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Small changes in whole-body corticosterone content affect larval *Rana pipiens* fitness components

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Abstract

In amphibians, large changes in tissue corticosterone content (caused by treatment with large doses of hormone) alter tadpole growth and development, but the effects of smaller changes on growth, development, behavior, and morphology are unknown. In the current study, we exposed pre-metamorphic *Rana pipiens* tadpoles to moderate doses (62 and 125 nM) of exogenous corticosterone by adding it to the rearing water. We then analyzed effects on growth, development, behavior, morphology, and the endogenous corticosterone response to exogenous adrenocorticotrophic releasing hormone (ACTH). A 50% elevation in whole-body corticosterone content was associated with slowed growth and development, increased tail muscle depth, and a diminished corticosterone response to ACTH. Behavior was unaffected by corticosterone administration. Treatment with the corticoid synthesis inhibitor metyrapone (MTP) reduced whole-body corticosterone content by 50% and was associated with increased size at metamorphosis but no change in time to metamorphosis. Our findings support the hypothesis that corticoids can mediate growth, developmental, and morphological responses of tadpoles to changing environmental conditions. Our results also demonstrate that even small changes in corticosterone content can have important implications for amphibian fitness. © 2002 Elsevier Science (USA). All rights reserved.

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1. Introduction

Corticosterone, a principal corticoid in amphibians (see Kikuyama et al., 1993), is known to affect tadpole growth and development when administered at large doses (enough to elevate whole-body corticosterone content at least 2–3-fold; Gray and Janssens, 1990; Hayes, 1995; Hayes et al., 1993; Kikuyama et al., 1993; Leloup-Hatey et al., 1990). However, effects on growth and development of smaller elevations in tissue corticosterone content, elevations nearer the physiological range, are unknown. Furthermore, whether corticoids can influence tadpole behavior and morphology is unknown.

Exogenous corticoids accelerate metamorphosis when administered with exogenous thyroid hormone (Frieden and Naile, 1955; Gray and Janssens, 1990;

Hayes, 1995; Kikuyama et al., 1983, 1993). In one study, administration of corticosterone alone to prometamorphic *Bufo boreas* accelerated metamorphosis (Hayes et al., 1993). Because most studies had found that administration of corticoids alone to prometamorphic tadpoles did not influence metamorphosis, Hayes et al. (1993) suggested that the effect of corticosterone in their study was likely due to synergy of the corticoid with rising endogenous thyroid hormone concentrations.

By comparison with the studies described above in which prometamorphic animals were treated with hormones, exposure to exogenous corticoids during pre-metamorphosis (before or at the hindlimb bud stage) tends to inhibit growth and development. Kobayashi (1958) first showed that treatment of premetamorphic *Bufo vulgaris formosus* or *Rana japonica* with corticoids inhibited hindlimb growth and metamorphosis, possibly through inhibition of the thyroid axis. Hayes and colleagues (Hayes, 1995; Hayes et al., 1993) later found similar effects of corticoids in *Bufo boreas*. Wright et al.

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(1994) found that treatment of premetamorphic *Rana pipiens* tadpoles with exogenous corticoids inhibited hindlimb growth and epidermal cell proliferation but did not alter hindlimb differentiation.

It should be noted that the doses of corticoids used in the studies described above (for both pre- and prometamorphic animals) might be considered high (ranging from 0.29 to 4.4 μM). Hayes and Wu (1995) found that treatment with 0.55 μM corticosterone in the aquarium water elevated whole body corticosterone content by 3-fold in *Bufo boreas* tadpoles (the duration of treatment and time after dosing at which animals were sacrificed for hormone measurements were not reported). We have found that treatment of *Xenopus laevis* tadpoles with roughly the same dose of corticosterone in the rearing water (0.5 μM) significantly elevates whole body hormone content but does so with complex kinetics (Denver and Farley, unpublished data). For example, whole body corticosterone content was elevated \sim 23-fold 2 h after immersion in the hormone solution, but declined thereafter to reach \sim 4-fold elevation by 24 h.

Tadpole growth, development, behavior, and morphology all are known to change in response to environmental conditions. For example, growth and development are slowed in the presence of competitors, and both behavior and tail morphology are altered in the presence of predators (Brockelman, 1969; Berven and Chadra, 1988; McCollum and Van Buskirk, 1996; Scott, 1990; Smith, 1987; Smith and Van Buskirk, 1995; Wilbur, 1977; Wilbur and Collins, 1973). However, the physiological mediators of these trait changes have not been identified. Corticosterone content increases in *Scaphiopus hamondii* tadpoles subjected to decreasing water levels (Denver, 1998) and in *R. pipiens* tadpoles exposed to intraspecific competitors (Glennemeier and Denver, 2002). Corticosterone therefore is a potential physiological mediator of varied tadpole responses to their environments.

In the current study, we examined the potential for physiologically relevant increases in corticosterone tissue content and decreases in corticoid biosynthesis to affect tadpole growth, development, behavior, and morphology, as each of these is known to respond to environmental conditions. We treated *R. pipiens* tadpoles with low doses of exogenous corticosterone to produce elevations in whole body corticosterone content that were within the physiological range for this species (unpublished data). We then measured effects on growth, development, behavior, and morphology. We also measured the effect of pre-treatment with corticosterone on adrenocorticotrophic hormone (ACTH)-induced whole-body corticosterone content, to determine the potential for small elevations in corticosterone to exert feedback on interrenal responsiveness. To examine the contribution of endogenous corticosterone to

R. pipiens fitness, we analyzed the effects of blocking corticoid biosynthesis with metyrapone on tadpole growth and development.

2. Methods

2.1. Animal husbandry and hormone treatments

Rana pipiens eggs were purchased from Carolina Biological Supply (Burlington, NC). For all experiments, tadpoles were raised in environmental chambers, at 22 °C, and were fed 10–15% of body mass per day (based on the mean mass of animals per tank) of a 3:1 mixture of Purina Rabbit Chow: Tetramin Fish Flakes. Tank water was changed weekly.

For hormone analysis, tadpoles were rapidly euthanized by immersion in 0.01% benzocaine and snap frozen. Time between entry of the investigator into the environmental chamber and snap freezing did not exceed 5 min. Tadpoles were stored frozen at -20°C until extraction and analysis of whole body corticosterone content by radioimmunoassay (RIA; see below).

To test effects of exogenous corticosterone on fitness components, corticosterone treatments were begun at Gosner stage 25 (Gosner, 1960). Tadpoles were placed into 4 L deionized water in plastic tanks, with 10 tadpoles per tank and six tanks per treatment (for a total of 24 tanks and 240 tadpoles). Corticosterone (ICN Biomedicals, Aurora, OH) was dissolved in ethanol and this solution added to the water to give water concentrations of 62 or 125 nM corticosterone. The volume of ethanol added to each tank was 0.0017% of total water volume. Vehicle-treated tanks received ethanol only, and control tanks received no treatment. Behavioral observations were conducted after 2, 4, 6, 8, and 14 days of treatment and were conducted prior to any feeding or corticosterone treatments for that day. Observations were made by the investigator standing next to the tanks and recording the number of tadpoles in each tank swimming, resting, and feeding at a given moment. After all tanks had been observed once, the procedure was repeated for a total of 10 observations per tank per day. Tanks were labeled such that the treatment was not visible to the investigator during observations.

After 18 days of treatment, three of the six replicate tanks from each treatment were randomly selected, and the tadpoles (30 in total) placed into a single tank. Tadpoles from this tank then were randomly assigned to either a morphological measurement group or a group for extraction and hormone analysis by RIA. Tadpoles for morphological measurements were anesthetized in 0.01% benzocaine and preserved in a 10% solution of formaldehyde. Morphological measurements of preserved tadpoles were taken using a Mitutoyo caliper. Gross morphology of mouthparts was qualitatively

assessed as well. Tadpoles for RIA were anesthetized in 0.01% benzocaine and then weighed, staged, and frozen for subsequent corticosterone extraction and RIA.

After 28 days of treatment, tadpoles from the remaining three replicate tanks were tested for ACTH responsiveness. All tadpoles from the three tanks within a treatment were combined into a single tank and then randomly assigned to uninjected, ACTH-, or vehicle (saline)-injected groups. Each group from each treatment was placed into a separate 4 L tank of water (no corticosterone present) and was undisturbed for 48 h prior to injection. Tadpoles in the ACTH treatment group were injected through the tail muscle into the dorsal peritoneum with 0.2 IU ACTH (Sigma–Aldrich, St. Louis, MO) per gram body mass, delivered in 20 μ l phosphate buffered saline (PBS; 0.02 M). Tadpole mass ranged from 500 to 900 mg. Vehicle-injected tadpoles were injected with PBS only. Five hours after injection, tadpoles were anesthetized in 0.01% benzocaine, weighed, and frozen at -20°C for later corticosterone extraction and RIA. Uninjected tadpoles also were collected, to determine basal corticosterone content. All animals were collected between 1500 and 1700 h, to minimize possible circadian variation in corticosterone content.

To determine effects of moderate reduction in whole-body corticosterone content, tadpoles were exposed to the corticoid synthesis inhibitor metyrapone (MTP). Tadpoles were housed and fed as described above, with 10 tadpoles per tank and three replicate tanks per treatment. MTP treatments were begun at Gosner stage 25. MTP (Sigma–Aldrich, St. Louis, MO) was dissolved in ethanol and this solution added to the water to give a water concentration of 44 μM MTP. This concentration was determined based on Hayes and Wu (1995), who found that 22 μM MTP reduced whole-body corticosterone content of toad tadpoles by 33%. Our goal was 50% reduction of *R. pipiens* whole-body corticosterone content. The volume of ethanol added to each tank was 0.0016% of total water volume. Control tanks received ethanol only. As tadpoles reached metamorphic climax (forelimb emergence; Gosner stage 42), they were collected, euthanized, weighed, and snap frozen for later extraction and RIA.

The interpretation of ACTH injection results is improved by information about the relative contribution of endogenous versus exogenous corticosterone to measured corticosterone content. We therefore examined short-term negative feedback on the activity of the interrenal axis (and the production of endogenous corticosterone), by treating premetamorphic tadpoles (Gosner stage 25) with the synthetic glucocorticoid dexamethasone (Sigma Chemical, St. Louis, MO) at 62 nM in the aquarium water for 48 h. Dexamethasone was first dissolved in ethanol before adding to the water,

and control tanks received ethanol only (ethanol concentration in the water = 0.0026%). After 48 h of treatment, tadpoles ($n = 4\text{--}7$) were euthanized and snap frozen for later extraction and RIA.

2.2. Whole-body extraction and corticosterone RIA

The extraction procedure is described by Hayes and Wu (1995) and Denver (1998). Briefly, tissues were homogenized in ethyl acetate and the extracts fractionated by thin layer chromatography (TLC) to separate corticosterone from other lipids. The region of the TLC lane containing the corticosterone (as determined by calibration with both radiolabeled and radioinert corticosterone; see Denver, 1998) was scraped and the silica collected into a borosilicate glass tube. The silica was extracted with ethyl ether, and the extract was dried under nitrogen and then resuspended in PBS-gelatin (PBS-G; 0.02 M, pH 7.3) for corticosterone RIA. The RIA was conducted as described by Licht et al. (1983). Anti-corticosterone serum was purchased from Endocrine Sciences (Calabasas, CA) and [^3H]-corticosterone from NEN Life Science Products (Boston, MA). Samples from a single experiment were analyzed in a single RIA or in multiple RIAs on a single day. Inter- and intra-assay coefficients of variation were 12% and 10%, respectively, and were monitored by including a quality control standard (pooled rat plasma) in each RIA. Dexamethasone, tested at doses as high as 40 $\mu\text{g}/\text{ml}$, did not crossreact in the corticosterone RIA.

2.3. Statistics

The effect of exogenous corticosterone treatment on whole-body corticosterone content was analyzed using one-way ANOVA of treatment versus corticosterone content, followed by Fisher's LSD pairwise comparisons between groups. Mass and stage data were analyzed by MANOVA of mass and stage versus corticosterone treatment, followed by individual ANOVAs and Fisher's LSD pairwise comparisons between groups. LSD tests compared each treatment group to the vehicle (ethanol) control group.

Behavioral data were recorded as the proportion of the total number of animals in a tank engaged in each activity and were analyzed by repeated measures ANOVA of treatment versus proportion resting or feeding (proportions were arcsine square root transformed), repeated for the five observation dates. One-way ANOVA of treatment versus proportion time (square root arcsine transformed) was used to analyze data within individual observation dates, followed by Fisher's LSD comparisons between specific groups. The 10 observations of each tank on a given day were averaged to provide the mean value for that tank on that day. These

means were then used to calculate a treatment mean and standard error among the six replicate tanks for each treatment, giving a sample size of six (not 60) for statistical analyses. The 10 observations were conducted to provide an accurate estimate of the activity within a tank and were not considered separate replicates in a statistical sense.

Tail fin depth and tail muscle depth were corrected for body mass differences by dividing each value by the body mass for that tadpole. Linear regressions of body mass versus tail fin depth ($R^2 = 0.97$; $p < 0.00005$) and body mass versus tail muscle depth ($R^2 = 0.94$, $p < 0.00005$) demonstrated linear relationships between the respective variables. The ratio of tail muscle depth to tail length was calculated to assess morphological proportions within the tail (linear regression of tail muscle depth versus tail length $R^2 = 0.97$; $p < 0.00005$). Data were analyzed using MANOVA of treatment versus the three measurements. This test was followed by individual ANOVAs and Fisher's LSD pairwise comparisons of treatment groups to the ethanol-treated group.

ACTH-injection data were analyzed using two-way ANOVA, with corticosterone content as the response variable and corticosterone treatment and ACTH manipulation as treatments. Individual ANOVAs then were performed within each treatment group and manipulation followed by Fisher's LSD pairwise comparisons between specific groups.

MTP treatment data were analyzed using nested MANOVA, with days to and mass at metamorphic climax as response variables and MTP exposure as treatment, with tanks nested within treatments. Whole-body corticosterone content at metamorphic climax was analyzed using Student's *t* test. Dexamethasone treatment data were analyzed using Student's *t* test, with corticosterone content as the dependent variable.

3. Results

3.1. Growth and development

Treatment of premetamorphic (Gosner stage 25) *R. pipiens* tadpoles with 125 nM corticosterone added to the aquarium water resulted in a 50% elevation in whole-body corticosterone content; whereas, treatment with 62 nM corticosterone did not alter whole-body corticosterone content (Fig. 1, panel A; ANOVA $F_{3,24} = 3.5$, $p = 0.032$). This 50% elevation in corticosterone content with the 125 nM dose tended to reduce body mass, although this effect was not significant (Fig. 1, panel B; ANOVA: $F_{3,34} = 2.6$, $p = 0.071$). However, treatment with 125 nM corticosterone significantly slowed development (Fig. 1, panel C; ANOVA: $F_{3,34} = 3.0$, $p = 0.045$).

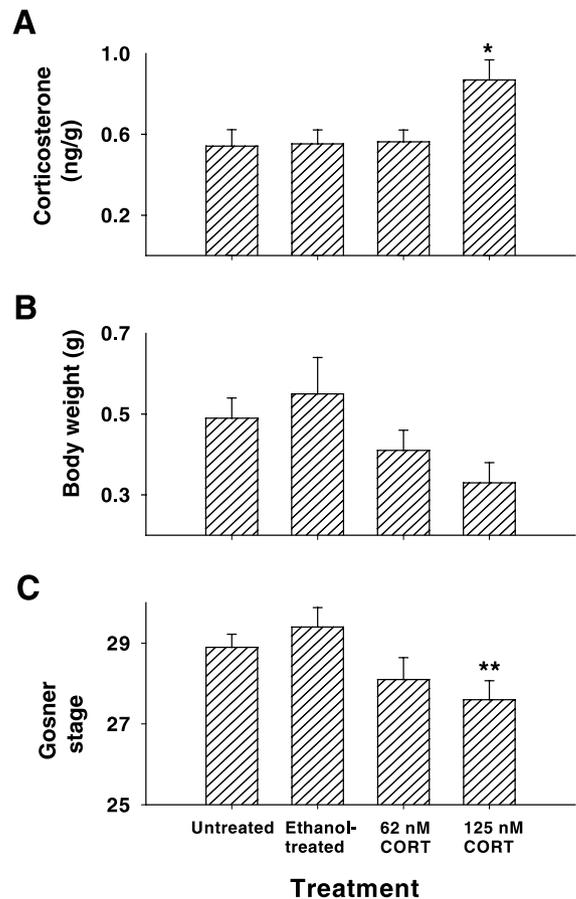


Fig. 1. Whole-body corticosterone content (panel A), wet body weight (panel B), and developmental stage (panel C) of *R. pipiens* tadpoles treated with exogenous corticosterone (CORT) or ethanol vehicle for 18 days. Treatments began at Gosner stage 25 (Gosner, 1960). Asterisks (*) indicate significant difference from ethanol-treated group (* $p < 0.05$; ** $p < 0.001$). Error bars represent standard errors of the mean of individual tadpoles (body weight and stage $n = 10$; corticosterone content $n = 10, 5, 4$, and 9 for control, ethanol, 62 nM corticosterone, and 125 nM corticosterone treatments, respectively).

3.2. Morphology

Relative tail muscle depth was increased by corticosterone treatment (Fig. 2, panel C). MANOVA of treatment versus the three measurements showed a significant effect of treatment on the ratio of tail muscle depth to tail length (ratio $F_{3,34} = 7.0$; $p = 0.001$). This ratio was significantly greater in the 62 and 125 nM corticosterone groups compared with the vehicle control group (LSD: $p = 0.003$ and < 0.0005 , respectively). Neither tail fin depth nor tail muscle depth was significantly different among the treatment groups, although there was a trend toward increased tail muscle depth with corticosterone treatment (fin $F_{3,34} = 1.7$, $p = 0.18$; muscle $F_{3,34} = 2.3$, $p = 0.09$). Mouthparts showed no gross, qualitative differences among groups. All groups contained intact, keratinized mouthparts and a full complement of tooth rows.

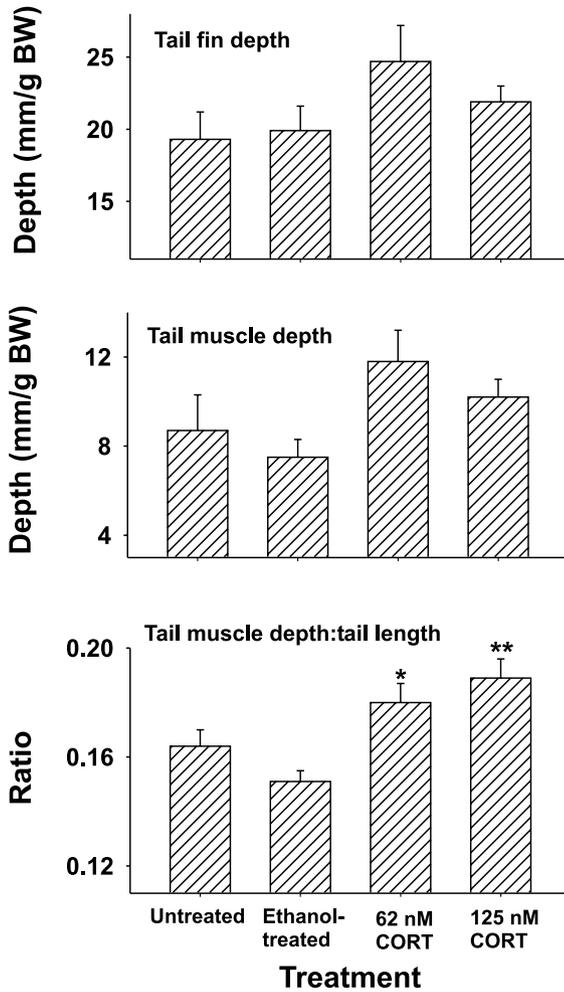


Fig. 2. Morphological measurements for *R. pipiens* tadpoles treated with exogenous corticosterone (CORT) or ethanol vehicle. Tail fin (top panel) and tail muscle depths (middle panel) were corrected for body mass by dividing depth (mm) by body weight (g). The bottom panel shows tail muscle depth divided by tail length. Treatments began at Gosner stage 25 and continued for 18 days before animals were anesthetized and preserved in 10% formalin. Asterisks (*) indicate significant difference from ethanol-treated group (* $p = 0.003$; ** $p < 0.0005$). Error bars represent standard errors of the mean of individual tadpoles ($n = 10$).

3.3. Behavior

Treatment with corticosterone had little effect on *R. pipiens* behavior (Fig. 3). ANOVAs of swimming time for individual dates showed a significant treatment effect only on the second date (treatment day 4), with the 125 nM corticosterone treatment showing significantly higher swimming activity than the control and 62 nM groups but no other differences among treatments on this date ($p = 0.003$ and 0.001 relative to control and 62 nM groups, respectively; $\alpha = 0.008$ to account for multiple comparisons). Repeated measures ANOVA of proportion time spent swimming for all dates showed significant effects of corticosterone treatment ($F_{3,20} = 4.23$; $p = 0.018$) and date ($F_{4,80} = 21.7$; $p < 0.00005$) and

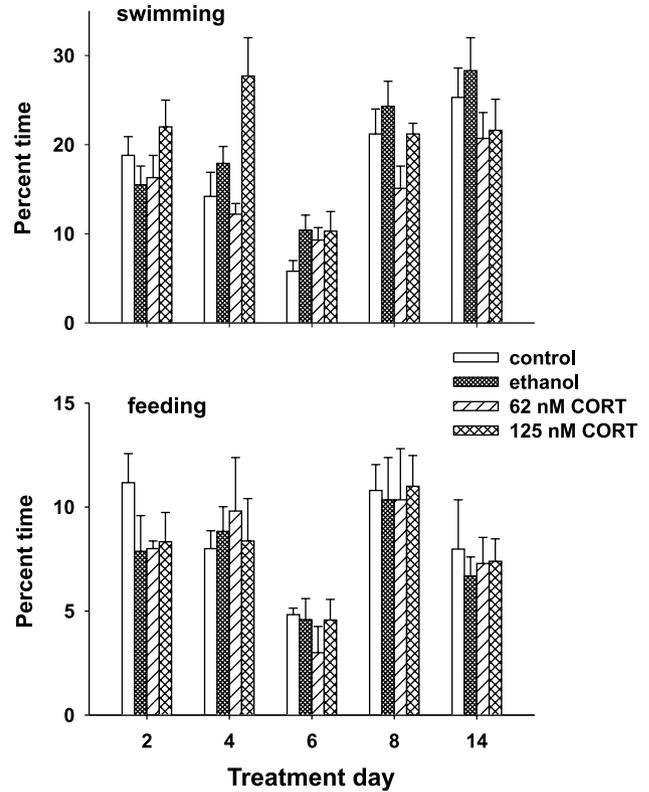


Fig. 3. Percent time swimming (top panel) or feeding (bottom panel) by *R. pipiens* tadpoles treated with exogenous corticosterone (CORT) or ethanol vehicle. Treatments began at Gosner stage 25. Error bars represent standard errors of the mean of six replicate tanks (see Section 2).

a significant interaction between the two ($F_{12,80} = 2.00$; $p = 0.035$).

Repeated measures ANOVA of proportion time spent feeding for all dates showed a significant date effect ($F_{4,80} = 11.6$; $p < 0.00005$) but no significant treatment effect ($F_{3,20} = 0.62$; $p = 0.61$) or interaction between the two ($F_{12,80} = 0.54$; $p = 0.88$). The fact that behavior differed on only one of five dates suggests either that the observed behavioral effects of corticosterone are transient or that there may not be a causative link between the corticosterone treatment and our observations of tadpole behavior.

3.4. ACTH response

Tadpoles treated with corticosterone showed a decreased response to ACTH compared controls (Fig. 4, panel A). Two-way ANOVA showed significant main effects of corticosterone treatment ($F_{3,66} = 9.88$; $p = 0.00002$) and ACTH manipulation ($F_{2,66} = 123$; $p < 0.00005$) and a significant interaction between the two ($F_{6,66} = 11.9$; $p < 0.00005$). Pairwise comparisons revealed the following differences between the individual groups. Within all treatment groups, ACTH injection resulted in significantly increased corticosterone content

over basal levels ($p < 0.00005$ for all treatments). Saline injection did not increase corticosterone content significantly in any treatment group.

No significant difference in basal corticosterone content was measured among treatment groups. The ethanol-treated group showed a significantly higher corticosterone content after both the saline and ACTH injection compared to the control group ($p = 0.022$ and 0.010 , respectively), suggesting an effect of the vehicle on the HPI axis. Saline-injected animals from the ethanol-treated, 62 or 125 nM corticosterone groups did not

differ in their corticosterone content. However, corticosterone content after ACTH injection was significantly lower in both corticosterone treatment groups compared with the ethanol-treated group ($p < 0.00005$ for both treatments). Fig. 4, panel B illustrates the ratio of corticosterone content after ACTH injection to corticosterone content after saline injection. Because each tadpole could be analyzed only for either post-ACTH or post-saline corticosterone content, not both, this ratio was calculated using the mean corticosterone values from the ACTH and saline groups shown in panel A of Fig. 4. The variance of each ratio was estimated using the variances of the respective means for that ratio (see Kish, 1965 for formula).

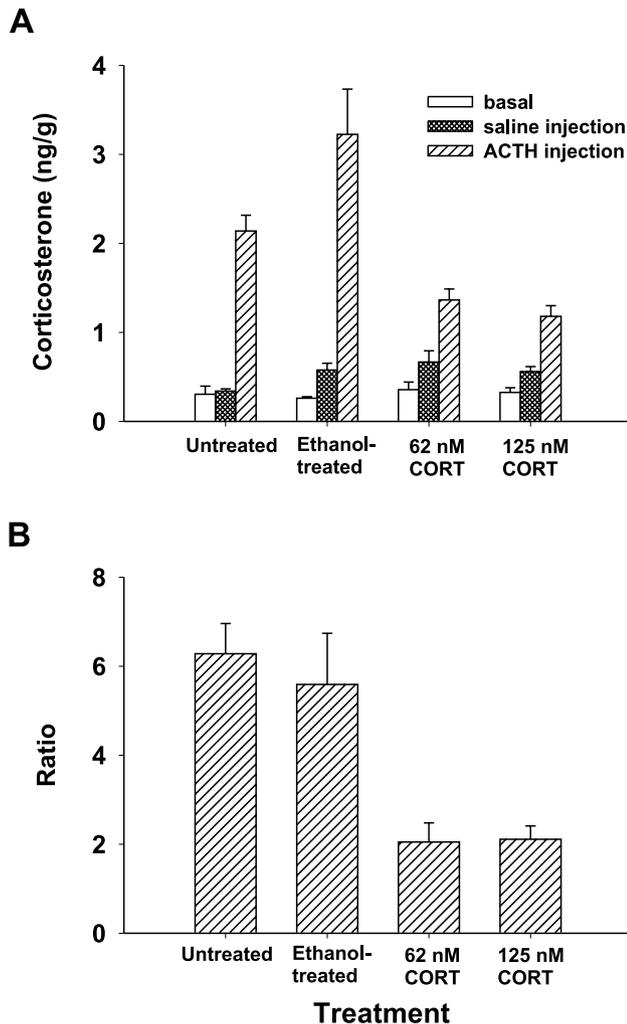


Fig. 4. Chronic treatment of premetamorphic tadpoles with low doses of corticosterone (CORT) reduces interrenal responsiveness to ACTH injection. Panel A: whole-body corticosterone content of *R. pipiens* tadpoles treated with exogenous corticosterone or ethanol vehicle for 28 days, followed by injection with saline or 0.2IU ACTH. Animals were collected five hours after injection for analysis of corticosterone content. Uninjected animals were collected at the same time as injected animals for determination of basal corticosterone content. Error bars represent standard errors of the mean of individual tadpoles ($n = 8$). Panel B: ratio of post-ACTH injection to post-saline injection corticosterone content, using data shown in panel A. Error bars were estimated as in Kish (1965).

3.5. MTP treatment

Chronic exposure to 44 μ M MTP reduced whole-body corticosterone content at metamorphic climax by 50% (Fig. 5, panel A; $t = 3.6$, $p = 0.005$). This reduction was associated with significantly increased mass at metamorphic climax but no effect on days to metamorphic climax (nested MANOVA: mass $F = 7.9$, $p = 0.007$; days $F = 0.065$, $p = 0.80$; nested tank effects not significant).

3.6. Effects of short-term dexamethasone treatment on whole-body corticosterone

Exposure of tadpoles to dexamethasone (62 nM) in the aquarium water for 48 h reduced whole-body corticosterone content from 200 to 20 pg/g ($p = 0.007$; Fig. 6).

4. Discussion

Chronic exposure to a moderate dose of exogenous corticosterone affected development, morphology, and the endogenous corticosterone response to ACTH injection. The highest corticosterone dose used here (125 nM) was approximately half that used by Wright et al. (1994) and four-or-more times lower than that used in other studies of the effects of exogenous corticosterone on tadpole growth and development (cf: Frieden and Naile, 1955; Gray and Janssens, 1990; Hayes, 1995).

4.1. Growth and development

Treatment with 62 nM exogenous corticosterone did not result in an elevation of whole-body corticosterone content in *R. pipiens* tadpoles, perhaps due to suppression of endogenous corticosterone production (Axelrod and Reisine, 1984; Keller-Wood and Dallman, 1984; Munck et al., 1984). Treatment with 125 nM exogenous corticosterone resulted in a 50% increase in *R. pipiens* whole-body corticosterone and was associated with a

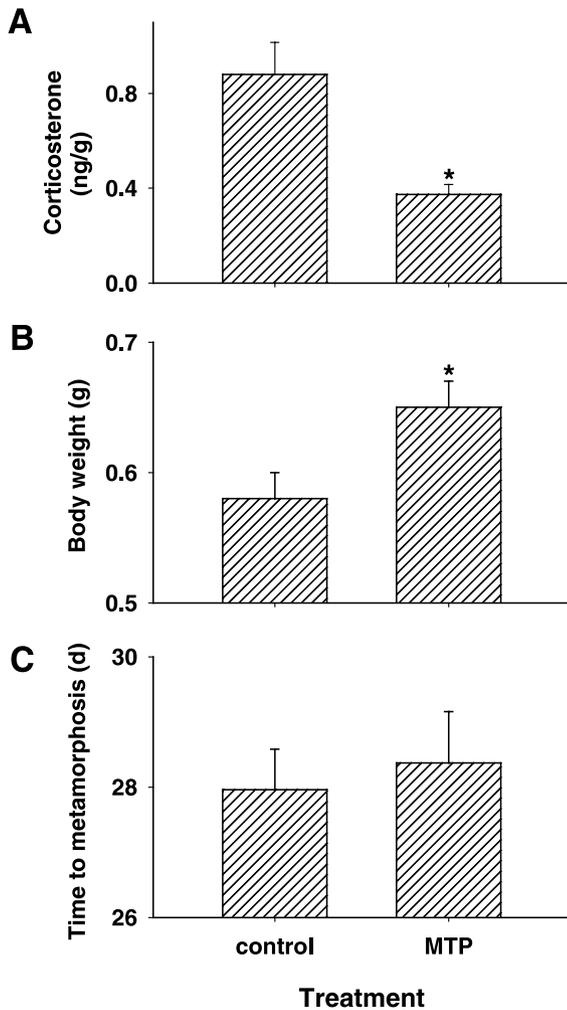


Fig. 5. Effects of chronic treatment with the corticoid synthesis inhibitor metyrapone (MTP; 44 μ M in the aquarium water; tadpoles treated from Gosner stage 25 [premetamorphosis] through metamorphic climax [stage 42]) on whole-body corticosterone content (panel A), wet-body weight (panel B), and days to metamorphic climax (panel C). Asterisk (*) indicates significantly different from controls ($p < 0.005$). Error bars represent standard errors of the mean ($n = 7$ –10 animals/treatment).

non-significant 30% decrease in body mass and significantly retarded development of the hindlimbs. The fitness consequences of such changes in tadpole growth and development have been well studied. Both a longer larval period and a smaller size at metamorphosis can delay adult reproductive maturity, decrease size at first reproduction, and in some cases decrease adult survival to first reproduction (Berven and Gill, 1983; Semlitsch et al., 1988; Smith, 1987). Decreased growth rate and longer time to metamorphosis also may increase exposure time to aquatic predators (Werner, 1986; Wilbur, 1980) or decrease the chance of metamorphosing before a quickly drying pond disappears (Newman, 1992).

Other studies have found slowed growth in tadpoles of ranid and bufonid species with exogenous corticosterone treatments of 290, 550 nM, or greater, where

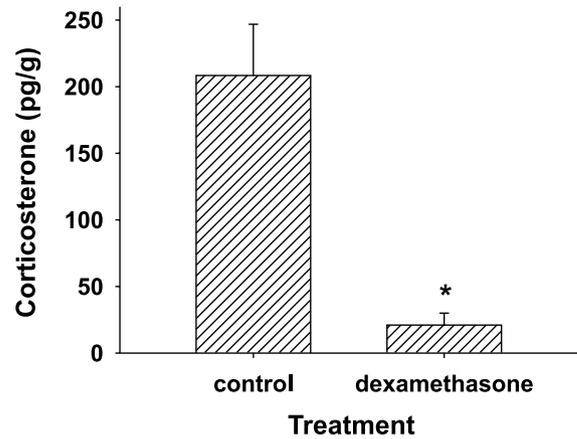


Fig. 6. Whole-body corticosterone content of premetamorphic (Gosner stage 25) *R. pipiens* tadpoles treated with 62 nM dexamethasone for 48 h. Asterisk (*) indicates significantly different from control ($p = 0.007$). Error bars represent standard errors of the mean of individual tadpoles ($n = 4$ –7).

550 nM exogenous corticosterone elevated whole-body corticosterone content approximately 3-fold over controls (Hayes, 1995; Hayes et al., 1993; Wright et al., 1994). However, hindlimb development of pre-metamorphic tadpoles was unaffected by such corticosterone doses in these and other studies (see also Gray and Janssens, 1990). The discrepancy between these and the current study may result from the difference in corticosterone dose used. Whole-body corticosterone content is low but detectable in pre-metamorphic *R. pipiens* tadpoles (stages 25–28) and increases slightly (at 1.5-fold) at the beginning of prometamorphosis when hindlimb buds are developing (stage 29–30; Glennemeier and Denver, unpublished results). The magnitude of the increase in whole-body corticosterone content produced by immersion in 125 nM corticosterone in the current study (~1.5-fold; see Fig. 1, panel A) is likely more physiologically relevant to premetamorphic stages than are the large increases produced in other studies. The increased whole-body corticosterone content produced by other investigators with higher doses may approximate the increased corticosterone content seen at metamorphic climax (~4.5-fold over premetamorphic content in *R. pipiens*; Glennemeier and Denver, unpublished results). The effects of such high doses (e.g., inhibition of forelimb emergence as shown by Hayes et al. (1993) could represent a pharmacological action of the hormone.

4.2. Morphology

Hayes et al. (1997) reported shorter tails and bodies in *Kassina senegalensis* pro-metamorphic tadpoles treated with 1.1 μ M exogenous corticosterone, consistent with the current study and others (see references above) showing decreased growth in tadpoles treated with

exogenous corticosterone. However, the current study is the first to identify corticosterone effects on morphological proportions independent of changes in overall body size. Tail fin depth (corrected for body mass) was unaffected by corticosterone treatment, but the dorsal-ventral depth of the tail muscle was greater in corticosterone-treated tadpoles than in controls, especially when compared to tail length. Hayes et al. (1997) also found developmental malformations of the snout and mouthparts in corticosterone-treated *K. senegalensis* tadpoles (but not in several other species; Hayes, 2000), but lower corticosterone doses produced no mouthpart changes in *R. pipiens* in the current study.

Van Buskirk et al. (1997) have suggested that a deeper tail fin enhances a tadpole's ability to escape predators, and several studies have demonstrated that tail fin depth and tail muscle size increase in tadpoles exposed to predators (McCollum and Van Buskirk, 1996; Smith and Van Buskirk, 1995). Since corticosterone influenced tail morphology in the current study, this hormone could serve as a physiological mediator that translates an environmental predator cue into a morphological, adaptive response. It is unknown whether predator presence elicits a rise in corticosterone content in tadpoles. However, components of the hypothalamic–pituitary–interrenal (HPI) axis have been shown to mediate the developmental response of desert toad tadpoles to a drying pond (Denver, 1997, 1998), and corticosterone content increases in *R. pipiens* tadpoles subjected to resource competition (Glennemeier and Denver, 2002). Studies of the morphological and corticosterone responses to predation in amphibian species may illuminate the degree to which the HPI axis mediates morphological responses to predation.

4.3. Behavior

Feeding and swimming behaviors were largely unaffected by moderate elevation in whole-body corticosterone content, although the increase in swimming behavior seen on day 4 may represent a transient but significant corticosterone-mediated behavioral change. No other studies have examined the effects of corticosterone on tadpole behavior, but corticosterone is known to affect reproductive, foraging, and territorial behavior in other taxa (DeNardo and Sinervo, 1994; Moore and Zoeller, 1985; Silverin, 1986; Wingfield et al., 1997). Further study of the effects of the corticosterone or other neuroendocrine factors on tadpole behavior is needed. Predation and competition are known to affect tadpole behavior, and behavior profoundly influences tadpole growth, mortality, and species interactions (Anholt and Werner, 1995; Skelly, 1992). Such behavioral studies therefore would provide additional insight into the physiological factors that mediate tadpoles' responses to their environment.

4.4. ACTH response

Chronic corticosterone elevation attenuated the tadpoles' endogenous corticosterone response to ACTH. Such desensitization of the HPI axis is well documented (Keller-Wood and Dallman, 1984), and the current results demonstrate that even small increases in corticosterone content can suppress the normal interrenal responsiveness to ACTH. The ethanol vehicle alone, despite being present at very low concentration (0.0017%), also affected the tadpoles' response to injection stress and to ACTH, emphasizing the importance of testing vehicle effects directly and of minimizing vehicle doses.

The fitness consequences for such chronic HPI axis desensitization could be significant, given the role of this axis in mediating an animal's response to its environment. Environmental 'stressors' activate this axis (or the homologous HPA–adrenal axis in birds and mammals) and elicit a common cascade of hormone secretions in all vertebrates that have been studied (Wingfield, 1994). These hormones increase metabolic rate and mobilize fuel stores, a response that presumably enhances performance during emergency or stressful events. Denver (1997, 1998) demonstrated a role for the HPI axis in mediating the accelerated development that Western spadefoot toad tadpoles undergo in response to a drying habitat. We also have found elevated whole-body corticosterone content in *R. pipiens* tadpoles subjected to intraspecific resource competition (Glennemeier and Denver, 2002). While relatively little is known about the amphibian stress response, these studies suggest that the HPI axis may mediate tadpoles' responses to common changes in environmental conditions. Suppression of the HPI axis therefore could impair tadpoles' ability to acclimatize to such changes. Whether HPI suppression of the magnitude found in the current study is sufficient to significantly impair functional responses to pond drying, resource competition, or other environmental 'stressors' is unknown, but a response diminished one-third to that of controls (see Fig. 4, panel B) is likely to be physiologically significant. Given that many pollutants have been shown to alter corticoid levels to a greater degree than in the current study, it seems likely that wildlife functional responses to environmental conditions could be seriously impaired in polluted environments. In one example, trout chronically exposed to heavy metal pollution in contaminated streams were more susceptible to a subsequent toxic dose of metals than were fish from uncontaminated streams, a response attributed to possible suppression of the HPI axis (see Norris et al., 1999).

Endogenous corticosterone production in corticosterone-exposed tadpoles could be lower than that indicated in Fig. 4, panel A. Tadpoles in the current study were collected for corticosterone analysis after 48 h in

non-treated water (see Section 2). If exogenous corticosterone was not yet metabolized after this time, the RIA would have measured exogenous as well as endogenous corticosterone. In a separate experiment, exposure to the synthetic glucocorticoid dexamethasone for 48 h reduced whole-body corticosterone content one-tenth to that of controls (Fig. 6). Such decreased endogenous production is well known to occur in the presence of exogenous hormone (Axelrod and Reisine, 1984; Keller-Wood and Dallman, 1984; Munck et al., 1984). Subtracting a comparable amount from the values shown in Fig. 4 (panel A, corticosterone treatment groups) would alter the absolute corticosterone values and the ACTH/vehicle injection ratios slightly, but the qualitative results and the magnitude of difference from the controls would remain similar.

4.5. MTP treatment

A 50% reduction in whole-body corticosterone increased size at metamorphosis by more than 10% but did not affect the rate of metamorphosis. Considering that corticoids have been implicated in the synergistic control of metamorphosis with thyroid hormone (see Kikuyama et al., 1993), our results might be taken as evidence against such a role. However, this conclusion must be tempered by the fact that we did not produce a complete reduction in corticoid biosynthesis, and one could argue that even small amounts of the hormone are sufficient to support metamorphosis. With regard to body size at metamorphosis, Smith (1987) found that an approximate 25% increase in size at metamorphosis was associated with an increased chance of breeding in the first year after metamorphosis, thereby elevating overall fecundity. Moderate corticosterone reduction in tadpoles therefore could increase adult reproductive success. However, other factors such as the ability to mount a corticosterone stress response may be altered in a less advantageous direction. Our 50% corticosterone reduction simulates the weakest effects reported for pollution-induced corticoid alteration in wildlife (see Gendron et al., 1997; Hontela et al., 1995; Quabius et al., 1997). Stronger reductions might produce quantitatively or qualitatively different effects on tadpole fitness components. Moderate corticosterone reduction per se, however, did not negatively affect growth and development in this species.

5. Conclusions

This work highlights the need for further study into the role of the HPI axis in mediating developmental responses of tadpoles to a variety of environmental stimuli. We found that relatively small elevations in whole-body corticosterone content affected *R. pipiens*

development and tail morphology, traits that are known to respond to environmental conditions. Given the potentially negative effects on fitness of moderate alterations in corticosterone content, this study also adds to the growing body of evidence that sublethal effects of disturbance can have significant, negative impacts on wildlife populations.

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