Morphological Characteristics Of The Adrenals Of *Rattus norvegicus*: A Revisit By Scanning Electron Microscopy

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ABSTRACT

In response to a need for accurate labeling of minute structures of the adrenal tissue microenvironment, an ultra-structural study was conducted under the scanning electron microscopes (SEM). Paired adrenal glands surgically biopsied from adult female *Rattus norvegicus* specimens were used. All wet and dried specimens were inspected under the Phillips conventional SEM prior and post sectioning. Slides of adrenal sections were reviewed under the LEO VPSEM. Results obtained revealed that the cranial external surfaces of the glands were encased in a thick adipose tissue capsule with various microvascular features. The inferior surface that is related to the cranial pole of the kidney appears polished with indented crater-like surface from various prominences of the kidney superior pole. The cranial part of the left gland was observed to be triangular in shape with a protuberance. Other features include islands of blood smear or biofilm and hematopoietic cells. The cut section of the adrenals revealed well presented discrete zones of the cortex and the medulla. The microvessels within the cortex are present with numerous valves. This study revealed that the adrenal glands of *Rattus norvegicus* have unique micro-morphological features, especially the mentioned valvular-micro vessels complex. These features could be of relevance in comparative study involving defects of the glands.

INTRODUCTION

The laboratory rat *Rattus norvegicus* of the strain Sprague Dawley is one of the most popular choice of experimental animal for scientific endeavors (Koolhas, 1999). Established control data acting as baseline anatomic features is of great assistance in various areas of biomedical research. However, detailed information on endocrine glands, especially the adrenal glands are still lacking, especially with detail information obtained with electron microscopic studies. Prior to the 1980’s, the scanning electron microscopic studies (SEM) of adrenal glands are relatively few (Ketelbant-Balasse, 1980, Krstic, 1981). Gradually, however, many articles were published describing histophysiological phenomena of this gland (Bulut & Gursoy, 1998, Chen *et al.*, 1998, Janossy *et al.*, 1998). But, we have not seen any well-documented study of the morphological features of adrenal glands viewed either under the SEM or under the transmission electron microscope (TEM). Many of the studies on the structural features of the adrenal gland were only reviewed using gross techniques and via light microscopy (Koko *et al.*, 2004, Silva *et al.*, 2004).

On gross inspection, a thick connective tissue capsule covering the *Rattus norvegicus* adrenal gland was seen. The gland is said to be composed of two distinct anatomical demarcations: an outer cortex that is of mesodermal origin and an inner medulla of neuro-ectodermal origin (Chen *et al.*, 1998, Mitruka *et al.*, 1982). The adrenal cortex is subdivided into three distinct layers of epithelial cells (Rosol *et al.*, 2001). Firstly, the most superficial zone is the zona glomerulosa - a narrow zone consisting of small columnar-shape cells lying next to the surface connective
tissue capsule (Koko et al., 2004, Silva et al., 2004, Rosol et al., 2001, Yilmaz & Girgin, 2005). Beneath the zona glomerulosa is the broad zona fasciculata (Koko et al., 2004, Silva et al., 2004, Rosol et al., 2001). This region consists of cuboidal shape cells, arranged in long parallel cords and the presences of prominent fenestrated capillaries separate the adjacent cell columns. Thirdly, the innermost layer of cortical zone adjacent to the medulla is the zona reticularis. It consists of anastomosing cells forming a network surrounded by fenestrated capillaries (Rosol et al., 2001, Beamer et al., 1983). The central part of adrenal gland is the medulla, which consists of large polyhedral cell populations surrounded by venous sinusoids (Kierszenbaum, 2002).

The steroidogenic adrenal gland is an endocrine tissue characterized by an intense capillary network of highly permeable, often fenestrated vessels that allows the transportation of the endocrine hormones to the blood circulation (Kikuta & Murakami, 1982). Adrenal alteration may lead to various disorders such as Addison’s disease, involving an intrinsic alteration of the adrenal cortex, or adrenal failure attributable to hypophyseal or hypothalamic pathology (Mayenknecht et al., 1998). Electron microscopic observations of adrenocortical zone of male adult rats irradiated with 200kv x-rays exhibited mitochondrial membrane disruption and reduction of the number of cristae. Extravasated erythrocytes were also observed. The presence of these extravasated erythrocytes indicate that extensive local, small hemorrhages had occurred. The stress reaction appears to be hypothetically stimulated by local and whole-body irradiation (Longostrevi & Lombardi, 1962).

It is essential that the present study focus on accessing detailed morphology of the adrenal gland in order to provide a baseline data for which comparative study can be performed to see changes that can occur when the environment surrounding the glands has been changed. In addition, this organ is reported to be one of the most vulnerable organ to toxic substances (Rosol et al., 2001), thus the present investigation can be of utmost importance in providing normal SEM morphology of adrenal glands. A holistic approach to understand the microenvironments of the healthy adrenal gland observed under high-resolution microscope is crucial in order to harness relevant information from previous studies using light microscopy and provide directions for future studies. The general structural layout with related fine structural details of various components of the healthy adrenal structure can in future be correlated or be utilized for the purpose of comparison especially to look at the gland, physiologically and pathophysiologically.

MATERIALS AND METHODS

Animals

Female Rattus norvegicus specimens from the Sprague Dawley strain; weighing 185-190 g were used in this experiment. The animals were procured from the Animal House, Universiti Sains Malaysia Health Campus. They were healthy rats maintained according to the Institutional ethical guidelines. Experiments were performed under standard laboratory conditions (20±2ºC with 12 h light / dark cycle). They had free access to a standard diet and water ad libitum.

Paired adrenal glands were collected after the animals were sacrificed by CO₂ asphyxiation. Each side of the glands were respectively weighed and immediately fixed in fresh full strength Karnovsky’s solution at 8ºC for 4 hours. They were subsequently rinsed with sodium phosphate buffer solution (pH 7.4) with three changes hourly. The left adrenal gland of animal A (LA) underwent dehydration protocols with a series of ethanol of 1 hour each. The specimens underwent critical point drying for 2 hours and then transferred into sterile containers until viewed. Right adrenal gland from the same animal (RA) on the other hand was maintained in buffer without any further processing until the day of observation. The glands from the second animal (SB) were cut to 4µm thickness using rotary microtome (Microm, Germany) and kept (without staining) in slide boxes until evaluation.
Scanning electron microscopy

The dry LA specimen was thinly coated with gold of 60 nm in thickness using sputter coater (BIO-RAD, UK) for 2 minutes. The whole uncut specimens were examined under conventional scanning electron microscope (Phillips XL Series 30, Holand). The inner part was then viewed at various magnifications upon cutting them into two equal halves via a sagittal section.

The wet RA sample was only processed prior to viewing under the SEM. It was dehydrated through graded ethanols for 10 minutes each, critically dried (POLARON CPD 7501, UK) for half an hour and coated with gold. Finally, it was examined under the Phillips conventional SEM. Its inner part was also examined after cutting the sample into equal halves.

The prepared slides of adrenal sections (SB) were evaluated under the LEO VPSEM (LEO 450 VP, United Kingdom) after coating with gold.

This experiment was designed to allow for inspection and scrutinization of the overall morphology of the gland. Included in the study were outer surface and three different zones of the cortex and the medulla which were thought to be important for future work where the adrenals could be observed upon treatment with exogenous substances.

RESULTS AND DISCUSSIONS

The paired adrenal glands studied lied at the superior pole of each kidney. They were flat and pyramidal in shape. Their absolute weight were 0.0314 g (right) and 0.0198 g (left) whereas the total weight is 0.0512 g. Adrenal weight measurements can be crucial and of considerable importance since the literature has indicated that this gland can show prominent changes in their weights and atrophy or hypertrophy of its cells with certain xenobiotics or radiation therapies (Chen et al., 1998, Silva et al., 2004, Rosol et al., 2001). In addition, the enlargement in their size is a major indicator of stress (Nemeroff et al., 1992 & Siripapu et al., 2005). In this experiment, the height and width of the right (RA) and left adrenals (LA) were 3.12 mm and 2.31 mm and 2.57 mm and 2.18 mm respectively.

At low magnification, the glands were observed to be encased with an intact connection adipose tissue and membrane showing various microvascular features. The capsule is indented with various hematopoietic cell infiltration with some of the cells showing doughnut-shape presentations. The outermost surface of the adrenal is also presented with smooth patches or islands of blood smear or biofilms. At higher magnification, the adipose cells are present as homogenous raised globules or bulb-like prominences. Further, the inferior surface related to the cranial pole of the kidney is polished and indented with crater-like surface formed by various prominences of the kidney (Figure ). As illustrated in Figure 2, the cranial part of the left gland was observed to be triangular in shape and presented with a protuberance. The whole surface of this cranial part showed scallops-like features with prominent burrows. At higher magnifications, three important components were noticed on the capsule; globules of pebble-like structures, patches of plague-like materials (most probably blood serums) and hematopoietic cells (red blood cells and leukocytes) (Figure 3). These suggest that the glands are highly vascularized and the vessels penetrate the capsule through its surface rather than through a hilum (Henrikson et al., 1997).

The cut section of RA is demonstrated in Figure 4. This micrograph shows a section of the entire outer cortical and the inner medullary regions. The adrenal cortex is subdivided into three morphologically discrete zones. This can be easily distinguished. In the present study, we noticed that there are numerous microvessels in the cortex particularly in the zona fasciculata. The microvessels of the gland pose a thin tunica and a wide lumen. Red blood cells are seen in the microvessels. The microvessels seem to be orientated haphazardly or as if snakes crawling into the cortex (see figure 5). Well-defined and numerous prominent valves are seen transversing the whole length of the microvessels (Figure 5). These valves are known to be commonly found in terminal
Figure 1. Scanning electron photomicrograph showing right adrenal gland, x26. The gland was observed to have clean and smooth surface and presence of adipose tissue (AT) on the surface.

Figure 2. Scanning electron photomicrograph showing left adrenal gland, x30. The gland showed triangular in shape with protuberance (P) at the superior part. Its surface is rough and presence of scallops-like features (S) with burrows (B) at whole surface.
Figure 3. Scanning electron photomicrographs of the adrenal capsule revealing the presences of: (a) red blood cells and (b) plague-like materials.

Figure 4. Scanning electron photomicrograph illustrating cut section of adrenal gland, x25. Adrenal cortex (C) and medulla (M) are well presented. Clearly seen is the medulla central vein (V).
Figure 5. Scanning electron photomicrographs revealing numerous veins identified within the adrenal cortex depicting; (a, b) red blood cells and (c) valves present.

The ability to visualize accurately the micro-vascular network under normal and abnormal or diseased conditions is crucial for evaluating emerging therapeutic strategies (McDonald & Choyke, 2003). Technical constraints currently confine most micro-vascular imaging to two dimensions, but three-dimensional (3D) stacks of vascular image data are often preferable. Three-dimensional visualization reveals the detailed architecture of the micro-vascular network that determines the rheology and perfusion status of the tissue, provides more accurate data for quantitative assessment, and allows investigation of the poorly characterized or vascularized, treatment-resistant and deep portion of the tumor. Of particular relevance will be problems of therapeutic delivery (Padera et al., 2002, Jain, 2001). There is a study describing the blood supply of common tree shrew (Tupaia glis) adrenal gland under transmission electron microscopy and vascular corrosion cast/scanning electron microscopy techniques. The results noted that in these glands, blood supply from branches of the inferior phrenic, aorta and renal arteries are the major perfusion of vessels. These arteries then divide into cortical and medullary arteries when reaching the gland. The cortical arteries with the sub-capsular capillary plexuses partially enfold the clusters of cells in the zona glomerulosa (ZG). These structures appear as lobular-like microvascular networks before running among the cellular cords in the zona fasciculata (ZF) and zona reticularis (ZR) (Thongpila et al., 1998).
SEM result harness standard histological methods observed under upright light microscope. However, it is believed that this SEM procedure is simpler and allows shortening of the analysis time. The use of VPSEM allows animal tissue observations commonly done by using routine sample preparation for light microscopy to be undertaken easily.

CONCLUSION

The present study revealed unique ultrastructural features of *Rattus norvegicus* adrenal glands not seen previously. These unique morphological features may be of importance for the interpretation of results from other modes of studies. The cortical microvascular anatomy, which is seen presented here with well-defined valves is suggestive of micro-functional role of the hormone-blood circulation-effects relationship of the adrenal glands. It is therefore vital that studies involving effects of induced changes in the physiology of the adrenals take this observation into account.

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