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Light and electron microscopic observations on the structure of the porcupine (*Hystrix cristata*) adrenal gland

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ABSTRACT

The aim of this study was to investigate the structure of the porcupine adrenal gland by light and electron microscopic observations. For this purpose, six adrenals were collected from three adult porcupines (two male and one female). The tissues were processed for both light and electron microscopy. The adrenal gland consisted of capsula (1.2%), zona glomerulosa (6.6%), zona fasciculata (48.5%), zona reticularis (17.9%) and medulla (25.7%). At the cortico-medullar boundary cortical cells were observed, together with chromaffin cells. The epinephrine cells were smaller and more numerous than the norepinephrine cells. Also, the epinephrine granules were moderately electron-dense, while norepinephrine granules were highly electron-dense. Both epinephrine and norepinephrine granules were similar in size and generally formed little rods. The results of the present study are the first report on the structure of the adrenal gland of porcupine.

Key words: *Hystrix cristata*, rodentia, porcupine, adrenal gland, ultrastructure

Introduction

Rodents (Rodentia), the largest order of placental mammals, comprise more than half of the mammals known at present. The porcupine that is the subject of this study is from the Hystricidae family, which constitutes a small group of the order Rodentia (KAROL, 1963; KURU, 1987; WEICHERT, 1970). The adrenal gland is composed of two distinct portions: an outer cortex (mesodermal origin) and an inner medulla (neuroectodermal origin) (BANKS, 1993; DELLMAN, 1993; GUDE et al., 1982; JUNQUEIRA et al., 1995). The adrenal cortex is subdivided into three (DELLMAN, 1993; GUDE et al., 1982; JUNQUEIRA et al., 1995) or four

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(BACHA and WOOD, 1990) distinct zones of epithelial cells. The outermost zone is called zona glomerulosa in ruminants, in human (DELLMAN, 1993; JAMDAR and EMA, 1982) and in a few rodents (GUDE et al., 1982; SMITH and CALHOUN, 1968; COLBY et al., 1992) and is formed of irregular clusters and cords of cells in the horse, donkey, pig and carnivores. This zone is called zona arcuata because the cells are arranged into arcs (BANKS, 1993; DELLMAN, 1993; PRASAD and SINHA, 1981; TANYOLAC, 1993). The zona fasciculata, the widest zone of the adrenal cortex arc consists of radially arranged cords of cuboidal or columnar cells (BANKS, 1993; DELLMAN, 1993; JUNQUEIRA et al., 1995; KARADAG et al., 1995; PRASAD and YADAVA, 1975; TANYOLAC, 1993). The foamy appearance of the cells is caused by the presence of numerous vacuoles, when lipid is removed in processing (BACHA and WOOD, 1990; DELLMAN, 1993; GUDE et al., 1982; TANYOLAC, 1993). The zona reticularis consist of cells disposed as freely anastomosing cords. The cells are roughly the same in morphological features as the cells of the zona fasciculata, but their nuclei and cytoplasm have darker staining (BACHA and WOOD, 1990; BANKS, 1993; JUNQUEIRA et al., 1995; TANYOLAC, 1993). There are two types of chromaffin cells in the adrenal medulla (COUPLAND, 1965; COUPLAND and WEAKLEY, 1968, 1970; ELFVIN, 1967; YATES, 1964) and the granules of epinephrine cells are smaller and less electron-dense compared to norepinephrine cells (COUPLAND, 1965; COUPLAND and WEAKLEY 1968, 1970; DELLMAN, 1993; STEVENS and LOWE, 1991). The norepinephrine cells contain a large spherical nucleus (DELLMAN, 1993) while chromaffin cells contain a full complement of cytoplasmic organelles (COUPLAND, 1965). The entire gland is drained by a large central venule (DELLMAN, 1993; TANYOLAC, 1993). This study focused on the investigations of the light and electron microscopic structure in porcupines in order to make a contribution to this seldom investigated field.

Materials and methods

In this study, six adrenal glands were collected from three adult porcupines (two males and one female). The porcupines were trapped in Eastern Anatolia. Animals were anaesthetized with Pentothal (6 mg/kg) and adrenal specimens were taken. For light microscopy, 10% formaldehyde solutions were used to fix the tissue. After fixation, the specimens were rinsed with buffer; treated with ethyl alcohol and xylol and embedded in paraffin blocks. Sections (5-7 μm thick) were made by microtome (Leitz) and then stained with hematoxylin-eosin. Photomicrographs were taken with a camera (Nikon) attached to a microscope. Micrometric measurements and volume were carried out with ocular micrometer.

The specimens for the electron microscopy were fixed in 2.5% glutaraldehyde, and paraformaldehyde prepared in cacodylate buffer (0.1 M, pH 7.4) and adjusted to pH 7.2 for 24 hours at +4 ^{0}C. They were then rinsed with cacodylate buffer for 3 hours. For post-

fixation, sections were kept in osmium tetroxide fixative (2%) prepared in cacodylate buffer. Rinsed in cacodylate buffer, sections were treated with a series of ethyl alcohols. Then, the sections were blocked in embedding moulds with araldite CY 212, dodecenyl succinic andyrine (DDSA) and benzyldimethylamine (BDMA). Semi-thin sections were visualized under microscope after staining with toludine blue. The sections (300-700 A^{o} thick) were stained with lead citrate and uranyl acetate. Electronmicrographes were taken by electron microscope (EM 9 Carl Zeiss).

Results

The paired adrenal glands were located to the cranio-medial of the kidneys and both right and left adrenals were flat. On average, they were 1.55 cm (right) to 1.70 cm (left) long, 0.70 to 0.70 cm thick and, 0.85 to 0.90 cm wide and weighing 0.64 g to 0.78 g.

Table 1. Average micrometric measurements in µm of the adrenal components and their ratio

Thickness of Capsula µm	Thickness of cortex µm	Thickness of medulla µm	Ratio, capsula to adrenal gland %	Ratio, cortex to adrenal gland %	Ratio, medulla to adrenal gland %
25	1491	525	1.22	73	25.7

Micrometric measurements of some structural components of the gland are shown in Table 1.

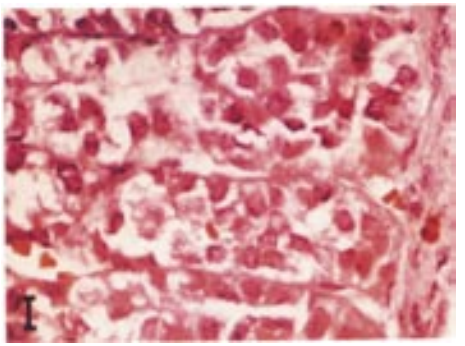

Fig.1. Light micrographs of zona glomerulosa of porcupine (*Hystrix cristata*) adrenal gland. H.E. ×200, scale bar = 25 µm

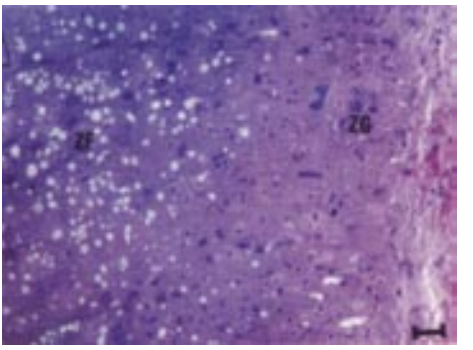

Fig. 2. Light micrograph of zona glomerulosa (ZG) and zona fasciculata (ZF) of porcupine (*Hystrix cristata*) adrenal gland. Toluidine blue ×100, scale bar = 50 µm.

The adrenal cortex is subdivided into three zones. The outermost zone was formed as zona glomerulosa. It contained prismatic cells that formed irregular clusters or cords and contained lipid vacuoles (Figs 1 and 2). The thickness of the zona glomerulosa was 135 μm and contained 6.6% of the adrenal gland. The zona fasciculata was 990 μm in thick and occupied about half (48.5%) of the adrenal gland. The zona fasciculata appeared foamy, because of the presence of numerous lipid vacuoles, especially in semi-thin sections stained with toluidine blue (Fig. 2). The cells of this zone contained irregularly shaped nuclei, endoplasmic reticulum, numerous mitochondria and lipid vacuoles. The zona reticularis appeared as irregular cords and smaller than other two zones of cortex. The zona reticularis was 366 μm in thickness and occupied 17.9% of the adrenal gland. Occasionally, collagen fibres appeared in intercellular spaces (Fig. 3).

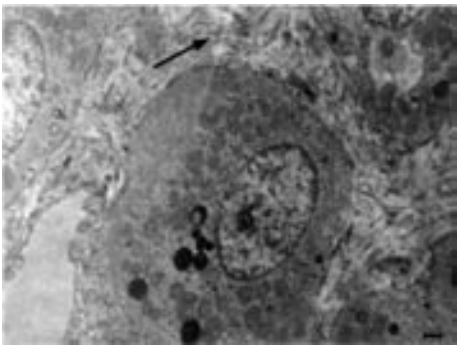

Fig. 3. Electron micrograph of cortical cells of porcupine (*Hystrix cristata*) adrenal gland. Arrow, collagen fibers. ×3000, scale bar = 2.5 μm

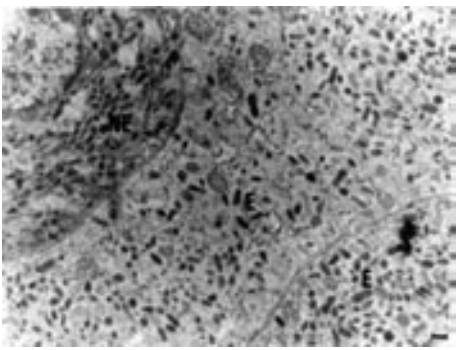

Fig. 4. Electron micrograph of chromaffin cells of porcupine (*Hystrix cristata*) adrenal gland. NE, norepinephrine-storing cell; E, epinephrine-storing cell. ×7000. scale bar = 1.1 μm.

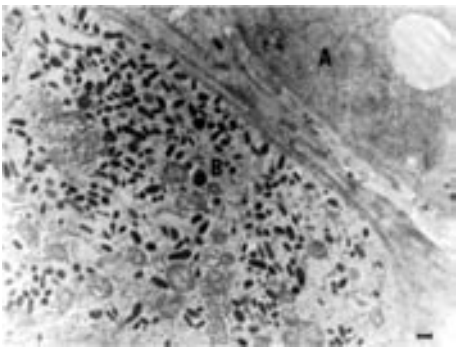

Fig. 5. Electron micrograph of cortico-medullary junction of porcupine (*Hystrix cristata*) adrenal gland. A, cortical cell; B, chromaffin cell. ×7000, scale bar = 1.1 μm.

Medulla. The adrenal medulla was about one quarter (25.7%) of the area of the gland. The chromaffin cells in irregular clusters, and at the cortico-medullary boundary cortical cells were observed. Epinephrine-storing cells (E-cells) were more numerous and smaller than norepinephrine-storing cells (NE-cells). The majority were moderately electron-dense E granules, but some were highly electron-dense NE granules. Ganglion cells were rarely observed. Numerous central sinusoidal vessels were noticed in place of a large central venule.

Both chromaffin cells contained a single large spherical nucleus. Nucleoli were well defined, and on occasions up to three nucleoli per cell were observed. E-cells were similar to the NE-cells in terms of size of granules and shape of nucleus (Fig. 4). The cytoplasm of chromaffin cells contained numerous mitochondria, lysosomes, endoplasmic reticulum associated with ribosomes. Cortical cells were observed together with chromaffin cells at the cortico-medullary boundary and their cytoplasma was mainly occupied by large rounded or ovoid mitochondria (Fig. 5).

Discussion

The cortex is divided into three zones in the guinea pig (COLBY et al., 1992; YUAN et al., 1997; EACHO and COLBY, 1983) and rat (SMITH and CALHOUN, 1968), two zones in the mouse (GUDE et al., 1982) in rodents, and te outermost zone forms glomerulosa. In the rabbit, as a near species, it is in the form of zona arcuata (KARADAG et al., 1995). Our results show that the cortex in porcupines comprises three zones, and the outermost zone is zona glomerulosa, as seen in other rodents.

Some investigators (BACHA and WOOD, 1990; BANKS, 1993; JUNQUEIRA et al., 1995; KARADAG et al., 1995; TANYOLAC, 1993) have reported that the zona fasciculata is the widest zone of the cortex and has a foamy appearance caused by the presence of numerous lipid vacuoles (BACHA and WOOD, 1990; DELLMAN, 1993; GUDE et al., 1982; JUNQUEIRA et al., 1995). The zona fasciculata forms 60% of the cortex (TANYOLAC, 1993) or 70.9% of the cortex in the rabbit (KARADAG et al., 1995). According to JUNQUEIRA et al. (1995) the zona fasciculate in human is 65% of the gland. Results of this study indicate that the zona fasciculata appears foamy and forms 66.3% of the cortex and 48.5% of the gland.

JAMDAR and EMA (1982) identified that the adrenal medulla in goat is about a quarter of the gland. According to JUNQUEIRA et al. (1995) the adrenal medulla is 13% of the human gland. Our results are similar to those reported in goat by JAMDAR and EMA (1982).

Previous reports described that the adrenal medulla contains both types of chromaffin cells (COUPLAND, 1965; COUPLAND and WEAKLEY, 1968, 1970; ELFVIN, 1967; JURECKA et al., 1978). Furthermore, at the cortico-medullary boundary, cortical cells were together with chromaffin cells (COUPLAND, 1965; COUPLAND and WEAKLEY, 1970). Epinephrine

cells were more numerous, smaller and contained less electron-dense granules than norepinephrine cells (COUPLAND, 1965; COUPLAND and WEAKLEY, 1968, COUPLAND and WEAKLEY, 1970; DELLMAN, 1993; STEVENS and LOWE, 1991). The results of this study on porcupines confirmed those previous findings.

Epinephrine and norepinephrine granules were round-shaped in the rat (COUPLAND, 1965), hamster (YATES, 1964), mouse (JURECKA et al., 1978) from the rodents and in the rabbit (COUPLAND and WEAKLEY, 1968, 1970). Our results show that in porcupines these granules are generally small and rod-shaped.

COUPLAND and WEAKLEY (1968) identified epinephrine granules as being bigger compared to norepinephrine granules. However, our findings differ from their observations. In the present study, we also noticed that there were numerous central venules. Earlier, one large central venule was reported in domestic animals (DELLMAN, 1993; TANYOLAC, 1993).

In conclusion, in the present study, despite a general resemblance in the structures of adrenal cortex and adrenal medulla, the findings related to shape and size of epinephrine and norepinephrine granules differed from that of other rodents. To the best of our knowledge, these findings represent the first study on the adrenal gland in porcupine.

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SAŽETAK

Cilj rada bio je istražiti građu nadbubrežne žlijezde dikobraza svjetlosnim i elektronskim mikroskopom. U tu je svrhu šest nadbubrežnih žlijezda bilo uzeto od tri odrasla dikobraza (dva mužjaka i tri ženke). Tkiva su usporedno pretraživana svjetlosnim i elektronskim mikroskopom. Žlijezdu je činila čahura (1,2%), zona

glomerulosa (6,6%), zona fasciculata (48,5%), zona reticularis (17,9%) i moždina (25,7%). Na prijelazu kore u moždinu ustanovljene su stanice kore zajedno s kromafinskim stanicama. Epinefrinske stanice bile su manje i brojnije od norepinefrinskih. Epinefrinska zrnca bila su umjerene, a norepinefrinska velike elektronske gustoće. I epinefrinska i norepinefrinska zrnca bila su podjednako velika i općenito su oblikovala male štapiće. Ovo je prvi opis građe nadbubrežne žlijezde dikobraza.

Ključne riječi: *Hystrix cristata*, glodavci, dikobraz, nadbubrežna žlijezda, mikroskopska građa