Steroid Hormones and Endocrine Disruptors: Recent Advances in Receptor–Mediated Actions

Dono Indarto* and Masao Izawa

Department of Biosignaling, Tottori University Faculty of Medicine, Yonago 683-0826 Japan

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It has been accepted that receptor-mediated action of steroid hormones depends on both the receptor and the hormonal level. The mechanism of transcription by steroid receptors is mediated by cofactors, which function as co-activators or co-repressors, while their non-genomic actions depend on receptors localized to the cell membrane. Recently, a number of environmental chemicals, which are now termed as endocrine disruptors, have been identified, and their unwanted effects on our lives have become serious problems all over the world. Their adverse effects on endocrine systems in animals, mostly estrogenic or anti-estrogenic, have resulted in reproductive malfunction and developmental disorders. Although aryl hydrocarbons exhibit estrogenic or anti-estrogenic activity through specific interaction with aryl hydrocarbon receptors, other chemicals seem to interact directly with estrogen receptors, α and β forms. In this paper, we surveyed the most recent understanding of endocrine disruptors from the viewpoint of steroid receptor systems. We suggest two potential mechanisms of action for endocrine disruptors. Endocrine distruptors i) directly associate with steroid receptor systems and/or ii) associate with the growth factor or the neurotransmitter receptor systems, and then upregulate the mitogen-activated protein kinase signaling cascades, leading to the ligand-independent activation of steroid receptor systems. Using these steroid receptor-dependent mechanisms, it appears that endocrine disruptors disorder our endocrine systems. We have proposed future suggestions to further understand endocrine disruptors from the viewpoint of steroid receptor systems.

Key words: endocrine disruptors; receptor-mediated actions; steroid hormones

Since the cloning of human glucocorticoid receptor (Hollenberg et al., 1985), a number of nuclear receptors including steroid receptors have been identified (Mangelsdorf et al., 1995; Pfaff et al., 1997), and are now understood as transcription factors included in a nuclear receptor superfamily (Freedman, 1999). Recently, estrogen receptors (ERs) have been shown to localize to the cell membrane, and their role in non-genomic actions have been demonstrated as well (Chen et al., 1999; Goetz et al., 1999).

A number of environmental chemicals have been identified as endocrine disruptors, and their unwanted effects on our lives have become serious problems all over the world. At present, their adverse effects on endocrine systems in animals, mostly estrogenic or anti-estrogenic, have resulted in reproductive malfunction and developmental disorders (Keith, 1997). Among endocrine disruptors identified so far, aryl hydrocarbons, dioxins, exhibit estrogenic or anti-estrogenic activity through specific inter-

Abbreviations: AhR, aryl hydrocarbon receptor; AR, androgen receptor; ARE, androgen responsive element; DES, diethylstilbeterol; EGF, epidermal growth factor; ER, estrogen receptor; ERE, estrogen responsive element; IGF, insulin-like growth factor; LBD, ligand binding domain; MAP mitogaen-activated protein; PR, progesterone receptor; PRE, progestin responsive element; PHA, polycyclic aromatic hydrocarbon

* Present address: Sebelas Maret University, Surakarta 57126, Indonesia. Short-term student exchange promotion program, Tottori University, supported by AIEJ 2000.

action with aryl hydrocarbon receptors (AhRs) (Osteen et al., 1997), while most other chemicals seem to interact directly with the ER system.

In this paper, we give a brief overview of the recent understanding of the receptor-mediated actions of steroid hormones, and discuss the receptor-mediated actions of endocrine disruptors.

Recent advances in the mechanism of action of steroids

Nuclear receptors including steroid receptors have been understood as transcription factors, which mediate extracellular signals to the nucleus. A carboxy-terminus region of steroid receptors, the ligand binding domain (LBD), specifically interacts with cognate ligands, while the AF-2 domain recruits cofactors (Freedman, 1999; also see Fig. 1). Once associated with estrogens, ER is activated and recruits co-activators such as those from the SRC-1 family and TIF2/GRIP1/NCoA2, to interact with the estrogen responsive element (ERE) in responsive genes (McKenna and O'Malley, 2000; Freedman, 1999). When bound by an estrogen antagonist, ER recruits N-Cor and SMRT, co-repressors, and inhibits the transcription of estrogen responsive genes (McKenna and O'Malley, 2000). The concept of ER-dependent genomic actions has been accepted for other steroid receptor systems. Recently, growth factors, neurotransmitters and cyclins have been demonstrated to crosstalk with steroid receptor systems. Epidermal growth factor (EGF), insulin-like growth factor (IGF) (Kato et al., 1995; Bunone et al., 1996) and dopamine (Mani et al., 1994) could activate ERα via the mitogen-activated protein (MAP) kinase pathway in a ligand-independent manner. Cyclins directly interact with the unliganded ERα (Zwijsen et al., 1997). Progesterone receptor (PR) is also activated by dopamine through cAMP-dependent phosphorylation, and associates with SRC-1e, a co-activator specific for PR, to interact with the progestin responsive element (PRE) (Bai et al., 1997; Brossens et al., 1999). On the other hand,

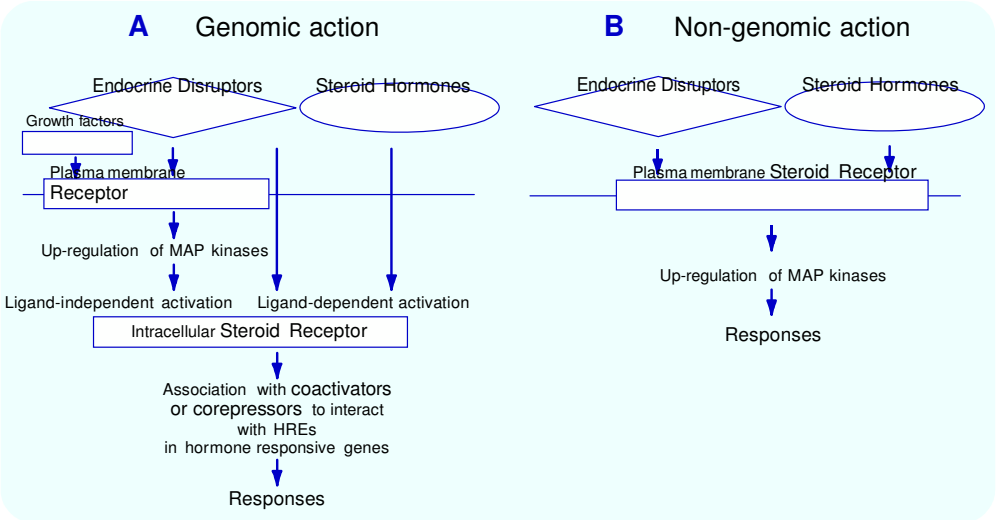

Fig. 1. Mechanism of action of steroid hormones and endocrine disruptors. **A:** Genomic action. Steroid hormones and endocrine disruptors diretly interact with intracellular steroid receptors. This leads to the recruitment of cofactors for specific interaction with hormone responsive elements (HREs) in hormone responsive genes. On the other hand, endocrine disruptors interact with receptors for growth factors or neurotransmitters to up-regulate mitogen-activated protein (MAP) kinase signaling. This leads to the ligand-independent activation of steroid receptors for the association with cofactors as described above. **B:** Non-genomic action. Steroid hormones and endocrine disruptors associate with steroid receptors localized to the cell membrane. This leads to the intracellular signaling pathways.

androgen receptor (AR) is activated by EGF, IGF and keratinocyte growth factor (KGF) through MAP kinase cascades, and binds to AP-1 and Ets, a co-activator specific for AR, to interact with the androgen responsive element (ARE) (Klocker et al., 1999; Culig et al., 1994).

Along with the ligand-dependent and the ligand-independent genomic actions, non-genomic actions through the plasma membrane receptor have recently been demonstrated to play impotant roles in hormone responsive cells (Chen et al., 1999; Goetz et al., 1999). In summary, the steroid receptor system is not only a ligand-induced transcriptional enhancer, but also a mediator of common intracellular signaling pathways in multiple cell types.

Endocrine disruptors and the mechanism of action

Adverse effects of pharmaceuticals, industrial waste and environmental pollutants (now all acknowledged as endocrine disruptors) on our lives have become a serious problem in the world. They disorder the endocrine system in animals directly or indirectly. It is obvious that they exert their effects in a receptor-dependent manner (Brouwer et al., 1998; Chia, 2000; Fenner-Crisp, 2000).

Endocrine disruptors, which seemed to be closely related to ER systems, could be classified into three groups: estrogen mimics, anti-estrogens and anti-androgens (Sonnenschein and Soto, 1998; Gray et al., 1999a). An initial target for these compounds is ER (Nishikawa et al., 1999). Diethylstilbesterol (DES), a synthetic estrogen, behaves as an estrogen agonist (Hunter et al, 1999), and exposure to DES during the fetal period induces urogenital abnormalities in males (Kuiper et al., 1998; Spearow et al., 1999), oligospermia (Krishnan et al., 1993; Vom Saal et al., 1997), the increased incidence of undescended testis (Kuiper et al., 1998; Spearow et al., 1999) and hypertrophy of the prostate (Newbold, 1995). Polycyclic aromatic hydrocarbons (PAHs) have an anti-estrogenic activity through AhR (Brouwer et al., 1998; Safe et al., 1998; Loaiza-Perez et al., 1999), and decrease the estrogen level in the blood (Kohn et al., 1993; Alvarez et al., 2000), the ER level in the uterus (De Vito et al., 1992; Alvarez et al., 2000) and the number of matured follicles associated with the upregulation of the follicle-stimulating hormone (Alvarez et al., 2000). Endocrine disruptors, which seemed to be closely related to AR systems are Flutamide, vinclozolin, p,p'-DDE and procymidone, anti-androgens, which disorder prostate maturation (Gray et al., 1999b), and induce undescended testis (Gray et al., 1999b), hypospadia (Gray et al., 1999b; Ostby et al., 1999) and permanent nipples (Gray et al., 1999b; Ostby et al., 1999) during sex differentiation. In adult animals, anti-androgens increase the serum levels of androgens, luteinizing hormone and estrogens (Ladics et al, 1998; Monosson et al., 1999), and induces spontaneous abortions, stillbirths, reproductive malfunction, neuroendocrine alteration, and disorder of immune systems (Goldman, 1997; Palanza et al, 1999; Alvarez et al., 2000).

As demonstrated above, disorder of steroid receptor-mediated signaling systems by endocrine disruptors leads to abnormal conditions in animal physiology. It is important to note that some endocrine disruptors exhibit potential activities: (i) to interact directly with endogenous steroids, (ii) to inhibit steroid receptor binding, non-competetively or (iii) to destroy receptor molecules or endogenous steroids (Sonnenschein and Soto, 1998; Soto et al., 1998).

Conclusion

Ligand-dependent and -independent signaling pathways by steroid receptors seem to provide insights into the understanding of the molecular basis of endocrine disruptors as summarized in Fig. 1. We also summarized representative endocrine disruptors identified so far in Table 1. Although a number of endocrine disruptors in our daily lives have been identified, little is known about their molecular basis. It becomes an urgent subject to further understand the molecular basis of the diverse actions of endocrine

Table 1. Representative endocrine disruptors

Compound	Use	Receptor	Reference
DES	Medicine	ERα, ERβ	23, 30
Bisphenol A		ERα, ERβ	23, 37
Nonylphenol		ERα, ERβ	23, 37
PAHs:HCB, HCH	Pesticide	AhR	25, 36
o,p'-DDT		ERα, ERβ	23
p,p'-DDT		ERα, ERβ	23
Methoxychlor		ERα, ERβ	23
Endosulfan		ERα, ERβ	23
Chlordecone		ERα, ERβ	23
p,p'-DDE		AR	14, 15, 32
Linuron		AR	14, 15, 32
Vinclozolin		AR	14, 15, 32
Procymidone		AR	14, 15, 32
PCBs	Industrial waste	ERα, ERβ	15, 23
Bisphenol A		ERα, ERβ	23, 37
4 Tert-octyphenol		ERα, ERβ	23, 37
4-Octyphenol		ERα, ERβ	23, 37
TCDD		AhR	36

AhR, aryl hydrocarbon receptor; AR, androgen receptor; DES, diethylstilbesterol; ER, estrogen receptor; HCB, hexachlorobenzene; HCH, hexachlorohexane; op'/pp'-DDT, dichlorodiphenyltrichloroethane; PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated hydroxybiphenyl; p,p'-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

disruptors. Understanding endocrine disruptors from the viewpoint of steroid receptor-mediated signaling may allow us to overcome these global problems. In addition, it is obviously important to keep up international cooperation.

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Correspondence: Masao Izawa, PhD
Department of Biosignaling
Tottori University Faculty of Medicine
86 Nishi-machi, Yonago 683-8503 JAPAN
Tel and Fax: +81-859-34-8042
E-mail: 1mizawa@grape.med.tottori-u.ac.jp