Comparative Biochemistry and Physiology, Part A 161 (2012) 153–158

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Elevated plasma corticosterone increases metabolic rate in a terrestrial salamander

Corina L. Wack [a,*], Sarah E. DuRant [b], William A. Hopkins [b], Matthew B. Lovern [c], Richard C. Feldhoff [d], Sarah K. Woodley [a]

[a] Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA
[b] Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, VA 24061, USA
[c] Department of Zoology, Oklahoma State University, Stillwater, OK, 74078, USA
[d] Department of Biochemistry and Molecular Biology, University of Louisville Health Sciences Center, Louisville, KY 40292, USA

ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form 16 October 2011
Accepted 19 October 2011
Available online 25 October 2011

Keywords:
Amphibian
Corticosterone
Glucocorticoid hormone
Metabolic rate
Oxygen consumption
Salamander

ABSTRACT

Plasma glucocorticoid hormones (GCs) increase intermediary metabolism, which may be reflected in whole-animal metabolic rate. Studies in fish, birds, and reptiles have shown that GCs may alter whole-animal energy expenditure, but results are conflicting and often involve GC levels that are not physiologically relevant. A previous study in red-legged salamanders found that male courtship pheromone increased plasma corticosterone (CORT; the primary GC in amphibians) concentrations in males, which could elevate metabolic processes to sustain courtship behaviors. To understand the possible metabolic effect of elevated plasma CORT, we measured the effects of male courtship pheromone and exogenous application of CORT on oxygen consumption in male red-legged salamanders (*Plethodon shermani*). Exogenous application of CORT elevated plasma CORT to physiologically relevant levels. Compared to treatment with male courtship pheromone and vehicle, treatment with CORT increased oxygen consumption rates for several hours after treatment, resulting in 12% more oxygen consumed (equivalent to 0.33 J) during our first 2 h sampling period. Contrary to our previous work, treatment with pheromone did not increase plasma CORT, perhaps because subjects used in this study were not in breeding condition. Pheromone application did not affect respiration rates. Our study is one of the few to evaluate the influence of physiologically relevant elevations in CORT on whole-animal metabolism in vertebrates, and the first to show that elevated plasma CORT increases metabolism in an amphibian.

1. Introduction

Glucocorticoid hormones (GCs) are released by the adrenal cortex during stimulation of the hypothalamic–pituitary–adrenal axis (Norris, 2006). Plasma concentrations of GCs may change diurnally, seasonally, and in response to exposure to acute and chronic stressors (e.g. Moore et al., 1991; Coe and Levine, 1995; Wingfield et al., 1998; Romero, 2002). Basal concentrations of GCs are believed to support basic processes, such as maintenance of vascular tone and blood sugar levels (Norris, 2006). Seasonal changes in GCs (e. g. Licht et al., 1983) may function to support behavioral and/or energetic changes associated with cyclic life history events, to suppress processes incompatible with a particular life history event (e.g., reproduction), and/or to prepare an organism for potentially stressful events (Romero, 2002). Finally, exposure to unpredictable threats or challenges (stressors) typically causes an elevation in plasma GCs. Stress-induced elevation of plasma GCs has been linked to behavioral, immunological, and metabolic responses to stressors (e.g. Kitaysky et al., 1999; Amaral et al., 2010; e.g. French et al., 2006; Martin, 2009; Ricciardella et al., 2010).

In temperate-zone amphibians, GCs tend to be elevated during the breeding season, and in response to social cues and stressful stimuli (Leboulenger et al., 1979; Licht et al., 1983; Pancak and Taylor, 1983; Zerani and Gobbetti, 1993; Homan et al., 2002). For example, plasma corticosterone (CORT; the primary GC in amphibians) concentrations were increased in male toads after mating with females compared to single males (Orchinik et al., 1988), in calling male anurans compared to non-calling anurans (Mendonca et al., 1985; Hopkins et al., 1997; Emerson and Hess, 2001; Leary et al., 2004, 2008), and in male red-legged salamanders exposed to pheromones (Schubert et al., 2009). Furthermore, exposure to stressors typically elevates plasma levels of GCs in amphibians (Herman, 1992). Confinement stress, food restriction, handling, and exposure to contaminants increased plasma CORT concentrations in amphibians (Moore and Zoeller, 1985; Hopkins et al., 1997; Glennemeier and Denver, 2002; Crespi and Denver, 2004).

Elevations in CORT have been linked to changes in behavior and immune function in amphibians (Moore and Miller, 1984; Leary, et al. 2006; Martin, 2009), but the effect of CORT on whole animal metabolism is unknown in this class of vertebrates. CORT is directly or indirectly involved in a number of intermediate metabolic pathways

* Corresponding author. Tel.: +1 252 398 6215.
E-mail address: wackc@chowan.edu (C.L. Wack).

1095-6433/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2011.10.017

that involve glucose, amino acid, and lipid metabolism (Mommsen et al., 1999; Norris, 2006). Through each of these pathways, CORT increases blood glucose concentrations in order to meet energetic demands. In the absence of compensatory mechanisms, changes in intermediary metabolism may be reflected in whole-animal metabolism. For example, both acute and chronic increases in CORT resulted in elevated whole-animal metabolic rates in lizards (DuRant et al., 2008; Preest and Cree, 2008). Thus, it is possible that increases in plasma CORT concentrations also influence whole-animal metabolism in amphibians.

Plethodontid salamanders are an excellent amphibian model for examining the function of acute increases in plasma CORT. They are abundant, available for study most of the year, and are amenable to both field and laboratory studies. Plasma levels of CORT increase in plethodontid salamanders in response to capture and/or handling (Schubert et al., 2009; Ricciardella et al., 2010; Woodley and Lacy, 2010), as well as social cues (Schubert et al., 2009). A previous study found that exposure to mental gland pheromones increased plasma levels of CORT in male red-legged salamanders (Schubert et al., 2009). Although mental gland pheromones are typically associated with courtship interactions between males and females (Houck and Verrell, 1993; Rollmann et al., 1999; Houck and Arnold, 2003), the CORT response elicited in males suggests that mental gland pheromones may have additional functions, including upregulation of metabolic processes important for supporting male–male interactions.

In the current experiment, we tested whether elevated plasma CORT might mediate metabolic changes in male red-legged salamanders (*Plethodon shermani*). We tested the hypothesis that courtship pheromones alter metabolic rate via an increase in CORT. We predicted the following: (1) courtship pheromones increase oxygen consumption, (2) courtship pheromones increase plasma CORT concentrations, and (3) elevation of plasma CORT increases oxygen consumption.

2. Materials and methods

2.1. Animal collection and husbandry

Adult male red-legged salamanders (*P. shermani*) were caught by hand in Macon County, North Carolina (83° 33′ 37″ N longitude; 35° 11′ 13″ W latitude) in August 2009, during their breeding season. *P. shermani* are in breeding condition from August to mid-October in the wild. However, their propensity to mate decreases as their time in captivity increases (personal observation). Animals were collected with appropriate permits from the North Carolina Wildlife Commission and US Forest Service. Throughout the experiments, animals were individually housed in 16×16×5 cm plastic boxes lined with moist paper towels, maintained on a 14:10 light:dark cycle at 16 °C, and fed wax worms.

2.2. Experimental design

The effects of male mental gland pheromone and exogenous CORT on oxygen consumption and plasma CORT were examined in two separate experiments in fall 2010. In the first experiment, oxygen consumption was measured using a closed-circuit respirometer at Virginia Tech. After measurement of oxygen consumption at Virginia Tech was completed, animals were transported to Duquesne University. Two weeks later, the effects of male pheromone and exogenous CORT on plasma CORT concentrations were measured in a second experiment. Methods were approved by Duquesne University's and Virginia Tech's Institutional Animal Care and Use Committees.

2.3. Experimental treatments

For each experiment, animals were randomly placed into one of three treatment groups: vehicle control (n = 16), pheromone (n = 16), or CORT (n = 15). Depending on the treatment group, pheromone or PBS vehicle was delivered to the nares of salamanders, and CORT or sesame oil vehicle was delivered transdermally via a minimally-invasive patch that was placed on the dorsum of the salamanders (Wack et al. 2010). Thus, animals in the control group had an oil patch placed on the dorsum and had vehicle (PBS) delivered to the nares, animals in the pheromone group had an oil patch placed on the dorsum and had pheromone delivered to the nares, and animals in the CORT group had a CORT patch placed on the dorsum and had vehicle (PBS) delivered to the nares. Treatments to the nares and the dorsum were delivered simultaneously and took approximately 45 min to apply.

2.3.1. Preparation and application of pheromone

Pheromone consisted of extract from male mental glands (Wirsig-Wiechmann et al., 2002; Schubert et al., 2006). To obtain mental gland extract, a separate group of males was captured and mental glands were removed (for surgical details see Wirsig-Wiechmann et al., 2002; procedure approved by Oregon State University ACUP to Dr. Lynne Houck). Mental glands from multiple males were pooled and pheromone was extracted with acetylcholine-chloride. A pheromone concentration of 1 μg/μL was used because it elevated plasma CORT concentrations (Schubert et al., 2009) and activated vomeronasal sensory neurons in male *P. shermani* (Wirsig-Wiechmann et al., 2002; Schubert et al., 2006). A volume of either 5 μL of pheromone or PBS vehicle was pipetted onto the nares every 5 min for 45 min for a total of 10 applications, following procedures of Schubert et al. (2009).

2.3.2. Application of CORT via dermal patches

We used dermal patches containing CORT to elevate plasma CORT. Patches consisted of a 1.5×3 mm rectangle cut from filter paper (Cat No. 1820–070 from Whatman) that was placed onto the dorsum between the two front legs of the salamander with forceps. Using a pipette, 0.625 μg of CORT was applied to the patch in a volume of 1.25 μL (Cat. no. Q1550-000, Steraloids Incorp.; 0.5 mg/mL). This amount of CORT was half of a quantity previously shown to elevate plasma CORT to high physiological concentrations in *P. shermani* (Wack et al., 2010). Controls received vehicle pipetted onto the patch. Patches were removed with forceps approximately 45 min after initial application.

2.4. Plasma CORT concentrations

To determine the effects of treatments on plasma CORT, treatments were delivered between 1515 and 1545, and trunk blood was collected from animals at 2, 4, or 10 h after patch removal. Blood was centrifuged and the plasma portion was frozen at −20 °C until assayed. CORT concentrations were measured by the Endocrine Services Laboratory at the Oregon National Primate Research Center. Briefly, a double ether extraction was performed on approximately 3 μL of plasma, and CORT concentrations were then determined by standard radioimmunoassay procedures (Resko et al., 1980; Gruenewald et al., 1992). The intra-assay coefficient of variation was 8.5%.

2.5. Oxygen consumption

To determine the effects of treatments on whole-animal metabolism, a computer-controlled, closed circuit respirometer measured the rate (mL/h) of oxygen consumption (hereafter V_{O_2}). Oxygen consumption was measured in salamanders that were individually placed in glass respiratory chambers (150 mL) lined with a Kimwipe moistened with ddH_2O. Incurrent air passed through columns of Drierite® to absorb water before passing into individual respirometry chambers. Air leaving respirometry chambers was dried again using magnesium perchlorate before passing into the oxygen sensor containing an