Neuroendocrinology of context-dependent stress responses: vasotocin alters the effect of corticosterone on amphibian behaviors

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Abstract

The ability of an animal to respond with appropriate defensive behaviors when confronted with an immediate threat can affect its survival and reproductive success. In the roughskin newt (Taricha granulosa), exogenous corticosterone (CORT) rapidly blocks and vasotocin (VT) enhances reproductive behaviors (mainly clasping behavior). Electrophysiological studies have shown that pretreatment of male Taricha with VT counteracts the inhibitory effects of CORT on neuronal activity in the medulla. To test whether similar interactions between VT and CORT influence reproductive behaviors in Taricha, we recorded the time spent and incidence of clasping in males injected with VT or vehicle at 60 min and then CORT or vehicle at 5 min before presentation of a female. This study found that clasping behavior is suppressed in males that received vehicle and then CORT, but is not suppressed in males that received VT and then CORT. Considering these results and the possibility that the performance of clasping behaviors might cause increases in endogenous VT activity, we tested whether the suppressive effects of CORT administration on clasping behavior would occur in males that had recently clasped females. The study found that, in contrast to males that had been isolated from females, CORT administration did not suppress clasping behavior in males that had been allowed to clasping females for 60 min prior to the hormone injection. Our results suggest that, at least in this amphibian and perhaps in other animals, the neuroendocrine regulation of alternative behavioral responses to threats involves functional interactions between corticosteroids and VT-like peptides.

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An animal’s survival and reproductive success depend on its ability to respond appropriately when confronted with an immediate threat. Most animals respond to a threat with specific defensive behaviors, expressing the most appropriate behavior for the animal’s specific situation. For example, adult killdeer without young will respond defensively by flying away from potential predators; whereas killdeer with young are more likely to present broken-wing displays that attract the attention of potential predators away from the nest (Ehrlich et al., 1988). Similarly, solitary male newts are more likely to respond defensively by swimming away from an approaching person, than are male newts that are engaged in sexual behaviors, such as clasping (unpublished observation). Thus, appropriate behavioral responses to stressors (sensory stimuli that activate the hypothalamo–pituitary–adrenal axis) depend on the animal’s context—its surrounding environment and current physiological and behavioral states. The corresponding neuroendocrine mechanisms underlying these context-dependent behavioral responses are poorly understood.

The roughskin newt, Taricha granulosa, has been used to examine the neuroendocrine mechanisms regulating the physiological and behavioral responses to stressors. In this amphibian, males respond to handling stress with elevated plasma corticosterone (CORT) concentrations and decreased expression of sexual behaviors (Moore and Miller, 1984). Administration of CORT rapidly and strongly inhibits male sexual behaviors, specifically inhibiting clasping behavior (Moore and Miller, 1984; Orchinik et al., 1991). Furthermore, treating male Taricha with metyrapone (a drug that blocks corticosteroid synthesis) tends to block the
stress-induced suppression of clasping behavior (Moore and Miller, 1984). Electrophysiological studies with Taricha show a corresponding suppressive effect of CORT administration in medullary neurons that respond to clasp-triggering cloacal stimulation and show clasping-related firing (Rose et al., 1993, 1995, 1998). Thus, in Taricha, acute stress results in elevated CORT levels, which in turn influence sensorimotor coordination of clasping behavior.

Acute stress is known to inhibit reproduction-related behaviors in many vertebrates. For example, acute stress decreases territorial marking and ultrasonic courtship vocalizations of male mice (Lumley et al., 1999), suppresses estrous behaviors in mice (Marchlewksa-Koj et al., 1994), decreases lordosis behavior in rats (Hulse and Coleman, 1983), and suppresses sexual behaviors in primates (Habib et al., 2000). Furthermore, acute stress rapidly elevates plasma corticosteroid levels in a variety of vertebrates including birds (Heiblum et al., 2000), rats (Graessler et al., 1989), frogs (Coddington and Cree, 1995; Lich et al., 1983), and reptiles (Moore et al., 1991). High plasma corticosteroid concentrations have been shown to modify behavioral responses in birds (Breuner et al., 1998; Silverin, 1986; Wingfield and Silverin, 1986), rats (Kavaliers and Ossenkopp, 2001; Haller et al., 1998; Sandi et al., 1996), hamsters (Hayden-Hixson and Ferris, 1991), and shrews (Schiml and Rissman, 1999).

Taricha have also been used to examine the behavioral and neurophysiological function of arginine vasotocin (VT). The effects of VT on behaviors in Taricha are consistent with studies on behavioral effects of VT and vasopressin (VP) (mammalian homologue of VT) in other vertebrates (for reviews see Bass and Grober, 2001; Goodson and Bass, 2001; Moore and Rose, 2002; Emerson and Boyd, 1999; Iwata et al., 2000). Specific examples of reproduction-related behaviors enhanced by VT/VP include spawning behavior in killifish (Pickford and Streek, 1977), sexual receptivity and phonotaxis in female frogs (Diakow, 1978; Boyd, 1994), sexual receptivity in female rodents (Human and Albers, 1993; Sodersten et al., 1983), and sexual behaviors in male birds (Kihlstrom and Danninge, 1972; review see Panzica et al., 2001). VT/VP administration also enhances scent marking and olfactory communication in hamsters (Albers and Rawls, 1989) and newts (Iwata et al., 2000), parental behavior, pair bonding and mate choice in prairie voles (Wang et al., 2000; for review of mammals see Young et al., 2001; Insel and Young, 2001), courtship and sperm transfer in newts (Iwata et al., 2000), and courtship behaviors of a teleost (Semsar et al., 2001; for review also see Bass and Grober, 2001; Goodson and Bass, 2001).

There is strong evidence that VT enhances reproductive behaviors in Taricha (for reviews see Moore and Rose, 2002; Moore, 1987, 1992). Administration of VT agonists increases the incidence of male clasping behavior; whereas administration of VT antagonists suppresses this behavior (Moore and Miller, 1983; Moore and Zoeller, 1979). Behavioral studies show that VT administration enhances ap-

petitive responses of males to visual and olfactory sexual stimuli (Thompson and Moore, 2000). Electrophysiological studies with Taricha also reveal that VT administration enhances the firing rates of medullary neurons that respond to clasp-triggering somatic stimulation of the cloaca (Rose et al., 1995). Taken together these studies suggest that VT and CORT have opposite effects on medullary neurons and reproductive behaviors in male Taricha.

Electrophysiological studies have revealed an interesting and complex interaction between VT and CORT in Taricha (Rose et al., 1995). CORT administration typically decreases the responses of medullary neurons to cloacal stimulation, whereas VT typically enhances the firing rate in these sensory-responsive neurons. However, if VT is administered prior to CORT, there is an overall potentiation of medullary neuronal firing in response to clasp-triggering tactile stimulation. Thus, pretreatment with VT appears to reverse the suppressive effects of CORT on neuronal activity.

These interactions between VT and CORT on neuronal activity suggested that these two hormones might interact in a similar manner to affect whole-animal behavioral responses. Therefore, we hypothesized that pretreatment with VT will block the inhibitory effect of CORT administration on clasping behavior of male Taricha. Furthermore, considering that the performance of clasping behavior might cause increased endogenous VT activity, we hypothesized that prior exposure to clasping would decrease the inhibitory effects of CORT on clasping behavior. Results from the studies described in this article are consistent with these two hypotheses.

Materials and methods

Animals

Sexually active adult newts (Taricha granulosa) were collected locally during the breeding season (March 2000) from permanent ponds in the Coast Range (Benton County). Newts were housed in an environmentally controlled room with natural photoperiod (13L:11D) and temperature (average 13°C). Males collected from the perimeters of ponds using a dip net were held together for 24–48 h in tanks of dechlorinated water and fed an excess mixture of red worms and beef liver each evening. Males weighed, on average, 14 ± 0.5 g. Females were captured during migration to breeding ponds in pit traps placed along drift fences. Females were housed individually for 24–72 h in small containers with damp moss, leaf litter, and an abundant supply of red worms and crickets. These collecting strategies allowed for capture of males in breeding condition and the capture of unmated females that are sexually attractive to males (Moore, 1978; Propper, 1989).
Behavioral testing

All behavioral testing was performed between 1400 and 2000 h. Individual males were tested in circular tanks (27 cm diameter) filled to a depth of 6 cm with dechlorinated water. These testing tanks were kept in low-light conditions within a specified arena (3 × 3 m) enclosed by a curtain of black plastic hung from the ceiling to the floor. Individual males were transferred from holding tanks into separate testing tanks at least 30 min prior to any injections and behavioral testing. Incidence of clasping and time spent clasping were determined from videotape recorded with a low-light video camera. Incidence of clasping was analyzed as proportion of males observed clasping at 15, 30, and 60 min after a female was added to the arena.

Hormone administration

Newts were injected intraperitoneally (ip) with 40 μg CORT/0.1 ml or 0.1 ml vehicle alone and 100 μg VT/0.1 ml or 0.1 ml vehicle alone. Because the sensitivity of Taricha to CORT varies seasonally, we conducted a dose–response study to determine the dose of CORT required to inhibit Taricha clasping behavior during the height of the breeding season. Of the different doses (0, 2, 11, 20, 30, or 40 μg CORT/0.1 ml), the only dose of CORT that reliably inhibited male clasping behavior was 40 μg/0.1 ml (Kruskal–Wallis = 26.2, P < 0.001).

CORT and VT were purchased from Sigma–Aldrich Company (St. Louis, MO). CORT solution was prepared with 99.8% amphibian Ringer’s and 0.2% DMSO (dimethyl sulfoxide; Sigma–Aldrich), and stored at 4 °C. Vehicle to control for CORT effect was prepared simultaneously with 99.8% amphibian Ringer’s and 0.2% DMSO. VT solution was prepared with amphibian Ringer’s in silicone-coated glassware (Sigmacoat, Sigma–Aldrich), divided into 1-ml aliquots, snap-frozen in dry ice, and stored at −80°C until use. A different vehicle control solution (amphibian Ringer’s) for VT effect was prepared simultaneously and stored in the same manner as VT stock.

Experiment 1: vasotocin pretreatment

This experiment examined whether prior exposure to VT might modulate the effect of CORT on clasping behavior. Male newts were randomly assigned to one of four treatment groups (12 males per group). The four treatments were as follows: VT followed by CORT (VT/CORT), VT followed by vehicle (VT/vehicle), vehicle followed by CORT (vehicle/CORT), and vehicle followed by vehicle (vehicle/vehicle). Individual males were randomly assigned to each test tank at time 0. At 30 min all males received an intraperitoneal injection of either VT or vehicle, and at 90 min all males received an intraperitoneal injection of either CORT or vehicle. Five minutes after the second injection one female was placed with each male, behavioral observations began immediately lasting 1 h.

Experiment 2: sexual experience pretreatment

This second experiment examined whether immediate previous sexual experience (the performance of clasping behavior) alters the effect of CORT on the incidence of clasping behavior. Individual males were randomly assigned to each treatment group (eight males per group). The four treatments were as follows: males that clasped a female for 60 min and then received an injection of CORT (CLASP/CORT), males that clasped a female for 60 min and then received an injection of vehicle (CLASP/vehicle), males that remained in isolation (without any females) in the testing arena and received an injection of CORT (NO CLASP/CORT), and males that remained in isolation in the testing arena and received an injection of vehicle (NO CLASP/vehicle). Treatments were organized such that all males received the injections 90 min after the start of the experiment and behaviors were observed during the same 60 min (95–125 min).

Statistical analysis

Nonparametric statistical tests were used because data from Experiment 1 and 2 were skewed and variances were heterogeneous. The “time spent clasping” data were analyzed using Kruskal–Wallis (KW) nonparametric analysis of variance (α = 0.05), and after finding significant differences with KW tests, comparisons among groups were evaluated with Dunn’s multiple comparisons test (α ≥ 0.05). The number of males in each group that were clasping at different times (15, 30, and 60 min of testing) was expressed as a proportion and then transformed using the arcsine transformation function. These transformed data sets were analyzed with within-treatment comparisons (repeated measures from individuals at times 15, 30, and 60 min) and between-treatment comparisons using Friedman’s nonparametric analysis of variance with repeated measures (α = 0.05). If Friedman’s nonparametric analysis revealed significant differences, then differences between pairs of treatment groups or time periods were determined using Mann–Whitney pairwise comparisons (α = 0.05).

Results

Experiment 1: vasotocin pretreatment

Pretreatment with VT counteracts the suppressive effect of CORT administration on male clasping behaviors. Over-
all, treatment significantly influenced the time males spent clasping (KW = 32.38, $P < 0.0001$) (Fig. 1A). Pairwise analysis revealed that VT/CORT males spent approximately the same amount of time clasping as vehicle/vehicle males ($P > 0.05$) and a significantly longer amount of time clasping than vehicle/CORT males ($P < 0.05$). VT/CORT males spent significantly less time clasping than VT/vehicle males ($P < 0.01$) (Fig. 1A). There was a significant difference in the proportion of males clasping within all of the treatments over time (at 15, 30, and 60 min) (Friedman’s = 29.52, $P = 0.0019$) (Fig. 1B). The proportion of VT/CORT males clasping increased over time (Friedman’s = 6.0, $P = 0.0278$), whereas the proportion of vehicle/CORT males clasping decreased over time (Friedman’s = 6.0, $P = 0.0278$). No changes in proportion of males clasping were observed in the other treatments (Fig. 1B).

**Experiment 2: sexual experience pretreatment**

Pretreatment with immediate sexual experience also counteracts the suppressive effect of CORT administration on male clasping behavior. Performance of clasping behavior and injections of CORT significantly influenced the time males spent clasping (KW = 19.74, $P = 0.0002$) (Fig. 2A). Pairwise analysis revealed that males that had clasped a female for 60 min prior to administration of CORT (CLASP/CORT) spent the same amount of time clasping as the vehicle-injected males (CLASP/vehicle, NO CLASP/vehicle) ($P = 0.05$) (Fig. 2A). In contrast, NO CLASP/CORT males spent significantly less time clasping compared with all of the other treatment groups (NO CLASP/vehicle, CLASP/CORT, or CLASP/vehicle) ($P < 0.05$). There were no differences in the proportion of males ob-
served to be clasping across time within each treatment group (Friedman’s = 19.2, $P > 0.05$) (Fig. 2B). CORT administration to males without immediate sexual experience (NO CLASP/CORT) resulted in significantly lower proportions of males observed clasping at 15 min ($KW = 6.857, P < 0.0001$) and 60 min ($KW = 6.237, P = 0.0095$) compared with CLASP/CORT or CLASP/vehicle males. Figure 2B illustrates that the percentage of CLASP/CORT males clasping was 100% at 15 and 60 min after hormone administration.

**Discussion**

The present study demonstrates that the immediate neuroendocrine (VT) or behavioral state (clasping) of an animal can modify the effects of CORT administration on subsequent behavior. The present study confirms that in *Taricha* CORT administration suppresses and VT administration enhances courtship clasping. The novel observation is that CORT administration does not suppress clasping behavior in males that have either recently performed this behavior or been injected 60 min earlier with VT. These results suggest that interactions between VT and CORT are instrumental in the regulation of context-dependent behavioral responses to stress.

**Pretreatment with VT blocks the suppressive effects of CORT**

In male *Taricha* pretreated with VT, CORT administration had no observable suppressive effects on male clasping behavior. These results are noteworthy because, in the absence of VT pretreatment, CORT administration potently suppressed clasping behavior in *Taricha*. The results of this study are consistent with electrophysiological studies in *Taricha* showing that pretreatment with VT counteracts the suppressive effects of CORT administration on firing rates of medullary neurons (Rose et al., 1995). It is important to note that electrophysiological studies also indicate that CORT administration to males pretreated with VT results in an enhancement of neuronal activity (Rose et al., 1995). The current study did not find any further enhancement of clasping behavior in VT/CORT-treated males, perhaps because the vehicle/vehicle control males showed such a high incidence of clasping already.

The results of the present study, showing that a behaviorally active neuropeptide can override the suppressive effects of a stress hormone on reproductive behaviors, have not been reported previously. However, a recent article described the effects of concurrent injections of VT and CORT on calling behaviors of green treefrogs (*Hyla cincta*) (Burmeister et al., 2001). They found that VT administration increases calling compared with vehicle-treated controls. However, because vehicle-treated and CORT-treated frogs had equally low levels of calling, the study did not find any suppressive effects of CORT on calling. Interestingly, frogs injected with CORT plus VT did significantly less calling than frogs injected with only VT. Burmeister’s observations are consistent with the electrophysiological studies in *Taricha*, because VT must be administered at least 10–17 min prior to CORT to counteract CORT’s suppressive effects on medullary neurons (Rose et al., 1995).

Although the present study and the Burmeister et al. (2001) study are among the first to examine behavioral modification by VT and CORT interactions, there is a more classic and perhaps familiar role of VT and VP in which VT-like peptides act at the level of the pituitary modulating hypothalmo–pituitary–adrenal axis (HPA) function (Rivier and Vale, 1983; Baker et al., 1996; Aguilera and Rabadang–Diehl, 2000). However, VT/VP are known to alter sexual behaviors by acting on neurons in the central nervous system rather than on the pituitary gland (Sodersten et al., 1983). Furthermore, based on electrophysiological and behavioral studies in *Taricha*, it seems likely that the major site where VT and CORT interact to alter sexual behavior is on reticular neurons in medulla.

**Prior performance of clasping behavior blocks CORT inhibition**

In Experiment 2, the suppressive effects of CORT on clasping behavior were completely blocked in male *Taricha* that had clasped for an hour prior to receiving CORT administration. These results, when considered with the results from Experiment 1, suggest that the performance of clasping behaviors stimulates the secretion of endogenous VT and that the elevated VT activity in an unknown brain site counteracts the suppressive effects of CORT on reproductive behaviors. This suggestion is consistent with the proposed mechanism through which vasopressin influences pair-bonding behaviors in male prairie voles (Young et al., 2001). Nevertheless, regardless of whether or not the performance of clasping activates the VT system, results from Experiment 2 are consistent with our field observations of *Taricha*. Newts respond differently to potential predators depending on their behavioral state; solitary males typically retreat quickly into deep water, but clasping males remain clasping and exhibit fewer escape responses (unpublished observation).

**Context-dependent stress responses**

The behavioral and physiological states of an animal are important parameters that influence how an individual responds to specific external sensory stimuli. There are many examples of variation in behavioral responses that depend on the animal’s condition or context. Shyness and boldness of pumpkinseed fish are expressed across different situations in the same individual (Coleman and Wilson, 1998). The aggressive behavioral response of mockingbirds to
song playback varies according to breeding context (Logan, 1988). The predator avoidance response of voles to an owl call, to freeze or flee, depends on previous behavioral experiences (Eilam et al., 1999). The probability that a Capuchin monkey chooses novel food items depends on the social context, the incidence increasing when in a social situation (Visalberghi and Fragaszy, 1995).

Literature on the behavioral effects of corticosteroids reveals that across different species stress steroids can have suppressive or enhancing effects on social behaviors. For example, CORT administration rapidly suppresses reproductive behaviors in newts (Moore and Miller, 1984) and female mice (Kavaliers and Ossenkopp, 2001). In contrast, injection of corticosteroids facilitates lordosis behavior of female mice (Kavaliers and Ossenkopp, 2001). In contrast, injection of corticosteroids facilitates lordosis behavior of female mice (Kavaliers and Ossenkopp, 2001). Indeed, it has been suggested that a rise in corticosteroid levels in sexually primed animals can enhance reproductive behaviors (Orchinik et al., in press). This suggestion is consistent with a number of studies that reveal high plasma corticosteroid levels coincident with the onset of courtship and mating behaviors. For example, high corticosteroid levels have been associated with behavioral displays of courtship of breeding season in Bufo marinus (Orchinik et al., 1988), Triturus carnifex (Zerani and Gobbetti, 1993), musk shrews (Schiml and Rissman, 1999), boars (Liptrap and Raeside, 1983), and rams (Borg et al., 1991; Borg et al., 1992).

Results from the present study suggest that the specific effect of stress steroids on behaviors is dependent on the neuroendocrine and behavioral context; therefore the observed species differences in the above studies may reflect differences in neuroendocrine context, rather than immutable species differences in the functions of stress steroids.

The ability of an individual animal to respond with appropriate behaviors to a potential threat is essential to its survival and reproductive fitness. Animals have a repertoire of different behavioral responses and which one is expressed depends on the animals’ environmental, physiological, and behavioral state. In this way, these are context-dependent behavioral responses. This study provides evidence that a peptide known to enhance reproductive behaviors (VT) can block the suppressive effects of a stress hormone (CORT). This finding suggests that the neuroendocrine regulation of context-dependent behavioral responses to acute stress can be explained in part by functional interactions between vasotocin-like peptides and corticosteroids.

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References


