Impacts of Xenobiotics on Crustacean Molting: The Invisible Endocrine Disruption

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SYNOPSIS. Aquatic pollution has led to the accumulation of various xenobiotics in crustaceans. A number of these environmental chemicals have been found to interfere with molting of crustaceans. Results of initial mechanistic studies with Uca pugilator suggest that the disruption of molting results from the disturbance to the Y-organ-ecdysteroid receptor (EcR) axis by xenobiotics. Such disturbance to the Y-organ-EcR axis can be caused by interference with epidermal ecdysteroid signaling and/or alterations in ecdysteroidogenesis and/or ecdysteroid disposition. Because the adverse impacts on crustacean molting cannot be readily seen in the wild, the disruption of molting represents an invisible form of endocrine disruption.

INTRODUCTION

Because of agricultural and industrial activities, aquatic environments are increasingly contaminated with various kinds of pollutants, many of which can interfere with hormonal signaling in vertebrates. Through feeding and direct uptake from water and sediments, these endocrine-disrupting contaminants have been found to accumulate in crustaceans living in various aquatic environments. Because of the generally high lipophilicity of organochlorine compounds, these organic contaminants can readily accumulate in the fatty tissue, such as hepatopancreas, of crustaceans. For instance, Mattig et al. (1997) reported the accumulation of organochlorine compounds, including polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethanes (DDTs), hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB), in the shore crab, Carcinus maenas, and the sand shrimp, Crangon crangon. Organochlorine contaminants, including toxaphene, chlordane and metabolites, HCHs, HCB, dieldrin, and PCBs, were found in the opossum shrimp, Mysis relicta, from the great lakes (Kucklick and Baker, 1998). Heptachlor epoxide, dieldrin, endosulfan, chlordane, DDT and metabolites, and HCHs were found to accumulate in the burrowing crab, Chasmagnathus granulata (Menone et al., 2000). Borga et al. (2002) detected DDTs, PCBs, chlordane, HCB, and HCH in several amphipods from the Arctic Ocean. PCBs, DDTs and metabolites, endosulfan, heptachlor epoxide, HCHs, and HCB were detected in the tissues of the amphipod, Gammarus lacustris, inhabiting a high-altitude lake (Blais et al., 2003). Like organochlorines, polycyclic aromatic hydrocarbons (PAHs), the pollutants from activities of petroleum and smelter industries, are also highly lipophilic and can easily accumulate in crustaceans tissues (Kayal and Connell, 1995; Eickhoff et al., 2003). Besides, cadmium, a metal capable of disrupting estrogen functions in vertebrates (Le Guevel et al., 2000), has been found to accumulate in the tissues of crustaceans in appreciable quantities (Paez-Osuna and Tron-Mayen, 1996; Mattig et al., 1997; Mouneyrac et al., 2001; Turoczy et al., 2001).

Earlier, the possibilities of the adverse effect of environmental chemicals on crustacean molting had been investigated. Weis and Mantel (1976) found a stimulatory effect of p,p’ DDT on molting of Uca pugilator. Fingerman and Fingerman (1977) reported the inhibitory effect of the PCB mixture Aroclor 1242, on molting of Uca pugilator. Schimmel et al. (1979) found that blue crabs, Callinectes sapidus, fed oysters contaminated with kepone, molt less frequently than crabs fed kepone-free oysters. Aromatic hydrocarbons benzene (Cantelmo et al., 1981) and dimethylnaphthalene (Cantelmo et al., 1982) were found to delay the molting of juvenile Callinectes sapidus. In recent years, with the emergence of a new subdiscipline of ecotoxicology, endocrine disruption, also called environmental signaling (McLachlan, 2001), new attention is being paid to the disrupting effects of environmental chemicals on crustacean molting. More molt-interfering chemicals have been identified and the pervasive-ness of molt-inhibition by xenobiotics has been reexamined. Zou and Fingerman (1997a), in their study on the effects of vertebrate endocrine disruptors on sex differentiation in Daphnia magna, found that estrogenic agents diethylstilbestrol (DES) and endosulfan do not affect male differentiation but delay the molting of this cladoceran. A further study by these investigators found that other environmental chemicals, such as Aroclor 1242, 2,4,5-trichlorobiphenyl (PCB29), and diethyl phthalate, can also inhibit molting of Daphnia magna (Zou and Fingerman, 1997b). Baer and Owens (1999) found that the pesticide methoxychlor delays the molting of Daphnia magna. Snyder and Mulder (2001) reported that exposure of Homarus americanus larvae to the cyclodiene pesticide heptachlor can cause a delay in molting. Molt-inhibition by heavy metal cadmium has also been reported in the estuarine crab, Chasmagnathus granulata (Rodriguez Moreno et al., 2003).

ACTION ROUTES OF MOLT-DISRUPTING XENOBIOTICS

As is mentioned above, a number of xenobiotics that readily accumulate in the tissues of crustaceans can...
adversely impact molting in crustaceans. Now, the question is: how is crustacean molting adversely affected by environmental chemicals? To seek an answer to such a question, we must first examine the endocrine control of molting in crustaceans. Since the endocrine system for regulation of molting in decapods is best understood, only the endocrine control for molting of these animals is described.

Molting in crustaceans is regulated by a multi-hormonal system, but is under immediate control of the steroid hormones called ecdysteroids (Chang et al., 1993). In decapods, ecdysteroids are produced in the Y-organs whose activity is held in abeyance during the intermolt stage by the molt-inhibiting hormone (MIH) from the X-organ-sinus gland complex (Fig. 1). When the animal enters premolt stage, this inhibition of Y-organ activity by the MIH is lifted and ecdysteroidogenesis in the Y-organs intensifies. Ecdysteroid titer in the hemolymph is, as a result, elevated. Ecdysteroids regulate gene activities at the transcriptional level through interacting with the ecdysteroid receptor (EcR), which then heterodimerize with crustacean retinoid X receptor (RXR) (Durica and Hopkins, 1996; Chung et al., 1998). This EcR/crustacean RXR dimer binds to the DNA response elements of the genes regulated by the molting hormones.

In theory, any event (MIH synthesis and release, ecdysteroidogenesis, EcR binding, etc.) in the endocrine cascades of molting regulation (Fig. 1) could be the target of environmental chemicals. To unravel the mechanisms for molt-inhibition, Zou and Fingerman (1999a, b) have taken a backtracking approach. These investigators reasoned that wherever a xenobiotic attacks on this hormonal system the impacts should be manifested at the terminal event, the expression of the genes regulated by the molting hormones. Thus, alterations in expression of these molting hormone-regulated genes reflect the overall impacts of an environmental agent on the endocrine system for molting control.

Among the products of ecdysteroids-regulated genes are the enzymes responsible for exoskeleton degradation. Chitobiase, also known as N-acetyl-β-glucosaminidase, is one of the two chitinolytic enzymes found in the molting fluid, which are required for complete degradation of exoskeletal chitin. Chitobiase has been proven to be a product of the gene regulated by the molting hormones in a crustacean. Chitobiase activity was found to correlate well with the profiles of ecdysteroids during the molting cycle of *Uca pugilator* (Table 1). Injection of the molting hormone 20-hydroxyecdysone resulted in a significant increase in epidermal chitobiase activity (Fig. 2). In their endeavors to elucidate the mechanisms for molt-inhibition by xenobiotics, Zou and Fingerman (1999a, b) investigated the alterations in chitobiase activity following exposure to various molt-inhibiting agents. The inhibition

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**Table 1. Chitobiase activity in the epidermis and ecdysteroid titer during the molting cycle of *Uca pugilator.***

<table>
<thead>
<tr>
<th>Molt stage</th>
<th>Number of animals</th>
<th>Epidermal chitobiase activity ± SD</th>
<th>Ecdysteroids (pg/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>5</td>
<td>197.3 ± 58.0</td>
<td>15</td>
</tr>
<tr>
<td>C</td>
<td>8</td>
<td>107.6 ± 36.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15</td>
</tr>
<tr>
<td>D&lt;sub&gt;0&lt;/sub&gt;</td>
<td>8</td>
<td>114.8 ± 63.1&lt;sup&gt;h,a&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td>D&lt;sub&gt;1&lt;/sub&gt;</td>
<td>8</td>
<td>127.0 ± 44.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30–40</td>
</tr>
<tr>
<td>D&lt;sub&gt;1±4&lt;/sub&gt;</td>
<td>6</td>
<td>214.5 ± 77.9&lt;sup&gt;b,e&lt;/sup&gt;</td>
<td>60</td>
</tr>
</tbody>
</table>

<sup>**</sup>Data sharing a common letter are significantly different from each other. <sup>a</sup>, <sup>b</sup> and <sup>d</sup>,<sup>P</sup> 0.05; <sup>c</sup>, <sup>e</sup> and <sup>f</sup>,<sup>P</sup> 0.01. Data from Zou and Fingerman (1999c).

<sup>1</sup>Enzymatic activity presented as nmol methylumbelliferone liberated · mg protein<sup>–1</sup> · 10 min<sup>–1</sup>.<sup>2</sup>Hopkins (1983).

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**Fig. 1.** Hormonal control of molting in decapod crustaceans.
of chitobiase activity appears to be a general response of *Uca pugilator* to the exposure to molt-interfering agents. Seven-day exposure of *Uca pugilator* to molt-inhibiting agents Aroclor 1242, PCB29, DES, endosulfan, and diethyl phthalate resulted in decreased chitobiase activity in the epidermis (Figs. 3 and 4). This inhibition of epidermal chitobiase activity can at least partly explain the slowing of molting caused by these xenobiotics because this enzyme is indispensable for complete digestion of exoskeletal chitin. Zou and Fingerman (1998, 1999b) proposed that the inhibition of chitobiase activity by these chemical agents strongly suggests that exposure to these xenobiotics can disturb the Y-organ-EcR axis in view of the fact that chitobiase is apparently the product of the gene regulated by the molting hormones and that none of these xenobiotics are capable of inhibiting chitobiase activity in vitro (Fig. 5).

The backtracking approach adopted by Zou and Fingerman (1999a, b) has shed some light on the mechanisms for inhibition of crustacean molting by xenobiotics.

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**Fig. 3.** Effects of three and seven day exposure to Aroclor 1242, endosulfan and diethylstilbestrol (DES) on chitobiase activity in the epidermis of *Uca pugilator*. Enzymatic activity is presented as nmol methylumbelliferone liberated mg protein^{-1} min^{-1}. *P < 0.05; **P < 0.01. Error bars represent standard deviation. N is shown in parentheses. Data from Zou and Fingerman (1999a).

**Fig. 4.** Effects of three and seven day exposure to diethyl phthalate and PCB29 on chitobiase activity in the epidermis of *Uca pugilator*. Enzymatic activity is presented as nmol methylumbelliferone liberated mg protein^{-1} min^{-1}. *P < 0.05; **P < 0.01. Error bars represent standard deviation. N is shown in parentheses. Data from Zou and Fingerman (1999b).

**Fig. 5.** Effects *in vitro* of Aroclor 1242, endosulfan, diethylstilbestrol (DES), PCB29, and diethyl phthalate on activity of chitobiase prepared from the epidermis of *Uca pugilator*. Controls contain 0.7% v/v acetone used as the carrier. Enzymatic activity is presented as nmol methylumbelliferone liberated mg protein^{-1} min^{-1}. Error bars represent standard deviation. N = 4.
biotics. Now the question becomes how the Y-organ-EcR axis can be disturbed by xenobiotics. There are basically two possible action routes, one being that environmental chemicals act through interfering the ecdysteroid signaling in the epidermis and the other is the alterations in ecdysteroidogenesis and/or ecdysteroid disposition by environmental chemicals.

Xenobiotics may interfere with ecdysteroid signaling in the epidermal cells through direct binding to the EcR owing to the fact that the EcRs have been shown to be not strict about their ligands and can be activated by other steroid compounds (Spindler et al., 1992) and even by nonsteroidal chemicals (Wing, 1988; Sohi et al., 1995). This possibility has recently been proven by the finding of Dinan et al. (2001). Using an ecdysteroid-responsive cell line from Drosophila melanogaster as the assay system, combined with an EcR binding experiment, these investigators found that di-ethyl phthalate, a chemical capable of slowing molting of Daphnia magna and inhibiting chitobiase activity in Uca pugilator, is an antagonist of the EcR. Thus, the antagonistic actions on the EcR can at least partly account for the molt-disrupting effects of diethyl phthalate. More screenings are needed to examine whether other xenobiotics, particularly those frequently accumulating in crustacean tissues, can also interact with the EcR.

Ecdysteroid signaling in the epidermis could also be disrupted by environmental chemicals through other mechanisms than direct interactions with the EcR. Besides activation by their respective natural ligands, vertebrate steroid hormone receptors, such as androgen receptor (AR) (Darne et al., 1998), estrogen receptor (ER) (Ignar-Trowbridge et al., 1992) and progesterone receptor (PR) (Bai et al., 1997), can also be activated in a ligand-independent manner. Recently, it has been demonstrated that β-HCH, an organochlorine with no affinity to the estrogen receptor (ER), is capable of activating the ER through a signaling pathway involving the membrane-bound receptor tyrosine kinase (RTK) c-ErbB2 and p44/42 MAP kinase (Hatakeyama et al., 2002). Such ligand-independent activation mechanisms may also exist in crustaceans considering the conservativeness of major pathways of signal transduction, such as the insulin signaling pathway (Claeys et al., 2002), in animals. Although their crustacean counterparts have yet to be characterized, the membrane-bound RTKs, such as Torso (Perrimon et al., 1995), Sevenless (Raabe, 2000) and the Drosophila epidermal growth factor receptor DER (Shilo, 2003), have been reported in insects. Investigations on the likelihood of crustacean EcR being activated by environmental chemicals in a ligand-independent manner would only be possible after the delineation of signaling pathways leading to EcR activation.

The disturbance to the Y-organ-EcR axis may also occur as a result of alterations in ecdysteroidogenesis in the Y-organs and/or ecdysteroid disposition by xenobiotics. Very little is known about the direct impacts of environmental chemicals on the synthesis of ecdysteroids in the Y-organs. Rodriguez Moreno et al. (2003) recently found that exposure of the eyestalk-ablated Chasmagnathus granulata to cadmium inhibits molting of this crab, which presumably results from the direct attack of this metal on the Y-organs. Activities of Y-organs could also be affected indirectly by changes in synthesis and/or release of the MIH from the X-organ sinus gland complex. If a xenobiotic stimulates the synthesis and release of the MIH, the ecdysteroidogenesis would be expected to decrease, whereas an inhibitory effect of an environmental agent on the X-organ-sinus complex would result in an intensification of the Y-organ activities.

Attempts have been made to look into the effects of xenobiotics on enzymatic system responsible for ecdysteroid metabolism. Exposure of Daphnia magna to nonphyphenol, while they are also exposed to testosterone, can inhibit the metabolic elimination of glucosel and sulfate-conjugated testosterone, thereby leading to increased accumulation of testosterone (Baldwin et al., 1997, 1998). Oberdörster et al. (1998) found that sub-lethal exposure of Daphnia magna to tributyltin does not affect molting but increases the production of hydroxylated, reduced/dehydrogenated, and glucose-conjugated metabolites of exogenous testosterone. However, it is still not known whether the testosterone-metabolizing enzymes are also involved in ecdysteroid metabolism in crustaceans. Snyder (1998) reported a new cytochrome P450 member, CYP45, in the lobster Homarus americanus, whose expression in the hepatopancreas correlates well with the profile of ecdysteroid titer during the molting cycle. Hepatopancreatic CYP45 was found to be inducible by the exogenous molting hormone, suggesting the possible involvement of this enzyme in ecdysteroid metabolism and other molting-related events. Snyder and Mulder (2001) observed that exposure of Homarus americanus larvae to heptachlor results in modulations in CYP45 expression and ecdysteroid levels. This shift in ecdysteroid profile is attributed to the slowing of larval molting caused by this pesticide.

CONCLUSION AND FUTURE DIRECTIONS

Unlike the disrupting effects on sexual development, which are usually easily seen, the adverse effects of environmental contaminants on crustacean molting are not readily visible and may have been going on in the wild unnoticed in view of the fact that aquatic environments are increasingly contaminated with various kinds of xenobiotics and many of these contaminants can accumulate in crustacean tissues. Therefore, the disruption of molting by xenobiotics represents an invisible form of endocrine disruption.

Molting is a very important physiological process for crustaceans because it not only allows for growth and development for these animals bearing a rigid, confining exoskeleton but is also tied to metamorphosis during the early stages of the life cycle and reproduction during the adult stage. More environmental xenobiotics should be tested for their abilities to interfere...
with crustacean molting. In this regard, a screening assay that can be used to detect molt-interfering effects of xenobiotics is urgently needed. Mechanistic studies, especially ecdysteroid signaling disruption in the epidermal cells and alterations in ecdysteroid synthesis and disposition, should also be carried out.

REFERENCES


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