Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i4.18444

Histological and immunohistochemical study of the thyroid gland of the broad-snouted caiman (*Caiman latirostris*)

Clarice Machado-Santos[1*], Monalisa Jales Teixeira[2], Armando Sales[3] and Marcelo Abidu-Figueiredo[3]

*[1]Departamento de Morfologia, Instituto Biomédico, Universidade Federal Fluminense, Rua Professor Ernani Melo, 101, 24210-130, Niterói, Rio de Janeiro, Brazil. [2]Médica Veterinária Autônoma, Rio de Janeiro, Rio de Janeiro, Brazil. [3]Departamento de Biologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil. *Author for correspondence. E-mail: clahisto@gmail.com*

ABSTRACT. The broad-snouted caiman, *Caiman latirostris*, is widely distributed in countries of South America. In Brazil it is considered an endangered species because of natural habitat destruction and illegal hunting. In reptiles, the thyroid gland plays an integral part in ecdysis, reproduction, tail regeneration, growth, endocrine function and metabolism rate. The aim of the present study was to characterize the thyroid gland morphology of *C. latirostris*, based on histochemical and immunohistochemical techniques. The thyroid glands were fixed in Bouin's fluid and serial cross sections were stained with hematoxylin-eosin, Mallory's trichrome, periodic acid-Schiff (PAS) and Alcian blue (AB pH 1.5 and 2.5). The immunohistochemical technique for 5-HT-IR cells was used. The thyroid gland has a dense irregular connective tissue forms a capsule enveloping the gland. There are several follicular acini of varying size lined by simple cuboidal or columnar epithelium in the thyroid gland. The follicles are connected by interfollicular connective tissue which contains blood vessels. We observed the presence of lymph nodes around the entire gland. There was a positive PAS reaction and negative AB reaction in the colloid. 5-HT-IR cells were detected around the follicle cells. No striking morphological differences were observed between *C. latirostris* and other domesticated mammals.

Keywords: thyroid, histology, reptiles, 5-HT.

Estudo histológico e imuno-histoquímico da glândula tireoide do jacaré do papo-amarelo (*Caiman latirostris*)

RESUMO. O jacaré do papo-amarelo, *Caiman latirostris*, é amplamente distribuído nos países da America do Sul. No Brasil este é considerado uma espécie em extinção pela destruição de seu habitat natural e caça ilegal. Em répteis, a glândula tireoide desempenha um papel fundamental na ecdise, reprodução, regeneração da cauda, crescimento, função endócrina e na taxa de metabolismo. O objetivo do estudo foi caracterizar a morfologia da glândula tireoide de *C. latirostris*, com base em técnicas de histoquímica e imuno-histoquímica. As tireoides foram fixadas em Bouin e seções transversais corados com hematoxilina-eosina, Tricrômico de Mallory, ácido periódico de Schiff (PAS) e Alcian blue (AB pH 1,5 e 2,5). A técnica de imuno-histoquímica para detecção de células imunoreativas a 5-HT foi utilizada. A glândula tireoide possui uma cápsula de tecido conjuntivo denso não modelado. Há numerosos ácinos foliculares de tamanho variáveis revestidos por epitélio cúbico ou cilíndrico simples. Entre os folículos observa-se o tecido conjuntivo ricamente vascularizado. Observamos a presença de linfonodos ao redor de toda a glândula. No coloide observou-se uma reação PAS positiva e AB negativa. Células imunoreativas para 5-HT foram detectadas em torno das células foliculares. Não foram observadas diferenças histológicas marcantes na tireoide de *C. latirostris* quando comparadas a outros mamíferos domésticos.

Palavras-chave: tireoide, histologia, reptil, 5-HT.

Introduction

The broad-snouted caiman, *C. latirostris*, is widely distributed in countries of South America. In Brazil, it occurs naturally in the São Francisco and Paraná rivers, and is considered an endangered species because of natural habitat destruction and illegal hunting (GROOMBRIDGE, 1987). The Gators have been present on Earth for at least 200 million years, and their study is important, considering they are, with the exception of birds, the only living creatures directly related to the large group of dinosaurs (JIN et al., 1992).

The thyroid is the endocrine gland responsible for the synthesis and secretion of the hormones thyroxine (T4), triiodothyronine (T3) and calcitonin. These hormones are present in the vertebrate class and have diverse functions. The thyroid hormone actions include the regulation of nutrient absorption, metabolism, calorigenesis,

growth and development (EALES, 1979; MC NABB, 1992), as well as energy balance regulation and maintenance of normal reproductive function in mammals (BOSWELL et al., 1994; SCHWARTZ et al., 1996; SHI; BARREL, 1992). In reptiles, the thyroid gland plays an integral part in ecdysis, reproduction, tail regeneration, growth, endocrine function and metabolism rate (DELLOVADE et al., 1995; SCHMIDT-NIELSEN, 1990). Thyroid deficiency, which can occur by embryogenic effects, leads to limited development of the skeletal, muscle and central nervous systems (MC NABB, 1992).

The serotonin or 5-hidroxitriptamina (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan, serotonin is widely distributed in the central nervous system, gastro-entero-pancreatic system (EL-SALHY et al., 1985) and platelets, of animals including humans. The 5-HT appears to have several functions, such as direct control on the release of certain hormones. The presence of 5-HT was demonstrated in *C. latirostris* in esophagus (MACHADO-SANTOS et al., 2011) and trachea (SANTOS et al., 2011). The thyroid, although the primary regulation is done through the TSH hormone released by the hypophysis gland, 5-HT is able to modulate thyroid activity in reptiles (SCIARRILLO et al., 2001).

The thyroid gland has been the subject of much research in different reptilian species, such as the characterization of seasonal changes in thyroid gland morphology in *Chalcides ocellatus* (VIRGILIO et al., 2004) and changes in normal thyroid morphology in *Alligator mississippiensis* exposed to environmental contaminants (HEWITT et al., 2002). There are also several review articles on the clinical endocrinology of the thyroid and parathyroid (RIVERA; LOCK, 2008).

The aim of the present study was to characterize the thyroid gland morphology in *Caiman latirostris*, based on histochemical and immunohistochemical techniques, to provide basic information about normal tissues, and thus contribute to the general knowledge of this species.

Material and methods

Ten adult male specimens of *Caiman latirostris,* raised for meat production, were obtained from the Bonsucesso Farm (Nossa Senhora do Amparo, Barra Mansa, Rio de Janeiro, Brazil). They were slaughtered in the Acquanature abattoir (Itaguaí, Rio de Janeiro State, Brazil). The breeder is registered with the Brazilian federal environmental agency (IBAMA) and has authorization from the local government, Rio de Janeiro state environmental agency (FEEMA) and regional veterinary medicine board. The animals were submitted to hypothermia and slaughtered by hypovolemia. After that, they were dissected and their glands removed.

Tissue preparation

The thyroid glands were fixed in Bouin's fluid for approximately six hours. The tissues were then washed in 70% ethanol overnight, dehydrated through a graded ethanol series and embedded in paraplast. Serial cross sections were cut at 5 μm, separated into parallel series, mounted on slides and stained by hematoxylin-eosin (HE), Mallory's trichrome, periodic acid-Schiff (PAS) and Alcian blue (AB) pH 1.5 and 2.5. The sections were observed using an Olympus light microscope (model CH30) coupled to a Nikon digital camera (Coolpix 4300).

Immunohistochemistry

The sections were dewaxed and rehydrated following the routine protocols. They were incubated in a methanol solution containing 3% hydrogen peroxide for 15 minutes to block any endogenous peroxidase activity. The sections were washed in three drops of PBS, incubated in a humid chamber at 37ºC for 30 minutes with 1% goat serum and then incubated overnight at 4ºC with rabbit polyclonal anti-serotonin (S 5545 - Sigma-Aldrich, Inc.) diluted to 1:6000. The sections were then incubated with biotinylated "Universal" secondary antibody diluted to 1:200 (PK 7200, Vector Laboratories, Inc., U.K.) for 30 minutes, then with ABC, diluted to 1:200, for 30 minutes (both from PK 6200, Vector Lab. Inc.). The sections were again washed in three drops of PBS and revealed by treatment with 3,3'-diaminobenzidine tetrahydrochloride (003222 - DakoCytomation, California, USA) prepared according to the kit instructions, washed in distilled water, dehydrated in an increasing concentration series of ethanol solutions and mounted using Entellan (Merck).

Results

The thyroid gland, when macroscopically analyzed, is a single-stranded structure of discontinuous type which crosses the middle of the trachea, having an average of 0.8 cm length.

A dense irregular connective tissue forms a capsule that envelops the gland and sends branches which form a network that surrounds the follicles (Figure 1A).

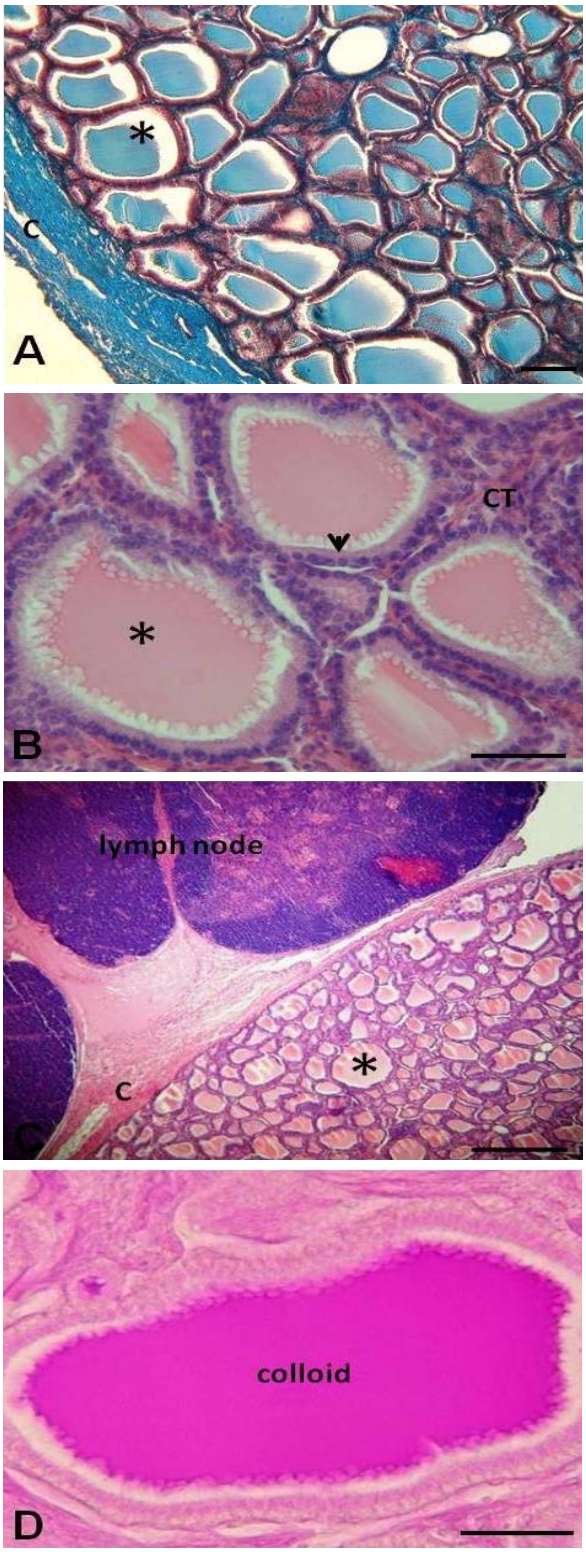

Figure 1. Photomicrography of a section in thyroid gland of *C. latirostris*. A. Connective tissue capsule and follicles (*). Mallory's trichrome. Scale bar = 50 μm. B. Follicles (*) composed of follicular cells (simple columnar epithelium), which are separated by scant interfollicular connective tissue (CT). HE. Scale bar = 50 μm. C. Note lymph node and connective tissue capsule (C) and follicles (*). HE. Scale bar = 100 μm. D. Colloid PAS positive in follicular lumen. PAS. Scale bar = 25μm.

The functional unit of the thyroid gland is its follicle. There are several follicular acini of varying size lined by simple cuboidal or columnar epithelium (Figure 1B). The follicles are filled with colloid, which is produced by the follicular cells. The follicles are connected by interfollicular connective tissue which contains blood vessels. In the interfollicular area there are a large number of cells, such as fibroblasts and parafollicular cells ("C" cells), which produce calcitonin. We also observed the presence of lymph nodes around the entire gland (Figure 1C).

The histochemical techniques revealed a positive PAS reaction (Figure 1D) and negative AB reaction in the colloid, which indicates the presence of a glycoprotein rich in neutral glycosaminoglycans. 5-HT-IR cells was observed around the follicle cells (Figure 2A and B).

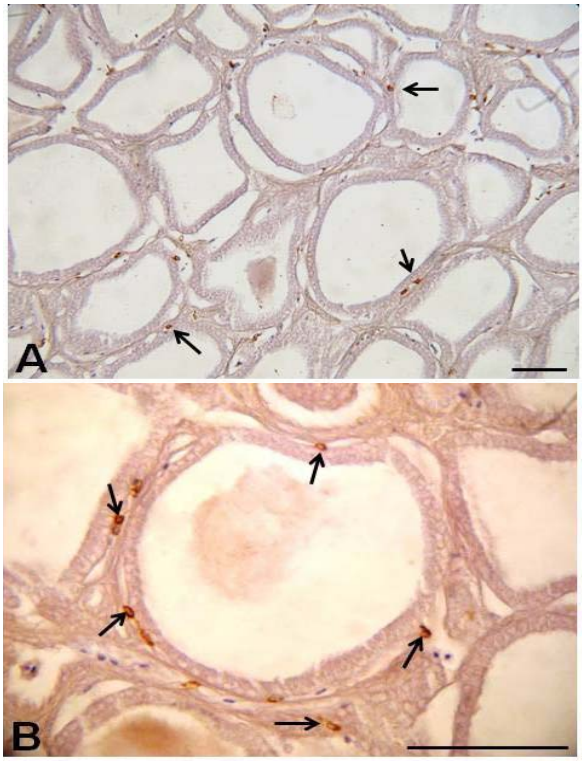

Figure 2. Photomicrography of serotonin-immunoreactive cells. A. Note the presence of serotonin-IR cells (arrows) in connective tissue. Bar = 50 µm. B. Serotonin-IR cells (arrows) around the follicle. Scale Bar = 50 µm.

These cells showed a higher frequency of the spherical shape, but there were also pyriform cells with the presence of a cytoplasmic process.

Discussion

The thyroid and parathyroid glands are intimately involved with many basic metabolic

functions. These glands have been the subject of extensive research in reptilian species. In lizards and crocodilians, the thyroid gland is located in the ventral cervical region (RIVERA; LOCK, 2008).

In most mammals and reptiles, the thyroid gland is composed of a large number of thyroid follicles formed by follicular cells, where their secretion product is stored. These secretions are precursors of hormones and other substances which form gelatinous colloid, located inside the follicles. The glandular epithelium becomes more cuboidal or columnar during periods of increased activity of the thyroid, and squamous during periods of reduced activity (FRYE, 1991). In *Caiman latirostris*, the follicular cells are more cuboidal or columnar indicating periods of glandular activity.

The follicles show seasonal changes in the dimensions of epithelial cells, extent of enclosed colloid material, presence of desquamated epithelial and blood cells, number of colloid droplets, and varying staining properties of colloid. In soft-shelled turtles, the low environmental temperature inhibits thyroid activity and high temperature stimulates gland activity (RIVERA; LOCK, 2008).

It is thought that the environmental temperature acts via the hypothalamo-hypophysial axis, which in turn alters thyroid function in turtles (CHUI et al., 1986; SENGUPTA et al., 2003). Thyroid hormone levels also vary with season and feeding (KAR; CHANDOLA-SAKLANI, 1985; KOHEL et al., 2001). Thyroid function is mediated centrally via thyrotropin-releasing hormone from the hypothalamus and thyroid-stimulating hormone (TSH) released from the hypophysis gland.

The central lumen of the thyroid follicle contains colloid, which is a substance rich in thyroglobulin, an iodinated glycoprotein, detected by the positive PAS reaction (KIERSZENBAUM; TRES, 2012), as observed in *C. latirostris*.

The secretion of thyroid hormones is regulated by a complex interplay between hormonal and neural signals, many of which have not yet been identified. One of these signals is sent by 5-hydroxytriptamine (5-HT) (serotonin). The TSH action on the thyroid depends on the presence of endogenous amines in the gland (NUNEZ; GERSHON, 1978). This influence on the thyroid metabolism is corroborated by the literature. The presence of this biogenic amine (5-HT) was demonstrated in this immunohistochemical study. These endogenous amines were also demonstrated in *Podarcis sicula*, which were located in the colloid and in the apical portion of the follicular cells.

The presence of such amine has also been verified in the parafollicular cells (PC) of sheep. Thyroid gland has neural crest derivatives that synthesize and release the biogenic amine serotonin (5-HT) as well as the hormone calcitonin (BERND et al., 1981). Similar results were observed in *C. latirostris*, where this amine was detected on the base of the follicular epithelium, the location of the parafollicular cells, as well as between the thyroid follicles.

Conclusion

Previous research has shown that the thyroid hormone parameters of reptiles are different from those observed in mammals. These differences are related to the different pattern of body temperature regulation (exothermic and endothermic, respectively) and the degree of activity of the gland, which is considered less active in reptiles (RIVERA; LOCK, 2008). However, these metabolic differences do not appear to interfere with the histology of this gland. No striking morphological differences were observed between *C. latirostris* and other domestic mammals. More studies should be conducted on this species to better understand the thyroid gland morphophysiology.

Acknowledgements

We thank Dr. Glenn Collard of Bonsucesso Farm (Nossa Senhora do Amparo, Barra Mansa, Rio de Janeiro) and Diogo Marcus Leibão Salles of Acquanature.

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Received on August 29, 2012.
Accepted on February 22, 2013.