General and Comparative Endocrinology 178 (2012) 64–74

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

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

Characterizing adrenocortical activity in zoo-housed southern three-banded armadillos (*Tolypeutes matacus*)

Jennifer A. Howell-Stephens [a,c,*], Joel S. Brown [a], David Bernier [b], Diane Mulkerin [b], Rachel M. Santymire [a,c]

[a] Department of Biological Sciences, University of Illinois, Chicago, IL 60607, USA
[b] Animal Care Department, Lincoln Park Zoo, Chicago, IL 60614, USA
[c] Davee Center for Epidemiology and Endocrinology, 2001 N. Clark St., Lincoln Park Zoo, Chicago, IL 60614, USA

ARTICLE INFO

Article history:
Received 18 August 2011
Revised 22 March 2012
Accepted 9 April 2012
Available online 17 April 2012

Keywords:
Southern three-banded armadillo
Adrenocortical activity
Fecal glucocorticoid metabolite analysis
ACTH challenge

ABSTRACT

Improving the husbandry in the southern three-banded armadillo (*Tolypeutes matacus*) through gaining knowledge of its stress physiology is imperative to maintaining a healthy, zoo-housed population. Our objectives were to: 1) validate the use of fecal hormone analysis for monitoring adrenocortical activity using both an adrenocorticotropic hormone (ACTH) challenge and biological events; and 2) characterize longitudinal adrenocortical activity in male and female southern three-banded armadillos. An ACTH injection was given intra-muscularly to one male (4 IU/kg; 5.6 IU total) and one female (5.5 IU/kg; 8 IU total) southern three-banded armadillo. Fecal samples were collected 1 day pre- and continued 5 days post-ACTH to capture the physiological response measured by elevated fecal glucocorticoid metabolites (FGM) to validate these techniques. Additionally, natural and routine events, including pairing individuals for breeding and veterinary procedures/handling, were used to biologically validate these techniques. To characterize adrenocortical activity, fecal samples (~3025 total; $n = 275$/animal/yr) were collected from 11 (5 males; 6 females) southern three-banded armadillos 5–7 times a week for 1 year at Lincoln Park Zoo (Chicago, IL). A cortisol enzyme immunoassay was used for FGM analysis. The ACTH challenge in the male resulted in a twofold increase of FGM (1123.2 ± 36.2 ng/g dry feces) above baseline (675.7 ± 10.0 ng/g dry feces) at approximately 54–94 h post- injection. The female exhibited a twofold increase (1635.4 ng/g dry feces) over baseline FGMs (608.5 ± 12.3 ng/g dry feces) approximately 30 h post-injection. Reproductive behaviors and veterinary procedures resulted in elevated FGM concentrations from all individuals except for one male. The longitudinal characterization demonstrated that sex and season did not influence ($P < 0.05$) FGM concentrations. Individuals were highly variable with mean FGM concentration of 2010.1 ± 862.4 ng/g dry feces (range, 816.3–7889.1 ng/g dry feces). Mean FGM baseline concentration was 878.5 ± 201.8 ng/g dry feces (range, 475.2–1955.5 ng/g dry feces) with a mean elevated FGM concentrations of 2694.3 ± 1111.4 ng/g dry feces (range, 1110.3–10,683.3 ng/g dry feces). This study provides the foundation for future research on how the environment directly affects the adrenocortical activity in this species of armadillo.

1. Introduction

Due to continual habitat loss and degradation, zoological institutions have become a safe haven for many of the 21 extant armadillo species [1,15,52], including the southern three-banded armadillo (*Tolypeutes matacus*), which has been housed in zoos for over four decades. The three-banded armadillo is a near threatened insectivore of the Family Dasypodidae, Order Cingulata, which inhabits parts of Argentina, Bolivia, Brazil and Paraguay [1,3]. In the wild, this species is in need of conservation and management due to the conversion of its natural habitat into agricultural lands and its frequent exploitation for food and the arts [29,32,39]. Although this species has been maintained in North American zoos for over 40 years, it still experiences limited reproductive success. The three-banded armadillo Population Management Plan® reports an offspring mortality rate of 47% (54% of males, 38% of females) across all North American institutions [5].

Improving the health and reproduction of *ex situ* populations is the goal of zoological institutions and wildlife managers [30,49]. To determine the factors that are limiting their success, stress physiology research is needed to understand how individuals respond to the environment [27,59]. In a stress response, a perceived threat to homeostasis triggers a neural signal that activates hypothalamic–pituitary–adrenal (HPA) axis. The hypothalamus releases corticotrophin releasing hormone (CRH), which stimulates the anterior pituitary to produce adrenocorticotropic hormone (ACTH).

* Corresponding author at: Department of Biological Sciences, University of Illinois, Chicago, IL 60607, USA. Fax: +1 312 742 7220.
E-mail address: howell.stephens@gmail.com (J.A. Howell-Stephens).

0016-6480/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2012.04.003

Then, ACTH acts on the adrenal cortex and causes the release of glucocorticoids which help the individual cope with the stressor [35,47]. Once the stressor is absent, the glucocorticoids are metabolized by the liver and kidneys. These steroid metabolites are excreted in urine and feces [35,36]. This response to minimal stressors can be healthy and positive for zoo-housed animals. However, chronic stress can lead to debilitating and negative results including reproductive failure, muscle wasting, decreased body condition and decreased immune system function [35,37].

Glucocorticoids and other steroid hormones can be directly measured in saliva and blood or their conjugates can be quantified in urine and feces [7,36]. Although feces is easy to collect, fecal hormone analysis requires validation to ensure that the hormonal metabolites are biologically relevant [42,55]. For example, an ACTH challenge can be used to stimulate the HPA axis, which replicates the physiological response that occurs in an individual. In this procedure, the animal is given an ACTH injection which stimulates the HPA axis causing the production of glucocorticoids. These are eventually metabolized and excreted in the feces. The ability of the hormonal assay to determine the increase of the FGMs in response to the ACTH validates the method of analysis. Biological events, including parturition, veterinary visits, handling and medical procedures, can also be used to validate the hormonal analysis procedure [55].

The aim of this study was to increase our knowledge of the three-banded armadillo stress physiology and gain a better understanding of how the environment is influencing their biology. Our specific objectives were to: 1) validate the use of fecal hormone analysis for monitoring adrenocortical activity using both an ACTH challenge and biological events; and 2) characterize longitudinal adrenocortical activity in the three-banded armadillo to lay the foundation for future research on how environmental factors influence their success.

2. Material and methods

2.1. Adrenocortical activity validations

2.1.1. Physiological validation

For the ACTH challenge, a 10 year old male and 6 year old female three-banded armadillo from Lincoln Park Zoo (LPZ) were used. The ACTH (corticotrophin; Monument Pharmacy, Monument, CO) dosages were: 4 IU/kg (5.6 IU total) for the male (# 20243) and 5.5 IU/kg (8 IU total) for the female (# 21310), which was determined by the veterinarian. Dosage amounts varied because the female was slightly larger and could be given an increased amount of ACTH to ensure a physiological response. This species' carapace makes access to its limbs difficult; therefore the ACTH injections were given intramuscularly into the neck muscles. For the ACTH challenge, fresh fecal samples were collected once a day for 1 day prior to ACTH injection, twice (pre- and post-injection) on the injection day and twice a day for 5 days post-injection. This procedure was approved through LPZ's Research Committee.

2.1.2. Sample collections

From the ACTH challenge procedure, it was determined that the three-banded armadillo defecates approximately twice daily. However, for the both the biological event validations and the adrenocortical activity characterizations, fecal samples (approximately 3025 total; 275/animal/yr) were collected once a day in the morning for 5–7 days per week during routine enclosure cleaning procedures. Samples were collected for 1 year from November 2007 through November 2008.

2.1.3. Biological validation

Five instances of biological events were used to validate the FGM analysis. The first three events were reproductive events including pairing, mating and pregnancy. The first reproductive event was a copulation observed between a male (# 6474) and female (# 20727) three-banded armadillo. These individuals underwent daily introductions and were separated at night, starting on December 23. On January 2, copulation was observed in this pair. The second reproductive occurrence was a pairing for mating (on April 2), which was halted due to aggression exhibited by the male (# 20202) towards the female (# 21310). The three-banded armadillos were separated to prevent injury of the female. The last reproductive event used to biologically validate our methods was a pregnancy (female #21310 from May 22 – Sept 12).

The two non-reproductive events used for validation involved veterinary procedures, which require inhalant isoflurane anesthesia delivered by chamber induction method. The animals are then maintained under anesthesia by facemask until recovery. The first veterinary procedure (February 3rd) involved a female (# 9338) which had been under treatment for ocular irritation for 4 days prior to the procedure. Following anesthesia, the animal received a full physical examination, had blood collected from the ventral tail vein and was examined by radiography to assess the severity of dental disease, which was suspected to be associated with the ocular presentation. Severe dental disease was identified and seven teeth were extracted. The female then was given oral anti-inflammatory for 72 h, eye drops for 4 days and oral antibiotics for 10 days post-procedurally. The second veterinary procedure (March 22nd) involved a male (# 20243) with a mandibular abscess. Following anesthesia, the animal received a full physical examination, had blood collected from the ventral tail vein and was examined by radiography and ultrasound to drain the mandibular abscess. During this procedure, the affected area was incised surgically and debrided. The individual then was given antibiotics parenterally or orally once daily for 10 days following the procedure.

2.2. Adrenocortical activity characterization

Eleven three-banded armadillos (5 males, 6 females) housed at LPZ were included in this portion of our study. Mean (± SEM) age was 11.0 ± 4.4 years old (range, 3–31 years old) for females and 12.7 ± 4.1 years old (range, 7–29 years old) for males. Animals were housed individually and exposed to natural and artificial light throughout the year. Individuals were fed Mazuri insectivore® diet (PMI Nutrition International) plus chopped vegetables and/or fruit. During the course of the study, two females experienced pregnancies (# 20727 and 21310). Samples were not collected from these individuals directly following parturition and throughout the weaning of their offspring to limit disturbance of mother and infant. One individual (# 20204) was also relocated to another zoological institute during the course of the study, resulting in fewer samples collected.

2.3. Fecal sample processing

Samples were stored in sealed bags at −20 °C prior to processing and analysis. All fecal samples were processed at the LPZ Endocrinology Laboratory. Fecal samples were lyophilized (Labconco Lyophilizer, Kansas City, MO) and steroids extracted using methods modified from previously described procedures [43]. Dried samples were pulverized and 0.02 g (±0.002 g) of fecal powder was briefly vortexed with 0.5 ml of 90% ethanol:distilled water. After vortexing, samples were shaken (Glas-col mixer, Terre Haute, IN, setting 60, 30 min) and then centrifuged (1500 rpm, 20 min). Extracts were poured off into a clean test tube. Fecal pellets were re-suspended in 0.5 ml of 90% ethanol:distilled water and vortexed