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Annual Review

INTERRENAL DYSFUNCTION IN FISH FROM CONTAMINATED SITES: IN VIVO AND IN VITRO ASSESSMENT

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Abstract—Cortisol, synthesized in the interrenal cells of teleost head kidney, has a major role in the physiologic response to physical and chemical stressors. Plasma levels of cortisol increase in physiologically competent fish acutely exposed to stressors such as cadmium or mercury. The effects of chronic low level exposures are less well understood. We have diagnosed an endocrine impairment characterized by a reduced capacity to elevate plasma cortisol levels in response to an acute standardized capture stress in yellow perch (*Perca flavescens*) and in northern pike (*Esox lucius*) sampled at sites contaminated by mixtures of pollutants (heavy metals, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls), by heavy metals, or by bleached kraft mill effluent. Our studies with fish, as well as with amphibians at contaminated sites, demonstrated that low level chronic exposures impair secretion of corticosteroids. We have developed new tests for assessment of the functional integrity of teleost and amphibian interrenal tissue by using an adrenocorticotropic hormone (ACTH) challenge, in vivo and in vitro. The reduced ability to respond to ACTH indicates that the normal neuroendocrine response to stressors may be disrupted and that the ability to cope with biotic and abiotic stressors in the environment may be significantly reduced in the impaired animals.

Keywords—Corticosteroids Interrenal Xenobiotics Fish Amphibians

INTRODUCTION

The endocrine system regulates hormone-dependent physiologic functions necessary for survival of the organism and the species. This system is a potential target of xenobiotics; its vulnerability resides in part in the finely tuned mechanisms through which the endocrine control operates [1]. Minute changes in hormone levels, their receptors, and various related biochemical signals effect significant changes in the activity of target cells and tissues. Xenobiotics can have either direct adverse effects on the endocrine glands and tissues, or their effects can be indirect through alterations of homeostasis and activities of nonendocrine organs [2,3]. The current interest and concern about chemicals that alter the normal endocrine function and physiologic status of animals is rapidly giving rise to an important development of ideas and techniques highly relevant to environmental toxicology. Recent studies in fish endocrine toxicology make an important contribution to an already impressive body of evidence concerning the physiologic and biochemical responses of fish to xenobiotics [4–7].

We have, over the last few years, investigated the effects of xenobiotics on corticosteroid hormones, which together with the catecholamines epinephrine and norepinephrine, are the endocrine effectors of the physiologic response to stressors [8,9]. Although we have worked mainly with fish and amphibians, similarities of the neuroendocrine stress response in all vertebrates [1,3] make our data relevant to other groups of animals. In mammals, the adrenal gland has already been shown to be an important target of specific xenobiotics [3,10].

TELEOST HYPOTHALAMO–PITUITARY–INTERRENAL (HPI) AXIS

Cortisol, the major corticosteroid of teleost fish, is synthesized and secreted by the interrenal tissue situated in the head kidney or pronephros. Both steroidogenic cells and the chromaffin cells that secrete catecholamines are intermingled in islets situated along sinuses of the cardinal vein (Fig. 1). The principal stimulant of cortisol secretion by the steroidogenic interrenal cells is adrenocorticotropic hormone (ACTH) released from the pituitary gland. The activity of pituitary corticotropes, cells that synthesize ACTH, is regulated by corticotropin-releasing hormone (CRH) and other hypophyseal peptides [11]. Photoperiod and temperature are some of the environmental cues that modulate the activity of the HPI axis in fish [9]. A negative feedback effect exerted by cortisol at the level of the hypothalamus and the pituitary also regulates the production of ACTH [12]. A similar organization of the HPI axis is evident in amphibians, although the major corticosteroid secreted is corticosterone and the interrenal tissue, organized in islets, lies on the ventral surface of the kidney [13]. Cortisol and corticosterone are synthesized from cholesterol, the precursor of all steroid hormones. Synthesis of corticosteroids involves first a cleavage of the cholesterol side chain, then hydroxylations mediated by several enzymes, including cytochrome P-450 [1].

FUNCTIONS OF CORTICOSTEROIDS

Receptors for cortisol have been identified in gills, liver, brain, and intestines of teleost fish [14–16]. Corticosteroids have metabolic effects enabling the animal to increase plasma glucose to fuel homeostatic mechanisms activated during exposure to stressors (Figs. 2 and 3). Corticosteroids stimulate gluconeogenesis using amino acids as substrates and they promote lipolysis through permissive actions with other hormones [9]. In lower vertebrates, corticosteroids also exert osmoregulatory effects on ion fluxes, counteracting osmotic perturbations [17,18]. High plasma levels of corticosteroids have

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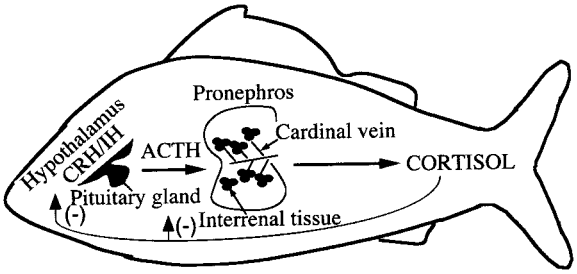

Fig. 1. Hypothalamo–pituitary–interrenal axis in teleost fish. Secretion of cortisol, synthesized by the interrenal tissue, is regulated by pituitary adrenocorticotropic hormone (ACTH) and hypothalamic corticotropin-releasing hormone (CRH), both subjected to a negative feedback effect exerted by cortisol.

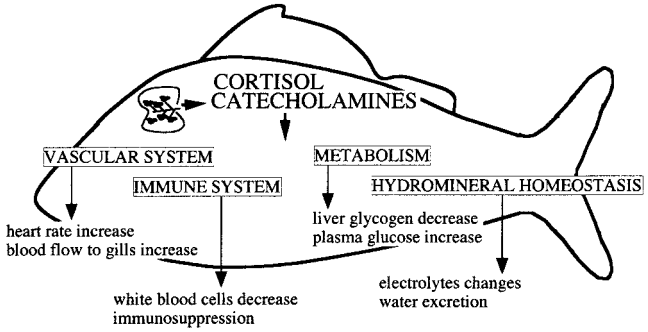

Fig. 3. Neuroendocrine and physiologic responses to stressors. Cortisol and catecholamines activate a suite of biochemical and physiologic responses that facilitate maintenance of homeostasis.

immunosuppressive effects in fish and other vertebrates [19,20]. In addition to permissive actions with metabolic hormones such as glucagon, growth hormone, and thyroxine [1,21], some evidence exists for antigonadal effects of cortisol in fish [22].

ADAPTIVE SIGNIFICANCE OF THE CORTISOL STRESS RESPONSE IN ACUTE EXPOSURES

Corticosteroid hormones, together with catecholamines released from the adrenergic chromaffin tissue, enable animals to cope with stressful situations. They activate a suite of biochemical and physiologic responses [8,9] including a decrease in liver glycogen, an increase in plasma glucose, an increase in heart rate, an increase in blood flow to the gills, and a change in plasma electrolytes (Fig. 3). Acute laboratory exposures to chemical stressors such as cadmium (Cd) and mercury (Hg) elevate plasma levels of cortisol in rainbow trout as well as other fish species [23,24]. Similar responses were observed following acute exposures of fish to constituents of pulp and paper mill effluents [25,26] or to soluble fractions of fuel or crude oil [27,28]. Although substantial evidence exists for elevated plasma cortisol in fish acutely subjected to a wide range of xenobiotics, effects of long-term low-level exposures to chemical stressors are still poorly understood [1]. Several extensive long-term studies were carried out with fish subjected to Cd but even these laboratory studies did not provide conclusive evidence about the status of the HPI axis in the exposed fish. Although several authors reported a decrease in plasma cortisol after several weeks of exposure to Cd, following an initial cortisol peak, it has not been established thus far whether the low levels reflect a complete acclimation to the chemical stressor or an exhaustion of the HPI axis [29–33].

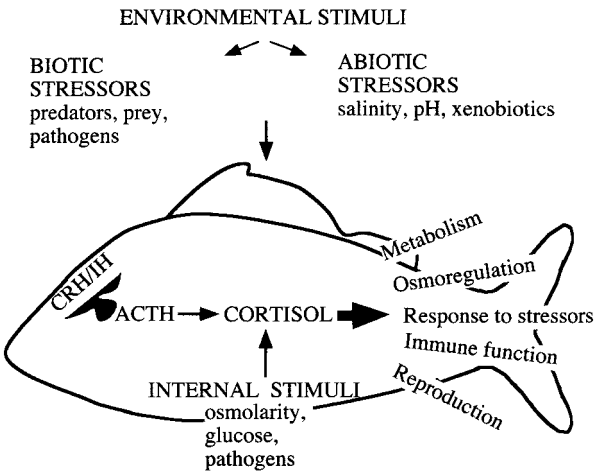

Fig. 2. Functions of cortisol. Plasma levels of cortisol increase during acute exposures to various stressors, including some xenobiotics. Cortisol has a role in the regulation of metabolism, osmoregulation, reproduction, and immune function.

IMPAIRED CORTISOL STRESS RESPONSE IN FISH FROM CONTAMINATED SITES

To characterize the effects of truly chronic exposures to environmental levels of contaminants, we investigated the endocrine and physiologic status of fish sampled at polluted sites. Functional tests that challenge the HPI axis were used to determine whether fish chronically exposed to xenobiotics were able to mount the normal hormonal stress response. In the first series of experiments, we used standardized capture as a challenge to activate the HPI axis, to test the hypothesis that chronic exposures to xenobiotics have an adverse effect on the function of the HPI axis and impair the ability of the fish to respond to additional stressors. Protocols for standardized capture included carefully timed seining, transport to shore, and manipulations of the fish, all executed at about the same time of day, on consectutive days, at contaminated and reference sites [1]. We used mostly yellow perch (*Perca flavescens*) in our field studies because it is a relatively abundant species in north eastern North America, and it spawns and feeds within a well-defined local habitat [34], thus reflecting well the contamination profile of its environment [35–37].

Our experimental protocol using capture as a challenge to the HPI axis revealed a functional impairment of the axis by comparing plasma cortisol levels in fish sampled at contaminated sites and at a matched reference site from the same ecological region. Thus far, we have sampled perch in systems of three different contamination profiles and the plasma cortisol levels in response to a standardized capture stress were consistently lower in fish from the contaminated site compared to the reference site (Fig. 4). These differences were observed in perch at sites contaminated by mixtures of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals in the St. Lawrence River [35,37–39], a site receiving bleached kraft mill effluent (BKME) [40,41], and sites contaminated mainly by heavy metals [36]. Impaired cortisol stress response was also diagnosed in northern pike (*Esox lucius*) sampled at sites contaminated by BKME and by mixtures [38,40]. Similar findings were reported previously in pike from a Canadian Shield lake heavily contaminated by Hg [42]. A positive relationship between body bur-

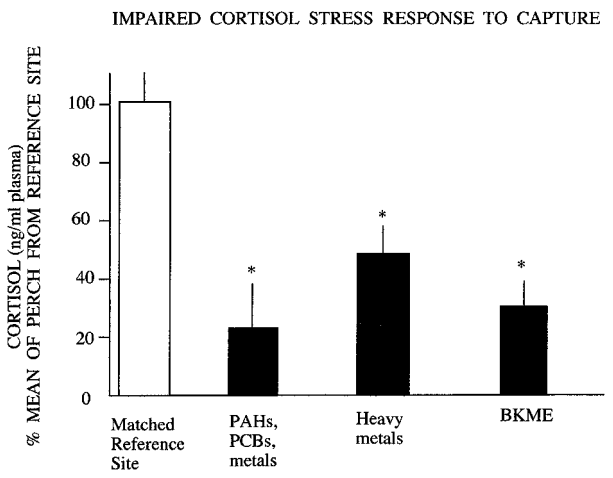

Fig. 4. Impaired cortisol stress response in aquatic vertebrates chronically exposed to xenobiotics. Plasma cortisol levels elicited by capture were lower in perch from contaminated sites compared to those from matched reference sites. Studies were done at sites in the St. Lawrence River system (polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], and metals), upstream and downstream from La Tuque on St. Maurice River (bleached kraft mill effluent [BKME]), and at sites around Rouyn–Noranda (metals). Modified from [35–40].

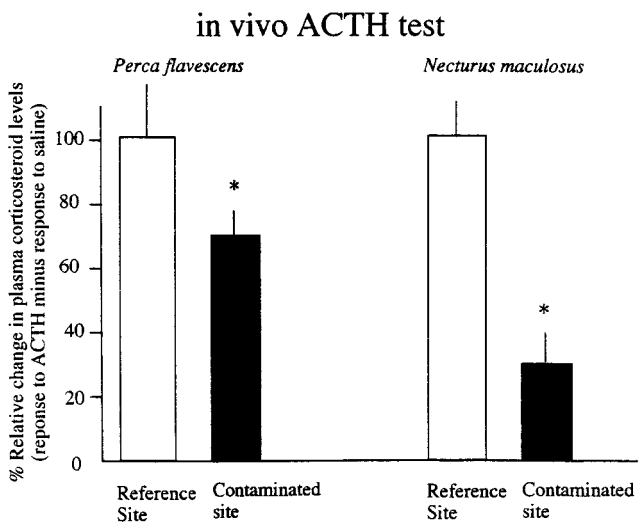

Fig. 5. In vivo adrenocorticotropic hormone (ACTH) test. Plasma cortisol levels elicited by ACTH administered intraperitoneally were lower in a teleost fish, *Perca flavescens*, and an amphibian, *Necturus maculosus*, sampled at contaminated sites, compared to those from a matched reference site. Modified from [13] and [37].

dens of heavy metals and degree of impairment of the cortisol stress response to standardized capture was demonstrated in yellow perch from lakes contaminated by heavy metals [36]. Perch and pike also exhibited structural alterations characteristic of cellular atrophy in the interrenal tissue and pituitary corticotropes [38,40].

The experiments using capture as a challenge to the HPI axis revealed that fish subjected to chronic low-level contamination in their habitats have a significantly reduced ability to elevate their plasma cortisol levels in response to an acute stress. Because the elevation of plasma cortisol is one of the neuroendocrine mechanisms that should facilitate the response to stressors, our present working hypothesis is that cortisol-impaired fish are physiologically compromised and will react inappropriately to various biotic and abiotic stressors encountered in their environment.

NOVEL IN VITRO AND IN VIVO METHODS TO ASSESS INTERRENAL FUNCTION

To evaluate quantitatively the extent of impairment of the interrenal tissue and to identify the cellular site where the HPI axis is disrupted in wild species chronically exposed to xenobiotics, we have developed functional tests using ACTH stimulation in vivo and in vitro.

In vivo ACTH challenge test

The in vivo ACTH test is designed to evaluate the capacity of the animal to respond to a standardized dose of ACTH given by intraperitoneal injection. Adrenocorticotropic hormone is the endogenous stimulant of cortisol secretion and ACTH is released during exposure to a stressor [9,12]. Thus, in vivo treatment with ACTH provides a framework in which the ability of the interrenal tissue to respond to a stimulation, mimicking exposure to a potent environmental stressor, is assessed. The test relies on standardized handling and a challenge with a weight-adjusted dose of $ACTH^{1-39}$, a commercially available peptide (Sigma Chemical Company, St. Louis, MO, USA). The protocol, described in detail elsewhere [37], is simple to execute. Fish are captured by seine and they are kept for 24 h in an experimental floating enclosure in situ. Following the rest period, which facilitates the return of plasma cortisol to precapture levels, fish are injected with saline (100 μl/100 g body weight) or ACTH (4 IU/100 μl/100 g body weight), and their blood is sampled 2 h later. The in vivo ACTH test was used in yellow perch at sites where impairment of the cortisol stress response was previously diagnosed with capture stress [38]. Fish from the polluted site had a significantly reduced ability to respond to a standardized dose of ACTH (Fig. 5) and seasonal and sex differences in the response were also revealed [37]. Even though the dose of the injected ACTH was maximal, cortisol impairment could not be reversed by the ACTH treatment. These results indicate that an interrenal rather than pituitary dysfunction may be responsible for the reduced capacity to secrete cortisol and that cortisol synthesis by the interrenal cells is disrupted at a site downstream from the ACTH receptor. A significantly reduced ability to respond to an in vivo challenge with ACTH was also observed in a species of aquatic amphibian, the mudpuppy (*Necturus maculosus*) [13]. Animals sampled at sites heavily polluted by organochlorinated chemicals had lower plasma corticosterone levels 2 h postinjection with ACTH compared to animals from a reference site (Fig. 5).

These results suggest that the impairment of corticosteroid secretion in chronically exposed animals may be an endocrine dysfunction that occurs in various classes of aquatic vertebrates. It is important to note that once the interrenal function has been assessed using the in vivo ACTH test, animals can be released into the environment [13,37] and possibly sampled again later. Use of nondestructive markers of exposure or effects is becoming an important issue in environmental toxicology and in environmental monitoring, particularly when endangered species are concerned [43].

In vitro ACTH challenge test

To further standardize the assessment of the interrenal function in organisms exposed to xenobiotics and to provide an experimental system in which the mechanisms of action of

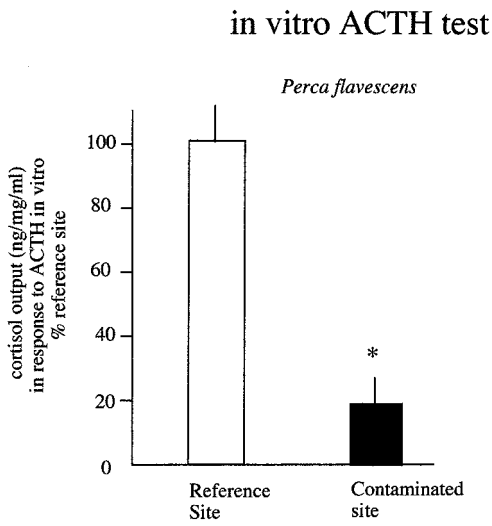

Fig. 6. In vitro adrenocorticotropic hormone (ACTH) test. Cortisol released from isolated perch interrenals in response to ACTH in vitro was lower in tissues from perch sampled at a contaminated site (Lake Osisko) in the Rouyn–Noranda region compared to perch from a reference site (Lake Dasserat). Modified from [36].

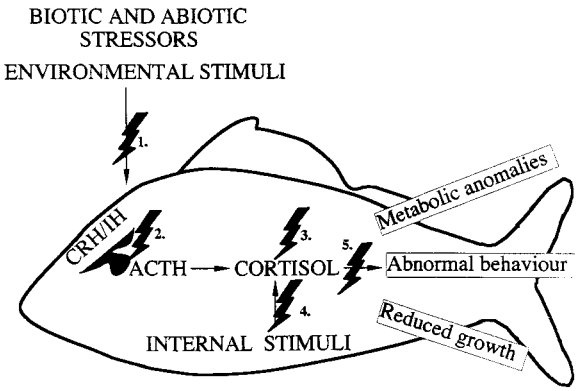

Fig. 7. Consequences of cortisol impairment. Abnormal metabolism, abnormal behavior, and reduced growth are the predicted adverse effects of cortisol impairment. The jagged arrows indicate sites of potential disruption of normal processes. (**1.**) Perception of stimuli. (**2.**) Synthesis and secretion of adrenocorticotropic hormone (ACTH). (**3.**) Synthesis and secretion of cortisol. (**4.**) Perception of internal stimuli. (**5.**) Physiologic functions.

xenobiotics can be investigated, we have developed in vitro methods and protocols that can be used both in the field and in the laboratory. A flow-through perifusion system was designed in which the interrenal tissue is maintained in a complete incubation medium and its response to an ACTH pulse is assessed in vitro [39]. The detection of the cortisol signal in response to a standardized dose of ACTH, relative to basal levels, is facilitated by holding the fish for 24 h postcapture in an enclosure, as is done for the in vivo ACTH test, and by perifusing the isolated interrenals for 90 to 110 min prior to the stimulation with ACTH. A lower response of the interrenal tissue to ACTH was shown in vitro (Fig. 6) in fish from sites contaminated by mixtures of PAHs, PCBs, and metals in the St. Lawrence River [39] and by heavy metals in northern Québec sites [36]. These results yielded further evidence that cellular processes within the interrenal tissue may be impaired by chronic environmental exposures to xenobiotics.

The standardized functional tests using ACTH provide a monitoring framework for quantitative assessment of the interrenal function of aquatic wildlife. The tests make it possible to differentiate between animals that are acclimated to environmental stressors, including xenobiotics, and have a full ability to respond to additional stressors, and animals that have a functionally impaired interrenal and a compromised ability to respond. Thus far, experimental evidence provided by our studies gives support to the latter phenomenon.

CONSEQUENCES OF CORTISOL IMPAIRMENT

Studies in progress are investigating the effects of cortisol impairment on the physiologic status and health of aquatic species exposed to xenobiotics. Carefully matched reference and contaminated sites and/or populations must be used in these studies because cortisol secretion is influenced by various biotic and abiotic factors, including xenobiotics. Acute exposures to stressors that elevate plasma cortisol, are also well documented to increase plasma glucose and decrease liver glycogen [1,23–25,44], facilitating maintenance of homeostasis [8,9,45]. However, the consequences of chronic exposures to environmental stressors, including xenobiotics, on the secretion of glucocoticosteroid hormones and the physiology of aquatic fauna are less well understood [1,17,45]. We have postulated that animals exhibiting an impaired cortisol stress response, a significantly reduced ability to elevate plasma cortisol, are physiologically compromised and are at a disadvantage in coping with environmental stressors (Fig. 7). So far, we have reported that cortisol-impaired fish from contaminated sites had abnormal carbohydrate metabolism, lower condition, and smaller gonads [35,37]. Because cortisol has a role in mobilization of energy substrates, we also hypothesized that the capacity for growth should be altered by an impairment of cortisol secretion. Growth and growth efficiency, which takes into consideration the amount of food the animal consumes, have been estimated in perch from contaminated and reference lakes and preliminary results indicate that cortisol-impaired fish grow less (Sherwood et al., in preparation). Future studies will attempt to elucidate the link between cortisol impairment in animals chronically exposed to pollutants and altered physiologic status. The ability of the cortisol-impaired fish to cope with abiotic and biotic stressors will be investigated through laboratory and field studies.

The neuroendocrine stress response, release of catecholamines and corticosteroids, and the ensuing fight or flight sequence [8,9], are integral components of the physiologic homeostatic mechanisms evolved and conserved in all vertebrates. Impairment of cortisol secretion through chronic exposures to xenobiotics may adversely affect the animal's ability to cope with stressors in its environment and eventually lead to reduced survivorship. It is important to identify and quantitatively assess phenomena that disrupt the normal endocrine processes, particularly in wildlife [46]. Adrenal toxicology is a discipline that has the potential to make an important contribution to our understanding of the impact of chronic low-level exposures to pollutants on wildlife populations.

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