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Morphology and Histology of the Atlantic Bottlenose Dolphin (*Tursiops truncatus*) Adrenal Gland with Emphasis on the Medulla

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With 16 figures and 1 table

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Summary

This study provides the first detailed description of the Atlantic bottlenose dolphin (*Tursiops truncatus*) adrenal gland with emphasis on the medulla. Thirty-one dolphins of varying age and sex were used in this study. No statistical differences were found between the right and left gland mass, however, the left was typically greater. Mean mass for the right and left adrenal glands were 4.99 ± 0.513 and 5.36 ± 0.558 g, respectively. No statistical differences were found between average gland mass and sexual maturity or sex. The average cortex/medulla ratio was 1.22 ± 0.060 meaning approximately 48% is cortex, 41% is medulla, and 11% was categorized as other (i.e. blood vessels, connective tissue, etc.). The cortex contained pseudolobules and the typical zonation. A medullary band, consisting of highly basophilic staining cells was found at the periphery of the medulla. Projections of the medulla to the gland capsule were noted. Immunolabelling with polyclonal antibodies against the enzymes dopamine β hydroxylase and phenylethanolamine N-methyl transferase indicated that noradrenaline producing cells are found throughout the medulla including the medullary band while adrenaline producing cells are only found within the medullary band. Transmission electron microscopy confirmed the presence of two distinct cell populations within the medullary band and a single cell population throughout the medulla.

Introduction

Species specific structural and functional adaptations of the adrenal medulla have been described in a variety of different mammals, including the rat (Nemes, 1976), pig (Suzuki and Kachi, 1994), and various small mammals (Suzuki and Kachi, 1996). Interestingly, very little attention has focused on cetacean adrenal glands. Previous studies have been restricted to general descriptions of its gross anatomy and/or cortical histology (Jacobsen, 1941; Bourne, 1949; Harrison, 1969; Simpson and Gardner, 1972; Miyazawa and Usui, 1983; Zhongjie, 1988; Kirby, 1990; St Aubin, 2002). This lack of information on the cetacean adrenal gland is unfortunate given that it is known to play a major role in the cetacean alarm reaction (Geraci and St Aubin, 1979; Turnbull and Cowan, 1998; St Aubin and Dierauf, 2001; Cowan and Curry, 2002). This physiological response to stressful stimuli involves the massive release of endogenous catecholamines from the adrenal medulla and often leads to cell and tissue injury in other tissues such as cardiac and skeletal muscles (Cowan and Curry, 2002). This response is clinically important in that it often hampers the successful rehabilitation of stranded marine mammals (Turnbull and Cowan, 1998; St Aubin and Dierauf, 2001).

In an effort to provide a more thorough understanding of the structure and function of the cetacean adrenal gland, we examined the general morphology and the cellular and subcellular structures of the Atlantic bottlenose dolphin (*Tursiops truncatus*) adrenal gland. We placed particular emphasis on the poorly described adrenal medulla.

Materials and Methods

Collection area and animals used

The samples used in this study were collected by the Texas Marine Mammal Stranding Network, under the auspices of National Marine Fisheries Service, from stranded bottlenose dolphins (*T. truncatus*) in the north-western Gulf of Mexico. These animals either: (i) beach-stranded alive and died shortly afterwards, (ii) washed onto the beach dead, (iii) were accidentally net captured, or (iv) died or were killed during rehabilitative efforts. All animals in this study had no signs of chronic diseases. The collection area included the entire Texas Gulf Coast and part of Western Louisiana. Collection of specimens spanned more than 10 years, lasting from March 1992 to December 2002.

Thirty-one bottlenose dolphins were examined for this study, including twelve sexually immature males, five mature males, thirteen immature females, and one mature female. Sexual maturity was determined by evidence of spermatozoa production in males and ovarian follicular development in females. Age was determined by counting growth layer groups (GLGs) in the dentine of decalcified, sectioned, and stained teeth following established methods (Myrick et al., 1983; Hohn et al., 1989; and Turner, 1998). Age determination of animals in this study ranged from neonates to 19 years.

Sample collection

The paired adrenal glands were removed from each animal, weighed on a Sartorius electronic platform scale (model 4800P) to the nearest 0.01 gram, and an approximate 0.5 cm cross-section was removed from the middle of each gland. Adrenal gland sections were placed in 10% neutral buffered formalin and stored for several days at room temperature or until well fixed.

General tissue sectioning and staining

After fixation, the tissue samples were embedded in paraffin wax, sectioned at 5–7 μ, and stained with haematoxylin and eosin (H&E) or haematoxylin, phloxine, and saffron (HPS), following standard procedures. Up to three H&E or HPS histological slides were made for each gland (right and left) which were used for cross-sectional analysis of cortex and medulla ratios.

Immunohistochemical staining

Immunohistochemical (IHC) staining was performed on a subset of animals to provide an understanding of the different chromaffin cell populations (adrenaline and noradrenaline) and their relative location within the medulla. Commercially available polyclonal antibodies (Chemicon International, Inc., Temecula, CA, USA) were used to stain against dopamine β hydroxylase (DBH), the enzyme that converts dopamine to noradrenaline (NE), and phenylethanolamine N-methyl transferase (PNMT), the enzyme that converts noradrenaline to adrenaline (E), to identify the location and concentration of these cells. IHC staining was performed using the typical three-step-labelled avidin-biotin technique (Warnke and Levy, 1980). Both antibodies (DBH and PNMT) were diluted as indicated by the manufacture to 1/1000. Antigen retrieval methods described by Cowan and Gatalica (2002) and Kumar and Cowan (1994) were also used to unmake the effects of formalin fixation. This technique of antibody labelling and antigen retrieval has proven to be effective using various bottlenose dolphin tissues, including adrenal glands (Cowan and Gatalica, 2002; and Kumar and Cowan, 1994). These controls were run for each staining series and consisted of: omission of primary antibody, omission of secondary antibody, and omission of the streptavidin conjugate.

Morphometric measurements

Morphometric measurements were made using the point-counting technique to determine the cross-sectional ratio of the cortex and medulla (Weibel, 1963; Dunnill, 1968; Abdalla and Ali, 1989). A 25-point hexagonal lattice reticle was used for this analysis as described by Weibel (1963) and Hennig (1959). Cross-sectional measurements were made on each histological slide, with up to three slides per adrenal gland (right and left), and the average of those measurements were used for quantitative analysis. All sections were examined using a Nikon Eclipse E400 compound microscope (Nikon Corporation, Tokyo, Japan) with a Q-Imaging Micropublisher digital camera and Meta Vue (version 6.0r5) Digital Imaging Software.

Transmission electron microscopy

Samples for transmission electron microscopy (TEM) analysis were collected along with the other histological samples taken at the time of necropsy. TEM samples were preserved in cold 2% glutaraldehyde and processed following standard EM protocols. Briefly, after fixation, tissues were rinsed in 0.1 M cacodylate buffer at pH 7.4, postfixed for 1 h in 1% osmium tetroxide, rinsed in distilled water, stained with 2% uranyl acetate, dehydrated through a graded series of alcohols, and embedded in epon. Sections were then cut on a Leica Ultratome and imaged using a Phillips 201 transmission electron microscope (FEI Company, Eindhoven, The Netherlands). All TEM samples and images were processed by the University of Texas Medical Branch, Galveston, TX, USA.

Statistical analysis

Total body length (cm) was used for nearly all statistical analyses in this study instead of body mass. However, total body mass (kg) was used to compare adrenal mass to body mass ratios. Emaciation is a common problem with stranded cetaceans as many have been sick or have had debilitating injuries for an extended period of time. Cowan et al. (unpublished data) and Cowan (1966) indicate that total body mass were not considered reliable as an accurate indicator of an animal's age and thus did not permit for comparisons across age or maturity groups.

Nonparametric one-sample Kolmogorov–Smirnov tests were run before any analyses to check for normality (i.e. normally distributed data). Paired *t*-tests were used to test for differences between gland mass and cortex to medulla ratios of the left and right adrenal glands. ANCOVA tests were used to compare average adrenal gland mass, and cortex and medulla ratio, between sexes and sexual maturity. Total body length (cm) was used as a covariate to correct for the effects of age. ANOVA, with Bonferroni *post hoc* test, was performed to test differences between cortex and medulla ratios within a single gland. Data is presented as mean $\pm$ 1 SE. All statistical analyses were performed using SPSS for Windows, version 11.5.0, and significance was assumed at $P < 0.05$.

Results

The location of these paired glands being found several centimetres cranial to the kidneys and along the dorsal surface of the abdominal cavity has been previously described (Rommel and Lowenstine, 2001; Reynolds *et al.,* 2002), so we will not elaborate on this. Being retroperitoneal, adrenal glands of bottlenose dolphins can often be mistaken for lymph nodes. Adrenal glands of *T. truncatus* are roughly triangular in cross-section with an outer cortex and inner medulla, following the typical mammalian configuration (Fig. 1).

Morphology

Gland mass

All data sets and measurements were checked for normality and all were found to be normally distributed (i.e. all *P*-values were >0.05). Adrenal gland mass for the right gland ($n = 28$) ranged from 1.44 to 12.20 g with the mean mass being 4.99 $\pm$ 0.513 g and for the left gland ($n = 31$) ranged from 1.56 to 15.50 g with a mean of 5.36 $\pm$ 0.558 g. There was no significant difference in mass of the right and left adrenal glands: however, the left gland was typically larger (paired *t*-test: $t_{27} = -1.772$, $P = 0.088$; Fig. 2). As there were no mass differences found between the left and right adrenal glands, both masses were averaged together for the remaining analyses (average gland mass = AGM).

As organs/glands continue to grow throughout life but growth slows after the onset of sexual maturity (Cowan et al., unpublished data; Cowan, 1966), total body length (cm) was

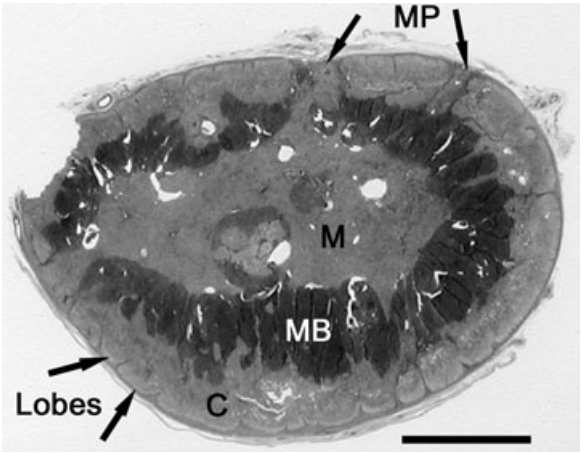

Fig. 1. Cross-section of an adrenal gland. Lobes, connective tissue bands invaginating deep into the cortex giving the appearance of lobulation; C, cortex; M, medulla; MP, medullary protrusions; MB, medullary band; Stain = HPS, Scale bar = 0.5 cm.

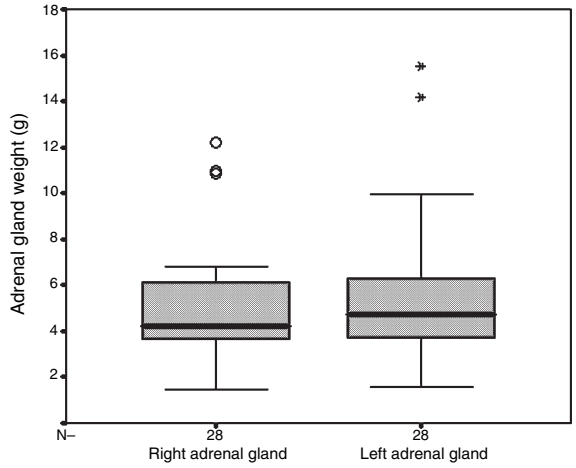

Fig. 2. Box-plot illustrating the gland mass (g) of the right and left adrenal glands from bottlenose dolphins (*Tursiops truncatus*). Dark line represents mean gland mass and bars indicate standard error. No statistical differences were found between the right or left adrenal glands.

used as a covariate to correct for age related effects. No significant differences were found when comparing AGM to sexual maturity and sex (ANCOVA, $F_{1,31} = 0.627$, $P = 0.436$, and $F_{1,31} = 0.941$, $P = 0.341$, respectively; Fig. 3). However, smaller sexually immature animals ($n = 25$) had a mean AGM of 4.33 ± 0.32 g, whereas, larger sexually mature animals ($n = 6$) had a mean AGM of 8.85 ± 1.63 g. A summary of adrenal gland masses are listed in Table 1.

Adrenal gland mass (g) to total body mass (kg) ratios were calculated for males, females, immature, and mature animals (Table 1). Male ratios ranged from 0.08 to 0.21 g/kg, while females ranged from 0.05 to 0.19 g/kg. No statistical differences were found when comparing adrenal mass to body mass ratios against sex or sexual maturity (ANCOVA, $F_{1,24} = 0.106$, $P = 0.748$, and $F_{1,24} = 1.558$, $P = 0.226$, respectively). Overall, average adrenal gland mass to body mass ratios for both sexes were 0.13 ± 0.008 g/kg.

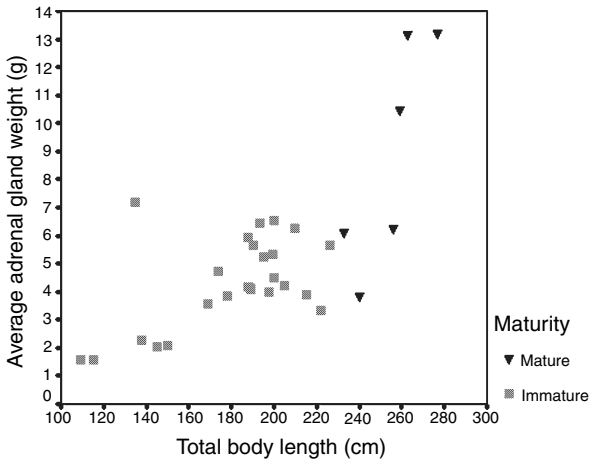

Fig. 3. Scatterplot of bottlenose dolphin (*Tursiops truncatus*) average adrenal gland mass (g), compared with total body length (cm) and sexual maturity.

Table 1. Average adrenal gland mass, adrenal gland mass to body mass ratios, and average cross-sectional ratios for males, females, immature and mature animals

Sex	Sexual maturity	Number of animals	Average adrenal mass (g)	Adrenal mass to body mass ratio (g/kg)	Average cross-sectional ratio
Males	Immature	12	4.55 ± 1.55	0.13	1.23 ± 0.26
	Mature	5	9.40 ± 4.19	0.10	1.62 ± 0.47
Females	Immature	13	4.13 ± 1.68	0.14	1.72 ± 1.12
	Mature	1	6.10 ± 0.00	0.08	1.14 ± 0.00

No statistical differences were found between any category.

Cortex to medulla ratios

When comparing cortex to medulla cross-sectional ratios (cortex/medulla = CM) using the point-counting technique, no statistical differences were found between the 14 pairs of adrenal glands (paired *t*-test: $t_{13} = -0.172$, $P = 0.866$). The left gland had an average CM ratio of 1.61 ± 0.219 (range = 0.71–4.42) and the right gland had an average CM ratio of 1.49 ± 0.141 (range = 0.74–2.61). One whole gland was sectioned approximately every 0.5 cm ($n = 13$ sections) to check for CM ratios differences along the entire length of the gland. Once the first two anterior and the last two posterior sections of the gland were removed, the middle sections (nos 3–10) had no significant differences in CM ratio (ANOVA, $F_{8,17} = 1.967$, $P = 0.167$). As there were no statistical differences between right and left CM ratio or throughout the central portion of the gland, the right and left ratios were averaged together for the remaining analyses (average cortex/medulla = ACM). The overall ACM ratio was 1.54 ± 0.171, indicating that the cortex comprised approximately 48% and the medulla comprised approximately 41% of the overall cross-sectional ratio. The remaining 11% were categorized as 'other', indicating blood vessels and connective tissue found within the adrenal gland as well as the gland capsule itself. Using length as a covariate, no significant differences were found between ACM ratio and sexual maturity or sex (ANCOVA, $F_{1,24} = 0.014$, $P = 0.906$, and $F_{1,24} = 0.008$, $P = 0.930$, respectively). A summary of the cortex to medulla ratios are

listed in Table 1 for males, females, immature, and mature animals.

Histology, general staining, H&E and HPS

The microscopic anatomy of the bottlenose dolphin adrenal gland contained an outer capsule, consisting of collagen fibres, surrounding the surface of the gland. Several areas of this capsule penetrate deep into the cortex giving a pseudolobulated appearance (Figs 1 and 4). The cortex contained the typical zonation of mammalian adrenal glands with the zona glomerulosa being the outermost zone followed by the zona fasciculata and then the zona reticularis (Fig. 5). The zona glomerulosa contained primarily cuboidal cells with some exhibiting more columnar. The inner part of the zona glomerulosa consisted of cords of cells with blood sinusoids separating them. The zona fasciculata contained mainly columnar cells running in cords and appeared to exhibit a greater abundance of blood sinusoids than the zona glomerulosa. Cells of the zona fasciculata appeared to gradually merge into the zona reticularis making it difficult to differentiate the zonal change. A further increase in the number of blood sinusoids was noted in the zona reticularis, making it the most vascularized of the three zones. Cells of this innermost layer were mainly cuboidal. The junction of the cortex and medulla was easily differentiated by a band of highly vascularized connective tissue.

The glandular cells of the medulla were primarily cuboidal and often appeared in a pinwheel or circular fashion surrounded by connective tissue with a central vein. These cells of the central medulla contained a translucent cytoplasm with a darker staining nucleus. In contrast, those cells of the outer medulla stained intensely with haematoxylin. These darker staining cells at the periphery of the medulla were of the same shape (cuboidal) as those of the central medulla but contained a more basophilic staining cytoplasm. This darker staining peripheral band of cells, referred to as the medullary band, varied in thickness and accentuated the cortical medullary junction (Figs 1 and 6). This

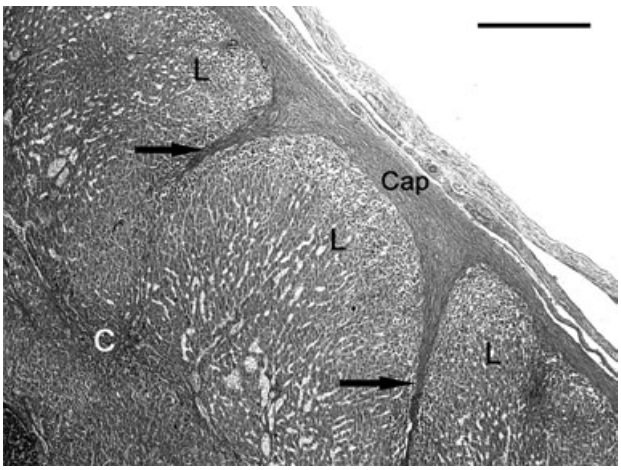

Fig. 4. Adrenal gland cortex showing pseudolobulation. Pseudolobules are separated by connective tissue bands (arrows) that invaginate deep into the cortex. C, cortex; L, lobes; Cap, gland capsule; Stain = HPS, Scale bar = 500 μm.

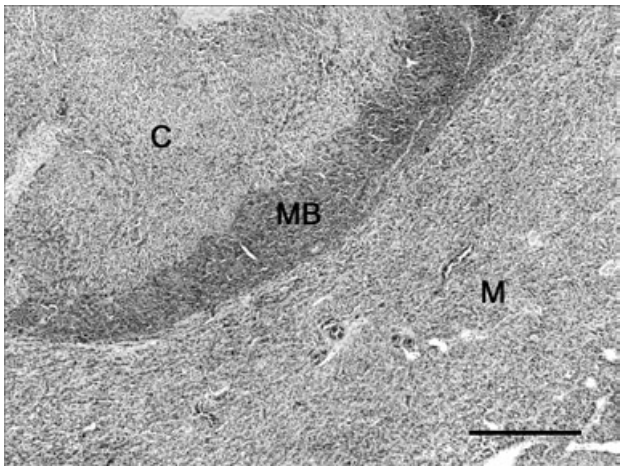

Fig. 6. Zonation/banding of the adrenal medulla. Note the distinct band of intense staining in the periphery of the medulla, known as the medullary band (MB). C, cortex; M, medulla; Stain = H&E, Scale bar = 500 μm.

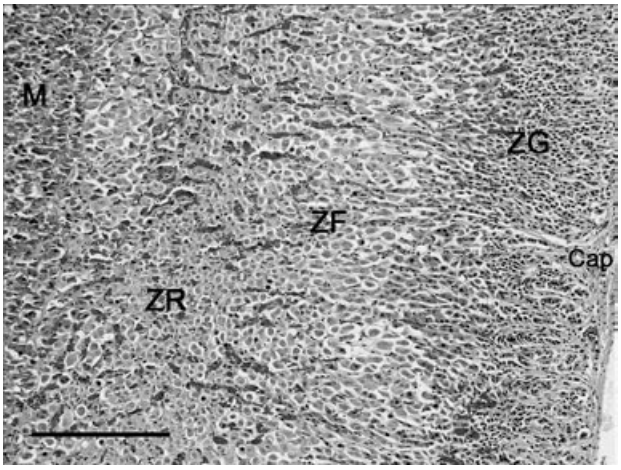

Fig. 5. Adrenal gland cortex with the three cortical zones (ZG, zona glomerulosa; ZF, zona fasciculata; and ZR, zona reticularis). M, medulla; Cap, gland capsule; Stain = H&E, Scale bar = 500 μm.

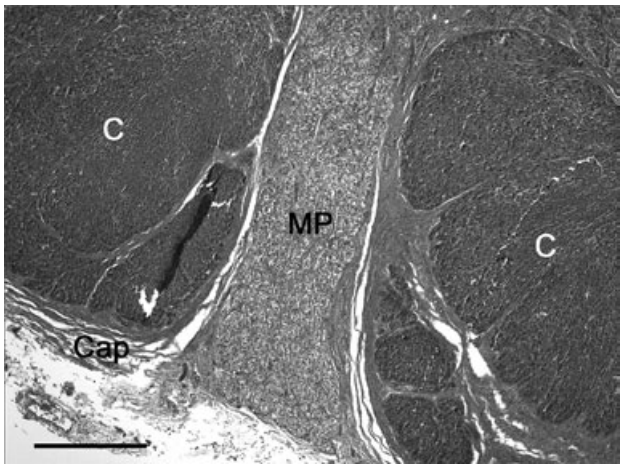

Fig. 7. Image of a medullary protrusion through the cortex to the gland capsule. C, cortex; MP, medullary protrusion; Cap, gland capsule; Stain = HPS, Scale bar = 500 μm.

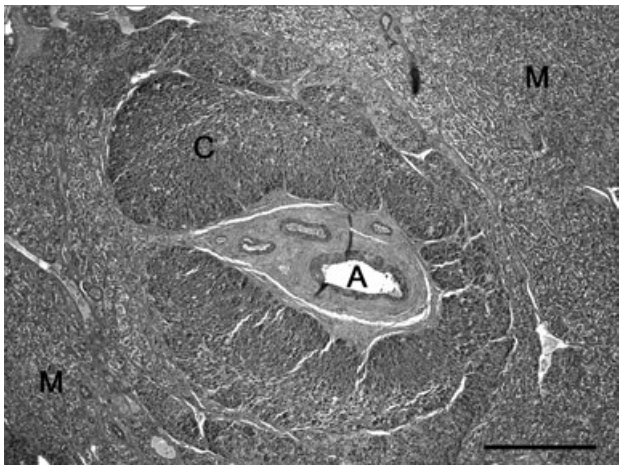

Fig. 8. Artery extending from cortex through the medulla with all three cortical zones surrounding the artery itself. C, cortex; M, medulla; A, artery; Stain = HPS, Scale bar = 500 μm.

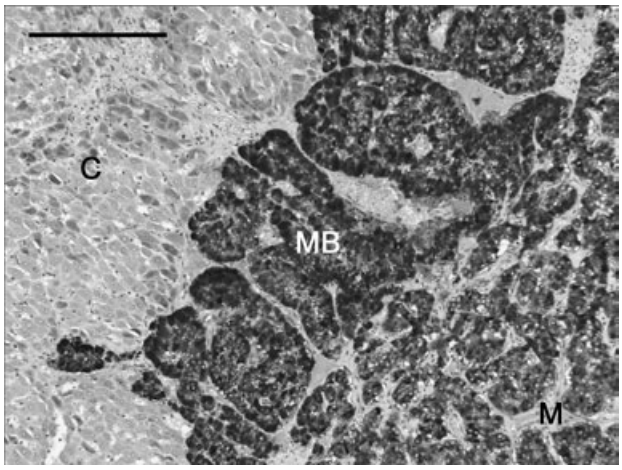

Fig. 9. Positive staining for the enzyme dopamine β hydroxylase in the adrenal medulla, indicating the location of noradrenaline producing cells. Note the noradrenaline cells are distributed throughout the medulla (M) including the medullary band (MB). C, cortex; Scale bar = 250 μm.

banding of the medulla was similar in appearance to the zonation of the cortex. Venules and arterioles were commonly found throughout the central medullary region but not within the medullary band or the cortex.

The corticomedullary junction is usually thought to be uniform, however, in the majority of animals examined, one to three projections of the medulla extended through the cortex to reach the capsule (Figs 1 and 7). Also, on occasion, an artery (or several arteries) appeared to extend through the cortex and into the medulla. A layer of cortex consisting of all three cortical zones appeared to encompass the arteries into the medulla (Fig. 8).

Immunohistochemistry

Immunohistochemical staining of bottlenose dolphin adrenal glands for DBH (dopamine β hydroxylase), the enzyme that converts dopamine to noradrenaline (NE), and PNMT (phenylethanolamine N-methyl transferase), the enzyme that converts NE to adrenaline (E) were completed on 15 animals (males $n = 6$, females $n = 9$), following methods by Kumar and Cowan (1994) and Cowan and Gatalica (2002). Positive staining for the enzyme DBH was detected throughout the entire medulla (Fig. 9), indicating a widespread distribution of NE producing cells. In contrast, positive staining for the enzyme PNMT was only detected in the medullary band, indicating a more limited distribution of E producing cells in the adrenal medulla (Fig. 10). In areas of medullary protrusion through the cortex, only DBH and not PNMT staining was detected (Figs 11 and 12 respectively). This latter observation suggests a widespread distribution of NE producing cells throughout the medulla and a restriction of E producing cells to the medullary band. All control slides for DBH and PNMT staining were negative (data not shown).

Transmission electron microscopy

Within the central medulla, a single population of glandular cells was observed (Fig. 13). Cells of this population were polyhedral, possessed roughly circular nuclei, and were filled with membrane bound granules. The granules contained a flocculant material of variable electron density. Many granules

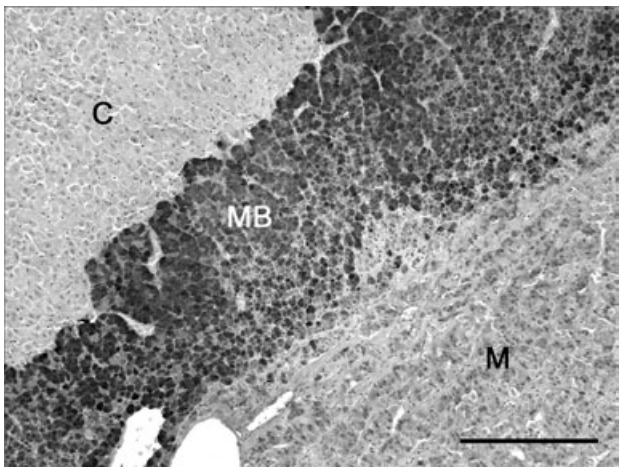

Fig. 10. Positive staining for the enzyme phenylethanolamine N-methyl transferase indicating adrenaline producing cells. Adrenaline cells are only located at the periphery of the medulla. C, cortex; MB, medullary band; M, medulla; Scale bar = 500 μm.

exhibited eccentrically located, electron dense cores. Cytoplasm intervening between the granules was moderately electron dense (Fig. 14). Based on our IHC data described above, we interpreted this glandular cell population to be NE producing cells. Other cell types identified in the central medulla included support cells and Schwann cells associated with nerve axons (data not shown).

Within the medullary band, two populations of glandular cells were noted (Fig. 15). One group appeared virtually identical to the glandular cells of the central medulla (described above) and we interpreted these cells to be NE producing cells. Cells of the second group shared characteristics with the NE producing cells, including being polyhedral, spherical nuclei and the presence of numerous membrane bound granules, but they also displayed some differences. The cytoplasm of these cells was less electron dense than that of the NE producing cells. Moreover, the membrane bound granules of these cells, while very prominent, were less tightly packed

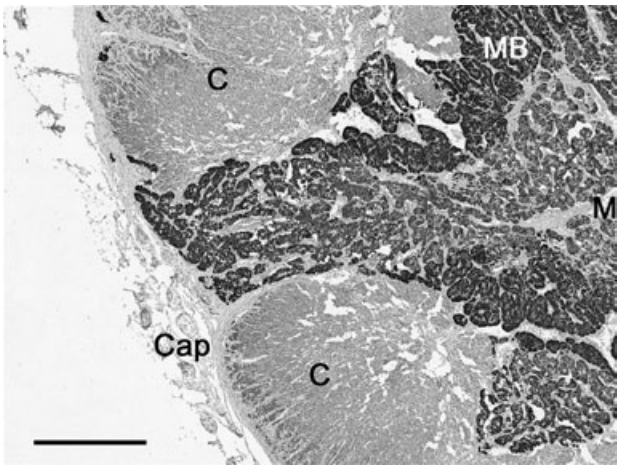

Fig. 11. Positive staining of the medullary protrusion for the enzyme dopamine β hydroxylase indicating noradrenaline producing cells are found within the medullary protrusion as well as throughout the medulla. C, cortex; M, medulla; MB, medullary band; Cap, gland capsule; Scale bar = 500 μm.

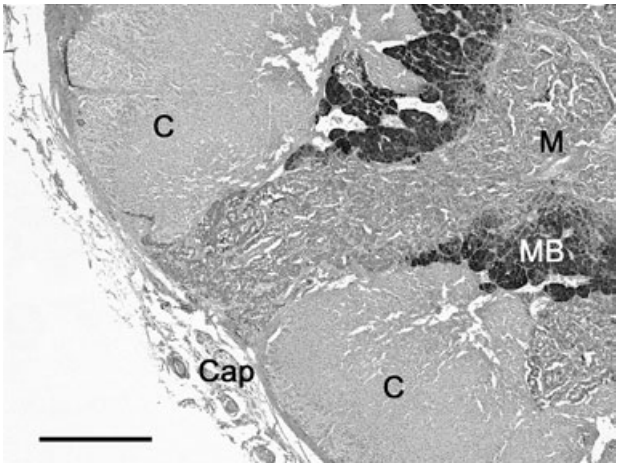

Fig. 12. Positive staining for the enzyme phenylethanolamine N-methyl transferase indicates the medullary protrusion does not consist of adrenaline producing cells. C, cortex; M, medulla; MB, medullary band; Cap, gland capsule; Scale bar = 500 μm.

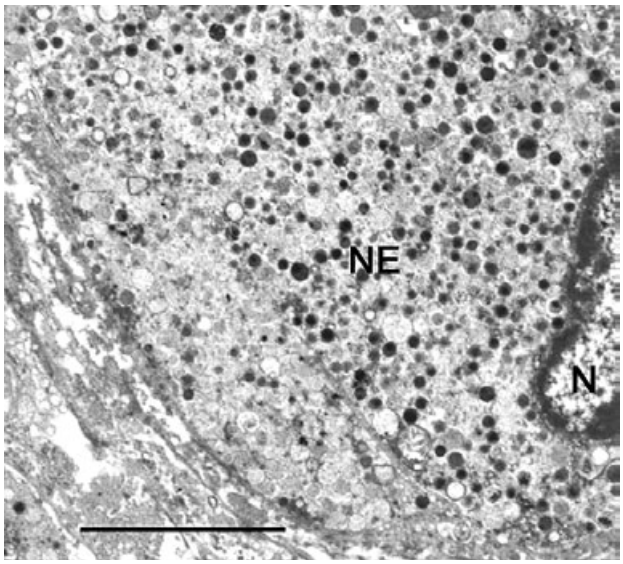

Fig. 13. Transmission electron micrograph of a noradrenaline (NE) producing cell located within the central medulla. N, nucleus. Scale bar = 5 μm.

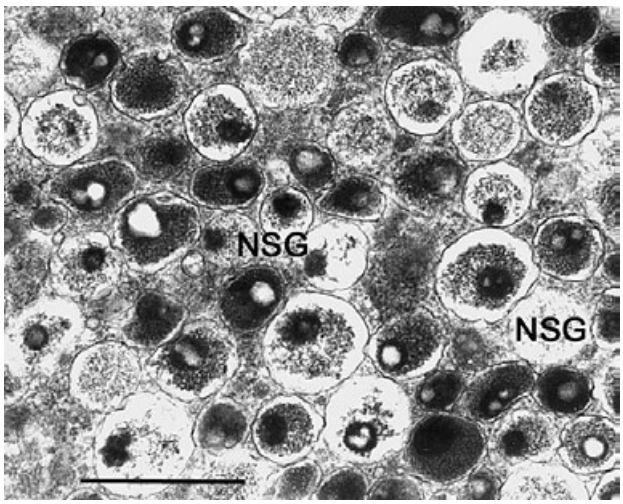

Fig. 14. Higher magnification of noradrenaline producing cell with electron dense cytoplasm and an abundant amount of electron dense granular material. The granular material of noradrenaline cells is typically eccentrically located. NSG, noradrenaline storing granule. Scale bar = 0.1 μm.

than in the NE producing cells. Individual granules displayed electron dense cores, but these were not observed as frequently as in the NE producing cells (Fig. 16). We interpreted this second cell population to be the E producing cells of the medullary band, demonstrated to be present in this region by immunohistochemistry (see above).

Discussion

In this study, we examined the general morphology, histology, and subcellular structures of the Atlantic bottlenose dolphin (*T. truncatus*) adrenal gland. We found no statistical difference in AGM between sexes. *T. truncatus* from the Gulf of Mexico are not considered to be sexually dimorphic so this finding was expected. Adrenal gland mass and gland mass to body mass ratios of bottlenose dolphins in this study were similar to that of other delphinids described by Harrison (1969). However, Zhongjie (1988) reported gland mass to body mass ratios much higher (0.10 – 0.48 g/kg) for the Chinese river dolphin (*Lipotes vexillifer*) than those reported here for *T. truncatus*. This higher ratio in the Chinese river dolphin might be explained by having a much greater cortex to medulla ratio as discussed below.

As the cortex to medulla ratios of *T. truncatus* did not change with age, sex, or sexual maturity status, this suggests that both the adrenal cortex and medulla develop at the same rate. Also, as the cortex to medulla ratio did not significantly change along the center half of the gland, it is safe to assume that sampling (i.e. cross-section) anywhere within the central portion of the adrenal gland will yield consistent ratios/information. For animals in this study, the adrenal glands had an approximate 1:1 ratio of cortex to medulla. Similarly, in Sei

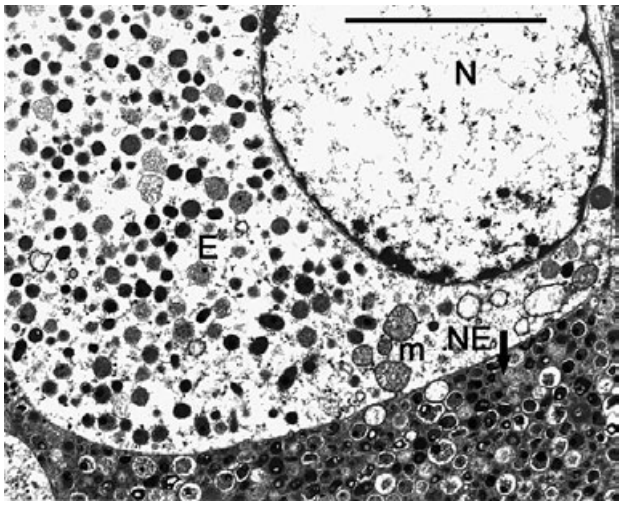

Fig. 15. Transmission electron micrograph of adrenaline (E) and noradrenaline (NE) producing cells located within the medullary band. N, nucleus; m, mitochondria. Scale bar = 0.5 μm.

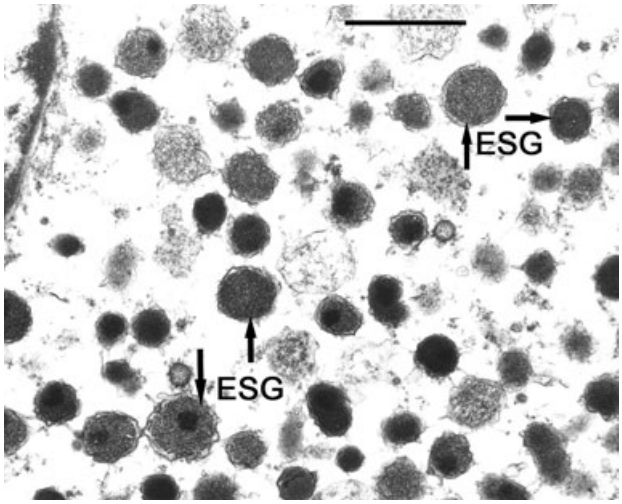

Fig. 16. Transmission electron micrograph of an adrenaline producing cell with finely granular and moderately electron dense granules. These granules typically have a thin symmetrical halo surrounding the granular material. ESG, adrenaline storing granules. Scale bar = 0.1 μm.

whales (*Balaenoptera borealis*), the cortex to medulla ratios were much closer to that of *T. truncatus* with a range of 1.09–10.1 with an approximate average of 2.65 (Miyazawa and Usui, 1983). These ratios are extremely different compared with those of other mammals. In humans, for example, the cortex comprises approximately 90% of the entire gland (Kirby, 1990). Similarly, Abdalla and Ali (1989) reported high cortex to medulla ratios in the camel (*Camelus dromedarius*) with an average ratio of 4:1, Zhongjie (1988) indicated ratios of 6.59 in the Chinese river dolphin, and Carballeira et al. (1987) reported that the cortex comprised more than 80% of the adrenal gland in two species of cetaceans (*Kogia breviceps* and *Mesoplodon europaeus*). The functional significance of these higher ratios is not completely understood, but it could be explained by the increased need to regulate water and electrolyte balance. As it is known that the adrenal cortex produces hormones (glucocorticoids and mineralocorticoids) that aid in water and electrolyte balance, these higher ratios (i.e. larger cortex) may be needed for increased production of these hormones in mammals that live in areas where water conservation is extremely important (i.e. dry/aired environments or freshwater rivers and lakes).

Structurally, bottlenose dolphin adrenal glands appeared similar to other mammalian adrenal glands (Bourne, 1949; Abdalla and Ali, 1989; Suzuki and Kachi, 1996), including those of other cetaceans (Jacobsen, 1941; Bourne, 1949; Harrison, 1969; Simpson and Gardner, 1972; Britt and Howard, 1983; Zhongjie, 1988; Kirby, 1990; St Aubin and Dierauf, 2001; St Aubin, 2002). These glands had the typical outer cortex, with three zones (zona glomerulosa, zona fasciculata, and zona reticularis), and an inner/central medulla. In *T. truncatus,* the capsule penetrates into the cortex at multiple locations giving the appearance of pseudolobulation. This pseudolobulation of the cortex has also been identified in other cetaceans (Blue and Fin whales, Burn et al., 1951; porpoise, Britt and Howard, 1983; pygmy sperm whale and Gervais' beaked whale, Carballeira et al., 1987; Chinese river dolphin, Zhongjie, 1988).

We noted numerous projections of the medulla through the cortex. These medullary protrusions extended completely through the cortex to the gland capsule. In the majority of animals we examined, at least one to three protrusions were identified. Similar protrusions have also been identified in the camel (Abdalla and Ali, 1989) and opossum (Carmichael et al., 1987) as well as in a few other cetaceans (Carballeira et al., 1987; Kirby, 1990). Carmichael et al. (1987) indicated that in the opossum, these medullary protrusions were located at the hilus of the adrenal gland. Unfortunately, because of sampling and tissue processing techniques of this study, we were unable to determine the precise location of these protrusions. The functional significance of these medullary protrusions in cetacean adrenal glands is currently unknown.

In several specimens examined, it appeared that arteries extended through the cortex and into the medulla and that these arteries were completely ensheathed by all three cortical layers. In histological sections, this gave the appearance of random units of cortex occurring within the medulla. This invagination of the cortex around an artery was also noted in the camel (Abdalla and Ali, 1989). Interestingly, we identified this cortical layering only around medullary arteries and not around medullary veins. Abdalla and Ali (1989) noted a similar arrangement in the camel and suggested that it may be because of the embryological development of these structures.

Functionally, the adrenal medulla is known to play an important role in the production and release of the catecholamines noradrenaline and adrenaline. Previous studies have indicated that the pattern of noradrenaline and adrenaline producing cells (i.e. chromaffin cell populations) may be species specific (Suzuki and Kachi, 1996). For example, Al-Lami (1970) reported that in the hamster NE producing cells are localized to the periphery of the medulla while E producing cells are found centrally. In contrast, hoofed mammals such as the pig (Suzuki and Kachi, 1996) and the sheep, horse and cow (Thompson et al., 1981) are reported to have a chromaffin cell arrangement opposite to that of the hamster (i.e. E producing cells peripherally and NE producing cells centrally). Our data indicate that the chromaffin cell

distribution in *T. truncatus* is closest to that found in hoofed mammals, an observation that could be explained by the evolutionary link between cetaceans and their ungulate ancestors. We did note some subtle but important differences between chromaffin cell distributions in *T. truncatus* and hoofed mammals; namely, E and NE producing cells both appear to be present in the periphery of the adrenal medulla (i.e. the medullary band).

Our general histological staining data further support the conclusion that E producing cells are concentrated in the medullary band of *T. truncatus*. Coupland (1965) and Kobayashi and Coupland (1993) both reported that adrenaline producing cells contain large amounts of basophilic substances and thus stain intensely with basic dies such as haematoxylin. On all H&E and HPS slides examined in the study, the medullary band appeared as a zone of strong basophilic staining as would be predicted if E producing cells are present. The functional significance of E producing cells being localized to the medullary band could be related to the blood flow within the medulla. The adrenal medulla receives a dual blood supply, one supply from the medullary artery and another from the sinusoidal network of the cortex which empties into the medulla (Long, 1977). As E producing cells are located around the periphery of the medulla and receive blood from the cortex, it is believed that the conversion of noradrenaline to adrenaline by the enzyme PNMT is induced by glucocorticoid hormones (Long, 1977).

In general, the ultrastructural features of the cells and organelles within the medulla of *T. truncatus* are similar to that described in other species (Kobayashi and Coupland, 1993). Based on ultrastructural features, we were able to identify two different populations of glandular cells which we interpreted to be NE and E cells. These cells differed from one another mainly in the density of their cytoplasm and the appearance and abundance of their cytoplasmic granules. According to previous reports (Coupland and Hopwood, 1966; Honore, 1971), the granules found within E and NE producing cells are most likely adrenaline and noradrenaline respectively, or their immediate precursors. An unusual intermixing of both cell populations was observed in the medullary band. A single cell population of glandular cells, interpreted as NE producing cells, was detected in the central medulla. The distinguishing cellular feature in the medulla of *T. truncatus* appears to be the overlapping of NE and E producing cells in the medullary band rather than in the ultrastructural features of the individual cell types.

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