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## **A Novel Mechanism of Endocrine Disruption: Inhibition of Steroidogenic Acute Regulatory Protein Transcription by Aryl Hydrocarbon Receptor Activation**

### **DIOXIN TOXICITY**

One detriment to human industrial society is the production and subsequent release of environmental pollution and contaminants. Organochlorines are a large group of persistent organic pollutants of industrial origin that are widespread in ecosystems all across the world including the Great Lakes [1] and the Canadian arctic [2]. Many organochlorines are not only environmentally persistent but they bioaccumulate or biomagnify in the food chain posing serious reproductive concerns [3]. A subset of organochlorine compounds are the dioxins, a class of compounds comprising polychlorinated dibenzo dioxins (PCDDs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo furans (PCDFs) which all have a common spectrum of biologic responses mediated via binding to a specific high-affinity cellular protein, the aryl hydrocarbon receptor (AhR) [4]. Once bound, the AhR, which is a cytosolic protein, dimerizes with arnt (AhR nuclear translocator) and this complex then binds to specific xenobiotic response elements (XREs) present in target genes, of which the best characterized is the cytochrome P450 1A1 (CYP1A1) gene [5]. Dioxin-like compounds are identified by their ability to trigger the induction of CYP1A1 protein via AhR [5] and all of these pollutants have become ubiquitously present in both marine and freshwater systems. The prototype chemical for this class is dioxin [2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD)] which has the potential to disrupt multiple endocrine pathways [4]. All of these compounds are toxic to animals, producing endocrine and immune dysfunction and carcinogenesis [6-9] with the potential to disrupt multiple endocrine pathways, but their mode of action is still unclear.

### **STEROIDOGENESIS IN FISH**

The steroid hormones are a key component of the vertebrate endocrine system and are comprised of three separate groups known as the mineralocorticoids (aldosterone), glucocorticoids (cortisol) and sex steroids (estrogen and testosterone). Aldosterone is the primary mineralocorticoid and its function relates to the control of hydromineral balance by regulating the retention of sodium and potassium. Aldosterone is produced in the zona glomerulosa of the adrenal gland in mammals but fish lack an adrenal gland. The current scientific consensus is that there is no reliable evidence of aldosterone in teleosts despite the presence of mineralocorticoid receptor homologs which have a high affinity for aldosterone [10]. Cortisol is the primary glucocorticoid in fish and it is produced in the interrenal cells of the anterior kidney (head kidney) where its release is stimulated by adrenocorticotrophic hormone (ACTH) from the pituitary [11]. Cortisol is involved in the regulation of numerous physiological processes including intermediary metabolism, ion regulation and immune responses [11] and the secretion of cortisol is considered a classic response to acute stressor exposure in teleostean fishes [12]. The sex steroids, testosterone and estrogen, are preferentially produced in males and females, respectively, and are primarily synthesized in the gonads (testis and ovary). The sex steroids play key roles in sexual differentiation, maturation and reproduction [13] by binding to their respective transcriptional receptors, estrogen (ER) and androgen (AR). Cholesterol is the primary substrate in both steroid and corticosteroid biosynthesis and its transport from the outer to the inner mitochondrial membrane represents a rate-limiting step in steroidogenesis [14]. The steroidogenic acute regulatory (StAR) protein, an intracellular cholesterol transport protein, has

been shown to play a key role in steroidogenesis and is therefore a target for endocrine disruptors [15].

### ENDOCRINE DISRUPTION BY DIOXINS

TCDD reproductive toxicity has been studied extensively, but the results are contradictory and the mechanisms are elusive. Anti-estrogenic effects following TCDD exposure include a reduction in rat uterine weights [16], a reduction in hepatocyte vitellogenin synthesis [17-19], a downregulation of ER-signaling in porcine oviduct cells [20] and a decrease in egg production and spawning success in zebrafish [21]. A number of studies have also shown estrogenic effects of TCDD which include reduced ejaculated sperm numbers in prenatally exposed male rats [22], a gene expression response similar to estrogen in rat uterus [23], abatement of gene expression responses in mice liver after given an anti-estrogen [24] and feminization of male fish gonads [25]. Finally, Grochowalski *et al.* [26] found both estrogenic and antiestrogenic responses in rat ovarian follicles based on cell type, only adding to the already confusing and contradictory results.

The effects of dioxin-like compounds on the hypothalamus-pituitary-interrenal (HPI) axis in fish are not well-characterized and, again, there are somewhat contradictory results. Well before the StAR protein was identified and characterized [27], it was observed that TCDD caused a decrease of mitochondrial cholesterol in mammalian adrenal cortical cells, the site of corticosteroidogenesis in mammals [28, 29]. Since that time, studies have identified various effects of dioxin-like compounds on the HPI axis including a lower cortisol response in PCB fed tilapia [30], a lower cortisol response in fasted PCB dosed Arctic charr [31, 32], an abolishment of ACTH stimulated cortisol production in rainbow trout after treatment with an AhR agonist ( $\beta$ -naphthoflavone) [33], and lastly an increase in cortisol production in human adrenocortical cells after PCB dosing [34]. The difference between the mammalian and fish cortisol responses to PCBs indicates species or at least tissue-specific differences in the mechanisms of PCB toxicity. And while the mechanisms are unknown, it is obvious that dioxin-like compounds are modulating both steroidogenesis and corticosteroidogenesis.

### ENVIRONMENTAL AND ECOLOGICAL SIGNIFICANCE

While the purpose of this work is to gain understanding into mechanisms associated with AhR activated disruption of steroidogenesis, there are sincere applications and implications in the field of environmental science and ecology. The end points of steroid biosynthesis pathways include cortisol and the steroid hormones, testosterone and estrogen. Cortisol once released, directs, or redirects, energy for optimal performance under conditions where homeostasis may be at risk. Its primary targets are gills and liver where its major actions are regulation of hydromineral balance and energy metabolism, respectively [56]. Cortisol promotes the differentiation of the chloride cells, the main ion-transporting cells of the gills, and increases the specific activity of ion-transporting enzymes ( $\text{Na}^+$  - $\text{K}^+$  -ATPase) in gills, intestine and kidneys [57]. Cortisol also induces hyperglycemia resulting from gluconeogenesis and glycogenolytic pathways [11]. Thus, a disruption of corticosteroidogenesis in fish would result in an alteration of the cortisol response and the associated biochemical and physiological responses critical for establishing homeostasis after stress. This in turn could lead to physiological dysfunction or even mortality as the organism's ability to maintain homeostasis would be severely compromised. In regions where environmental pollution (PCBs, dioxin, etc.) is particularly severe, fish show signs of a decreased ability to respond to environmental stressors [31], giving

importance to this work in the government regulatory and fisheries management fields. While these types of dysfunctions have implications for individual survival, reproductive impairment via a disruption of steroidogenesis not only threatens the individual survival [58], but also threatens the reproductive continuity. Testosterone and estrogen are the key reproductive steroid hormones and most, if not all, of the physiological changes associated with sexual maturation and reproduction are derived from their effects. A disruption of steroidogenesis in fish would result in a loss of reproductive timing and ability, which has population level and even ecological effects [59].

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