CORTICOSTERONE STRESS RESPONSE IN TREE SWALLOWS NESTING NEAR POLYCHLORINATED BIPHENYL- AND DIOXIN-CONTAMINATED RIVERS

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Abstract—We assayed baseline and stress-induced corticosterone concentrations from adult female and nesting tree swallows, Tachycineta bicolor, from New England, USA, sites with different levels of contamination with polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Corticosterone was compared over 2 years from a highly contaminated PCB site along the Housatonic River (Berkshire County, MA, USA), a midrange contaminated site upstream, and a reference site. Adult females (n = 29), sampled only in 2003, showed an inverted-U association with PCBs, with higher stress-induced corticosterone with midrange contamination than at the high-contamination site. In nestlings, stress-induced corticosterone was highest for the highly contaminated site compared with the other sites in 2003 (n = 53, 29 nests), with no difference among sites in 2004 (n = 93, 27 nests). In 2004, we began testing mechanisms underlying these changes in nestlings at the high- and low-PCB sites. Corticosterone response to dexamethasone injection (used to test negative feedback) was not different between sites, but stress-induced corticosterone was reduced at the contaminated site after adrenocorticotropin hormone injection (used to test adrenal responsiveness), suggesting an inhibited ability to mount a stress response. We also compared nestlings from a stretch of the Woonasquatucket River, Rhode Island, USA, heavily contaminated with TCDD (n = 80, 43 nests) with nestlings from an upstream site that had lower levels of TCDD and the Berkshire County reference site. Although there were no stress-induced differences, baseline corticosterone was lower at the higher TCDD site than at the reference site. Altogether these findings suggest that tree swallows chronically exposed to high PCB and TCDD levels exhibit altered baseline and stress-induced corticosterone responses, but the patterns of alteration might not be predictable.

Keywords—Corticosterone Stress Contaminants Tree swallows

INTRODUCTION

Measuring contaminant concentrations in blood and other tissues in wildlife living in polluted sites is important for determining the level of exposure to contaminants but provides limited information about the effects on wildlife health. To demonstrate an effect on health, it is important to identify and measure suitable health parameters or biomarkers. Furthermore, contaminants and other environmental stressors could have synergistic effects, so measurement of suitable biomarkers can identify effects not necessarily predicted by contaminant levels [1,2]. Considerable attention has been given to evaluating the effects of contaminants on the endocrine system in free-living vertebrates, particularly on reproduction and development (e.g., [3,4]) and, to a lesser degree, on the adrenocortical stress response (e.g., [5,6]).

Vertebrates, including humans, respond to noxious stimuli (stressors) by evoking the stress response, which involves a suite of behavioral and physiological responses necessary for coping with adverse environmental conditions [7]. However, when the stress response is inappropriately initiated or maintained (i.e., chronic stress), the health of the animal deteriorates. Chronic stress increases overall energy consumption, which reduces energy available for other energetically expensive functions. Several important processes are affected, including glucose metabolism; musculoskeletal health; growth, tissue repair; and immune, cardiovascular, reproductive, and neurologic functions [8–10]. Therefore, a measurable indicator of the stress response can provide information about the potential health of an animal.

One of the main systems activated during the stress response is the hypothalamic–pituitary–adrenal axis that culminates in glucocorticoid release (i.e., cortisol in humans, corticosterone in birds) several minutes after exposure to a stressor. Glucocorticoids (GCs) are secreted into the bloodstream from the adrenal or interrenal glands, and secretion is regulated by adrenocorticotropin hormone (ACTH) released by the pituitary. Adrenocorticotropin hormone release, in turn, is regulated by additional secretory hormones from the hypothalamus. Classic negative feedback loops then turn off GC release. Glucocorticoids are influential in many physiological processes and have multiple effects on the body [10]. Measurement of GCs allows for analysis of relative stress levels between individual animals and animal populations within a species [11,12], which has been inferred to indicate differences in relative health [13,14].

An excellent model species for studying contaminant effects on the stress response is tree swallows (Tachycineta bicolor).

Tree swallows forage close to their nests, eat mainly emergent aquatic insects, and readily occupy nest boxes placed in appropriate habitats, allowing for easy sampling of incubating females and nestlings [15]. For these reasons, tree swallows are used widely as indicators of local contamination (e.g., [16–19]). Currently, little is known about how local contamination...
affects the health of these animals. Our major goal with this study was to investigate whether the corticosterone stress response can be used as a bioindicator of contaminant-induced health effects.

Our main study sites were two heavily contaminated areas. The first sampling area was located downstream from a Superfund Site in Pittsfield, Massachusetts, USA, where construction and repair of electrical transformers occurred for much of the mid-20th century [20]. Tree swallow eggs collected along the Housatonic River in Lenox, Massachusetts, USA (directly downstream from Pittsfield), have some of the highest concentrations of total polychlorinated biphenyls (PCBs) reported, with means ranging from 31.5 to 100.9 μg/g wet weight [16]. A negative relationship between hatching success and PCB concentrations has been documented at this site [16].

The other main site was along the Woonasquatucket River at a Superfund Site northwest of Providence, Rhode Island (USA). Concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in tree swallows nesting in this area in 2000 and 2001 were some of the highest ever reported in avian tissue [19]. The source of contamination was from textile and chemical manufacturing plants and a drum recycling operation. Custer et al. [19] found that at the two most contaminated ponds, Allendale and Lyman, mean concentrations in eggs ranged from 300 to >1,000 μg/g. Accumulation rates and contaminant concentrations in diet samples from nestlings imply that the contamination was accumulated locally. As with PCB contamination at the Lenox site, hatching success was negatively associated with concentration of TCDD in eggs [19].

We used these two main sites, along with lesser contaminated and reference sites, to investigate the effect of chronic contaminant exposure on the corticosterone stress response in tree swallows. Furthermore, we attempted to identify the underlying mechanisms regulating changes in corticosterone release. We injected dexamethasone (dex, a synthetic GC) to investigate whether negative feedback is altered. Dexamethasone should inhibit endogenous corticosterone secretion by binding to corticosterone receptors, and the lack of a response indicates impaired negative feedback. We hypothesized that feedback would be impaired (i.e., decreased response to dexamethasone) at contaminated sites. We also injected ACTH to assess adrenal responsiveness. Corticosterone levels should increase after ACTH injection, and the lack of a response indicates impairment at the level of the adrenal gland [21].

MATERIALS AND METHODS

In 2003, we sampled adult females and nestlings (11–13 d old, nestlings were not sexed) from nest boxes located in an area highly contaminated with PCBs on the Housatonic River, downstream of the primary polychlorinated biphenyl contamination source (Lenox Road, Lenox); an upstream tributary site with lower contaminant levels (Southwest Branch, Pittsfield); and a reference site located in the Housatonic River basin (Threemile Pond, Great Barrington, MA, USA). Nestlings were also sampled in 2003 from a stretch of the Woonasquatucket River highly contaminated with TCDD (Allendale Mill and Lyman Mill Ponds, northwest of Providence) and an upstream reference site on Woonasquatucket Reservoir (called Fire Station). Both reference areas had much lower concentrations of either PCBs or TCDD than their respective contaminated sites [16,19]. With few exceptions, two nestlings were sampled per nest box in 2003. Only nestlings from the Housatonic River and Threemile Pond reference site were sampled in 2004 (see further description below).

Sampling methods for corticosterone analysis were as follows: Adult females were captured in nest boxes while incubating eggs. Nestlings were sampled at 11 to 13 d of age. For each sample, approximately 50 μl of upwelling blood was collected in heparinized microhematocrit tubes after lancing the wing vein with a sterile needle. Initial blood samples were collected from both adults and nestlings within 3 min of capture to reflect precapture (baseline) levels [22]. Birds were restrained in cloth bags for 30 min, except while measurements were being taken. Another blood sample was collected at the end of the 30 min of restraint, before release back into the nest, to measure corticosterone secretion resulting from the stress of capture and restraint. This sample reflects the animal’s ability to mount a physiological stress response [7].

In 2004, we sampled nestlings from the highly contaminated PCB site (Lenox Road) and the reference site (Threemile Pond). Sampling focused on trying to identify a mechanism to explain significant findings in nestlings from 2003. After baseline sample collection, nestlings were injected with one of three treatments: 10 μl saline subcutaneously (SC) as a control, 20 μg dex in 10 μl saline SC [23] to evaluate negative feedback, or 2.5 IU ACTH in 10 μl saline SC [23] to assess adrenal responsiveness. The number of nestlings per nest receiving each treatment depended on brood size. For nests that did not contain multiples of three nestlings, treatments were randomly distributed so that an even number received the dex and ACTH treatments. The saline treatment was applied to additional nestlings. Nestlings were restrained in cloth bags except while measurements were being taken. A second blood sample was collected after 30 min of restraint before being returned to nest boxes.

Samples were stored on ice in the field and centrifuged in the laboratory, and the plasma was separated and frozen at −4°C until assayed. Plasma samples were assayed by radioimmunoassay following the methods of Wingfield et al. [24]. Briefly, tritiated corticosterone was added to all plasma samples for determination of recoveries. After equilibration, samples were extracted with redistilled dichloromethane. The supernatant extracts were dried and resuspended in phosphate buffer for assay. Dextran-coated charcoal was used to separate bound from unbound steroid. Samples were assayed in duplicate and compared with a standard curve, with concentrations adjusted by the recovery (ng/ml of plasma). We used antibody B3-163 from Esoterix Laboratory (Calabasas Hills, CA, USA) that has 3.3% cross-reactivity with desoxycorticosterone and <1% cross-reactivity with all other steroids. The assay was validated for tree swallows by demonstrating that the serial dilution of pooled plasma sample paralleled the standard curve (data not shown). Interassay and intra-assay variability were <8% and <12%, respectively, as determined with a standard pool in each assay. Accuracy of measurement of a standard sample was 96%, and the sensitivity was 0.6 ng/ml.

Statistical analyses

All data were tested for normality (Shapiro–Wilk W goodness of fit) and equal variances (Bartlett’s test) and were found to satisfy the assumptions for analysis of variance (ANOVA) (p > 0.05 for all tests). For evaluating responses to PCB exposure in adult females, we compared plasma corticosterone concentrations between sites by ANOVA with post hoc Tukey tests for pairwise comparisons. To control for sampling more
than one nestling per nest box, mixed model ANOVAs were used with all nestling data, with nest box as a random effect. For 2004, baseline corticosterone from all nestlings was included in the analysis, but the 30-min corticosterone comparison consisted only of saline controls. The effect of year on site was examined by two-way mixed model ANOVA for Massachusetts nestlings from Lenox Road and Threemile Pond only because nestlings were not sampled from Southwest Branch in 2004. For 2004 nestlings, a two-way mixed model ANOVA was used to compare 30-min corticosterone concentrations by site and treatment (saline, dex, and ACTH). Post hoc comparisons were the differences in least square means. Allendale Mill and Lyman Mill Ponds were combined for statistical analyses because of low sample size at Lyman and are hereafter referred to as LymAllen. LymAllen was compared with both the PCB and TCDD reference areas.

**RESULTS**

For adult female tree swallows sampled in 2003, baseline plasma corticosterone levels were not different between the highly contaminated (Lenox Road) and reference (Threemile Pond) sites ($F = 1.38, p = 0.26, df = 1, 15$; Fig. 1A). Insufficient baseline samples were available to include the upstream lesser contaminated site (Southwest Branch) in the analysis. Stress-induced concentrations were significantly higher at the Southwest Branch than Lenox Road, with Threemile in between ($F = 6.98, p = 0.004, df = 2, 27$; Fig. 1B).

In PCB-exposed nestlings from Massachusetts, baseline concentrations were not significantly different between sites for 2003 or 2004 ($F_{2,26} = 0.23, p = 0.79, df = 2, 26$ for 2003; $F = 2.51, p = 0.12, df = 1, 73$ for 2004; Fig. 2A). Stress-induced corticosterone concentrations were higher in nestlings at the Lenox Road site in 2003 compared with the other two sites ($F = 6.55, p = 0.005, df = 2, 26$; Fig. 2B). In 2004, stress-induced concentrations did not differ significantly ($F = 0.41, p = 0.53, df = 1, 17$; Fig. 2B). The interaction between year and site for stress-induced but not baseline corticosterone was significant ($F = 13.16, p = 0.0004, df = 1, 120$). Response to treatment with saline, ACTH, or dex differed within each site (Fig. 3), with ACTH significantly augmenting and dex significantly decreasing the corticosterone response to saline.

Furthermore, the response to ACTH at the highly contaminated Lenox site was significantly reduced compared with the response to ACTH at the Threemile reference site (treatment $F = 91.84, p < 0.0001, df = 2, 154$; site $x$ treatment $F = 5.38, p = 0.006, df = 2, 154$; Fig. 3).

When comparing TCDD contamination, baseline corticosterone was lower at LymAllen compared with Threemile, but...


DISCUSSION

The overall results from this study suggest that local exposure to contaminants can significantly affect both circulating baseline and stress-induced corticosterone concentrations. These results are consistent with a number of previous studies showing that the GC stress response can be altered as a result of anthropogenic environmental perturbation. For example, GC measurement has been used to study the effects of habitat degradation on spotted salamanders [25], ecotourism on Galapagos marine iguanas and Magellanic penguins [26–28], snowmobile activity on wolves and elk [14], and logging activities on northern spotted owls [13]. More specifically related to this study, contaminants appear to affect the GC pathway. For example, Wikelski et al. [6] found increased baseline and stress-induced GC concentrations in marine iguanas (Amblyrhynchus cristatus) after 7 d acute exposure to an oil spill. Fowler et al. [29] observed significantly higher circulating corticosterone concentrations in oiled than nonoiled female but not male Magellanic penguins (Spheniscus magellanicus) 3 weeks after an oil spill. Analyses of herring gull (Larus argentatus) yolk sac organochlorine concentrations and plasma corticosterone concentrations showed a significant inverse relationship with polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans, total PCBs, non-ortho PCBs, and 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents [30]. Consequently, the results of this study add to a growing literature indicating that GC concentrations are sensitive to contaminant exposure and that direction of effects can vary across taxa.

Our results did not reveal a consistent or predictable pattern in contaminant effects. A close analysis of these results suggests four main themes. Except for stress-induced corticosterone concentrations in 2003 nestlings, there is an overall damping of corticosterone responses with increasing PCB and TCDD contamination. Polychlorinated biphenyls and TCDD appear to alter corticosterone concentrations in different ways. Apparently, how PCBs affect corticosterone release varies yearly. The effects of living on a site contaminated with PCBs might be different in adult females and nestlings.

Several aspects of this study support the overall damping of both baseline and stress-induced corticosterone responses in birds sampled at PCB- and TCDD-contaminated sites. Baseline corticosterone concentrations clearly decreased at the TCDD-contaminated sites and stress-induced concentrations showed a similar trend. Adult females exposed to PCBs also demonstrated significantly lower stress-induced concentrations as contamination increased from moderate to high levels, although there was a nonsignificant increase from low to moderate levels. These results are consistent with the fish and amphibian literature showing decreased response to capture stress with contaminant exposure [5,31–34], although baseline GCs usually increase in these species. In a study on tree swallows sampled in 2004. Baseline plasma corticosterone concentrations (A) were measured within 3 min of capture and removal from nest boxes, and stress-induced concentrations (B) were measured 30 min after capture. Threemile Pond = reference site, located in the Housatonic River basin (Great Barrington, MA, USA). Fire Station = upstream from the Superfund Site on Woonasquatucket Reservoir (Rhode Island, USA). LymAllen = Allendale Mill and Lyman Mill Ponds downstream from the Superfund Site on the Woonasquatucket River. Each bar represents the mean ± SE for the sample sizes indicated. Different letters represent significant differences by post hoc comparisons.
lows, Martinovic et al. [35] measured corticosterone in nestlings from several sites contaminated with chlorinated hydrocarbons. Although they did not discover any relationship between contaminants and 10-min poststressor corticosterone concentrations, they did find differences in baseline concentrations among sites with different levels of contaminants and a negative correlation between polychlorinated dibenzofurans and baseline corticosterone. We found a similar trend.

The mechanism for an overall decrease in corticosterone release appears to be a direct effect on the secretory ability of adrenal tissue. No site-specific differences appeared after dexamethasone administrations, suggesting no alterations in negative feedback, but response to ACTH was decreased in nestlings at the Lenox site in 2004 (Fig. 3). The decreased response to ACTH is consistent with a growing literature in fish and amphibians. Hopkins et al. [33] observed decreased responses to ACTH injection in southern toads (Bufo terrestris) exposed to coal combustion wastes. Similarly, mudpuppies (Necturus maculosus) exposed to chlorinated hydrocarbons (PCBs and organochlorine pesticides) showed lower GC responses to ACTH stimulation [32]. A number of studies in fish exposed to a variety of pollutants (heavy metals and organic compounds) have shown a reduced GC response to ACTH administration [5,36]. Overall, the damped corticosterone responses suggest a possible decreased ability to respond to environmental stressors. In contrast, Bowerman et al. [37] found a positive association between increased response to ACTH and PCB concentrations in nestling bald eagles (Haliaeetus leucocephalus), but the sample size was quite small. Given the wide variety of contaminants that have been studied, as well as the mix of contaminants in field conditions, it is not surprising that some studies report contradictory results.

The observation that PCBs and TCDDS alter corticosterone release in different ways is a result of the direct comparison between nestlings. The contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin was associated with reduced baseline, but not stress-induced, corticosterone concentrations, whereas PCBs were associated with increased stress-induced, but not baseline, corticosterone concentrations in 2003. Given the discussion above, the increase in stress-induced concentrations in relation to PCBs was unexpected. However, contaminant-associated increases have been documented elsewhere. For example, GC concentrations were higher in marine iguanas (Amblyrhynchus cristatus) exposed to an oil spill [6] and in oiled female Magellanic penguins (Spheniscus magellanicus) [29].

The difference between years in nestlings exposed to PCBs seems to be driven by the highly contaminated site in 2003. Three plausible scenarios could explain these results. First, hydrological and weather variation could have resulted in yearly differences in exposure levels, although neither year was noticeably out of the ordinary. Ideally, we would have collected our data at the same time contaminant levels were determined so that direct comparisons could have been made. Second, exposure to contaminants can vary through the season, apparently on the basis of emergence patterns of prey that differ in levels of contamination [38]. However, we controlled for this as best we could by collecting samples in both years at identical times of the tree swallow breeding cycle. Third, it is possible that a secondary unidentified stressor was acting on the nestlings at the high-PCB site (Lenox Road) in 2003 and that the measurable alteration in the stress response occurred because of an additive effect. This effect is known to occur under laboratory conditions and is hypothesized to occur under field conditions [21]. Regardless of the mechanism, these results point to the stochastic nature of contaminant effects on corticosterone release and suggest caution when interpreting single-year studies.

Finally, the influence of contaminant stressors on adult females and nestlings appears to differ. The highest PCB levels were associated with a decrease in stress-induced corticosterone compared with mid-PCB levels in adult females, but with an increase in nestlings in the same year. It is difficult to predict specifically how effects might differ between adult females and nestlings, especially given the complexity of environmental exposure to contaminant mixtures rather than exposure to individual contaminants, as well as other contributing environmental factors that might affect nestlings and adults differently. Lorenzen et al. [30] found that organochlorines have an effect on corticosterone concentrations, even at the embryonic stage before hatching in herring gulls (Larus argentatus). In some species, younger nestlings are unable or less able to mount a stress response compared with adults, and the ability increases as the nestlings approach independence [39]. To the best of our knowledge, the age at which tree swallow nestlings are normally able to mount an adult stress response is not known. Our results show that the nestlings (all >11 d old) are mounting a definitive stress response that was elevated in relation to PCB exposure in 2003. The amount of corticosterone was less in adult females (range 0.05–19 ng/ml; Fig. 1) compared with nestlings (range 3–58 ng/ml; Fig. 2), which might have influenced corticosterone release when challenged with a contaminant stressor.

We have used PCBs and TCDD throughout the study as a surrogate for total organic contamination, and we recognize that specific causative agents of altered corticosterone concentrations are not confirmed. For example, among organochlorine contaminants along the Housatonic River, correlation is high. Total PCBs in tree swallow eggs are correlated with total dioxin and furan contamination (r = 0.783) and with the most common furan congener (r = 0.857) present along the Housatonic River [16]. The only way to determine causative agents and definitively address relative risks is through controlled experiments.

Further research is needed to understand the underlying mechanisms of contaminant action on glucocorticoid synthesis and regulation. Controlled laboratory studies to elucidate mechanisms of action of individual contaminants are needed to help make sense of the variation in responses seen in the field. However, laboratory studies alone are unlikely to fully address the more pressing concern of wildlife health and fitness in polluted ecosystems. We suggest that field investigations into the relationship between an altered stress response and survival and fitness indicators are also important for fully understanding the detrimental effects of pollution in wildlife and humans.
PCB and dioxin effects on corticosterone in tree swallows

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HL., eds, Biomarkers: Biochemical, Physiological, and Historical Markers of Anthropogenic Stress. Lewis, Boca Raton, FL, USA, pp 5–86.


