rat Mammary Gland

The development of mammary gland may be divided into five distinct stages; embryonic and fetal, prepubertal, pubertal, sexually mature adult, and pregnancy or lactation³⁵. Estrogen action does not appear to be essential in the embryonic and fetal stage of female mammary gland³⁵. However, aberrant exposure of the neonatal female mouse to estradiol, testosterone, or PRL has been reported to result in an apparent increased sensitivity of the gland to mammotrophic hormones during adulthood, leading to ductal growth and differentiation^{36, 37}. The last four stages of mammary gland development are strongly regulated by the endogenous ovarian steroid hormones. Upon pregnancy and the onset of lactation, the glands reveal dramatic differentiation with alveoli forming throughout the ductal network to produce milk protein. Thus, the mammary gland provides a unique system within which the interactions of gonadal and peptide hormone systems may be studied.

The rodent mammary gland, however differs from its human counterpart. Rodents have a particularly sensitive positive feedback mechanism between PRL secretion and estrogen (Fig. 3). That is, estrogens classically inhibit the hypothalamic secretion of dopamine, as a result of which PRL release is stimulated. PRL is luteotrophic in rats; therefore, this also leads to an increase in progesterone^{38, 39}. On exposure to chronic estrogen or EDCs, the three hormones, PRL, progesterone, and exogenous estrogen, act in synergy, leading to chronic overstimulation of mammary tissue, and then to mammary tumors^{38, 39}. PRL is not luteotrophic in primates; therefore, this does not occur in woman^{38–40}. However, a prolactin-releasing peptide was recently discovered in rat brain^{41, 42}. In addition, for risk assessment of EDCs using rats, there are several potential parameters, such as serum PRL concentration⁵. PRL is known to function not only in mammary gland development and maintenance in the reproductive state, but also in growth factor, in osmoregulation, or in synergism with steroids^{43, 44}. One should be aware of the synergistic effects of PRL on estrogenic action and/or EDCs risk assessment.

From our results, it is suggested that the regulation of the local expression of extrapituitary PRL and its PRL-receptor in the mammary gland has different mechanisms. As an example, extrapituitary PRL in mammary gland is not constitutively expressed in rats of the reproductive state, but highly expressed in mid-to late pregnancy and throughout lactation⁴⁵. The significance of local extrapituitary PRL in rat mammary gland has not been elucidated, without suggestion of an autocrine/paracrine manner^{43–48}. However, no PRL mRNA in rat mammary gland was detected following exposure to estrogen, in spite that the serum PRL levels and morphological change were similar to those in lactating mammary gland^{49, 50}. In addition, in hypophysectomized lactating rats, PRL mRNA was detected on the predominant involution of the mammary gland, in spite of extremely reduced serum PRL levels (Fig. 4). Thus, local extrapituitary

A. Regulation of prolactin secretion **B. Influence of estrogens on prolactin secretion in rodents**

Fig. 3. Regulation of prolactin secretion in the rat. A. Normal state. B. Functional state after stimulation by estrogens. From modified Neumann F. Mutat Res 1991, Ref.29

Fig. 4. Expression of PRL mRNA in normal and hypophysectomized lactating rats by *in situ* RT-RR. Left: Normal condition. Diffuse expression of PRL mRNA was observed. Right: Hypophysectomized condition. Diffuse expression of PRL mRNA was also observed in the involutive mammary gland.

PRL in rat mammary gland is suggested to be *de novo* synthesis without regulation of serum PRL levels. PRL-receptor, however, especially the long form of mRNA, may affect the serum PRL concentration^{43, 44, 51}. Therefore, the mechanisms at work in a peripheral sites including the mammary gland, ovary and uterus, provide primary insight into the functions of hormonal effects.

Conclusion

Still, the exact role played by the hormone and the hormone receptor complex in the final manifestation of hormonal effects has remained unclear. Therefore, one challenge confronting biologist and toxicologist has been to elucidate the exact and systematic manner in which hormones serve as regulators of biochemical processes in tissues of normal animals. In addition, an increased understanding of the mechanism, direct and/or indirect, of synergistic hormonal effects in animal studies, will continue to provide insight into aspects of significant hormonal function, as well as any possible cooperative roles hormones might play within the same tissue or even within the same cell.

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