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ORIGINAL ARTICLE

Histological Structure of the Adrenal Gland of the Bottlenose Dolphin (*Tursiops truncatus*) and the Striped Dolphin (*Stenella coeruleoalba*) from the Adriatic Sea

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Summary

The structure of the adrenal gland was studied in 11 bottlenose dolphins (*Tursiops truncatus*), and five striped dolphins (*Stenella coeruleoalba*). These species are legally protected in Croatia. All examined animals died of natural causes and were found stranded along eastern Adriatic coast. In both species the adrenal gland consists of a cortex and a medulla; the cortex is divided into three zones. Whereas in the bottlenose dolphin, there is a zona arcuata which contains columnar cells arranged in the form of arches; in the striped dolphin this zone is replaced by zona glomerulosa containing rounded clusters of polygonal cells. In both species, the zona fasciculata consists of radially oriented cords of polygonal cells, whereas in zona reticularis cells are arranged in branching and anastomosing cords. The adrenal medulla in both species contains dark, epinephrine-secreting cells and light norepinephrine-secreting cells. Epinephrine-secreting cells are localized in the outer part of the medulla, whereas norepinephrine-secreting cells are found in the inner part, arranged in clusters and surrounded by septa of thin connective tissue. The gland is surrounded by a thick connective-tissue capsule, from where thick trabeculae extend towards the interior. In the bottlenose dolphin, group of cells resembling both medullar and cortical cells can be seen within the capsule; whereas only groups of cells resembling cortical cells are found within the capsule of the striped dolphin. In the bottlenose dolphin invagination of the adrenal cortex into the medulla is obvious as well as medullary protrusions extending through cortex to the connective tissue capsule.

Introduction

The adrenal gland in mammals is typically made of two concentric layers of glandular tissue: the outer cortex and the inner medulla. These two parts differ from each other by origin, microscopic structure and function. Because of the differences in cell arrangements, the adrenal cortex is divided into three zones: either the zona glomerulosa or the zona arcuata; the zona fasciculata and the zona reticularis. The zona glomerulosa is found in humans and bovids whereas the zona arcuata is described in horses, donkeys, carnivores and pigs (Banks, 1993; Dellmann, 1993; Raviola, 1994; Junqueira and Carneiro, 2005). In marine mammals, examined so far, the adrenal cortex is also divided into the zona glomerulosa, the zona fasciculata and the zona reticularis (Simpson and Gardner, 1972; Zhongjie, 1988; Bragulla et al., 2004; Clark et al., 2005, 2008). It seems that there are significant differences in zone thickness in different marine mammal species, which may be related to the functional state of the gland (Simpson and Gardner, 1972).

The adrenal gland in marine mammals is surrounded by a connective-tissue capsule from which connective tissue septa spread at a right angle towards the interior, showing a pseudolobulated appearance to the gland (Simpson and Gardner, 1972). Pseudolobulation was described in various species of cetaceans (Zhongjie, 1988, Lair et al., 1997; St. Aubin, 2002; Clark et al., 2005, 2008); however, it seems that it was not found in pinnipeds (Simpson and Gardner, 1972). The degree of pseudolobulation of the adrenal cortex in the pan-tropical spotted dolphin and the spinner dolphin varied from minimal to highly lobulated (Clark et al., 2008). Lucić (2002) found stronger lobulation of the adrenal cortex in the bottlenose dolphin than in the striped dolphin from the Adriatic Sea.

The functional significance of the adrenal gland in marine mammals is reflected by the fact that hormones are secreted as an adequate defensive reaction in response to stress. In extreme or prolonged stress situations, a strong response of the body can be fatal for animals (Clark et al., 2005). The catecholamines of the adrenal gland are essential for oxygen reserve control (St. Aubin, 2002).

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 in 40 shoals during the winter period (Gomerčić et al., 1998). The striped dolphin (*Stenella coeruleoalba*) is not frequently found in the Croatian part of the Adriatic Sea and is considered a non-resident species in the area (Galov et al., 2009). Both species are 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 was to characterize the structure of the adrenal gland in the bottlenose dolphin (*T. truncatus*) and in the striped dolphin (*S. coeruleoalba*) from the Croatian part of the Adriatic Sea. Furthermore, we aimed to describe possible differences between these two species as well as differences with regard to the adrenal gland structure in domestic animals and human.

Materials and Methods

The study was carried out on 16 animals: 11 bottlenose dolphins, *T. truncatus* and 5 striped dolphins, *S. coeruleoalba*. All animals died of natural causes and were found stranded along the eastern coast of the Adriatic Sea. The dead animals were brought to the Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, and thoroughly investigated. During autopsy, tissue and organ samples were taken for diagnostics and research purposes. The age of animals was estimated by counting annual growth layer groups in tooth dentine from longitudinal and cross sections according to Slooten (1991).

Samples of the adrenal gland taken for histological examination were fixed with 4% neutral formalin, embedded in paraffin and cut into 6 μm thick sections which were routinely stained with haematoxylin and eosin (H&E). The Mallory stain and the Masson's trichrome staining method (Romeis, 1968) were used to demonstrate collagen fibres and the Pincus method (Romeis, 1968) was used for a differential staining of elastic fibres. Microscopic slides were analysed under a Nikon – Microphot FXA light microscope and a Nikon – SMZ-U stereomicroscope (Nikon Corporation, Tokyo, Japan), and photographed with a digital Sony – CCD-IRIS/RGB colour video camera (Sony Corporation, Tokyo, Japan).

Results

Estimated ages of bottlenose dolphins were from 2 to 21 years, whereas striped dolphins were from 11 to 23.

The bottlenose dolphins' (*T. truncatus*) adrenal gland was divided into the cortex and the medulla. The gland was surrounded by a connective tissue capsule from which clearly visible trabeculae spread towards the interior (Fig. 1).

The connective tissue capsule in the bottlenose dolphin was very thick. In the surrounding connective tissue, a supracapsular network of blood vessels and nerve fibres was detectable. Clusters of cells, which by their appearance

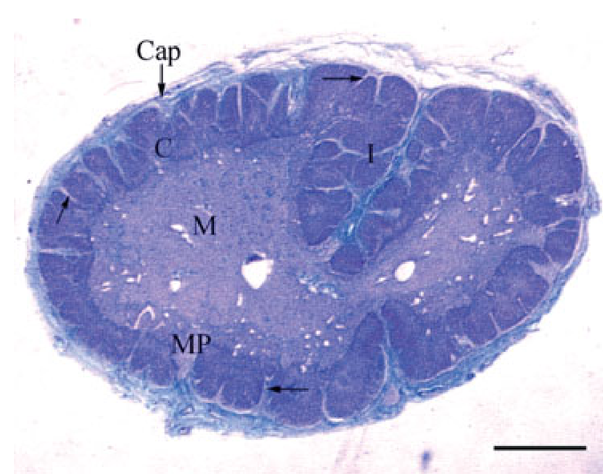

Fig. 1. The bottlenose dolphin. Cross-section of the adrenal gland. The gland is wrapped with a connective tissue capsule (Cap). Trabeculae (arrows) are spread from capsule towards the interior. The parenchyma is divided into the cortex (C) and the medulla (M). The cortical invaginations (I) and the medullary protrusions (MP) are visible; Stain = Masson trichrome, Scale bar = 0.5 cm.

and staining properties resembled the cortical cells, and clusters of cells which looked like medullar cells could be found within the capsule. From the connective tissue capsule thick trabeculae spread through the cortex. The trabeculae were especially thick at the beginning of their course, later they became somewhat thinner and reached the border between the cortex and the medulla as thin septa. The capsule and the trabeculae were mainly made of collagen fibres and a few elastic fibres. The arrangement of the cells in the cortex surface zone changed along the trabecular course (Fig. 2). Such an arrangement of cells along the trabeculae gave the adrenal cortex a look of an incomplete lobule.

The adrenal cortex was divided into three areas or zones, each with different cell arrangement. Below the capsule there was the zona arcuata, an area which was built of columnar cells arranged in arches with a convex side facing the capsule. Blood capillaries could be found within the arches and between the neighbouring arches. The columnar cells had an acidophilic cytoplasm and oval nuclei (Fig. 3). A small nucleolus could be seen within the nuclei. Between the zona arcuata and the adjacent zona fasciculata there was a layer of smaller cells with irregular, very dark nuclei. This zone had no continuity and possibly represented an intermediate zone, as described for domestic mammals. The zona fasciculata was built of radially orientated cords of cells. It seemed that there were one or two cell rows in each cord. The cells were large, polygonal shaped with the centrally located light nuclei. Very often binucleate cells could be seen. The zona reticularis presented the deepest layer of the adrenal cortex. It consisted of interconnecting, irregular cords of large, polygonal cells with dark nuclei.

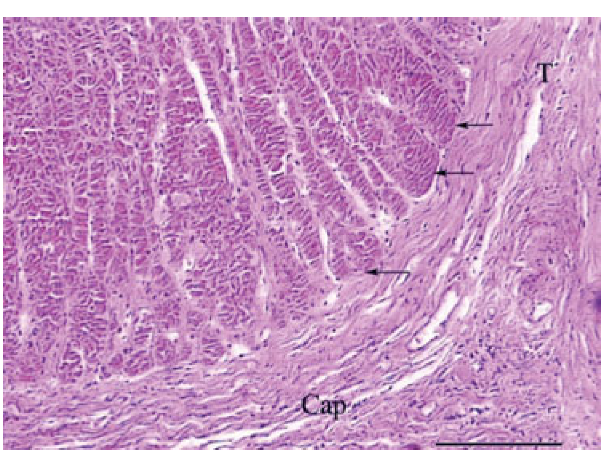

Fig. 2. The bottlenose dolphin. Connective tissue capsule (Cap) on the gland surface from which a thick trabecula (T) is separated. Zona arcuata (arrows) follows the course of trabecula; Stain = H&E, Scale bar = 200 μm.

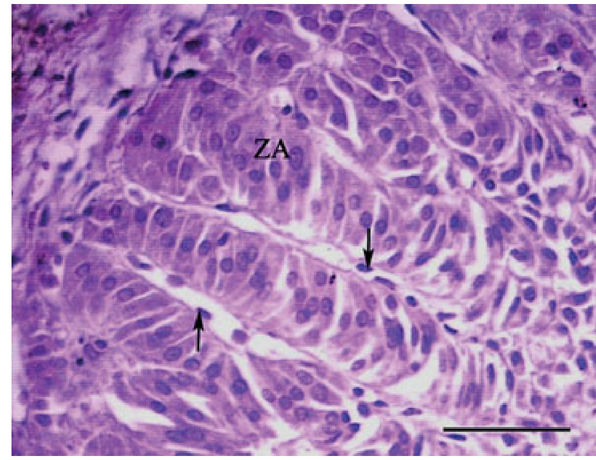

Fig. 3. The bottlenose dolphin. Columnar cells arranged in arches. Within arches and between neighbouring arches endothelial cells of the blood capillaries are present (arrows). Zona arcuata (ZA); Stain = H&E, Scale bar = 50 μm.

Between the cords of cells the capillaries often appeared dilated, contrary to the capillaries of the zona arcuata and the zona fasciculata (Fig. 4).

The border between the cortex and the medulla in the adrenal gland of the bottlenose dolphin was well-defined with conspicuous interdigitations. The cortex and the medulla were separated by a layer of connective tissue originating from the trabeculae (Fig. 5).

The outer part of the medulla was built of large, dark-stained polygonal cells with light, somewhat eccentrically located nuclei. The inner part was composed of clusters of cells surrounded by a clearly visible layer of connective tissue. The cells showed a translucent cytoplasm and dark nuclei. In the adrenal medulla, large blood vessels and

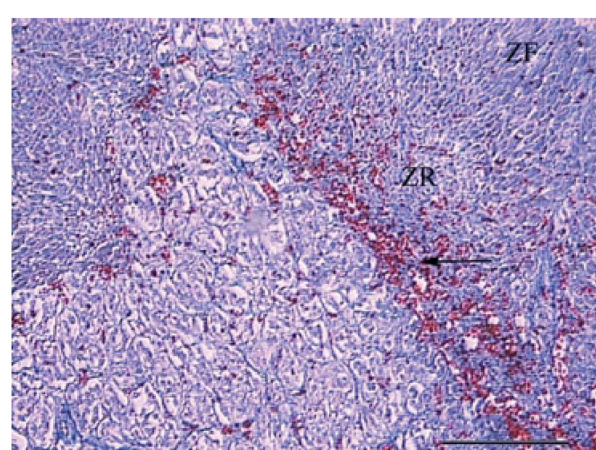

Fig. 4. The bottlenose dolphin. Branched capillaries of the zona reticularis (arrows). Zona fasciculata (ZF); Zona reticularis (ZR); Stain = Mallory, Scale bar = 200 μm.

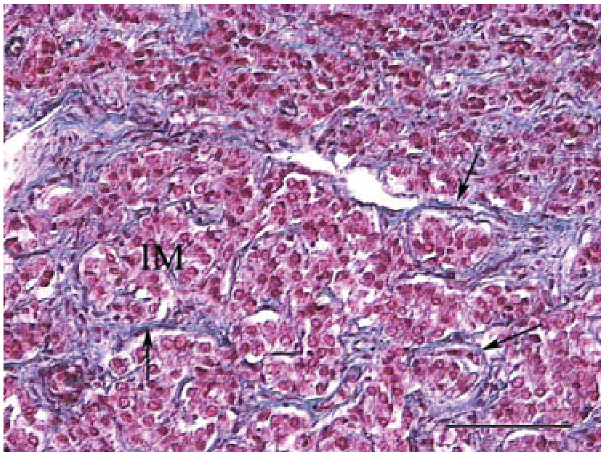

Fig. 5. The bottlenose dolphin. Cell aggregations in the inner part of the medulla (IM) surrounded with a connective tissue (arrows). Stain = Masson trichrome, Scale bar = 100 μm.

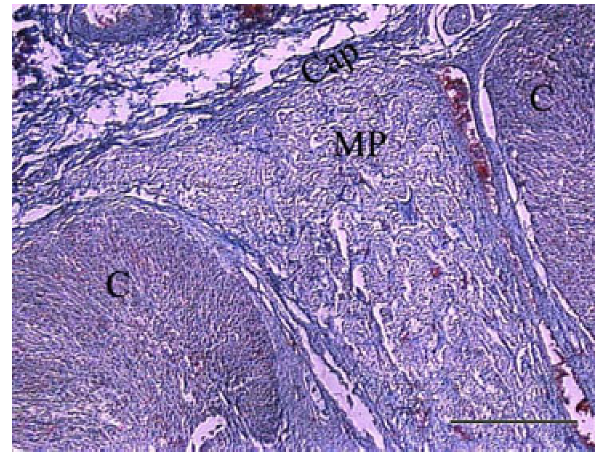

Fig. 7. The bottlenose dolphin. The medullary protrusions reach the connective tissue capsule. They are formed of clusters of cells which are surrounded by connective tissue, in the same way as in the inner part of the medulla. Medullary protrusion (MP); Cortex (C); Connective tissue capsule (Cap); Stain = Mallory, Scale bar = 500 μm.

long sinusoids were present, and were even more numerous in the outer part of the medulla.

Invagination of the adrenal cortex in the bottlenose dolphin is clearly visible (Fig. 1). It is formed around the blood vessel surrounded by the zona arcuata, the zona fasciculata and the zona reticularis of the adrenal cortex. From the outer side, the invagination was surrounded by cells of the outer part of the medulla (Fig. 6). Medullary protrusions reached the connective tissue capsule and were built by the tissue of the inner part of the medulla (Fig. 7).

The adrenal gland of the striped dolphin (*S. coeruleoalba*) from the Adriatic Sea was also divided into the cortex and the medulla (Fig. 8). On the gland surface, there was a connective tissue capsule from which strong trabeculae spread towards the interior, as in the bottlenose dolphins. Aggregations of cortical tissue could be found within the connective tissue capsule (Fig. 9).

The adrenal cortex in the striped dolphin was clearly divided into three zones. In place of zona arcuata, found in the bottlenose dolphin, the striped dolphin had the zona glomerulosa. The zona glomerulosa was made of polygonal cells with markedly dark nuclei. The cells were grouped in cords or clusters (Fig. 10). The zona fascicula-

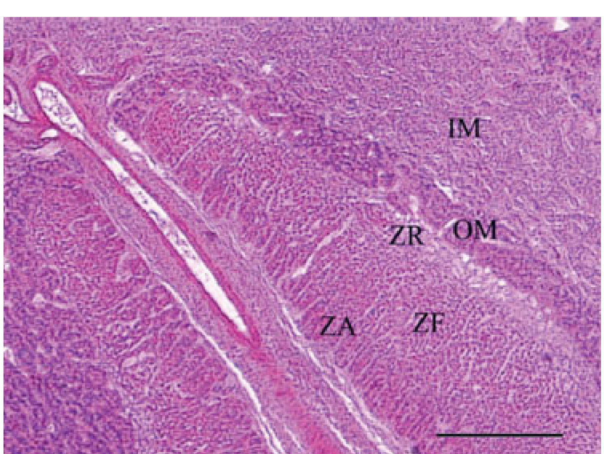

Fig. 6. The bottlenose dolphin. A blood vessel surrounded by the cortical tissue. Zona arcuata (ZA); Zona fasciculata (ZF); Zona reticularis (ZR); Outer part of the medulla (OM); Inner part of the medulla (IM); Stain = H&E, Scale bar = 500 μm.

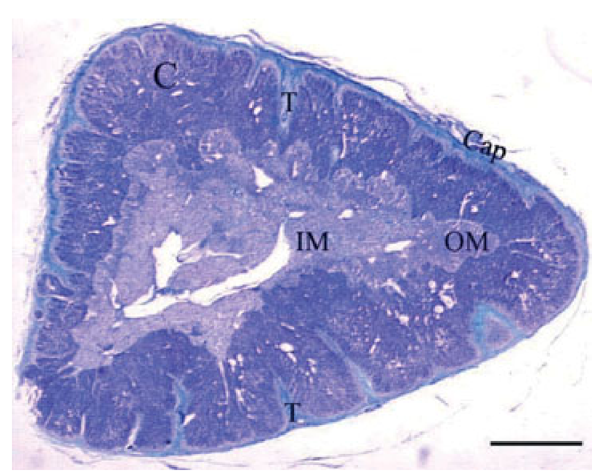

Fig. 8. The striped dolphin. Cross-section of the adrenal gland. Connective tissue capsule (Cap); Cortex (C); Outer part of the medulla (OM); Inner part of the medulla (IM); Trabeculae (T); Stain = Masson trichrome, Scale bar = 0.5 cm.

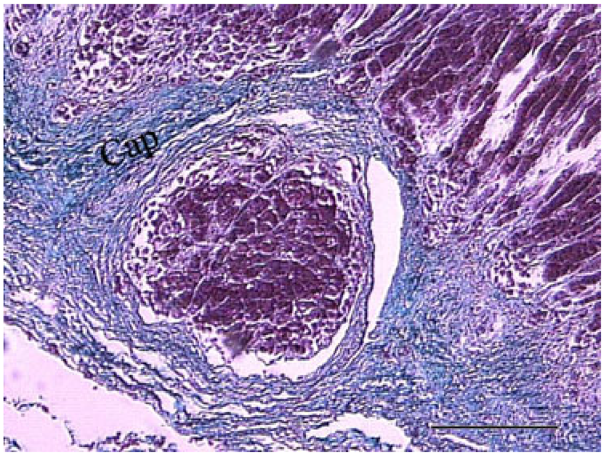

Fig. 9. The striped dolphin. Cortical cells aggregation within the connective tissue capsule (Cap). Stain = Masson trichrome, Scale bar = 200 μm.

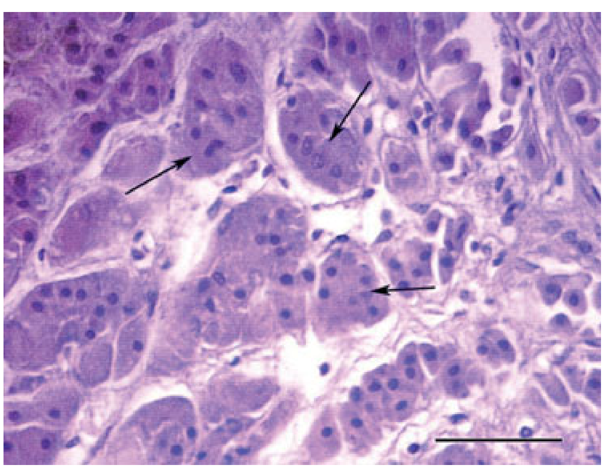

Fig. 10. The striped dolphin. Polygonal cells clusters in the zona glomerulosa (arrows). Stain = H&E, Scale bar = 50 μm.

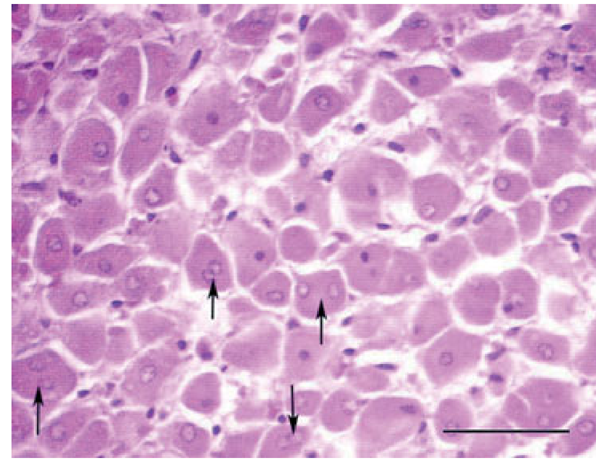

Fig. 11. The striped dolphin. Cells are with two nuclei (arrows) in the zona fasciculata. Stain = H&E, Scale bar = 50 μm.

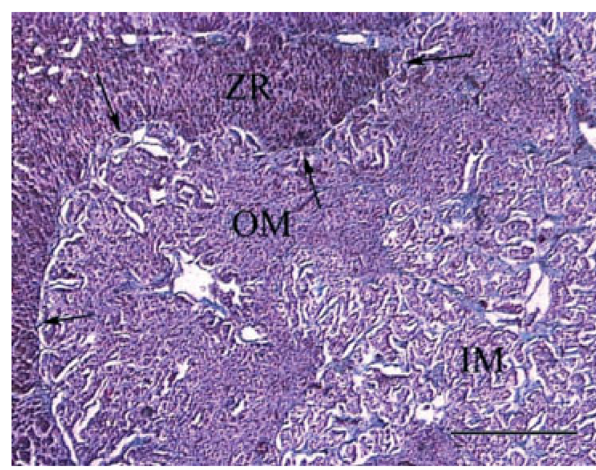

Fig. 12. The striped dolphin. The adrenal cortex and the medulla are separated by a layer of the connective tissue (arrows). The medulla is divided in outer, darker area (OM) and the inner, lighter area (IM). The inner area is built of cell aggregations surrounded by a connective tissue. Zona reticularis (ZR); Stain = Masson trichrome, Scale bar = 500 μm.

ta was made of radially arranged cords of polygonal cells which spread from the zona glomerulosa up to the zona reticularis. Binucleated cells in the zona fasciculata were usually present (Fig. 11). The cells in the zona reticularis were arranged in irregular cords which formed a network together with blood capillaries.

The adrenal cortex and the adrenal medulla of the striped dolphin were separated by a layer of connective tissue. In the outer part of the medulla there were dark, polygonal cells with light nuclei. The inner part was made of lighter cell aggregations surrounded by connective tissue, as in the bottlenose dolphins (Fig. 12).

In histological sections of the adrenal gland of the striped dolphin neither invaginations of the cortical tissue into the medulla nor medullary protrusions towards the connective tissue capsule were noticed.

Discussion

In this study, the structures of the adrenal glands of the bottlenose dolphin and the striped dolphin from the Adriatic Sea were studied. On the adrenal gland surface in both dolphin species there was a thick capsule made of fibrous connective tissue. In whales, capsule thickness displays great local variations (Simpson and Gardner, 1972) and the same was described for young common seals (Bragulla et al., 2004). In our investigation, we found no difference in capsule thickness between the bottlenose dolphin and striped dolphin. Dellmann (1993) describes thin trabeculae in domestic mammals which originate from the capsule, pass through the cortex and rarely enter the medulla. However, in the adrenal glands of the bottlenose dolphin and striped dolphin studied, connective tissue trabeculae extended to the layer of connective tissue between the cortex and the medulla and then spread as very thin septa through the entire medulla. A similar arrangement of connective tissue within the adrenal gland was described for different whale species by Simpson and Gardner (1972) and Clark et al. (2005, 2008).

The adrenal cortex of all marine mammals described so far was built of three zones: the zona glomerulosa, the zona fasciculata and the zona reticularis (Simpson and Gardner, 1972; Zhongjie, 1988; Lair et al., 1997; Lucić, 2002; Bragulla et al., 2004, Clark et al., 2005, 2008). However, our study showed that the adrenal cortex of the bottlenose dolphin contained the zona arcuata instead of the zona glomerulosa. Admittedly, Bragulla et al. (2004) described a zona arcuata in the adrenal gland of young common seals and believed that the arrangement of cells changed by the stress conditions, so that a zona arcuata changed to glomerulosa in the adult animals. Our study does not support the assumption that the zona arcuata modifies to zona glomerulosa during ageing. Among the investigated bottlenose dolphins that had an estimated age between 2 and 21 years, we did not notice any changes in structure of the zona arcuata of the adrenal cortex.

A thin layer of small cells situated below the zona arcuata in bottlenose dolphins could correspond to the zona intermedia which was found in most domestic mammals (Banks, 1993; Dellmann, 1993). A possible existence of zona intermedia cells in the adrenal cortex of the common seal was also noticed by Bragulla et al. (2004).

A zona fasciculata of columnar cell cords which gradually convert into cuboidal cells of a zona reticularis was described in the Atlantic bottlenose dolphin, by Clark et al. (2005). According to our research, cells in the zona fasciculata were polygonal with large, light nuclei and frequently had two nuclei. The foamy appearance of the cells, described for domestic animals (Banks, 1993; Dellmann, 1993) and humans (Ross et al., 1989; Junqueira and Carneiro, 2005) caused by the presence of numerous empty vesicles from the dissolution of lipid droplets during routine tissue processing, were not observed in the bottlenose dolphin and striped dolphin in this research.

The adrenal cortex and the adrenal medulla in the Adriatic bottlenose and striped dolphin species were separated by a clearly visible layer of connective tissue. The same was described by Clark et al. (2005) for the Atlantic bottlenose dolphin; by Clark et al. (2008) for the pan-tropical spotted dolphin and the spinner dolphin and by Amoroso et al. (1965) for a harbour seal male. However, a connective tissue layer between cortex and medulla of the adrenal gland for domestic mammals and humans was not described (Banks, 1993; Dellmann, 1993; Junqueira and Carneiro, 2005).

Previous researchers described that the adrenal medulla to be built of two types of cells which contain different granules. Cells which secrete epinephrine have smaller, less electron-dense granules which are regularly arranged within the cytoplasm, whereas cells which secrete norepinephrine have larger, electron-dense, irregularly arranged granules (Coupland, 1965; Coupland and Weakley, 1970; Fenwick et al., 1978; Scheuermann, 1993). Cells which secrete epinephrine stain intensely with basic dyes such as haematoxylin (Costa, 1968). Moreover, in horses, cows, sheep and pigs, the medulla is divided into two areas, the outer area, built of the more intensively stained epinephrine-secreting cells, and the inner area, built of norepinephrine-secreting cells that have a weaker stain affinity (Dellmann, 1993). Such a cell arrangement of the adrenal medulla was found in the Atlantic bottlenose dolphin (Clark et al., 2005) and in the pan-tropical spotted dolphin and the spinner dolphin (Clark et al., 2008), as well as in our study for both investigated species.

We observed medullary protrusions which reach the connective tissue capsule in the Adriatic bottlenose dolphin. Similar protrusions were described by Carballeira et al. (1987) and Clark et al. (2005, 2008). Clark et al. (2005) noticed an average of two protrusions per the histological section of the adrenal gland of the Atlantic bottlenose dolphin, whereas Clark et al. (2008) observed less than one or one protrusion per histological section of the adrenal glands of the pan-tropical spotted dolphin and the spinner dolphin. We did not observe medullary protrusions in the striped dolphin; however, the reason for this could be low number of animals examined. Functional significance of these medullary protrusions is unknown for the present.

Clark et al. (2005) described an artery in the bottlenose dolphin which extended over adrenal cortex into the adrenal medulla. This artery is completely ensheathed by three layers of cortex cells. This was explained by a specific embryonic development of the adrenal gland. In the common seal, Bragulla et al. (2004) found that the cortex invaginates into the medulla on the gland hilus forming the inverse cortex, which is functionally significant with respect to the angioarchitecture. We also observed an invagination of the adrenal cortex formed around the centrally located blood vessel in the bottlenose dolphin from the Adriatic

Sea. It is known that an abrupt growth of total body length in the bottlenose dolphin from the Adriatic Sea stops at the age of 9 years for both male and female animals. Total body length does not increase after 10–12 years in females, whereas male animals continue to grow slowly also after that age (Đuras Gomerčić, 2006). Organs and glands in Cetacea grow during the whole life, but the growth slows down with sexual maturity (Cowan, 1966). If organs grow permanently, then the invagination of the cortex in the adrenal gland hilus can be explained by an overgrowing with the gland tissue during development.

In domestic animals accessory aggregations of the cortical tissue can be incorporated within several organs, but they are most frequently tied to the adrenal gland capsule (Dyce et al., 1996). Therefore, our findings of cell aggregations which resemble the medullary cells, situated in the connective tissue capsule of the adrenal gland in the bottlenose dolphin can be considered an accessory adrenal tissue, whereas the cell aggregations which resemble the cortical cells in both investigated species can be considered as accessory inter-renal tissue.

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