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Interactions of endocrine-disrupting chemicals with stress responses in wildlife*

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Abstract: The extent to which nonreproductive aspects of the endocrine system are affected by environmental contaminants is to a large extent unknown. However, an emerging body of data demonstrates that the neuroendocrine stress response is a sensitive target for disruption by a range of environmental contaminants, at a number of discrete loci. Several mechanisms are responsible for generating and sustaining the corticosteroid response to a stressor, including synthesis of the steroid, negative feedback at the pituitary and hypothalamus, and clearance via metabolism and conjugation in peripheral tissues and the liver. Laboratory and field studies provide evidence that these elements of the stress response are susceptible to interference by endocrine active substances. The functional significance to the individual of interference with this important adaptive mechanism remains to be established.

INTRODUCTION

Animals within the natural environment, whether aquatic, aerial, or terrestrial, are confronted with adverse and challenging conditions that can present a significant threat to their well-being or at worst, survival. The severity of any threat will be exacerbated if the ability of the animal to deploy its normal suite of adaptive or protective responses is compromised. It is the intention of this article to consider to what extent endocrine active substances (EASs) may alter the performance capacity of wildlife via modulation of the mechanisms that are employed in combating environmental stressors.

Despite considerable concern worldwide regarding the extent to which wildlife is being affected by exposure to low, but biologically active levels of EASs [1–3], there are as yet relatively few examples of endocrine disruption (ED) for which causality is established [4] and fewer still where population level impacts have been observed [5]. Indeed some skepticism has been expressed that ED is as significant a threat to wildlife and humans as has been supposed [6–8]. Most (though not all) documented or suspected cases of ED in wildlife concern effects within the reproductive axis, and some well-defined biomarkers of disturbance exist for a limited range of species [9,10]. For most vertebrate endocrine systems, clear markers of disruption are not yet established, and the extent to which nonreproductive endocrine systems, including those involved in adaptive stress responses, are at risk of disruption is not fully understood [11]. Because these limitations apply even more markedly to invertebrates [12,13] this article will consider vertebrates only.

The interrelationship between EAS and non-EAS stressors is potentially reciprocal:

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- Exposure to EAS can modify the response of an animal to a non-EAS stressor.
- Exposure to a stressor may exacerbate effects arising from an existing EAS exposure.

Only the first of these possibilities will be considered in this article. This is because of the high level of uncertainty implicit in the second possibility. When considering ED in general, uncertainty lies in (i) making the link between measures of exposure to suspected EAS and physiological effects, (ii) interpreting biochemical and physiological effects in relation to whole organism fitness, and (iii) relating the relevance of responses in the individual to ecologically significant measures such as population sustainability [14]. These uncertainties are compounded and amplified where the interaction of two levels of disruption must be combined and interpreted. The primary response of vertebrates to most stressors is neuroendocrine in nature. Evaluating the likely interactions between alterations in the endocrine system that arise due to the stressor, and those that may be present due to exposure to EAS is a task that exceeds the scope of our existing knowledge base. Therefore, this article focuses on the mechanisms by which exposure to EAS can modify the response of an animal to a non-EAS stressor. That is, can EAS interfere with the adaptive capability of an animal?

What is a stressor?

The term stress is used in many different contexts leading to some confusion [15–17]. It is often employed in a generalized or nonspecific manner (e.g., ecosystem stress) or may be linked to a specific factor (e.g., pollutant stress). In a physiological, endocrine, or clinical context, stress is taken to have a very specific meaning (see below). A looser definition might include environmental factors that challenge the survival of the animal while not necessarily activating the neuroendocrine stress response. For example, exposure to organic or inorganic contaminants may evoke an adaptive (detoxification/elimination) response in the animal while not disturbing homeostasis sufficiently to activate the endocrine stress axis. For the purposes of this article, stressor will be employed to denote a destabilizing stimulus, the term stress response will define the primary response to the stressor, and exposure to a stressor with subsequent activation of the stress response will be considered to induce a state of stress in the animal. The destabilizing influence, or stressor, may be external or internal in origin.

Responses of wildlife to environmental stressors

Both aquatic and terrestrial animals are exposed to conditions that are potentially detrimental to their well-being. Adverse conditions can arise naturally, or through anthropogenic influences and may be rare, intermittent, or sustained. Four broad classes of stressor can be identified.

- Physical – (i) Abiotic: *temperature, winds, precipitation, flow regimes, suspended sediment, habitat alteration, UV exposure, e.g., [18–22].* (ii) Biotic: *conflict, predator damage, parasite damage e.g., [23].*
- Chemical – *alterations in dissolved oxygen or pH, direct or indirect exposure to pollutants, e.g., [24–26].*
- Physiological – *starvation, dehydration, salinity stress, disease (may arise as a consequence of exposure to physical or chemical stressors), e.g., [27–29].*
- Psychological – *threat of predation, intra- and inter-species conflict/competition, territoriality e.g., [30–33].*

Several constraints must be acknowledged when considering the interactions of physical, physiological, and psychological stressors with EASs in wildlife. The first and most frustrating of these is that we know relatively little about the physiological responses of wildlife to stressors in a natural environment. Because of the difficulties inherent in measuring physiological parameters that are altered by the process of measurement (capture, disturbance) there are few data that demonstrate the effects of

environmental stressors on wildlife. Furthermore, very little is known about natural variation of factors that may be tertiary consequences of stress, such as disease patterns [34], among free-living animals. In contrast to this, for chemical stressors there is a sizeable section of ecotoxicological literature that concerns the measurement of biomarkers of exposure to a range of contaminants. Some responses are specific to a group of compounds (e.g., P450 induction, acetylcholinesterase inhibition) while others may be more general (heat shock proteins, DNA adducts, lysosomal stability, DNA strand breakage, SOD induction etc.).

By inference, the response of wildlife to stressors can be classified within one or more of three response types:

- Neuroendocrine stress response: *(adaptive/maladaptive) – nonspecific in nature, i.e., the response is common to a wide range of stressors, including all those listed above.*
- Behavioral response: *(avoidance/escape; modification of normal behavior; inappropriate behavior) – specific or nonspecific in nature, i.e., the response may be highly appropriate to the stimulus or may be entirely redundant. May be accompanied by a neuroendocrine response.*
- Physiological/biochemical response: *(induction of detoxification processes; direct toxic effects on biochemical processes; respiratory or osmoregulatory dysfunction; activation of nonspecific or specific immune system) – specific in nature, i.e., the response is directly dependent on the identity of the stressor. May be accompanied by a neuroendocrine and/or behavioral response.*

EASs interact with the endocrine system via a limited number of mechanisms. These may be direct (receptor-mediated augmentation, suppression, or modification) or indirect (nonreceptor-mediated interference). Given that all three classes of stress response listed above (neuroendocrine, behavioral, and physiological/biochemical) are either endocrine in nature, or are subject to control or modulation by the endocrine system, it is clear that all are potentially susceptible to the influence of EASs. The potential for interaction between EASs and the response of animals to stressors will be considered on the basis of the best evidence available from appropriate field and laboratory studies. At the present time, most of the available evidence concerns the influence of contaminants on the neuroendocrine stress response and this will therefore be the focus of this article.

INTERACTION OF ENDOCRINE-DISRUPTING CHEMICALS WITH STRESS RESPONSES

EASs and the neuroendocrine stress response

Neuroendocrine stress response

It is a central tenet of stress biology that the stress response is part of an adaptive strategy to cope with a perceived threat to homeostasis [35–39]. In response to a stressor, the animal alters its behavior and physiology to best serve the goal of reestablishing or preserving homeostasis. The stress response is highly conserved throughout the vertebrate taxa, emphasizing its adaptive value [see 40: nonhuman mammals; 41,42: fish; 43: reptiles; 44: birds]. The brief description below is drawn from these and the reviews of Chrousos and Gold [35], Johnson et al. [36], and Weissman [45].

The neuroendocrine stress response is initiated by perception of a threat. Identification of potentially threatening stimuli occurs within the higher centers of the central nervous system, and habituation or acclimation may occur to benign but initially stressful stimuli. The response comprises two elements; one originating within the sympathetic nervous system (rapid), the other a wholly endocrine response centered on the hypothalamic-pituitary-adrenal (HPA; or interrenal, HPI) axis (slow). Activation of the sympathetic nervous system results in the release of the catecholamines epinephrine (adrenaline) and norepinephrine (noradrenaline) into the circulation, primarily from the adrenal medulla (chromaffin tissue in lower vertebrates) and sympathetic nerve terminals. The catecholamines act at sites within the cardiorespiratory system resulting in optimization of the oxygen carrying and delivery

capacity of the blood and of oxygen uptake from the environment. They also have positive effects on energy mobilization.

The endocrine response originates within the hypothalamus with the release of corticotropin-releasing hormone (CRH) that in turn stimulates the release of adrenocorticotropin (ACTH) from the pituitary. ACTH enters the general circulation and stimulates the synthesis and secretion of corticosteroids (cortisol or corticosterone, dependent on species) by the adrenal cortex (the interrenal in lower vertebrates) into the blood. These changes constitute the core or primary endocrine stress response, but stress also results in the increased or reduced secretion of several other pituitary hormones including prolactin, growth hormone, the gonadotropins, somatolactin, and the endorphins.

The stress response is conserved throughout the vertebrates, and must be assumed to offer significant adaptive value. It is therefore something of a paradox that activation of the stress response can also cause problems for the animal, that may be life-threatening in severity. Chronic or frequent activation of the response results in growth suppression, reproductive dysfunction, and immunosuppression. In higher vertebrates chronic or intermittent stress is linked to psychoneuroendocrine and emotional disruption, while in lower vertebrates normal behavior patterns can be disturbed.

Conventional (non-EAS) pollutants and the neuroendocrine stress response

It is well known that a neuroendocrine stress response may be initiated by exposure to toxic contaminants (e.g., in fish, see [46]) in addition to any specific detoxification mechanisms that might be activated. Apportioning the physiological consequences of this combined response is difficult because prolonged activation of the HPA axis is inherently harmful (as discussed above) and thus adverse effects of chronic stress may occur simultaneously with direct toxic effects of the contaminant. Although exposure to contaminants may elicit an endocrine response, this does not represent ED in the widely accepted sense; such a response can be considered to be a normal function of the intact HPA axis—a response to destabilizing influences (unless, of course, the endocrine response is itself damaged, in which case the non-EAS becomes an EAS). However, it would be difficult to discriminate between a stress response arising due to activation of the neuroendocrine cascade, and elevated corticosteroid levels that might arise due to direct stimulatory effects of contaminants on elements of the hypothalamic-pituitary-adrenal/interrenal cascade (see, e.g., [47,48]).

Endocrine-disrupting chemicals and the neuroendocrine stress response

In principle, all the elements of the neuroendocrine stress response are susceptible to interference by endocrine-disrupting contaminants, and therefore it must be assumed that EAS can affect the ability of an animal to mount an appropriate stress response. Although to date there have been no documented cases of direct interference with peptide signalling systems by xenobiotic hormone mimics it is likely that indirect effects occur, for example by interference in the biosynthesis of peptide hormones, or by alteration of regulatory mechanisms (e.g., steroid feedback on peptide secretion, a mechanism which operates extensively in the pituitary; see [49] for a possible example of disruption at this level). Certainly the distal portion of the HPA/I cascade is particularly susceptible to modulation because of its reliance on steroid hormone signalling. Several mechanisms are responsible for generating, sustaining and terminating the corticosteroid response to a stressor, including synthesis of the steroid, negative feedback at the pituitary and hypothalamus, and clearance via metabolism and conjugation in peripheral tissues and the liver. Laboratory and field studies have provided evidence that these elements of the stress response are susceptible to interference by EASs.

Effects of chemical contaminants on pituitary function

During the response to a stressor, the pituitary secretes ACTH following stimulation by CRH of hypothalamic origin. The ACTH-secreting cells (corticotropes) are also sensitive to negative feedback by corticosteroids. There are limited data that describe contaminant effects on pituitary function in this context. Exposure to various classes of organic contaminants results in the induction of those biotransforming enzymes that participate in both xenobiotic metabolism as well as endogenous substrate me-

tabolism [50]. A cytochrome P450 monooxygenase system is known to exist in the vertebrate brain and induction of P450 isoenzymes has been reported to occur in the pituitary of rats and rainbow trout (*Oncorhynchus mykiss*) [51,52] treated with β-naphthoflavone and other organics [53]. In trout, the enzymes were found in gonadotropic cells and exposure to β-naphthoflavone resulted in alterations of gonadotrope function. It is likely that corticotropes could be similarly affected. Depending on the substrate specificity of the induced enzymes, this is a potential route by which steroid metabolism within the pituitary, and therefore feedback regulation of pituitary hormone secretion might be modulated.

Corticotrope dysfunction has been reported to occur in fish exposed to xenobiotics. In pike (*Esox lucius*) and perch (*Perca flavescens*) that were recovered from sites polluted by polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and mercury, the corticotropes were atrophied compared to cells from fish at unpolluted reference sites. The fish that displayed abnormal corticotropes also failed to elevate blood cortisol levels in response to the stress associated with capture [54]. In this case, whether the atrophy was the result of direct toxic effects, or prolonged negative feedback suppression by corticosteroids, or the failure of some other regulatory mechanism, was unclear.

There are limited data that suggest that the bioactivity of key hormones associated with the stress response can be altered by exposure to organic contaminants. Rat anterior pituitary cells exposed to the aromatic hydrocarbon 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in vitro secreted ACTH, both constitutively and in response to CRH stimulation, which was less potent in stimulating corticosterone secretion from cultured rat adrenal cells than was equimolar quantities of ACTH. The authors interpreted these results to indicate that TCDD was altering the bioactivity of the secreted ACTH [55]. Subsequent studies with TCDD have not produced identical results, although evidence has been found that TCDD disturbed HPA function in male rats [56] and that TCDD stimulated the synthesis and secretion of ACTH by the anterior pituitary under basal (unstressed) conditions, but reduced pituitary responsiveness to CRH stimulation [57].

Effects of chemical contaminants on plasma corticosteroids

Corticosteroids are synthesized de novo in the adrenal cortex (or interrenal tissue in lower vertebrates) following stimulation by ACTH. Some short-loop feedback by corticosteroids may occur at this level [58]. Peripheral conversion of corticosteroids occurs together with significant levels of biotransformation and conjugation in the liver [59]. Effects on circulating levels of corticosteroids have been reported for many fish species exposed to organic and inorganic contaminants.

Plasma levels of cortisol were lower in white sucker (*Catostomus commersoni*) exposed to bleached kraft pulp-mill effluent (BKME) than in fish from unpolluted sites [60] and in eels (*Anguilla anguilla*) exposed to 50 % secondary-treated BKME normal cortisol secretion was prevented and cortisol accumulated within the interrenal tissue [61]. Similarly, in schelly (*Coregonus lavaretus*) exposed to untreated or treated BKME, the increase in plasma cortisol following air exposure was attenuated [62]. Eels exposed to diesel oil water-soluble fraction also displayed an attenuated stress response [63], and the cortisol response to air exposure of marbled sole (*Pleuronectes yokohamae*) collected from Tokyo Bay was reduced in comparison with that of fish from less polluted reference sites [64]. Yellow perch (*Perca flavescens*) sampled from sites polluted by multiple organic and inorganic pollutants displayed an attenuated plasma cortisol response to capture and to ACTH challenge relative to the response of fish from a reference site [65,66], and the ability of inorganic contaminants to induce interrenal dysfunction was further indicated by the reduced interrenal responsiveness to stressors exhibited by yellow perch from lakes contaminated with Zn, Cu, and Cd [67] and by brown trout (*Salmo trutta*) from metal-contaminated sites [68]. Administration of β-naphthoflavone to rainbow trout resulted in a lower increase of plasma cortisol than was observed in control fish following a brief disturbance [69] or heat shock [70] and the administration of PCB 126 to tilapia (*Oreochromis mossambicus*) and rainbow trout via the diet resulted in reduced cortisol responsiveness to a confinement stressor [71,72].

Similar effects to those described for fish have been reported to occur in the mudpuppy, an aquatic salamander (*Necturus maculosus*), exposed to organic pollutants. Animals from impacted sites dis-

played a significantly lower corticosterone response to capture and confinement, and to ACTH challenge, than animals from reference sites [73].

Effects on corticosteroid levels have also been observed in contaminant-exposed birds. In a study of herring gull (*Larus argentatus*) embryos environmentally exposed to organochlorine contaminants in ovo at various Great Lakes sites, basal plasma corticosterone concentrations were inversely related to concentrations of organochlorine residues in the yolk sacs [74]. The authors interpreted these findings as indicating that organochlorine contamination was adversely affecting the HPA axis in contaminant-exposed gulls.

In marked contrast to these data for amphibians, birds, and fish, juvenile alligators (*Alligator mississippiensis*) sampled from a severely contaminated (agricultural runoff, sewage, pesticides) lake and an uncontaminated lake showed no difference in their ability to mount a corticosteroid response to capture and confinement stress [75]. This was despite there being significant differences in blood androgen levels in males from the two lakes, indicative of disruption of the reproductive endocrine system. The authors interpreted this as evidence that gonadal steroidogenic capacity may be modified without necessarily altering overall steroidogenic ability of the animal. Arguably, these findings simply emphasize that the site of lesions within the endocrine system is specific to the identity of the contaminants to which the animals are exposed or to the species concerned. It is also worth bearing in mind the methodological problems associated with measuring levels of hormones that are extremely sensitive to disturbance. If it is not possible to standardize methods of capture the resultant variability in corticosteroid levels can mask underlying differences.

Effects of chemical contaminants on interrenal responsiveness to ACTH

Alterations in the responsiveness of the adrenal/interrenal tissue to ACTH is evidently a primary factor underlying the modification of stress responsiveness by xenobiotics, presumably via interference in the detection and transduction of the ACTH signal or from a reduced steroidogenic capacity of the adrenal/interrenal tissue. In tilapia, the DDT metabolite *o,p′*-dichlorodiphenyldichloroethane (*o,p′*-DDD) suppressed the response of interrenal tissue to ACTH both in vitro [76] and in vivo [77]. In rainbow trout, *o,p′*-DDD was found to be adrenotoxic, disrupting the 3′,5′-monophosphate (cAMP) generation step [78]. The adrenotoxicity of *o,p′*-DDD is discussed further in the next section.

Interrenal tissue from β-naphthoflavone (BNF)-treated rainbow trout was less sensitive to ACTH in vitro than that from control fish. Treatment with BNF in vivo did not significantly affect either turnover or tissue distribution of cortisol, leading the authors to conclude that cortisol clearance mechanisms were not affected [69]. Exposure of interrenal cells from rainbow trout to the organochlorine pesticide endosulfan in vitro decreased the ACTH- or dbcAMP-stimulated cortisol secretion in a concentration-dependent pattern. The doses required to disrupt cortisol secretion were significantly lower than those that were lethal to the head kidney cells [79]. Reduced interrenal responsiveness to ACTH and dbcAMP was also exhibited by yellow perch from metal-contaminated lakes [67,80,81]. The suppressive effects of dietary PCB 126 on the stress response of tilapia and rainbow trout was also linked to impairment of interrenal steroidogenic capacity with both ACTH and cAMP-stimulated cortisol release from interrenal tissue in vitro being lower in PCB-treated fish [71,72]. In these studies, the attenuated response observed in response to cAMP stimulation confirmed that disruption occurred downstream of the ACTH receptor itself. Similarly, the reduced cortisol response to stress observed in marbled sole from Tokyo Bay was associated with impairment of cortisol release from the head kidney following in vitro ACTH treatment [64].

The impairment of the stress response in perch from polluted sites has been linked to the age of the fish. The plasma cortisol elevation in response to capture stress was reduced in perch of 4 years and older relative to fish from uncontaminated sites, but was not affected in fish younger than 4 years [81]. As in other studies, failure of the interrenal tissue of 4+ fish to respond fully to ACTH appeared to contribute to the disparity in responsiveness. The authors suggested that it is the cumulative effects of lifelong exposure to polluted conditions that accounts for the age effect. Age of the animal and duration of

exposure are not factors that have been focused upon by other authors, and it may be that age is only of significance where the concentrations of contaminants to which animals are exposed are low, or exposure is intermittent.

Leopard frog (*Rana pipiens*) tadpoles exposed to a PCB congener 77-TCB showed decreased whole-body corticosterone content relative to controls both before and after injection with ACTH [82]. However, a study on toads (*Bufo terrestris*) exposed to coal combustion wastes provided results that contrast with most of the reports cited above. In exposed toads, corticosterone levels were high, and remained so after laboratory acclimation. Furthermore, injection of ACTH had no effect on circulating corticosterone levels in exposed toads, whereas an increase was observed in toads taken from reference sites [83]. The results suggest hyperactivation of the interrenal tissue, and given the considerable adverse effects of chronically elevated corticosteroid levels this response is likely to be more damaging to the individual than a dysfunctional stress response.

Effects of chemical contaminants on interrenal steroid synthesis and metabolism

The mechanisms by which steroidogenic capacity of the interrenal tissue might be altered by xenobiotics are not addressed in detail by most of the studies cited above. One mechanism may involve the P450 isoenzymes which are critical elements in the biosynthesis of steroids within the adrenal/interrenal tissue. There is potential for P450 enzymes, induced by the presence of a xenobiotic, to impact on the normal processing of steroid hormones of endogenous origin [84]. In rainbow trout treated with 3,3′,4,4′-tetrachlorobiphenyl (TCBP), hepatic uptake and catabolism of cortisol was increased, data interpreted by the authors to indicate that the fish may not be able to fully respond to a stressful stimulus [85]. Unfortunately, this was not investigated further (i.e., the fish were not subjected to a stressor) and the increased uptake and metabolism of cortisol was accompanied by plasma levels of cortisol in otherwise unstressed fish that were four-fold higher than those in control fish. Of course, it is almost impossible to obtain reliable estimates of plasma cortisol levels in unstressed free-living fish from a riverine or lacustrine environment, so we know nothing about baseline cortisol levels in fish from contaminant-exposed populations. There was no effect on cortisol production in the interrenal tissue of rainbow trout exposed to 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB) despite the conversion of progesterone to 17α-hydroxyprogesterone and 11-deoxycortisol being increased by HCB treatment [86]. The physiological implications of these observations are unclear. A further complication is presented by the fact that certain P450 isoenzymes in fish are modulated by estrogens and androgens [87,88], raising the possibility that estrogenic contaminants could exert effects on hepatic or interrenal steroid metabolism [89].

Several studies in mammals have suggested mechanisms by which contaminants may interfere with the function of the adrenal/interrenal tissue. In rats treated long-term with TCDD baseline corticosterone production by adrenal tissue was markedly reduced in vitro, although ACTH-stimulated release was comparable to that of controls, a result interpreted as indicating a reduction in the bioactivity of ACTH in the TCDD-treated animals [90]. In mouse adrenal tumor cells, cadmium chloride, acetate and sulfate inhibited basal steroid production and ACTH-stimulated steroid secretion in a dose-dependent fashion [91,92]. The authors postulated interference by Cd with both the transduction of the ACTH signal and elements of the steroidogenic pathway. Effects on basal secretion were clarified as being related to the inhibition of cholesterol and 25-hydroxycholesterol utilization [93], and both these and effects on ACTH-stimulated steroid secretion may be related to effects on adenyl cyclase activity [94]. Mercury treatment of rats caused dysfunction of adrenal steroid biosynthesis by inhibiting the activity of 21α-hydroxylase with the effect of lowering plasma levels of corticosterone [95]. A similar effect on plasma corticosterone levels was observed in rats exposed to Cu^{2+}. In this case, the reduction of corticosterone levels was attributed to effects on the cytochrome P450 enzymes responsible, in particular, for 11β-hydroxylation [96].

Perhaps the best characterized agents of adrenotoxicity are the DDT metabolites 3-methylsulfonyl-DDE and *o,p′*-DDD, both of which interfere with steroid synthesis in the adrenal cortex. The sulfone is a competitive inhibitor of 11β-hydroxylase, a key element of corticosteroid synthesis [97] and

has been associated with adrenal dysfunction in wildlife [98,99] and in rodent [100] and human cell lines [101]. The metabolite *o,p′*-DDD reduces the elevation of plasma cortisol following stress in rainbow trout [102] and the corticosteroid-inhibiting properties of this metabolite have even been exploited therapeutically in the treatment of Cushing's syndrome [103]. In contrast to these data, a recent study on Arctic charr (*Salvelinus alpinus*) failed to detect any effects of a single (force-fed) oral administration of *o,p′*-DDD on the ACTH and cortisol response to a handling stressor [104]. Whether this result reflects the prolonged period between dosing and imposition of the stressor (28 days) or represents a genuine difference in species sensitivity is uncertain.

In mice, oral administration of the chlorinated insecticide lindane led to significant reductions in circulating corticosteroid levels [105]. This effect is evidently related to the adverse effects of lindane on the expression of steroidogenic acute regulatory (StAR) protein [106]. StAR mediates the intramitochondrial transfer of cholesterol to the P450 (side chain cleavage; SCC) enzyme—the rate-limiting and acutely regulated step in hormone-stimulated steroidogenesis. Lindane-induced reduction in second messenger production may also contribute to suppression of steroidogenesis [107].

Effects of chemical contaminants on the sensitivity of target tissues to corticosteroids

Corticosteroids exert their effects at target tissues by interaction with a specific receptor protein that may be either internal or external to the cell. Internal cytosolic receptors mediate effects of the steroid at the genomic level whereas membrane-bound cortisol receptors mediate more rapid responses via a coupled G-protein mechanism (e.g., depolarization) [108]. Corticosteroid receptors are "self-regulated", that is, prolonged elevation of the ligand induces a reduction in receptor abundance within the cell [109]. Therefore, there is scope for a corticosteroid mimic both to evoke a target tissue response by interaction with the receptor and also to cause a down-regulation of receptor and thereby reduce the sensitivity of the tissue to the endogenous ligand. Up-regulation of the estrogen receptor in rainbow trout liver has been observed in response to estrogen mimics [110,111], and there is no reason why a similar effect should not occur as a consequence of exposure to corticosteroid mimics.

There are few studies that have examined the interactions of xenobiotics with the corticosteroid receptor. Unsurprisingly, a range of known estrogen mimics displayed no affinity for the rainbow trout cortisol receptor [112]. However, Johansson et al. [113] found significant interaction of persistent PCB metabolites with the human glucocorticoid receptor using a response element and reporter enzyme construct. They demonstrated that a number of methylsulfonyl PCBs displayed IC50s of 2.7 μM or greater and interpreted these results to be of functional significance.

Effects of chemical contaminants on the magnitude of the stress response

An animal may modify the magnitude and/or duration of the stress response in order to address changing circumstances. This is particularly true during reproductively active periods, and attenuation of stress responsiveness has been reported in fish [114], birds [115], and mammals [116]. Changes in magnitude of the response appear to be controlled by gonadal steroids with androgens generally exerting a suppressive effect and estrogens a stimulatory effect on the response [117]. It is therefore conceivable that exposure to environmental androgens, estrogens, or agonists/antagonists of these compounds will also exert a modulating effect on the stress response.

The life-long characteristics of the stress response in an individual can also be altered as a consequence of prenatal or antenatal exposure to elevated levels of corticosteroids with both endocrine and behavioral consequences [118,119]. These changes arise from alterations in the differentiation of neurons that are involved in the negative feedback regulation of the HPA axis and in particular changes in glucocorticoid receptor gene expression in the hippocampus and frontal cortex [120]. The possibility therefore exists that parental exposure to environmental corticosteroid agonists/antagonists might result in the modification of important adaptive traits in their offspring, or that exposure of juveniles to corticosteroid agonists/antagonists will modify this important adaptive mechanism.

EASs and the behavioral response to stressors

The stress response itself may contain adaptive behavioral elements and exposure to stressors can also result in behavioral modification [121]. In general, stress exerts an inhibitory effect on "nonessential" activities such as reproductive behavior [122,123]. This aspect of the stress response of animals is less well studied than the underlying neuroendocrinology of stress and there are inadequate data available to support extensive speculation on the likely effects of EASs in this context. However, it is well established for vertebrates that much behavior is susceptible to influence, or is controlled, by steroid hormones, particularly reproductive behavior [124] and it is likely therefore that any stress-related behavioral activities that are steroid dependent will also be susceptible to disruption or modulation by EASs. This certainly appears to be the case for reproductive behavior in fish, which is altered by exposure to environmental ethinylestradiol [125].

EASs and the physiological/biochemical response to stress

The adaptive responses that are adopted by organisms exposed to potentially toxic contaminants are extremely well documented, and, as indicated above some of these mechanisms are regulated or modulated by the endocrine system. It is beyond the scope of this short article to address the complex issue of how these responses might be affected by EASs.

RESEARCH NEEDS

Research effort and public and governmental concern have understandably focused on the reproductive and developmental implications of ED, and even here matters are by no means resolved. The extent to which nonreproductive aspects of the endocrine system are affected by environmental contaminants is largely unknown. There is an increasing body of evidence that demonstrates that the neuroendocrine stress response is a sensitive target for disruption by a range of environmental contaminants, at a number of discrete loci. However, it remains to be established precisely how this affects the performance capacity of the individual. The benefit provided to the animal by possession of a functional neuroendocrine stress response is difficult to quantify, but is assumed to be substantial because of the conservation of this response throughout vertebrate taxa. If the response is attenuated, as much of the data presented here suggest is likely in polluted environments, what is the cost to the individual? Or is the cost of the attenuated stress response subsidiary to the presence of other effects (reduced growth, immunotoxicity, etc.) that may be more intrinsically harmful to both the individual animal and the population? It is clear that a considerable amount of research is required to address these issues that cannot be satisfactorily answered with the information currently at our disposal. The questions that require consideration include:

- How broad is the range of chemicals that interact with elements of the neuroendocrine stress response?
- Are the concentrations at which effects on the functioning of the stress response are observed lower than existing regulatory limits?
- Are these chemicals ubiquitous enough in the environment to cause widespread effects or are they restricted to localized hot spots?
- What is the functional significance to the animal of interference with the normal functioning of the stress response?

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