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Environmental Stress as a Developmental Cue: Corticotropin-Releasing Hormone Is a Proximate Mediator of Adaptive Phenotypic Plasticity in Amphibian Metamorphosis

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Environmentally induced phenotypic plasticity allows developing organisms to respond adaptively to changes in their habitat. Desert amphibians have evolved traits which allow successful development in unpredictable environments. Tadpoles of these species can accelerate metamorphosis as their pond dries, thus escaping mortality in the larval habitat. This developmental response can be replicated in the laboratory, which allows elucidation of the underlying physiological mechanisms. Here I demonstrate a link between a classical neurohormonal stress pathway (involving corticotropin-releasing hormone, CRH) and the developmental response to habitat desiccation. Injections of CRH-like peptides accelerated metamorphosis in western spadefoot toad tadpoles. Conversely, treatment with two CRH antagonists, the CRH receptor antagonist α-helical CRH$_{(9-41)}$ and anti-CRH serum, attenuated the developmental acceleration induced by habitat desiccation. Tadpoles subjected to habitat desiccation exhibited elevated hypothalamic CRH content at the time when they responded developmentally to the declining water level. CRH injections elevated whole-body thyroxine, triiodothyronine, and corticosterone content, the primary hormonal regulators of metamorphosis. In contrast, α-helical CRH$_{(9-41)}$ reduced thyroid activity. These results support a central role for CRH as a neurohormonal transducer of environmental stimuli into the endocrine response which modulates the rate of metamorphosis. Because in mammals, increased fetal/placental CRH production may initiate parturition, and CRH has been implicated in precipitating preterm birth arising from fetal stress, this neurohormonal pathway may represent a phylogenetically ancient developmental regulatory system that allows the organism to escape an unfavorable larval/fetal habitat.

While the genotype determines the range of phenotypic possibilities for an organism, the phenotype generated is strongly influenced by the external environment (Stearns, 1989). Amphibian larvae exhibit extreme plasticity in phenotypic characters which is dependent on a complex array of biotic and abiotic factors present in the larval habitat. For example, environmental effects on amphibian development can involve switching between alternate morphologies (e.g., induction of carnivorous morphs in spadefoot toad tadpoles; Pfennig, 1990; predator-induced changes in tail morphology and behavior in several species; see Werner, 1992; McCollum and Van Buskirk, 1996) or alterations in the length of the postembryonic period (Newman, 1992). Such developmental plasticity is thought to be adaptive for species which inhabit unpredictable environments (Newman, 1992).

Desert amphibians tend to breed in ephemeral ponds which are sporadically filled by rain, and evolution has produced traits which likely maximize fitness in such unpredictable environments. Following rainfall, adult desert toads (*Scaphiopus* spp.) are prepared to breed within minutes to hours after emergence from their fossorial enclaves; i.e., they are ''explosive'' breeders (Newman, 1992; Bragg, 1965). Tadpoles have a short development time, metamorphosing in as little as 8 days from hatching (e.g., *Scaphiopus couchii;* Newman, 1989). Also, they can accelerate development in response to habitat desiccation (they exhibit phenotypic plasticity in development time).

A central paradigm of evolutionary biology is the idea that there are trade-offs between suites of traits that make up the organismal life history (Pease and Bull, 1988). For desert toad tadpoles there are obvious trade-offs between mortality in the larval habitat and size at metamorphosis (tadpoles in short duration ponds metamorphose at a smaller size and size at transformation is

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generally correlated with adult measures of fitness; i.e., terrestrial performance, size and age at first reproduction; Newman, 1989; John-Alder and Morin, 1990; Smith, 1987; Semlitsch *et al.*, 1988; Werner, 1986). Because of such trade-offs, having a plastic development rate in an unpredictable environment may confer higher fitness than a fixed fast or a fixed slow rate; that is, the plasticity may be adaptive (Stearns, 1989; Newman, 1992). A rigorous test of the assertion that phenotypic plasticity induced by environmental variability is an adaptive response, and an understanding of the evolution of adaptive phenotypic plasticity, requires information on the fitness trade-offs associated with size at metamorphosis, the proximal environmental signals, and the functional relationship of these signals to the physiological mechanisms controlling growth and development (Stearns, 1989; Newman, 1992).

While knowledge of environmental effects on physiological systems controlling amphibian development is limited, there is a considerable amount of tissue-level, mechanistic information on the endocrine systems which influence metamorphosis. Amphibian metamorphosis is controlled by several endocrine systems, but the primary morphogen is thyroid hormone. Thyroid hormone induces the entire suite of morphological and biochemical changes which occur in each of the tadpole's tissues during metamorphosis (Kikuyama *et al.*, 1993). Corticosteroids from the interrenal glands, the classical vertebrate stress hormones, can synergize with thyroid hormone to accelerate metamorphosis (Kikuyama *et al.*, 1993). Alterations in development rate in response to environmental change are likely mediated by changes in the activity of these two endocrine systems.

The tadpole's neuroendocrine system (hypothalamus and pituitary gland) controls the normal progression of metamorphosis by controlling the activity of the thyroid and interrenal glands (Kikuyama *et al.*, 1993; Denver, 1996). This system serves as an external and internal monitoring system, transducing environmental information into a physiological response, thus modifying the rate of development. Current evidence suggests that, in tadpoles, the secretion of both thyroid hormone and corticosteroids is controlled by the neuropeptide corticotropin-releasing hormone (CRH; see Denver, 1996). CRH of hypothalamic origin acts by stimulating the production of pituitary hormones that control the thyroid and interrenal glands [thyrotropin (TSH) and adrenocorticotropic hormone (ACTH), respectively]. Thyrotropin-releasing hormone (TRH), the primary stimulator of TSH release in mammals (see Morley, 1981) is not active in this regard in tadpoles (see Norris, 1989; Denver, 1996). We first showed that CRH stimulates the release of TSH by adult frog, tadpole, and turtle pituitary glands [frogs (TSH bioactivity): Denver, 1988; Denver and Licht, 1989a; turtles (TSH immunoreactivity by RIA): Denver and Licht, 1989b, 1991; CRH also stimulates the release of immunoreactive TSH from cultured pituitaries of tadpoles of *Xenopus laevis* (detected by Western blot using antiserum to human TSHβ which recognizes frog TSH; Malagon *et al.*, 1989, 1991); R. J. Denver, unpublished results]. Since then, CRH has been shown to stimulate TSH secretion in representatives of all vertebrate classes except mammals (salmon: Larsen *et al.*, 1994; frogs: Jacobs and Kuhn, 1989; Malagon *et al.*, 1991; Jacobs and Kuhn, 1992; salamanders: Jacobs and Kuhn, 1989; chickens: Meeuwis *et al.*, 1989; Geris *et al.*, 1995, 1996). Two CRH-like molecules have been described in amphibia: the 41-amino-acid-residue peptide from *X. laevis* brain shares more than 93% homology with mammalian CRHs (Stenzel-Poore *et al.*, 1992) while sauvagine, a 36-a.a. peptide isolated from the skin of *Phyllomedusa sauvagei* shares approximately 50% homology with the mammalian and frog CRHs (Montecucchi and Henschen, 1981). Corticotropin-releasing hormone is the central and primary neuroendocrine regulator of the vertebrate stress response, and activation of CRH neurons leads to an elevation in plasma corticosteroid levels (see Vale, 1992). Thus, one predicts that in tadpoles, stressful stimuli might accelerate metamorphosis by producing an elevation in plasma thyroid hormone and corticosteroid concentrations, the two primary metamorphic hormones.

I hypothesized that modulation of the stress hormone axis allows tadpoles of desert species to respond adaptively to habitat dessication by accelerating development. In order to elucidate the proximate, physiological mechanisms which function in the metamorphic response to environmental stress I subjected western spadefoot toad tadpoles (*Scaphiopus hammondii*) to artificial habitat desiccation in the laboratory. Tadpoles accelerated metamorphosis when the water level of their aquarium was reduced, and the developmental response was positively correlated with elevations in hypothalamic CRH-like peptide content. Injection of CRH-like peptides accelerated metamorphosis and elevated whole-body concentrations of thyroid hormones and corticosterone (CORT). By comparison, injection of CRH antagonists attenuated the developmental response to habitat desiccation and reduced whole-body thyroid hormone concentrations. These results support a central role for CRH in environmentally induced acceleration of metamorphosis.

METHODS

Animal husbandry. Adult spadefoot toads were collected at various times near Livermore, California and housed in the laboratory in cages filled with potting soil. Animals were maintained on a 12L:12D photoperiod, 18–22°C thermal environment and fed crickets dusted with DIAGLO vitamins (Syntex). Spawning was induced by injecting males and females with 1 μg gonadotropin-releasing hormone agonist (Sigma Chemical Co.). Eggs and newly hatched tadpoles were maintained in transparent polystyrene rat cages (45 × 24 × 20 cm) with distilled H_2O containing 10% Holtfreter's salt solution (see Rugh, 1962) and provided with airstones; water was changed every other day. Tadpoles were fed *ad libitum* with tadpole chow [a mixture of rabbit pellets, agar, and Knox gelatin (see Rugh, 1962)] boiled lettuce, and spinach. Water temperature ranged from 21 to 23°C and photoperiod was kept constant at 12L:12D. Gosner (1960) staging was used to determine the developmental stage of spadefoot toad tadpoles. Tadpoles were raised in stock tanks for 2 weeks after hatching during which time they achieved a minimum size for metamorphosis (see Wilbur and Collins, 1973) and a minimum developmental stage (stage 30–32) for responding positively to habitat dessication (manipulation of the water level prior to achieving this stage of development results in retardation of growth and development; Denver, unpublished observations). Animal care was in accordance with the institutional guidelines set by the Animal Care and Use Committee of the University of California, Berkeley and the University Committee on the Care and Use of Animals of The University of Michigan.

Experiments were initiated by placing stage 32 tadpoles in polystyrene rat cages containing 10 L of water (water temperature 21–23°C; 12L:12D). Animals were maintained with either a constant ''high'' water level (''controls'') or the water level was decreased daily (by 0.5–1 L; ''experimentals''); the rate of decline of the water level is indicated on the graphs. Appropriate perturbations of the water were made in the control tanks to mimic water removal in the experimental tanks.

Treatment with CRH-like peptides and anti-CRH serum. The synthetic peptides and the anti-CRH sera used in these studies were kindly donated by Drs. Jean Rivier and Wylie Vale, respectively, both of The Salk Institute for Biological Studies. Sauvagine (SV), *Xenopus* CRH (xCRH), and α-helical $CRH_{(9-41)}$ (αhelCRH$_{(9-41)}$) were dissolved in the injection vehicle 0.6% phosphate-buffered saline (PBS); aliquots were stored at −80°C. In the amphibian assays in which they have been tested (i.e., stimulation of bioactive pituitary TSH release *in vitro,* stimulation of thyroid activity *in vivo,* acceleration of metamorphosis) and at the doses used, the CRH-like peptides ovine CRH (oCRH), rat/human CRH (r/hCRH), xCRH, and SV exhibited roughly equal potencies (Gancedo *et al.*, 1992; Denver, 1993; R.J. Denver, unpublished observations; throughout this paper the term CRH is used generically to refer to CRH-like peptides). αhelCRH$_{(9-41)}$ is a potent CRH receptor antagonist in mammals (Rivier *et al.*, 1984) and amphibians (Lowry and Moore, 1991). The anti-r/hCRH serum used for passive immunization of tadpoles was raised in sheep (Vale *et al.*, 1983) and diluted 1:1 with 0.6% PBS before injection. Control animals were injected with normal sheep serum (NSS; from Antibodies, Inc.) prepared in the same manner as the antiserum.

Tissue extraction and radioimmunoassay for thyroxine, triiodothyronine, and corticosterone. Whole-body hormone concentrations were determined by radioimmunoassay following extraction as described by Denver (1993) for thyroid hormones and Hayes and Wu (1995) for CORT. Tadpoles were homogenized in methanol with 1 m*M* propylthiouracil and the homogenates were divided in half for either thyroid hormone or corticosteroid extraction. Recoveries were estimated by adding either [^{125}I]thyroxine (T_4) or [^{3}H]CORT to the homogenates. Pilot studies comparing the recovery of [^{125}I]T_4 and [^{125}I]triiodothyronine (T_3) revealed that they were roughly equal; therefore, only the recovery of [^{125}I]T_4 was monitored routinely. Recoveries ranged from 45 to 70% for [^{125}I]T_4 and 20 to 45% for [^{3}H]B. The thyroid hormone RIAs (T_3 and T_4) were as described by MacKenzie *et al.* (1978) and Denver and Licht (1988) and the RIA for CORT was as described by Licht *et al.* (1983). Primary antisera for T_3 and CORT were purchased from Endocrine Sciences and the antiserum for T_4 was purchased from Dr. Viggo Kruse (Denmark).

Tissue extraction and radioimmunoassay for CRH. Individual hypothalami collected from tadpoles were acid-extracted prior to RIA as described by Mastorakos and colleagues (1995). Recoveries, which were assessed by the addition of ^{125}I-oCRH (New England Nuclear) to tissues prior to homogenization, averaged 51%. The antiserum used in the RIA was produced in rabbits against xCRH conjugated to human α globulins following the methods of Vale and colleagues (1983). The tracer was high-specific-activity ^{125}I-oCRH (Dupont NEN), the standard was synthetic xCRH, and the RIA was done as described by Vale and colleagues (1983). Dilutions of both oCRH and tadpole hypothalamic extracts produced dilution curves that were parallel to the xCRH standard. The

sensitivity of the assay was 4 pg/ml extract and all samples were analyzed in a single assay with an intra-assay coefficient of variation of 3.9%. A second assay was performed where the primary antiserum was anti-oCRH (oC24; this antiserum cross-reacts with all CRH-like molecules; Vale *et al.*, 1983) and xCRH and oCRH were compared as standards. This assay gave results that were virtually identical to those obtained with the assay using anti-xCRH as the primary antiserum (i.e., suggesting the same level of cross-reactivity of both antisera with CRH-like peptides). Because the precise identities of the CRH-immunoreactive compounds in the spadefoot toad tadpole hypothalamus were not identified in this study, peptide content is referred to as CRH-like immunoreactivity. Protein assays (Bio-Rad) were done on aliquots of tissue extracts for normalization of tissue CRH content; i.e., CRH levels are expressed as pg peptide/μg tissue extract.

Statistical analyses. Data for developmental stage, body mass, hind limb length, and whole-body and hypothalamic hormone concentrations were analyzed for differences between treatment groups using the SOLO statistical package (BMDP Software, Inc.) Sample sizes are indicated in the figure legends. Data were $\log_{10}$-transformed to achieve homogeneity of variance. In experiments with multiple measurements which extended over the larval period, I used multivariate analysis of variance (MANOVA) to determine significant date-by-treatment interactions. If the date-by-treatment interaction was significant, I analyzed separate univariate contrasts (one-way ANOVA) to determine at which dates the treatments diverged. Duncan's multiple range test was used to separate means in experiments with greater than two variables. Student's *t* test was used in some cases (indicated in text) to test for significance between unpaired means.

RESULTS AND DISCUSSION

Tadpole metamorphosis was accelerated when I reduced the water level of the aquarium (hereafter referred to as habitat desiccation; Fig. 1). Tadpoles subjected to habitat desiccation diverged in developmental stage from controls at Day 26 posthatch ($P < 0.05$). The response obtained with *S. hammondii* tadpoles in this study resembles that observed with *S. couchii* tadpoles subjected to habitat desiccation in experimental outdoor ponds (Newman, 1989). By controlling this response in the laboratory, questions regarding the physiological mechanisms underlying the response to the changing larval environment can be addressed.

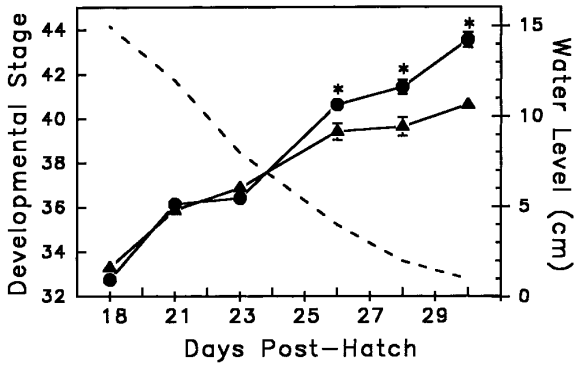

FIG. 1. Developmental acceleration in spadefoot toad tadpoles in response to experimentally induced habitat desiccation. Animals were maintained with either a constant "high" water level ("controls"; ▲) or the water level was decreased daily (by 0.5–1 L; "experimentals"; ●) as indicated by the dashed line on the graph. Treatments were replicated six times (i.e., six tanks) to control for possible tank effects. Points on the graph are the means with SEM. Metamorphic climax (forelimb emergence) is stage 42.

Because CRH can regulate tadpole thyroid and interrenal function (see Denver, 1993) and CRH is the only neuropeptide so far identified which possesses metamorphosis-accelerating activity (Denver, 1993, 1996; Gancedo *et al.*, 1992) I hypothesized that this molecule functions as a proximate mediator of the developmental response of spadefoot toad tadpoles to habitat desiccation. The CRH system may serve to transduce external environmental signals (e.g., pond drying) into an endocrine response leading to accelerated development. To test this hypothesis I manipulated the neuroendocrine axis of spadefoot toad tadpoles and examined the correlation between neuroendocrine function and phenotypic plasticity. First, I conducted a temporal study of the acceleration of metamorphosis by a CRH-like peptide (SV) in *S. hammondii.* Hormone treatment produced significant advancement in developmental stage, reduction in body weight, and increase in size-specific hindlimb length by 8 days after the injections were begun (developmental stage: $F(1, 22) = 5.14$, $P = 0.03$; body weight: $F(1, 22) = 4.69$, $P = 0.04$; hind limb length: $F(1, 22) = 4.77$, $P = 0.04$; Fig. 2). The experimental animals continued to diverge from the controls with continued peptide treatment and reached metamorphic climax at an earlier age. These results support previous findings that exogenous CRH-like peptides can accelerate tadpole metamorphosis (Gancedo *et al.*, 1992; Denver, 1993).

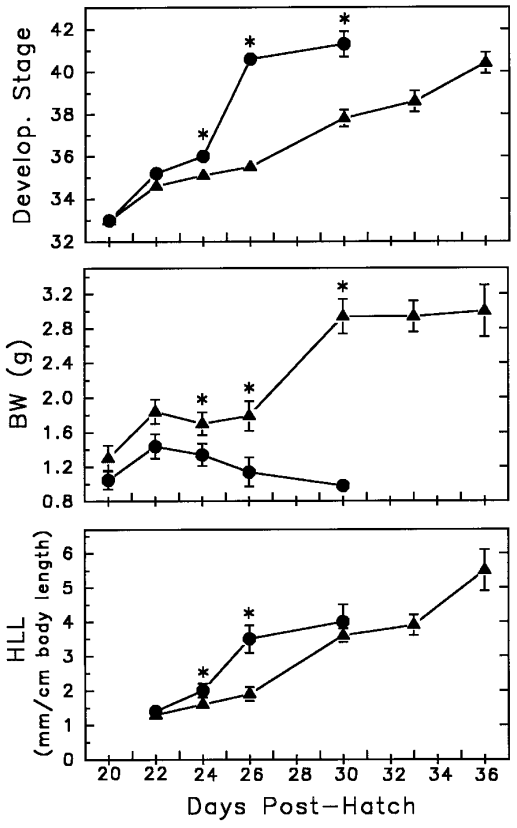

FIG. 2. Acceleration of tadpole metamorphosis by the CRH-like peptide sauvagine (SV). Injection of SV (●; ip; 2 μg/animal) or vehicle (0.6% saline; ▲) was begun at stage 32 (18 days posthatch; i.e., 2 days before the initial measurement shown on the graph at 20 days posthatch) and continued every other day for the next 10 days. The experiment included seven tanks per treatment (4-L water/tank) each with a starting density of 12 tadpoles/tank. One tank from each treatment was removed from the experiment at each time point, the animals were measured (BW: body weight; HLL: hindlimb length) and sacrificed for hormone measurements (see Fig. 4); each point on the graph represents the average of 10–12 animals with SEM. Asterisks designate a significant difference between the hormone- and saline-injected groups at the different time points ($P < 0.05$; ANOVA).

If CRH functions as a physiological regulator of metamorphosis, then the CRH gene should be expressed at the appropriate time during development. In support of this, CRH-like immunoreactivity appears in the median eminence of bullfrog tadpoles during prometamorphosis and increases during metamorphic climax, paralleling elevations in plasma thyroid hormone and corticosteroids (Carr and Norris, 1990). In the present study, the peptide was present in the hypothalamus of *S. hammondii* during the appropriate developmental stages (Table 1). Furthermore, exposure to habitat desiccation produced an elevation in hypothalamic CRH content at 23 days posthatch. Interestingly, this difference occurred 3 days before significant morphological divergence occurred between the experimental and control animals (see Fig. 2 and Table 1); significantly, the time of elevation in CRH content corresponds to the time point at which there are elevations in thyroid hormone and corticosteroid levels in the experimental tadpoles (Denver, submitted for publication). Thus, elevations in CRH and the peripheral hormones regulated by the peptide are correlated, and these increases precede external morphological change. Continued exposure to the desiccating environment resulted in a significant decline in CRH content, which could reflect accelerated peptide release or decreased biosynthesis (i.e., resulting from increased negative feedback by rising plasma corticosteroid levels).

To test more directly the hypothesis that CRH controls metamorphosis and the developmental response to environmental change, I blocked the activity or availability of the endogenous peptide during desiccation-induced metamorphosis using two approaches. First, I injected tadpoles with the synthetic peptide αhelCRH$_{(9-41)}$ to block CRH binding to its receptor. Second, I passively immunized tadpoles with antiserum to CRH to sequester and thus make unavailable the endogenous CRH (Denver, 1993). Both treatments significantly attenuated the developmental response to habitat desiccation (Figs. 3A and 3B). Saline-injected tadpoles subjected to habitat desiccation were significantly different from the saline-injected constant high-water tadpoles and the αhelCRH$_{(9-41)}$-treated tadpoles by Day 27 posthatch (7 days after injections were begun; $F(3, 28) = 9.83$; $P < 0.001$); they continued to diverge from the other groups throughout the experiment. Also, while 100% of the NSS-injected tadpoles subjected to habitat desiccation reached metamorphic climax by 30 days posthatch, none of the anti-r/hCRH tadpoles or the NSS-treated constant high-water controls had metamorphosed by 36 days posthatch when the experiment was terminated. Taken together, these results provide compelling support for endogenous CRH being a physiological mediator of adaptive phenotypic plasticity in metamorphosis.

The primary pathway by which CRH controls metamorphosis is likely to be through activation of the thyroid axis (Gancedo *et al.*, 1992; Denver, 1993; see Denver, 1996) since thyroid hormone is required for the metamorphic process (Kikuyama *et al.*, 1993). CRH should also activate the interrenal axis (Kikuyama *et al.*, 1993; Denver, 1996); however, corticosteroids are not sufficient to induce metamorphosis, although they can synergize with thyroid hormone (Kikuyama *et al.*,

TABLE 1

Hypothalamic CRH-like Peptide Content during Spontaneous and Desiccation-Induced Metamorphosis in *S. hammondii*

Days since hatching	Treatment		*P* value
	Constant high	Decreasing	
22	3.27 ± 0.87 (36.9; 5)	9.49 ± 1.74 (36.4; 5)ab	0.013
25	7.36 ± 2.33 (39.4; 6)	3.07 ± 0.61 (40.6; 6)c	0.078
27	9.55 ± 4.17 (39.6; 4)	3.41 ± 1.61 (41.4; 5)c	0.101
29	4.59 ± 0.51 (40.6; 6)	4.54 ± 0.52 (43.5; 5)bc	0.936
ANOVA:	$F_{(3,20)} = 1.44$, $P = 0.264$	$F_{(3,20)} = 4.33$, $P = 0.0193$	

Note. CRH was measured in individual tadpole hypothalami by radioimmunoassay and is expressed as pg peptide / μg protein. Tadpoles were subjected to the pond drying protocol as described in the legend to Fig. 1 (a parallel experiment was done for tissue collection). Days since hatching correspond to intervals in Fig. 1 beginning with the third measurement point; i.e., tissues were not collected for measurement of CRH content at the first two time points when morphometric analyses were done (19 and 21 days posthatch). The numbers in parentheses are the average developmental stage of the group of tadpoles at the time of measurement and the sample size. One-way ANOVA was performed within treatments to determine significant age effects (statistics presented at bottom of columns). Letters next to the Decreasing group measurements separate the means based on Duncan's multiple range test ($P < 0.05$). *P* values in the last column are from unpaired *t* tests comparing the means of the two treatments within an age group.

1993). Consistent with this prediction, injection of the CRH-like peptide SV produced an early (relative to the developmental stage) and sustained elevation in whole-body content of the thyroid hormones T_4 and T_3 and the interrenal steroid hormone CORT in *S. hammondii* tadpoles (measured 24 hr after injection; Fig. 4). Interestingly, SV has been thought to not influence ACTH secretion in amphibians (adult *Rana ridibunda;* Tonon *et al.*, 1986). The positive effect of SV on CORT content in this study could relate to species or developmental stage differences. In another experiment, injection of xCRH produced a rapid (by 6 hr), dose-related increase in whole body T_4, T_3, and CORT content (Fig. 5), which is consistent with the view that CRH acts directly on the pituitary to stimulate the release of TSH and ACTH (Denver, 1996). By contrast, treatment with αhelCRH$_{(9-41)}$ reduced whole-body T_4 and T_3 but not CORT (Fig. 6A), suggesting that the primary mechanism by which αhelCRH$_{(9-41)}$ decelerates metamorphosis is by reducing thyroid hormone production (CORT production may be controlled by alternate pathways, e.g., arginine vasotocin; reviewed by Denver, 1996).

The activity of hypothalamic CRH neurons in mammals is increased in response to external stress (see Vale, 1992). Because CRH is the primary neuroregulator of the vertebrate stress response, a neuroendocrine stress pathway may mediate the developmental acceleration induced by habitat desiccation. The response to stress in vertebrates results in elevations in plasma corticosteroids which mobilize stored fuels to allow the animal to cope with the stress (Ballard, 1979). Whether the tadpole perceives as stressful the loss of habitat by desiccation is not known; nevertheless, such loss results in a series of endocrine changes resembling a classical endocrinological stress response (i.e., increases in whole-body CORT content; Denver, submitted for publication). Because CRH regulates both thyroid hormone and corticosteroid production in the tadpole, the response to stress may also produce increased thyroid activity because CRH activates the thyroid axis. To test whether stress per se activates the tadpole thyroid axis I subjected animals to handling / injection stress, a stressor common to all vertebrates studied (Selye, 1976). When compared with undisturbed tadpoles, animals subjected to the handling / injection stress exhibited elevated whole-body T_4 content within 2 hr of treatment (Fig. 6B), supporting the view that neuroendocrine stress pathways influence the tadpole thyroid axis. Similar results were recently reported with bullfrog tadpoles (*Rana catesbeiana*) in which chronic injection stress resulted in elevations in thyroid hormone production (Wright *et al.*, 1996). The CRH-stress pathway may allow tadpoles of desert amphibians to monitor habitat quality and to mount an adaptive response by accelerating metamorphosis and thus reducing mortality in the desiccating larval habitat.

Corticotropin-releasing hormone may function at multiple levels in the developmental and behavioral response of spadefoot toad tadpoles to habitat desiccation. A neurotransmitter / neuromodulator function for CRH is suggested by the wide distribution of CRH-like immunoreactivity and mRNA expression in the vertebrate brain (mammals: see Sawchenko *et al.*, 1993; amphibians: Verhaert *et al.*, 1984; Gonzalez and Lederis, 1988; Olivereau *et al.*, 1987; Carr and Norris, 1990; Stenzel-Poore *et al.*, 1992; Gonzalez *et al.*, 1996). In addition

to the well-known hypophysiotropic role of hypothalamic CRH (i.e., stimulation of ACTH and TSH release), CRH-like peptides have been implicated in the control of several behaviors and autonomic functions (reviewed by Koob *et al.*, 1993; Fisher, 1993). For instance, CRH-like peptides are known to suppress appetite and feeding behavior in several vertebrate species (reviewed by Koob *et al.*, 1993; De Pedro *et al.*, 1993, 1995; Spina *et al.*, 1996) including tadpoles (Corpas *et al.*, 1991). *Scaphiopus* tadpoles reduce foraging behavior as the water level of their aquarium declines (before lateral movement is obviously restricted; Denver, unpublished observations). Furthermore, in tadpoles, there is a complex interaction between food level and metamorphic rate, and food restriction or starvation after a critical developmental stage accelerates metamorphosis (D'Angelo *et al.*, 1941; Newman, 1994; Denver, Mirhadi, and Phillips, submitted for publication). Thus, in desert tadpoles there could be a complex inter-

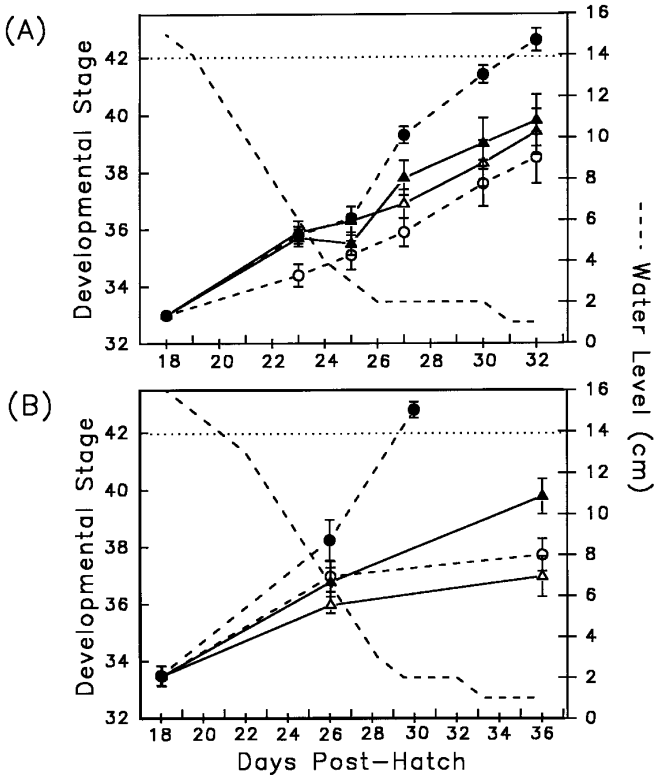

FIG. 3. Attenuation of the developmental response of spadefoot toad tadpoles to habitat dessication by antagonists of CRH. (A) Effects of αhelCRH$_{(9-41)}$ on habitat desiccation-induced metamorphosis in *S. hammondii.* Tadpoles (10/group) were subjected to a similar habitat desiccation protocol as described in the legend to Fig. 1 (change in water level is indicated by the dashed line). Dashed lines and circles indicate a decreasing water level while solid lines and triangles indicate a constant high (10-L) water level. Tadpoles were injected with either 0.6% saline (△, ○) or with αhelCRH$_{(9-41)}$ (▲, ●; ip; 2 μg/animal/day). Injections were begun on Day 20 posthatch and continued every day for 10 days. Each point represents the average of 8–10 animals with SEM. Metamorphic climax (stage 42) is indicated by the horizontal dotted line in the upper part of the graph. (B) Tadpoles (6–10/group) were given an ip injection of anti-CRH (αCRH; △, ○) serum or normal sheep serum (NSS; ▲, ●) starting at the onset of the pond drying protocol (see legend to Fig. 1). Serum (anti-r/hCRH raised in sheep) was diluted 1:1 with 0.6% saline and administered in a 50-μl injection volume. Injections were repeated 4 days after the initial injection. As in Fig. 3A, dashed lines and circles indicate a decreasing water level while solid lines and triangles indicate a constant high water level. Differences between treatment groups approached significance ($P = 0.07$) at Day 26 on the graph. At Day 36, the NSS-injected constant high-water group was significantly advanced in developmental stage ($P = 0.02$) compared with the remaining two groups.

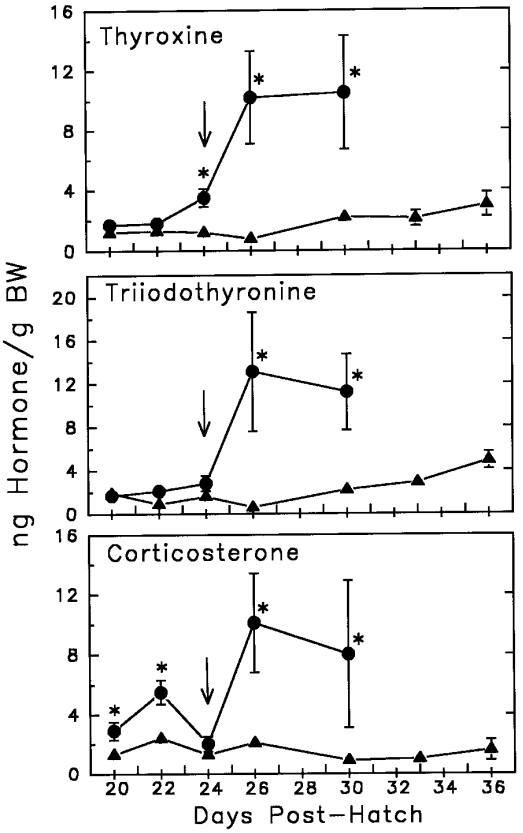

FIG. 4. Elevation of whole body content of thyroxine, triiodothyronine, and corticosterone in *S. hammondii* tadpoles treated chronically with the CRH-like peptide sauvagine (SV). The experimental protocol was as described in the legend to Fig. 2 (▲ 0.6% saline; ● SV 2 μg/animal). Whole body hormone content was determined 24 hr after injection in tissue extracts by radioimmunoassay. Each point is the mean with SEM for six animals. Asterisks indicate significant univariate contrasts ($P < 0.05$). Vertical arrows designate the time point at which a significant divergence in morphological characters was observed (see Fig. 2).

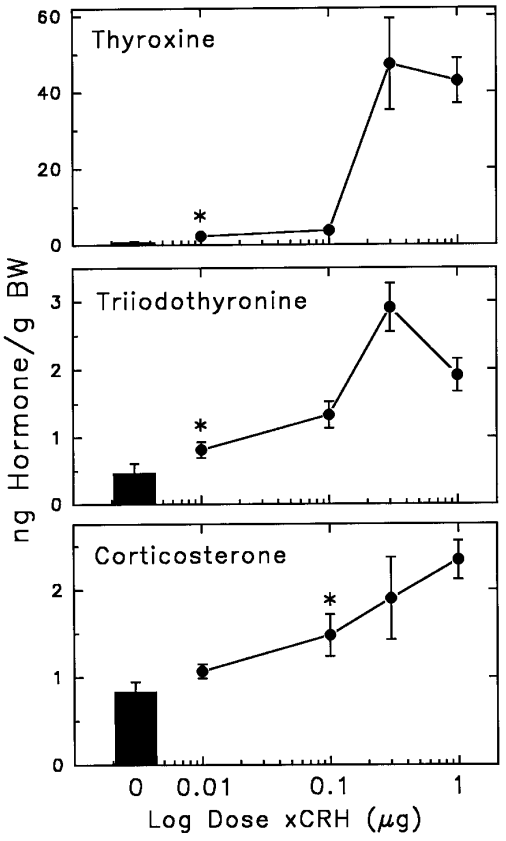

FIG. 5. Rapid, dose-related elevation in whole body content of thyroxine, triiodothyronine, and corticosterone following injection of xCRH. Stage 37–39 tadpoles ($n = 7$/treatment) were given a single ip injection of 0.6% saline (filled bars) or varying doses of xCRH (●). Tadpoles were sacrificed 6 hr later and extracted for hormone analyses (see legend to Fig. 4). Asterisks indicate the minimum effective dose; this dose and all doses higher were significantly different from the zero dose [$P < 0.05$ (Student's t test)].

action among food intake, behavior, and morphogenesis that is coordinated by the CRH neurons (see Fig. 7 for a model for regulatory interactions involving CRH in the desert toad tadpole). The coordinated suppression of feeding and acceleration of morphogenesis makes sense when one considers that the gut is undergoing dramatic remodeling at this time of tadpole development, transforming from an omnivorous/herbivorous morphology to a carnivorous morphology (Shi and Ishizuya-Oka, 1996).

A role for CRH as a developmental regulator in mammals is becoming increasingly apparent. Studies in sheep strongly support a role for fetal CRH secretion in the initiation of parturition (Brooks and Challis, 1988; McDonald and Nathanielsz, 1991). Recent data in humans also support a role for CRH (of either placental or fetal origin) in triggering parturition (McLean, 1995). Interestingly, in pregnancies complicated by preeclampsia and in several clinical situations that result in preterm births (i.e., fetal stress)

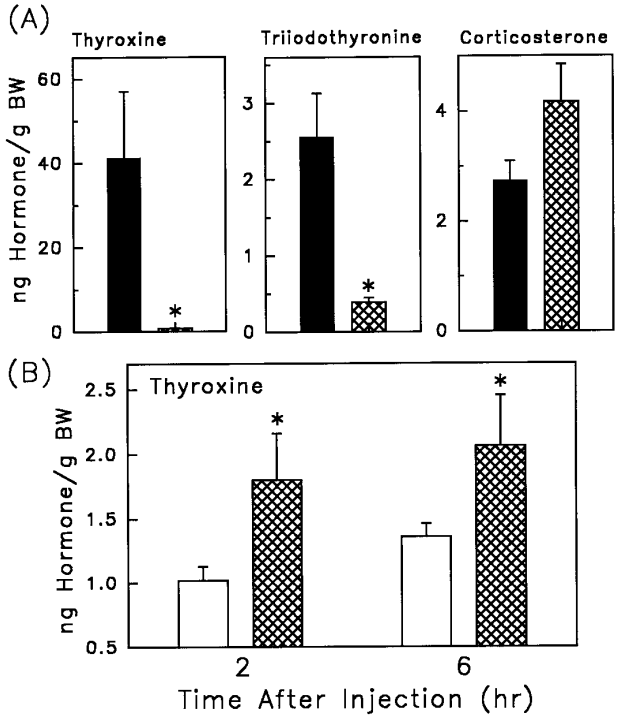

FIG. 6. Alterations in thyroid activity of prometamorphic *S. hammondii* tadpoles by injection of the CRH receptor antagonist αhelCRH$_{(9-41)}$ or 0.6% saline. Hormone content was determined by RIA (see legend to Fig. 4). Gosner stage 36–38 tadpoles were used in each of the experiments. (A) Alterations in whole body thyroxine, triiodothyronine, and corticosterone content by αhelCRH$_{(9-41)}$ in *S. hammondii* tadpoles. Tadpoles raised in a large volume (10 L) from hatching were transferred directly to a low-water environment (0.5 L) and given daily ip injections of vehicle (0.6% saline; ■) or αhelCRH$_{(9-41)}$ (1 μg/animal; BW @ 2.5 g; ▩) for 3 days. Tadpoles were sacrificed 6 hr after the last injection. Bars are the means ($n = 6$) with SEM and asterisks designate significant differences between vehicle- and αhelCRH$_{(9-41)}$-injected animals by hormone [$P < 0.001$ (Student's t test)]. Hormone levels of the saline-injected animals were significantly elevated (due to transfer to a low-water environment) compared with tadpoles maintained in a high-water environment; i.e., a rapid decline in water level results in rapid (within 2 days) activation of the endocrine axes (data not shown) which is attenuated by the CRH receptor antagonist. (B) Handling stress elevates whole-body thyroxine concentration in *S. hammondii* tadpoles. Tadpoles were distributed into tanks 24 hr before the experiment was begun. Two groups of 8 animals each were handled and given an ip injection of 0.6% saline at time 0; two other groups were left undisturbed. One handled group (▩) and one unhandled group (□) were sacrificed at 2 and 6 hr and processed for whole body thyroxine measurement (see legend to Fig. 4). Bars represent the mean of eight animals with SEM and asterisks indicate a significant difference from the control [unhandled; $P < 0.05$ (Student's t test)].

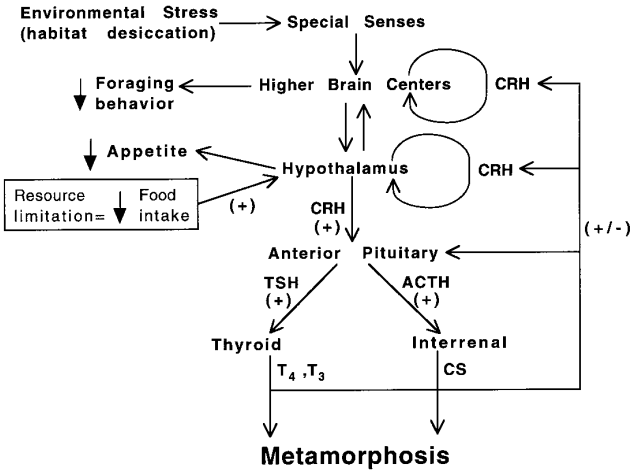

FIG. 7. A model for the multiple levels of corticotropin-releasing hormone (CRH) actions/interactions in the developmental and behavioral responses of spadefoot toad tadpoles to habitat desiccation. Tadpoles could either respond directly to the drying pond by sensing a change in the water level, or they might respond to environmental changes that are correlated with the decline in the water level (e.g., decreased resource availability due to increased population density and thus increased competition for resources; see box). The stress of habitat loss is perceived through special senses. This stress results in the activation of extrahypothalamic CRH neurons which then reduces foraging/exploratory behavior. Activation of CRH neurons in the hypothalamus (through descending pathways from higher brain centers) reduces appetite; alternatively, resource limitation with a resultant decrease in plasma amino acids and glucose could result in the stimulation of hypothalamic CRH biosynthesis/secretion (see box for alternative pathway; Sumitomo *et al.*, 1987; Suda *et al.*, 1988; Berkenbosch, 1989; Guillaume *et al.*, 1989). Hypothalamic CRH stimulates pituitary thyroid-stimulating hormone and adrenocorticotropic hormone secretion, which stimulate the production of peripheral hormones responsible for promoting metamorphosis (thyroid hormones: T_4 and T_3; corticosteroids: CS). The thyroid and the interrenal hormones exert both positive effects on the differentiation of the central nervous system and negative feedback on the production of hypothalamic and pituitary hormones (see Denver, 1996; Denver *et al.*, 1997).

maternal and fetal plasma CRH levels are dramatically elevated (McLean *et al.*, 1995). Upregulation of the CRH gene in humans may result in a cascade of regulatory events culminating in the delivery of the fetus from an unfavorable environment (Challis and Hooper, 1989). Because data from the sheep indicate the fetus as the point of decision in the process (McDonald and Nathanielsz, 1991), this mechanism may allow the developing organism to sense a deteriorating fetal habitat and to send an activational signal to the mother for the initiation of parturition (Challis and Hooper, 1989). The results from desert amphibians suggest that CRH could represent a phylogenetically ancient developmental regulator which developing organisms use to monitor and respond adaptively to deleterious changes in the larval/fetal environment.

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