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Anatomical and histological characteristics of the pituitary gland in the bottlenose dolphin (*Tursiops truncatus*) from the Adriatic Sea

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ABSTRACT

The structure of the pituitary gland was studied in 3 bottlenose dolphins (*Tursiops truncatus*) from the Adriatic Sea. This species is legally protected in Croatia. All examined animals died of natural causes and were found stranded along the eastern Adriatic coast. The pituitary gland is a rectangular body suspended at the base of the brain and situated in a depression of the sphenoid bone, clearly identified as sella turcica. The gland was divided into the adenohypophysis and the neurohipophisis, interconnected by thick layer of connective tissue. Pars distalis and pars tuberalis were observed in the adenohypophysis while the pars intermedia was lacking. The pars distalis was built of three main cell types: acidophils, basophils and chromophobes. Most of the acidophils were situated in the centre of the gland while chromophobes occurred dorsal and basophils ventral. Pars tuberalis was composed of cords of polygonal and vacuolated cells, which were in close association with numerous blood vessels. Large numbers of colloid-containing follicles were observed throughout the pars tuberalis. The neurohypophysis of the bottlenose dolphin consisted of unmyelinated nerve fibres among which numerous pituicytes were scattered.

Key words: pituitary gland, adenohypophysis, neurohypophysis, bottlenose dolphin, *Tursiops truncatus*, Adriatic Sea

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Introduction

The pituitary gland or hypophysis in domestic mammals is the master endocrine organ since it produces hormones that directly influence the activity of other endocrine glands. Its location as an appendage of the brain also points to its significance as the relay between the nervous and humoral mechanisms that jointly control certain functions. The pituitary gland is suspended below the hypothalamus by a narrow stalk and is positioned in a bony depression in the sella turcica of the sphenoid bone, hypophyseal fossa. Dura mater invests the gland and incompletely covers the depression, having an opening through which the hypophyseal stalk passes (DYCE et al., 1996).

The pituitary gland is divided into a glandular portion, the adenohypophysis and a neural portion, the neurohypophysis. The adenohypophysis develops from an evagination (Rathke´s pouch) of the oral ectoderm and the neurohypophysis develops from neural ectoderm as a downgrowth of the diencephalons. Both the adenohypophysis and the neurohypophysis are joined and encapsulated into a single gland. The adenohypophysis is further divided into pars distalis, which comprises the bulk of the adenohypophysis, pars tuberalis, and pars intermedia. The neurohypophysis is divided into pars nervosa and the infundibulum (JUNQUEIRA and CARNEIRO, 2005; DELLMANN, 1993; BANKS, 1993). The pars distalis and pars tuberalis are considered to be the anterior lobe of the gland, while the pars nervosa and pars intermedia are termed the posterior lobe (ROSS et al., 1989).

The pituitary gland of marine mammals is of particular interest, as it has been suggested that adaptation to maintain body temperature in a relatively cold aquatic environment includes modulation of metabolic rate and physical activity (COWAN et al., 2008). There is little published information on pituitary gland morphology in cetaceans. COWAN et al. (2008) stated that the cetacean pituitary gland consists of two distinct parts, the adenohypophysis and the neurohypophysis, which are separated by a thin fibrous membrane, in contrast to terrestrial mammals in which these parts are apposed and joined through a pars intermedia. In cetaceans, the pituitary pars intermedia and the hypophyseal cleft are completely absent (FLANIGAN, 1972; LUCIĆ, 2002; COWAN et al., 2008). The hormone produced by the pars intermedia in terrestrial mammals is produced by the pars distalis in cetaceans (FLANIGAN, 1972). The same author noted that due to the fact that the same adenohypophysis cell types found in other mammals are also found in cetaceans, there is no reason to think that the endocrine system is basically different.

Only one cetacean species resides in the Croatian part of the Adriatic Sea, the bottlenose dolphin (*Tursiops truncatus*). The number of animals is estimated to be 220, sorted into 40 shoals during the winter period (GOMERČIĆ et al., 1998). The species is under strict protection in Croatia (Rules on the Protection of Some Mammals, Law on Nature Protection, Republic of Croatia, 1995).

The aim of this study is to describe the anatomical and histological characteristics of the pituitary gland in the bottlenose dolphin (*Tursiops truncatus*) from the Croatian part of the Adriatic Sea.

Materials and methods

The study was carried out on three bottlenose dolphins, *Tursiops truncatus.* All animals died of natural causes and were found stranded in different places along the Croatian coast of the Adriatic Sea. The dead animals were brought to the Department of Anatomy, Histology and Embryology, the Faculty of Veterinary Medicine, University of Zagreb, and thoroughly investigated. The length and mass were recorded, among other external measurements. The age of animals was estimated by counting annual growth layer groups (GLG) in tooth dentine from longitudinal and cross sections, according to SLOTTEN (1991). During the autopsy, tissue and organ samples were taken for diagnostics and research needs.

The bottlenose dolphins studied were marked in the protocol as D141, D143 and D211.

Pituitary glands were collected after removal of the posterior calvarium and the brain.

The samples of the glands taken for histological examination were fixed with 4% neutral formalin, embedded in paraffin and cut into 6 μm thick sections, and were routinely stained with haematoxylin and eosin (H&E). The Masson´s trichrome staining method (ROMEIS, 1968) was used to demonstrate collagen fibres, the Congo red stain (SHEEHAN and HRAPCHAK, 1980) and the PFAAB, PAS, Orange G method (PEARSE, 1968) were used for differential staining of various cell types. Microscopic slides were analyzed under a Nikon - Microphot FXA (Nikon Corporation, Tokyo, Japan) light microscope and photographed with a digital Sony - CCD-IRIS/RGB Color Video Camera (Sony Corporation, Tokyo, Japan).

Results

The pituitary gland of the bottlenose dolphin (*Tursiops truncatus*) from the Adriatic Sea was a rectangular body, suspended at the base of the brain by a narrow, fragile stalk and was situated close to the optic chiasm (Fig. 1). It was three times longer from side to side than from the anterior to the posterior border (Fig. 2). The gland was situated in a shallow depression of the sphenoid bone at the cranial cavity floor. This depression was defined with a higher anterior and a lower posterior bony crest and could be identified as the sella turcica. A covering of dura extended from the margins of the sella turcica, roofed the depression and embraced the hypophysial stalk from all sides (diaphragma sellae). This arrangement preserved the pituitary gland in the sella turcica while the brain was removed at autopsy.

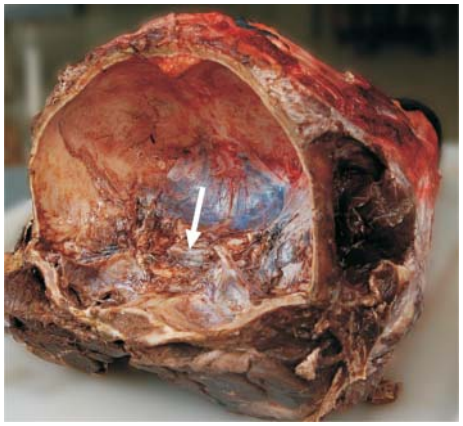

Fig. 1. The bottlenose dolphin (D211). Location of the pituitary gland in the depresion of the sphenoid bone.

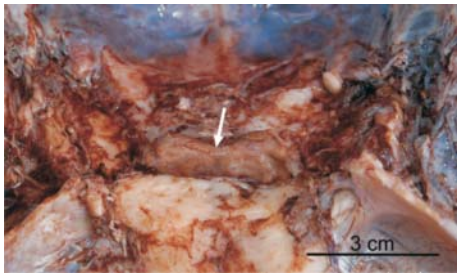

Fig. 2. The bottlenose dolphin (D211). Rectangular shape of the pituitary gland. The pituitary gland is three times longer from side to side than from the anterior to the posterior border.

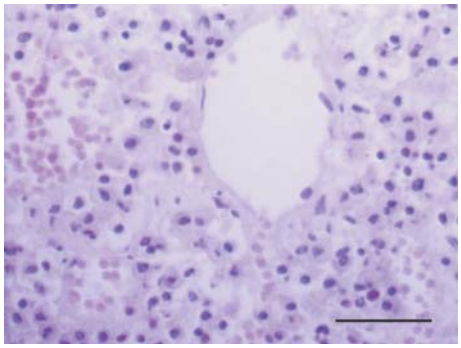

Fig. 3. The bottlenose dolphin (D 141). Large sinusoidal capilaries of the pars distalis. HE, objective ×20, scale bar = 100 μm.

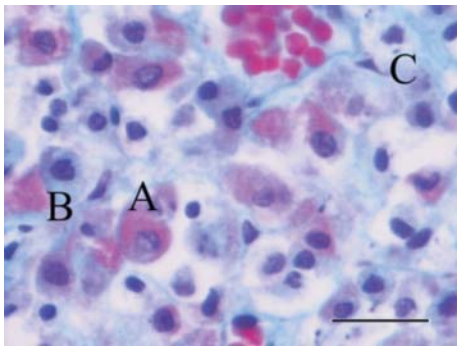

Fig. 4. The bottlenose dolphin (D 143). Pars distalis. A-acidophils, B-basophils, C-chromophobes. Masson trichrome, objective ×40, scale bar = 50 μm.

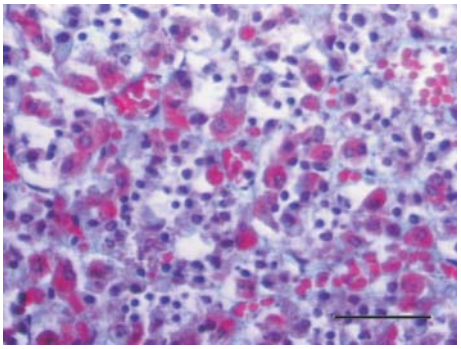

Fig. 5. The bottlenose dolphin (D 143). Pars distalis. Numerous acidophils in the center of the gland. Masson trichrome, objective ×20, scale bar = 100 μm.

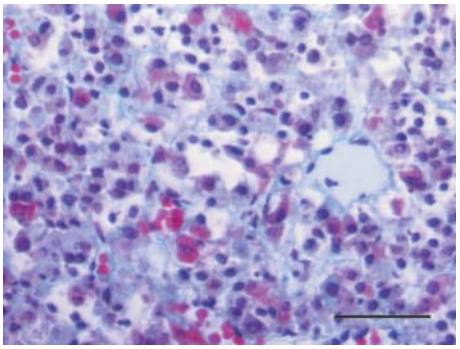

Fig. 6. The bottlenose dolphin (D 143). Pars distalis. Chromophobes in the dorsal part of the gland. Masson trichrome, objective ×20, scale bar = 100 μm.

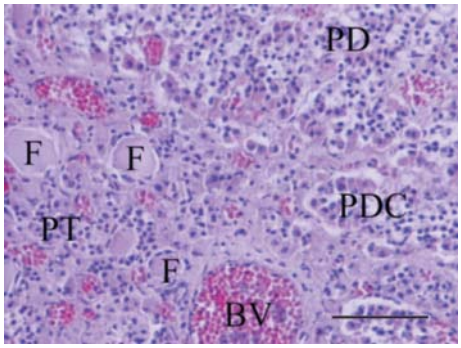

Fig. 7. The bottlenose dolphin (D 143). Pars distalis (PD). Pars tuberalis (PT). BV-blood vessels, F- colloid-containing follicles, PDC- islets of pars distalis cell. HE, objective ×10, scale bar = 200 μm.

The bottlenose dolphin pituitary gland was divided into the adenohypophysis and the neurohypophysis. The gland was surrounded by a common capsule of dense irregular connective tissue. The adenohypophysis and the neurohypophysis were interconnected by thick layers of connective tissue. The pars distalis and pars tuberalis were observed in the adenohypophysis.

The parenchymal components of the pars distalis were arranged as clusters or cords of cells. They were surrounded by a delicate connective tissue stroma, which contained large sinusoidal capillaries (Fig. 3). On the basis of staining properties, the cells of the pars distalis were divided into three main types: acidophils, basophils and chromophobes (Fig.

4). Acidophils were large and polygonal shaped, with heterochromatic nuclei and orange-red cytoplasm. Basophils were rounded or oval cells, slightly smaller than the acidophils. Dark rounded nuclei were noticeable in the centre of the cells. Chromophobes were weakly stained cells of the pars distalis. These cells had less cytoplasm than acidophils and basophils and very dark nuclei. The distribution of cell types varied throughout the pars distalis in the adenohypophysis of the bottlenose dolphin. Most of the acidophils were situated in the centre of the gland (Fig. 5). Chromophobes were situated mostly dorsal (Fig. 6) while basophils were ventral.

The pars tuberalis entered the gland from the anterior surface and penetrated the pars distalis. The boundary between these two parts of adenohypophysis was not entirely clear because few islets made of pars distalis cells could be seen in the pars tuberalis. Also, cell aggregations of the pars tuberalis could be found in the pars distalis.

The pars tuberalis of the adenohypophysis was composed of cords of polygonal and pale basophilic cells. Some of them were vacuolated. The cords of the cells were in close association with numerous blood vessels (Fig. 7). Colloid-containing follicles of various sizes were observed throughout the pars tuberalis. The follicles were lined by thin, flat cells which resembled simple squamous epithelium cells. Also, the same follicles were seen in the pars distalis.

The neurohypophysis of the bottlenose dolphin consisted of unmyelinated nerve fibres. Numerous cells corresponding to pituicytes were scattered among these fibres.

Discussion

The pituitary gland of the bottlenose dolphin (*Tursiops truncatus*) from the Adriatic Sea was located at the basis of the brain, on the sphenoid bone, as described for all domestic mammals (DYCE et al., 1996). The gland was located in a defined depression of the sphenoid bone called the sella turcica. Our results corroborate those of COWAN et al. (2008) who described the anatomical and histological features of the pituitary gland in numerous cetacean species and found that for most investigated species there was no true sella turcica, but a shallow depression in the sphenoid bone, with anterior and posterior ridges. The authors emphasised that in the Atlantic bottlenose dolphin the sphenoid bone has a poorly expressed sella turcica.

The pituitary gland of the bottlenose dolphin consisted of the adenohypophysis and the neurohypophysis, as described in domestic mammals (DELLMANN, 1993; BANKS, 1993; BACHA and BACHA, 2000) and humans (HOLMES and BALL, 1974; ROSS et al., 1989; JUNQUEIRA and CARNEIRO, 2005). The identical division of the pituitary gland in marine mammals had been described before (YOUNG and HARRISON, 1970; OELSCHÄGER and KEMP, 1998; COWAN et al., 2008). In this study we noticed that the neurohypophysis was

considerably smaller than the adenohypophysis, which confirmed previously published data by COWAN et al. (2008). They found that the adenohypophysis was much larger than the neurohypophysis in nearly all species, with the exception of *Kogia* spp. The dual origin of the pituitary gland, and the interrelation of the two components during the developmental and growth period, play a major role in the determination of the final form of the gland (HOLMES and BALL, 1974).

In this study we found that the adenohypophysis and the neurohypophysis were separated by a thick layer of connective tissue, whereas in domestic mammals the parts were in contact and joined through the pars intermedia (DELLMANN, 1993; BANKS, 1993; BACHA and BACHA, 2000). OELSCHÄGER and KEMP (1998) investigated the development of the sperm whale (*Physeter macrocephalus*) brain in embryos and early foetuses. The authors noticed that two components of the hypophysal rudiment were obvious in the youngest estimated embryo (9.5 mm crown-rump length). They also noticed that the pars intermedia of the pituitary gland did not develop. In the pituitary gland of the bottlenose dolphin from the Adriatic Sea no pars intermedia was observed. The absence of the pars intermedia in the pituitary gland of cetaceans has been described by YOUNG and HARRISON (1970), FLANIGAN (1972) and COWAN et al. (2008). HOLMES and BALL (1974) stated that the pars intermedia only developed if close contact between the epithelial and neural elements of the developing pituitary gland existed. Furthermore, differentiation only occurred in the epithelial component which was in such contact. In mammals in which connective tissue separates the adenohypophysis from the neurohypophysis, as in the cetaceans, no pars intermedia was found. FLANINGAN (1972) noted that the hormones ordinarily produced by the terrestrial mammals' pars intermedia were produced by the pars distalis in the cetaceans.

In this study, the pars distalis of the adenohypophysis consisted of three types of cells: acidophils, basophils and chromophobes. This classic tinctorial distinction and classification of various cell types according to the stain affinity of their secretory granules is useful for routine examinations. Using immunohistochemical methods, the various adenohypophysial cells can be classified according to the hormone they contain. COWAN et al. (2008) found that ACTH-positive cells were most abundant in the pars distalis of all investigated cetacean species. Using electron microscopy, YOUNG and HARRISON (1970) identified six cell types in the adenohypophysis of the *Delphinus delphis* differing in cell size, granule size and cell shape. They recognized lactotrophs and somatotrophs, which corresponded to acidophils, and gonadotrophs and tyrotrophs, which corresponded to basophils. Stellate cells were described as a distinct feature of the delphinid pars distalis. Also, an additional cell type was present, whose significance was obscure.

The distinct characteristics of the pars tuberalis of the bottlenose dolphin pituitary gland were colloid-containing follicles. Furthermore, a few follicles were also noticed

in the pars distalis. Similar follicles were described by COWAN et al. (2008). The authors observed many small to large follicles throughout the adenohypophysis, although they were most frequently found anteriorly, ventral to and extending into the stalk.

The structure of the neurohypophysis of the bottlenose dolphin was the same as described in domestic mammals (DELLMANN, 1993; BANKS, 1993; BACHA and BACHA, 2000) and humans (ROSS et al., 1989; JUNQEIRA and CARNEIRO, 2005). The neurohypophysis is not an endocrine gland per se, but rather is part of the hypothalamo-neurohypophysial system (DELLMANN, 1993).

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SAŽETAK

Istraživana je građa hipofize u tri dobra dupina (*Tursiops truncatus*) iz Jadranskog mora. Dobri dupin u Republici Hrvatskoj najstrože je zaštićena životinjska vrsta. Sve istražene životinje uginule su u prirodi i nađene su nasukane duž istočne jadranske obale. Hipofiza u dobroga dupina pravokutnoga je oblika, smještena na osnovici mozga i uložena u udubinu klinaste kosti, prepoznate kao sella turcica. Žlijezda je podijeljena na adenohipofizu i neurohipofizu koje su međusobno povezane debelim slojem vezivnog tkiva. Adenohipofiza dobroga dupina sastoji se od pars distalis i pars tuberalis, dok pars intermedia nedostaje. Pars distalis građen je od tri tipa stanica: acidofilnih, bazofilnih i kromofobnih. Acidofilne stanice u najvećem su broju smještene u središtu žlijezde, dok se kromofobne stanice nalaze dorzalno, a bazofilne ventralno. Pars tuberalis građen je od nizova poligonalnih i ponešto vakuoliziranih stanica koje su blisko vezane uz mnoge krvne žile. Uočen je i velik broj folikula ispunjenih koloidom. Neurohipofiza dobroga dupina građena je od nemijeliziranih živčanih vlakana između kojih se nalaze brojni pituiciti.

Ključne riječi: hipofiza, adenohipofiza, neurohipofiza, dobri dupin, *Tursiops truncatus*, Jadransko more