Comparative Biochemistry and Physiology, Part A 195 (2016) 65–72

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Adrenocortical function in cane toads from different environments

Sandra E. Hernández [a,b,*,1], Conrad Sernia [a,1], Adrian J. Bradley [a,1]

[a] School of Biomedical Sciences, The University of Queensland, Brisbane, 4072, Queensland, Australia
[b] Veterinary School, Universidad Nacional Autónoma de México, Mexico City 4510, Mexico

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 2 February 2016
Accepted 2 February 2016
Available online 11 February 2016

Keywords:
Rhinella marina
Amphibia
Stress response
ACTH
Dexamethasone

ABSTRACT

The adrenocortical function of cane toads (*Rhinella marina*) exposed to different experimental procedures, as well as captured from different environments, was assessed by challenging the hypothalamic–pituitary–adrenal (HPA) axis. It was found that restriction stress as well as cannulation increased plasma corticosterone (B) levels for up to 12 h. A single dose of dexamethasone (DEX 2 mg/kg) significantly reduced B levels demonstrating its potential for use in the evaluation of the HPA axis in amphibia. We also demonstrate that 0.05 IU/g BW (im) of synthetic adrenocorticotropic hormone (ACTH) significantly increased plasma B levels in cane toads. Changes in size area of the cortical cells were positively associated with total levels of B after ACTH administration. We also found differences in adrenal activity between populations. This was assessed by a DEX-ACTH test. The animals captured from the field and maintained in captivity for one year at the animal house (AH) present the highest levels of total and free B after ACTH administration. We also found that animals from the front line of dispersion in Western Australia (WA) present the weakest adrenal response to a DEX-ACTH test. The animals categorized as long established in Queensland Australia (QL), and native in Mexico (MX), do not shown a marked difference in the HPA activity. Finally we found that in response to ACTH administration, females reach significantly higher levels of plasma B than males. For the first time the adrenocortical response in cane toads exposed to different experimental procedures, as well as from different populations was assessed systematically.

1. Introduction

The adaptation of organisms to their physical environment is a major determinant of survival (Bennett, 1997). While the biological mechanisms underlying adaptation are still being elucidated, it is known that the neuroendocrine system, and its involvement in the stress response, has an important role in maintaining the homeostatic balance in animals (Wingfield et al., 2015). The differences in the stress response between individuals from the same species living in different environments are the result of environmental pressures shaping the physiological responses and optimizing fitness success (Silverin et al., 1997). These changes are directed toward a better outcome energetic tradeoffs during the life story stages in the living organisms (Wingfield et al., 2015). The neuroendocrine system acting via the hypothalamic–pituitary–adrenal (HPA) axis has an important role in maintaining the homeostatic balance in animals by the regulation of the adrenal hormones produced during the stress response (Sapolsky et al., 2000). Among these hormones, glucocorticoids (GCs) have attracted the most interest because of their involvement in chronic stress (McEwen and Stellar, 1993), immune suppression (Sternberg and Licinio, 1995), reproductive impairment (Hardy et al., 2005), metabolic diseases (Dallman et al., 1993), and central nervous system (CNS) damage (McEwen and Sapolsky, 1995). Therefore the activation of the HPA to environmental changes has an important role in integrating changes in behaviour and physiology by optimizing the energy expenditure during emergency life history stages (Wingfield, 2008; Wingfield and Kitaysky, 2002).

The cane toad has one of the widest distributions of any anuran species. It is native to the Americas but was introduced to Australia and several Pacific and Caribbean islands (Hero and Stonham, 2005). It is an opportunistic species and a dietary generalist (Zug and Zug, 1979). The presence of both endemic and introduced populations of cane toads in diverse geographical locations offers opportunity to studying adaptive mechanisms in this species which may underpin their apparent success vis-a-vis other amphibian species.

In amphibia, and in other vertebrates, the hypothalamic–pituitary axis modulates the hormone profile in response to stress. In mammals, following the exposure to a stressor, the corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus, and the arginine vasopressin (AVP) from the posterior pituitary, act in conjunction to promote the production of

* Corresponding author at: Grupo de Ecología de Enfermedades, Departamento de Etología, Fauna Silvestre y Animales de Laboratorio, Universidad Nacional Autónoma de México, Mexico City 4510, Mexico. Tel.: +52 55 5622 5941.
E-mail address: sandrahm@unam.mx (S.E. Hernández).
[1] These authors contribute equally to the study.

http://dx.doi.org/10.1016/j.cbpa.2016.02.001
1095-6433/

adrenocorticotropic hormone (ACTH) from the anterior pituitary (Carrasco and Van de Kar, 2003). However in anurans, the greatest CRH immunoreactivity and synthesis of the corticotropin releasing hormone (CRH) are localized in the regions of the anterior preoptic area (POA) and the external zone of the median eminence (Jorgensen, 1976) (Yao et al., 2004). The CRH pathway signals traverse through the median eminence and via a vascular route reach the pars distalis where the synthesis and release of ACTH are controlled (Capaldo et al., 2004; Ogawa et al., 1995).

In mammals, as well as in amphibians, ACTH is released into the circulation via the hypophyseal portal system and exerts its action in the adrenal gland. The function of ACTH in the adrenal gland is to regulate the production of glucocorticoids (GCs) (cortisol, corticosterone, aldosterone) (Janssens, 1970). In amphibia, the administration of ACTH stimulates the secretion of aldosterone and corticosterone by the cortical cell from the inter-renal tissue in vitro (Capaldo et al., 2004; Hanke, 1978; Vinson and Whitehouse, 1974) and in vivo (Dupont et al., 1976; Kemenade, 1968; Narayan et al., 2011). In response to ACTH stimulation, corticosterone is the glucocorticoid present at the highest concentration in blood (Capaldo et al., 2004; Glennemeier and Denver, 2002; Hanke and Weber, 1965).

Amphibian studies related to the properties of the corticosteroid binding globulin (CBG) are scarce. Under normal conditions in mammals, 70–85% of the glucocorticoids circulate bound to CBG (White, 2001). This increases their half-life by reducing metabolic clearance, and creating a circulating reservoir for easy access during the stress response. In amphibia plasma globulins show high affinities for glucocorticoid binding, and more than 90% of corticosterone has been reported to bind with high affinity to CBG with free levels below 5% (Glennemeier and Denver, 2002; Martin and Ozon, 1975; Seal and Doe, 1965).

The objective of this study was to assess the adrenal activity from wild cane toads exposed to different experimental procedures to compare the adrenal activity of cane toads exposed to different environments.

2. Methods

2.1. Capture and housing of toads

A total of 86 cane toads (66 males, 20 females), weighing between 200 and 500 g, were collected by hand during the wet season from 2009 to 2011. Two populations were located in Australia, one established in 1945 in Brisbane, Queensland (Hero and Stonham, 2005) ([QL, males = 56, females = 10]) and the other located in the front line of dispersion in Kununurra, Western Australia(Department of Environment and Conservation, 2013) [WA, males = 5, females = 5]). A third population was sampled in its native distribution at Los Tuxtlas, Veracruz, Mexico [MX, males = 5, females = 5]). All animals were captured during the night near urban areas and transported in plastic containers to a field laboratory.

To assess the effect of captivity, five males and five females from The University of Queensland Lakes, St Lucia, SE Queensland were kept in captivity for one year (The University of Queensland animal house [AH]). The groups of males and females were maintained separately in tanks (1.5 m^3) with a pond and a dry area containing sections of PVC pipe (120 × 300 mm) used as refuges. Environmental enrichment was provided constantly in the form of live prey and plastic aquatic plants. An ambient temperature of 22–24 °C was maintained with a light cycle of 12L:12D. Animals were fed three days a week with live prey (crickets, wood roaches, meal worms, earth worms) and dry food with 40% of protein ad libitum and supplemented with calcium (Olvera-Novoa et al., 2007). All procedures were approved by The University of Queensland Animal Ethics Committee (SBMS/437/09/URG/GOVTMEX/HSF/CFOC).

2.2. Experimental design

The male cane toads used for the following experiments (N = 46) were captured from The University of Queensland Lakes, St Lucia, SE Queensland.

2.2.1. Assessment of adrenocortical function

(a). Effect of movement restriction in corticosterone plasma levels in cane toads

To determine a corticosterone baseline level in plasma, five male cane toads were captured by hand and within 2 min of capture a blood sample (200 μl) was obtained by cardiac puncture with a 1 ml heparinized syringe (25 G × 1″ needle) (time 0). To ensure that cardiac puncture was only performed once on each animal, 15 male cane toads were captured and placed in a plastic container to restrict movement and divided into three groups of five animals each. The first group of five toads was kept in the plastic container for 1 h (time 1) before drawn a blood sample. Five toads were kept in the plastic containers for 2 and 12 h respectively (time 2 and 12) before collecting blood by cardiac puncture. An ambient temperature of 22–24 °C was maintained throughout the experimental period and all animals were euthanized with 100 mg/kg of pentobarbital (Lethabarb) after each time point.

(b). Effect of cannulation in corticosterone plasmatic levels in cane toads

The stress response to cannulation was determined in five male toads captured on the same night. The animals were transported to the laboratory where they were weighed, anesthetized with a dose of 200 mg/kg (BW) of ketamine combined with 0.2 mg/kg (BW) of diazepam (Hernández et al., 2012) and cannulated with a 24 gauge × 19 mm catheter (Introcan Certo, Braun, Germany) inserted into the ventral vein as described previously (Hernández et al., 2012). Flunixin meglubine 1 mg/kg IM (Finadyne; Schering-Plough Pty Ltd., Australia) (Wright, 2001) an analgesic non-steroidal anti-inflammatory, was administrated as post-operative pain management in the toads. Catheters were placed to facilitate blood sampling and to allow humane euthanasia at the end of the study period.

Once the placement of the catheter was completed, toads were housed individually to recover in 4 l plastic containers, with a wet bedding a depth of 2 cm of water and a dry refuge area. An ambient temperature of 22–24 °C was maintained with a light cycle of 12L:12D. Animals were fasted during this period. After 24 h of recovery, a first blood sample (100 μl) was collected (time 0), followed by consecutive blood samples (100 μl) at one hour (1 h), two hours (2 h) and twelve hours (12 h) after the first blood sample (time 0), to enable a determination of plasma corticosterone (B) concentrations. To reduce fluctuations in B levels due to daily cycles (Leboulenger et al., 1982), all experiments were carried out during the night and at the same starting time.

(c). Suppression of corticosterone levels by dexamethasone (DEX)

Five male cane toads captured on the same night were cannulated as explained above. After 24 h recovery, a first blood sample (100 μl) was collected followed immediately by an intravenous injection of 2 mg/kg dexamethasone sodium phosphate (Dexadreson, Intervet Pty Ltd. Australia) (Wright, 2001) in 100 μl 0.9% saline (sterile) via the catheter. Consecutive blood samples (100 μl) were collected at one hour (1 h) two hours (2 h), four hours (4 h) and twelve hours (12 h) after DEX administration.

(d). Response to exogenous ACTH (Synacthen)

The determination of an effective dose of ACTH in cane toads was performed in 16 male cane toads captured on the same night and