INTERSPECIES VARIATION IN THE ESTROGENICITY OF P,P'-DDE

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Introduction

Wildlife are exposed daily to environments contaminated with a variety of chemicals from industrial, agricultural and pharmaceutical origins. Over the last decade a growing number of studies have focused on the endocrine disruptive activities of these contaminants in wildlife populations[1]. Much of the literature on endocrine disruption in wildlife and humans has focused on the estrogenic or antiestrogenic actions of various contaminants[2]. Given the vital role estrogens play during embryonic development and adult reproductive activity, this focus is not unwarranted[3]. However, a number of studies now indicate that labeling various chemicals as simply estrogenic or antiestrogenic given their action in a single test species is inappropriate. Further, the potency of the response can also be altered given the species studied. Below, we examine these phenomena using reptilian sex determination as a model.

Reptilian Sex Determination

Sex determination in reptiles can follow two basic patterns: genetic sex determination (GSD) or environmental sex determination (ESD), where the principle factor appears to be the temperature of incubation during a specific time period of embryonic development; thus, the term temperature-induced sex determination (TSD)[4]. Of importance to this discussion, is the fact that TSD in reptiles can be altered by exogenous treatment with an estrogenic substance[5-7]. That is, if eggs from a reptilian species exhibiting TSD are incubated at a male producing temperature but treated prior to sex determination with an estrogenic substance, or an aromatizable androgen, than females are produced. Treatment of eggs incubated at a female temperature with an androgen does not produce males. Thus, TSD in reptiles provides an important model system to determine the estrogenicity of various environmental contaminants and further, allows cross species comparisons[8].

Contaminant-induced Sex Reversal in Reptiles

In 1994, Bergeron et al.[9] demonstrated that environmental contaminants, such as hydroxylated PCBs could sex reverse freshwater turtle embryos, producing females at male incubation temperatures. Since that report, numerous studies have begun to demonstrate that various persistent organochlorine pesticides or their metabolites are capable altering sex determination in reptilian embryos. For example, *trans*-nonachlor, *cis*-nonachlor, chlordane, and Aroclor 1242 all induce sex reversal in the red-eared slider turtle at ecologically relevant concentrations[10]. Likewise, *trans*-nonachlor, dicofol, and TCDD are capable of inducing sex reversal in the embryos of the American alligator[11, 12]. The estrogenic nature of o,p'-DDT has been known since 1950[13]. Likewise, metabolites of DDT have been known to have endocrine activity, such as the anti-steroidogenic actions of o,p'-DDD on the adrenal of some species[14]. It is the species selective nature of the anti-steroidogenic action of o,p'-DDD[15] that stimulated us to begin looking at

possible differences in the response of various species to the endocrine disruptive action of DDT and its metabolites.

Three metabolites of DDT, p,p'-DDD, o,p'-DDD and p,p'-DDE have been tested on various reptiles with TSD with varying results (Table 1). First, it should be noted these are not matched studies with the same dose range but the treatment procedure was very similar with eggs treated using the application of the exogenous agent onto the eggshell immediately prior to the period of sex determination.

Table 1: Reptilian species examined for sex reversal following treatment with DDT metabolites.

Species	Compound	Dose	Sex Reversal	Reference
Red Eared Slider	p,p'-DDD	0.8 mgKg	No	10
American Alligator	p,p'-DDD	0.1-10 mg/Kg	Yes	12
American Alligator	o,p'-DDE	0.1-0.3 mg/Kg	Yes	11
American Alligator	p,p'-DDE	1-10 mg/Kg	Yes/No	11
Red Eared Slider	p,p'-DDE	5.8 mg/Kg	Yes	10
Green Sea Turtle	p,p'-DDE	0.3-6.6 mg/Kg	No	16
Snapping Turtle	p,p'-DDE	0.05-0.65 mg/Kg	No	17

In the red eared turtle, p,p'-DDD was not estrogenic at a relatively low dose whereas in the American alligator it was estrogenic at similar doses. Likewise, p,p'-DDE exhibited mixed results having estrogenic activity in the red eared slider but no apparent estrogenic activity in the green sea turtle or North American snapping turtle. In the American alligator, p,p'-DDE displays mixed results, as this compound induces sex reversal in embryos at high doses (1 – 10 mg/Kg), can synergize with its isoform o,p'-DDE to produce 100% sex reversal and yet can act in an anti-estrogenic fashion when combined with ethinylestradiol[18]. We have shown previously that o,p'-DDE and p,p'-DDE exhibit an affinity for the alligator estrogen receptor and can displace estradiol-17β, suggesting it has the potential for estrogenic and anti-estrogenic activity[19].

Implications and Future Studies

Much of the current focus on endocrine disruption centers on the ability of various environmental contaminants to bind to specific receptors and induce or block gene expression. The data above, suggest that a fruitful direction could involve examining the sequences of the estrogen receptors in these species to determine what characteristics they have in common and which differ. That is, if the American alligator and Red eared turtle exhibit estrogen-dependent sex reversal following treatment with p,p'-DDE whereas the common Snapping turtle and Green sea turtle do not, a first approach would be to clone the receptors from these species. Second, constructs of these receptors transfected into stable cell lines with reporter genes would allow comparative analysis of the binding characteristics and gene expression abilities of these receptors following exposure to various contaminants. Studies of this nature, could help identify which aspects of the ligand and DNA binding regions of these receptors are important for their differential activity with environmental estrogens. Further, recent studies have also suggested that gene expression associated with estrogen action could occur via estrogen receptor-independent mechanisms [20]. Examining species where environmental estrogens give differing responses could allow examination of receptor and non-receptor mediated phenomena. Evolution has provided important

comparative groups that if studied in an appropriate fashion could provide important insight into the molecular and physiological mechanisms endocrine disruption in wildlife and humans as well.

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