General and Comparative Endocrinology 187 (2013) 23–31

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

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

The reproductive cycle of the male house gecko, *Hemidactylus flaviviridis*, in relation to plasma steroid concentrations, progesterone receptors, and steroidogenic ultrastructural features, in Oman

Issa S. Al-Amri [a,*], Ibrahim Y. Mahmoud [a], Colin P. Waring [b], Abdulaziz Y. Alkindi [a], Tabisam Khan [a], Charles Bakheit [c], Kamla M. Al-Mawali [d]

[a] Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, Oman
[b] Department of Biological Sciences, Institute of Marine Sciences, University of Portsmouth, Portsmouth, United Kingdom
[c] Department of Mathematics and Statistics, College of Science, Sultan Qaboos University, Al-Khod, Muscat, Oman
[d] Department of Histopathology, Sultan Qaboos University Hospital, Al-Khod, Muscat, Oman

ARTICLE INFO

Article history:
Received 6 October 2012
Revised 24 January 2013
Accepted 23 February 2013
Available online 22 March 2013

Keywords:
Lizard
Ultrastructural steroidogenic features
Sertoli cell
Leydig cell
Steroids
PR

ABSTRACT

The annual testicular cycle of the house gecko *Hemidactylus flaviviridis* in Oman was studied. Plasma testosterone (T), estradiol (E_2) and progesterone (P) concentrations were measured using a sensitive HPLC-MS/MS detection technique. The ultrastructural steroidogenic features in Sertoli and Leydig cells, which were the major source of steriodogenesis, were examined, using transmission electron microscopy (TEM). In addition, progesterone receptors (PR) were examined throughout the testicular cycle, using an immunohistochemical technique. The steroidogenic ultrastructural features were characterized by the presence of smooth endoplasmic reticulum (SER) in the form of cisternal whorls and tubular cisternae, presence of swollen vesiculated mitochondria, and association between SER, mitochondria and lipid droplets. The rise in plasma steroid concentrations was closely associated with the development of the ultrastructural features and PR expression in Leydig and Sertoli cells. During the active phase (November–May), there was a significant rise in plasma steroid concentrations ($P < 0.05$) related to well developed steroidogenic features and strongly expressed PR. During the quiescent phase (June–August) there was a significant decline in plasma steroid concentrations, undeveloped steroiodogenic features and weakly expressed PR. The Renal Sexual Segment (RSS) was fully developed during the active phase. The data provides strong evidence that these ultrastructural steroidogenic features were related to the plasma sex steroid concentrations during the testicular cycle.

1. Introduction

The seasonal testicular cycle in lizards has been described in several species. The majority of these studies have concentrated on testicular growth and development followed by regression during the reproductive cycle. The spermatogenic activities leading to spermiation and mating behavior were the main emphasis in these investigations (Flemming, 1993; Gribbins et al., 2011; Nkosi et al., 2004; Ramírez-Bautista et al., 1996). In addition, some of the morphological studies have attempted to link specific features related to testicular activities such as the development of specific histological and ultrastructural features of the testicular tissues (including germ cells, Sertoli and Leydig cells) in relation to the seasonal activities (Akbarsha et al., 2006; Rheubert et al., 2009; Sanyal and Prasad, 1966, 1967). Other studies emphasized the relationship between plasma steroid concentrations and testicular activities such as spermiation relative to courtship and mating behavior (Amey and Whittier, 2000; Ando et al., 1990, 1992; Johnson et al., 2011; Radder et al., 2001; Schuett et al., 1997).

In male squamates, seasonal fluctuations in plasma T concentrations are related to reproductive phases. Both plasma T concentrations and testicular mass increases during spermatogenesis, to peak in the final stages of gamete maturation, coinciding with mating, and falling rapidly thereafter (Ando et al., 1992; Kumar et al., 2011; Mahmoud et al., 1985; Radder et al., 2001).

However, the annual changes in plasma concentrations of 17β-estradiol (E_2) and progesterone (P) in males have only been investigated in a few species. These studies demonstrated the roles of E_2 and P in the regulation of the reproductive cycle (Edwards and Jones, 2001; Lindzey and Crews, 1988; Lindzey and Crews, 1992; Witt et al., 1994; Young et al., 1991).

* Corresponding author. Address: Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, P.O. Box 33, 616 Muscat, Oman.
E-mail addresses: issa.alamri@unizwa.edu.om (I.S. Al-Amri), ibrahim.younis@unizwa.edu.om (I.Y. Mahmoud), colin.waring@port.ac.uk (C.P. Waring), alkindi@unizwa.edu.om (A.Y. Alkindi), tabisam@unizwa.edu.om (T. Khan), sakib@squ.edu.om (C. Bakheit), kamla69@squ.edu.om (K.M. Al-Mawali).

0016-6480/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygcen.2013.02.040

Some studies have suggested that P may be important in stimulating the reproductive behavior in some male squamates (Lindzey and Crews, 1988, 1992; Witt et al., 1994; Young et al., 1991). Exogenous P has been shown to stimulate sexual behaviors in the male lizard *Cnemidophorus inornatus* (Lindzey and Crews, 1986). Restoration of sexual behavior in P-sensitive male whiptail lizards (*C. inornatus*) by synthetic progestin agonists indicated that P was producing this behavioral effect (Lindzey and Crews, 1988), and further, binding studies suggested that progesterone receptors (PR) mediated this response (Lindzey and Crews, 1993).

In the reptilian testis, steroidogenic ultrastructural features have been correlated with plasma sex steroid concentrations and spermatogenesis (Mahmoud and Licht, 1997). Specifically, Leydig and Sertoli cells are associated with the production of steroids such as T, E_2 and P which are associated with the increase in smooth endoplasmic reticulum (SER), free ribosomes, mitochondria with swollen cristae and the presence of SER in the form of cisternal whorls associated with lipids droplets and mitochondria (Licht et al., 1985; Mahmoud and Licht, 1997). However, few studies have examined these relationships and associations at the different stages of testicular development in the reproductive cycle.

The aim of this study was to measure plasma steroid concentrations including T, E_2 and P in relation to the development of ultrastructural steroidogenic features and PR expression associated with spermatogenesis during different phases of the annual testicular cycle in the house gecko, *Hemidactylus flaviviridis*, a species which is widely distributed in Oman, the Arabian Peninsula and part of East Africa.

2. Materials and methods

2.1. Study area

The study area is located in Barka, Al Batinah Region, Sultanate of Oman (23°39′42.31″N, 57°52′00.92″E). The area is a wooded habitat at sea level and consists mainly of palm trees with some mango and lime trees and scattered farm houses. Annual rainfall averages 30–100 mm, with temperature ranging between 13 °C and 47 °C. The rainy season occurs between December and April.

2.2. Animals

A total of 98 adult male house geckos [snout–vent length (SVL) ranged between 6 and 8.5 cm] were captured at night from the study area during 2008–2010, of which 22 were sacrificed during the postbreeding (quiescent) phase (June–July), 24 during the prebreeding (recrudescent) phase (August–October) and 52 during the breeding (active) phase (November–May).

2.3. Blood and tissue collection

The lizards were sacrificed immediately after capture to minimize stress which could alter the natural hormone values (Owens and Ruiz, 1980) and blood was collected in EDTA tubes. Tissue samples were collected from all the sacrificed lizards, fixed in appropriate solutions (see the methods of tissue fixation described below) and kept in ice until arrival at the laboratory, which took approximately 120–180 min.

In the laboratory, blood samples were centrifuged and the plasma samples were stored at −70 °C until analysis.

Tissue samples were taken from the testes, epididymis and kidney for examination using light and transmission electron microscopy. Tissue samples were also prepared for immunohistochemistry.

2.4. Effects of temperature on the activity of the gecko

Observations on feeding, mating, and movements based on 75 active males suggest that the geckos had limited activity during the cold nights (December–February) where temperature drops below 15 °C. During courtship and mating (March–April) the temperature ranged between 25–35 °C, and generally, the lizards were active during this period.

2.5. Analysis of sex and stress hormones by HPLC–MS/MS

Plasma concentrations of T, E_2, P and corticosterone (C) were measured with the highly specific and sensitive method of high performance liquid chromatography coupled with a tandom quadropole mass spectrometer (HPLC–MS/MS). Initial separation of the steroids from the biological media was performed by the HPLC separation column and individual components were quantified by the mass spectrometer.

The samples were prepared by protein precipitation; a 500 μL aliquot of acetonitrile was added to 500 μL of plasma. The sample was vortex mixed for 5 min and then centrifuged at 3000 rpm for 10 min in an Eppendorf (Hamburg, Germany) microcentrifuge. The precipitated plasma protein was discarded and the top layer containing the extract was analyzed by injecting a sample of 10 μL into the HPLC–MS/MS. A 1 pg/μL standard solution of E_2, P, T and C (Sigma Aldrich, UK) was used to tune the mass spectrometer for optimum sensitivity. The analysis was performed with a tandem quadrupole mass spectrometer (Quattro Ultima Pt, Waters Corp., MA, USA) in multiple reaction monitoring mode (MRM). Specific MRM transitions were optimized E_2 (271.3 > 145.2 m/z), P (429.2 > 313.1 m/z), T (289.2 > 97.0 m/z) and C (347.2 > 329.2 m/z). Both the collision energies and cone voltages were optimized to generate the highest possible sensitivity. The E_2, P, T and C were run in negative ion Electrospray and were separated using a High Performance Liquid Chromatography system (Agilent 1100, Palo Alto, CA) with an Xterra C18, 2.1 × 100 mm, 3.5 μm column (Waters Corp., MA). Acetonitrile/water (45/55, v/v) was used as the mobile phase (Sigma Aldrich, UK), at a flow rate of 0.3 μL/min. Validation of the extraction procedure and HPLC–MS/MS method was achieved by comparing extraction recoveries. The peak area in the chromatogram of a 10 pg/mL standards E_2, P, T and C solution in water/acetonitrile was compared with standards at the same concentration and with the spiked lizard plasma that had been through the protein precipitation extraction procedure. A blank plasma sample was run and subtracted from the spiked sample.

E_2, P, T and C levels in the blank were below the limit of quantification for the method and this was used to back-calculate the concentrations in the calibration curve standards and subsequent samples. The efficiency of recovery was 98%. The back-calculated concentration from the protein precipitation extraction method was used to evaluate the steroid concentrations in actual lizard plasma samples. Calibration curves were constructed in acetonitrile/water, first for validation only and then for the actual samples. In order to compare the same concentrations to verify the calibration concentration, the calibration line was constructed using known concentrations of standard at each calibration point e.g. 1 pg/μL. This known value was added (10 μL) to the raw plasma and extracted using the same extraction procedure as the samples. Thus a comparison of solutions of the same concentration was obtained. The calibration curve was constructed over a range of dilutions: 0.1 pg/μL–10,000 pg/μL. The calibration curve resulted in a correlation coefficient of greater than 0.99 for each component. The sensitivity of the lowest level concentration was evaluated at 0.1 pg/μL using a signal: noise ratio of 8:1 for all components.