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Topic 4.11

Effects of endocrine active substances in wildlife species: Genetic, biochemical, and physiological factors in variable susceptibility to endocrine disruptors*

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Abstract: Responses to endocrine active substances (EASs) in animals are various, and differences between the responses among individuals, populations, and species are well known. These differences are observed not only in EASs but in most environmental chemicals including synthetic and naturally occurring ones. The basic differences in sensitivity to EASs are attributed to that of affinity or specificity of the receptors to EASs at the cellular level. Although the nucleotide sequences encoding for estrogen receptor proteins have been documented in several species and the functions of the receptors are the same, the ability to bind the natural hormones and the estrogenic xenobiotics is not necessarily identical. The reproductive endocrine system is basically common among vertebrates, but chemical types of hormones, physiological roles of hormones, and the basal blood levels of hormones differ among each species, especially in sex steroids. These differences cause various types of responses and sensitivity to EASs among animal species. Xenobiotic metabolism is important for the genetical, biochemical, and physiological factors concerning the influence of EASs. Some EASs directly inhibit cytochrome P450 (CYP) activity as was reported in tributyltin that inhibits CYP19 (aromatase) activity causing imposex in neogastropods. Some organochlorines including dioxins stimulate arylhydrocarbon (Ah) receptor-mediated xenobiotic metabolism, and result in the metabolic disruption of steroid hormones such as estrogen as were reported in eggshell thinning in birds of prey and uterus occlusion in seals. CYP activity greatly differs among wildlife species in both terrestrial and aquatic organisms, and these differences are significantly responsible for the multiple effects or toxicity of EASs. Sex and age differences also cause different responses to EASs and are largely due to the differences in xenobiotic metabolizing activities.

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INTRODUCTION

Endocrine disruption has been postulated as the cause of a large number of adverse effects on the health of various wild animal species. The majority of cases involve reproductive abnormalities that might be linked to population decline. Data supporting cause–effect relationships between the biological effects and exposure to certain specific chemical agents are extremely limited to a small number of cases. The impact of chemical pollution on the reproductive success and population sizes of wildlife species is often difficult to predict because of the ecological factors including geographic range, specific habitat, food habits, etc. Moreover, physiological, biochemical, and genetical factors are closely related to the differences of endocrine disruption between species, sex, and age of animals.

Responses to endocrine active substances (EASs) in animals are various, and the differences of responses among individuals, populations, and species are well-known facts. In this paper, the genetic, biochemical, and physiological factors influencing the variable susceptibility of wildlife species to EASs are described with special references to species, sex, and age differences. These differences are generally observed not only with EASs, but with most chemicals including synthetic and naturally occurring ones.

PHYSIOLOGICAL FACTORS

Sex, sexual status, age, endocrine system, and endogenous levels of sex steroids are important physiological factors influencing the susceptibility of aquatic and terrestrial wildlife species to EASs.

Sex differences and sexual status influences the levels of organochlorines in striped dolphins (*Stenella coeruleoalba*)

Endocrine disruption in marine mammals has been reported in seals since the 1970s, and uterus occlusion [1] and lesions of skull bones [2] were clearly noticed in the individuals that accumulated high levels of organochlorines such as PCBs. Organochlorines in these mammals are likely to cause endocrine disruption, especially those affecting estrogen metabolism, directly or indirectly through xenobiotic metabolism. Sex differences or sexual status affect the accumulation and excretion of organochlorines in wild animals. For example, PCB concentration and burdens in the blubber of striped dolphins migrating along the Pacific coast of Japan, were markedly low in mature females (lactating, pregnant, or resting) compared to mature males and immature individuals [3]. This is because of the transportation of PCB from mother dolphins to newborns through lactation. More than 60 % of organochlorines accumulated in lactating females are mobilized to transport during the lactation period of about 6 months. From these findings, physiological factors such as age, sex, and sexual status are concluded to be largely responsible for the uptake, accumulation, and excretion of organochlorines. Consequently, some adverse effects, including reproductive and immunological toxicity, might occur in marine mammals [4]. In addition, lipid content and lipid composition are responsible for the accumulation of organochlorines. Most organochlorines are accumulated in blubber, being a subcutaneous thick fatty tissue. Apart from blubber, high levels of organochlorines were observed in the tissues of high triglycerides content such as mammary gland, kidney, and pancreas. Though brain tissues show high lipid content, the lipids are mainly comprised of phospholipid and total cholesterol, and consequently the levels of organochlorines are low [5]. In this way, physiological characteristics such as lipid content and lipid composition of tissues or organs in certain animals drive the accumulation and fates of organochlorines in the body.

Reproductive cycles and developmental stages of carp related to the level of vitellogenin and 17β-estradiol (E2)

Reproductive stages and season are important for the understanding of sex steroid hormone levels and specific markers such as the egg protein precursor vitellogenin in fish. In the immature stages of male and female carp (*Cyprinus carpio*) (1 to 2 years), significant differences in blood E2 levels between the sexes are not observed. However, maturing male carp show rather higher levels of E2 in autumn as compared to females. After that, E2 levels in mature females clearly increase. Vitellogenin content in blood increases in accordance with the increase of E2 during winter to spring in female carp [6]. Though the blood vitellogenin levels in wild male fish are considered to be a good marker for the exposure to estrogenic substances in aquatic environments, endogenous sex steroid levels are significantly responsible for the low levels of vitellogenin in males and modify the effect of exogenous EASs.

Endogenous estrogens and androgens in male and female fish living in freshwater and marine environments

Chemical types of sex steroids and their roles differ among vertebrate species. E2 is a common female sex steroid, and its major role in oviparous animals is in the production of vitellogenin. In most vertebrates, E2 is involved in the occurrence of female sex behavior, but this is not the case in fish [7]. Testosterone is known as a major androgen in mammals, but this steroid is produced both in the ovary and testis in fish (Table 1), and blood levels of testosterone are higher in females than in males in some fish species.

The major androgens in amphibians and fish are dihydrotestosterone and 11-ketotestosterone, respectively [7]. Since these androgens are nonaromatizable, their mode of action and effects are considered to be different compared to those of testosterone or 17α-methyltestosterone, which are aromatized and bind to the estrogen receptor at some target organs. Although testosterone or 17α-methyltestosterone are often used as standard androgens in bioassays, the effects of EASs on nonaromatizable androgens should be also considered and examined depending on which chemical type of androgens the animal species possesses.

Endogenous hormone levels seem to be one of the factors that cause differences in sensitivity to EASs. When a certain type of hormone is exogenously administered to two animals in which blood concentrations of the endogenous hormone are different, effects of the administered hormone would be expected to be higher in the animal with low blood levels than in the one with high levels due to the competition between the exogenous and endogenous hormone in binding to the receptor. Large variations in blood levels of hormones have been reported in vertebrate species. Blood E2 concentrations of sexually matured female mammals are known to range from 10 to 200 pg/ml. Blood E2 concentrations in fish are much higher, and the variation among species is also large. Blood E2 levels in most female marine fish species examined are 10–2000 pg/ml while the levels range from 1 to 10 ng/ml in female cyprinid species during vitellogenesis. In salmonid species, blood E2 levels rise as high as 10 to 50 ng/ml. Blood E2 levels in male fishes have not been studied to the extent of those in females, but it

Table 1 Blood levels of sex steroid hormones in sexually mature fish.

	17β-Estradiol		Testosterone		11-Ketotestosterone	
	Male	Female	Male	Female	Male	Female
Salmonids	~0.5	10~40	100~200	100~200	100~200	–
Cyprinids	~0.5	5~10	5~100	5~100	1~20	1~10
Marine species	–	1~10	1~5	1~5	1~5	–

ng/ml

is reported that the levels rise up to 1000 pg/ml in common carp (*Cyprinus carpio*) during testicular development [6,8,9]. It is possible that large amounts of endogenous hormones compete with EASs in binding to the receptor, and the effects of EASs might therefore vary depending on the blood levels of endogenous hormones.

BIOCHEMICAL AND GENETICAL FACTORS

The following differences are considered to be important for species differences in the effects caused by EASs in wild animals.

Species differences in enzyme activity toward xenobiotics

Large species differences have been reported in the activity of several enzymes that play an important role in xenobiotic metabolism including microsomal oxidase, epoxide hydrolase, *N*-acetyltransferase, UDP-glucuronyltransferase, etc. [10]. For example, activity of microsomal oxidases was nearly five orders of magnitude higher in cow compared to cat, when benzo(a)pyrene was used as a substrate. There exists a multiplicity of enzymes in different species and this emphasizes the need for extreme caution in extrapolating metabolic information across species even with structurally similar chemicals. Clearly, these differences can lead to significant species differences in the action of xenobiotic EASs.

Species difference in the 17β-estradiol transactivation of the luciferase gene

Over the last decade, cloning studies of estrogen receptors have been performed with various vertebrates. Figure 1 shows the analysis of species differences in estrogen receptor (ER) dependent transactivation with E2 using reporter-gene assays [11]. Full-length ER cDNAs from human, rat, chicken, caiman (*Caiman crocodilus*), whiptail lizard (*Cnemidophorus uniparens*), African clawed frog (*Xenopus laevis*), and rainbow trout (*Oncorhynchus mykiss*) were prepared from hepatic mRNA by the RT-PCR method and inserted into expression plasmids. Both expression and reporter plasmids were transiently transfected into HeLa cells, and the estrogenic activity was analyzed in terms of induction of luciferase activity [11].

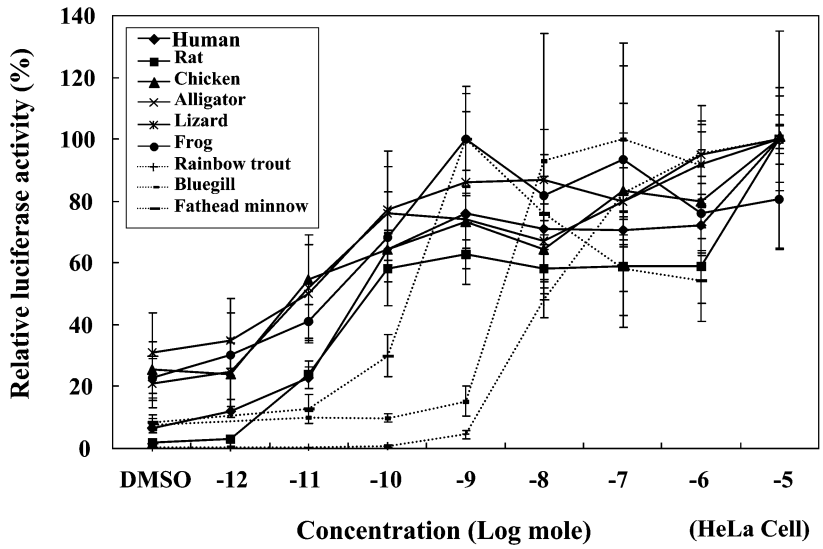

Fig. 1 Species differences in the transactivation of the luciferase gene (Receptor: ER, Ligand: 17β-estradiol).

No significant species differences in the transactivation by E2 were found among human, alligator, and lizard ER in Fig. 1. However, decreases in response to E2 were commonly observed in the fish species including bluegill, fathead minnow, and rainbow trout ERs in HeLa cells at 37 °C. The response of ER of rainbow trout to E2 was two-orders lower compared to that of human. This may be due to the temperature conditions of the reporter-gene assays, because thermo-dependent alteration in the affinity to ER has been reported in binding assays using ER of rainbow trout.

Figure 2 shows the elevation in response of rainbow trout ER to E2 in BF-2 cells derived and established from bluegill (*Lepomis macrochirus*) fry at the incubation temperature of 24 °C, compared to the result in HeLa cells at 37 °C [11]. This finding will be useful for assessing estrogenic effects of chemicals on wildlife species as well as humans.

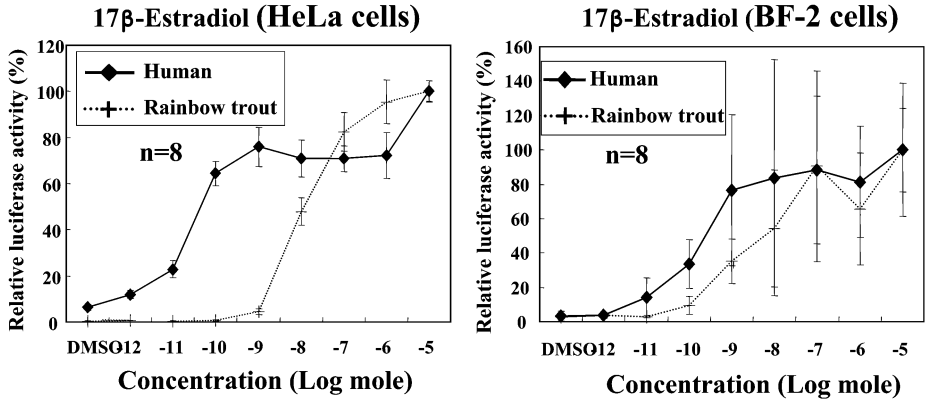

Fig. 2 Species differences in the transactivation of the luciferase gene: temperature effects (Receptor: ER, Ligand: 17β-estradiol).

Species differences in the amino acid sequences of cytochrome P450

Cytochrome P450 (CYP) and glutathione-*S*-transferase (GST) are important families of enzymes involved in the biotransformation of xenobiotics including EASs. A number of environmental pollutants are known as inducers of CYP and GST expression levels. Therefore, the induction of both enzymes by xenobiotics in wild life species is considered to be useful as a biomarker for monitoring levels of pollution [12]. The CYP family has been investigated in fish, amphibians, reptiles, gastropods, and crustaceans. Among these animals, studies of xenobiotic metabolism are highly advanced in fish, especially in rainbow trout (*O. mykiss*).

Species differences in the amino acid sequence of CYP has also been reported in several kinds of mammals relative to human [13], and the sequence of primates such as crab-eating macaque and marmoset are closely similar to human, while clear differences are reported between human and hamster or mouse.

Species differences in the amino acid sequences of ER-α

The ER is essential for the functional expression of estrogen. The ER is divided into A to F regions from the *N*-terminal, based on homology and function. The C-region being the DNA binding zone is important for the recognition of estrogen-responsive elements and for the binding of the receptor to DNA. As shown in Fig. 3, the amino acid sequences of the C-region are highly homologous (90 % more) among mammals (humans), birds (chickens), reptiles (lizards and alligators), amphibians (frogs), and fish (medaka). However, the amino acid sequence of the E-region which is important for the binding of es-

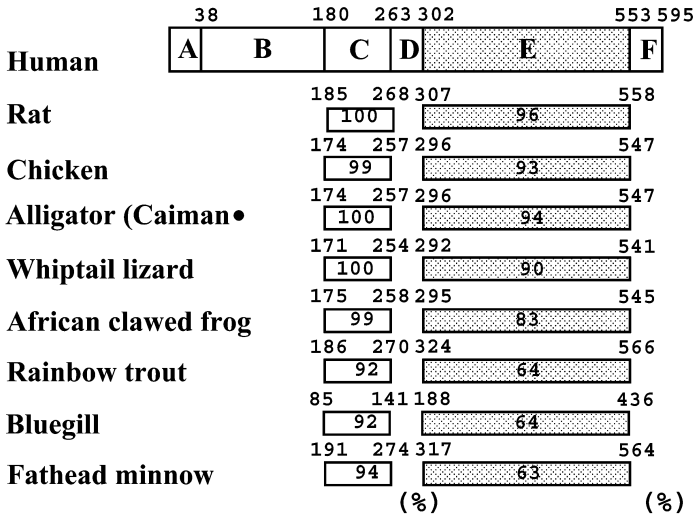

Fig. 3 Comparison of the amino acid sequence similarities of the individual C and E domains for ER.

trogen to ER is not so homologous among species, and the homology compared to humans decreases in the following order [14].

Bird > Reptile > Amphibian > Fish

In fish, the homology is only 60 % to human E-region of ER. This evidence shows the species difference in the E-region of ER.

FUTURE STUDIES

Steroid hormones are lipophilic and do not dissolve easily in water. A very small percentage of the hormones are free in the blood, and most are carried bound to serum binding proteins such as sex hormone binding globulin (SHBG). Hormones bound to SHBG are protected from degradation by the liver enzymes, and are also considered to function as a reserve of available hormones. The concentration of SHBG, and the rate of degradation and excretion from organisms regulate the availability of steroid hormones to their target tissues. To date, little research has been conducted on the interaction of EASs with SHBG, although most EASs circulate freely in blood. Certain chemicals such as diethylstilbestrol (DES), octylphenol and *o,p*-DDT seem to have very low affinity for SHBG, because estrogenic activity of these chemicals was poorly inhibited by the presence of SHBG or albumin (nonspecific serum binding protein) in an in vitro assay when compared to the total inhibition of E2 activity [15]. Another way that EASs could alter the availability of hormones in an organism is to induce the production of SHBG, binding more free hormones and reducing their bioavailability to the cells. Some plant compounds, such as lignans and isoflavonoids, seem to stimulate SHBG synthesis in the liver in vivo and in vitro [16]. Consequently, information on SHBG in wildlife species is required.

Finally, the authors would like to emphasize that physiological factors are closely related and linked to ecological and ethological ones. Unfortunately, many findings on the adverse effects of various naturally occurring and synthetic chemicals including EASs are mostly obtained by laboratory experiments alone. Behavioral ecotoxicology will therefore be one of the most important disciplines in the near future.

SUMMARY AND CONCLUSIONS

Genetic or biochemical differences in ER influence gene expression and the synthesis of estrogen mediated or dependent functional proteins.

Differences of xenobiotic metabolism including CYP and conjugating activity are also important factors affecting the susceptibility of wild animals to EASs.

It is possible that endogenous sex steroids such as estrogens and androgens compete with EASs and cause differences in sensitivity to EASs depending on their titres in blood, especially in fish.

RECOMMENDATIONS

Obtaining information on normal levels of hormones and physiological response caused by endogenous hormones in each wild species is important. These data are essential to know whether the responses observed in animals are caused exogenously by EASs or physiologically by endogenous hormones.

Research on the interaction of EASs with SHBG should be conducted for the evaluation of availability of steroid hormones in their target tissues.

Information on CYP activity in wildlife species is very sparse, and studies on differences between species, age, and sex should be carried out.

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