Ultrastructural and Morphometrical Studies on the Endothelial Cells of Arteries
Supplying the Abdomino-inguinal Mammary Gland of Rats during the Reproductive Cycle

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ABSTRACT. The ultrastructure of the endothelial cells of the deep circumflex iliac and caudal superficial epigastric arteries supplying the abdomino-inguinal mammary gland of female Wistar rats was studied throughout the reproductive cycle with an electron microscope and image analyzer and compared with that of mammary gland capillaries. The subendothelial space of the arteries was broader at the virgin and post-weaning stages but narrower in pregnancy and lactation. In some rats, the endothelial cells of the arteries radiated some blunt processes into the media through fenestrations in the internal elastic lamina. The density of pinocytic vesicles (PV) in the arteries (number of PV per \( \mu m^2 \) of endothelial cytoplasm) significantly increased during pregnancy and reached its maximum value during lactation, then subsequently decreased during the post-weaning stage. The mammary gland capillaries showed the maximum changes of PV during pregnancy and lactation, with twofold and fourfold increases during the respective periods. The density of mitochondria increased significantly in the capillaries during pregnancy and lactation. The length of the marginal folds and microvillous processes increased significantly during lactation in both the arteries and capillaries, and especially, increased twofold in the mammary gland capillaries. It is assumed that the ultrastructural changes of the endothelial cells of the arteries and capillaries are closely associated during reproductive cycle with the functional demand of the mammary glands. — KEY WORDS: artery, endothelial cell, mammary gland, morphometry, rat.


The fine structure of mammalian arteries has been extensively investigated, and the basic structures of various types of vessels have been well established [14, 20–22, 29]. Buck [3] described the fine structure of endothelial cells of large arteries (the thoracic aorta and splenic arteries) of rats, rabbits, puppies, cats and ferrets. The ultrastructure of the capillary endothelium has been studied electron microscopically by Palade [18] and Moore and Rusca [13].

On the other hand, it is well known that the mammary glands show marked development during pregnancy and lactation. The morphological relationship between the mammary parenchyma and the vasculature has been well investigated in mouse mammary glands [6, 12, 15, 27, 28]. For example, Matsumoto et al. [12] reported by morphometry the capillary endothelium in mouse mammary glands during pregnancy and lactation. Although we previously described that the deep circumflex iliac, caudal superficial epigastrics, and mammary arteries supplying the abdomino-inguinal mammary glands showed the maximum pattern of changes in the diameter and thickness during the reproductive cycle [1], no ultrastructural study on the endothelial cells of these arteries has yet been carried out.

Therefore, the present study was conducted to clarify the ultrastructural changes of the endothelial cells of these arteries and to compare the changes with those of mammary gland capillaries, as mammary parenchyma is known to show a cyclic proliferation pattern during the reproductive cycle.

MATERIALS AND METHODS

A total of 12 female Wistar rats at the stages of virgin (90-day-old), pregnancy (15 days), lactation (10 days) and post-weaning (10 days after weaning) was used in this study. They were bred and maintained as a closed colony in our laboratory. Three rats at each stage were studied. The day following an overnight mating was counted as day 1 of gestation. The rats were given a commercial diet (Oriental East Co., Ltd.) and water ad libitum. During lactation, each mother rat was housed together with her (8-12) pups. Under sodium pentobarbital anesthesia, the rats were perfused from the left ventricle with physiological saline solution followed by a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer. The deep circumflex iliac and caudal superficial epigastric arteries and the first abdomino-inguinal mammary glands were carefully dissected out in pieces. The tissues were then fixed in the same solution for 2 hr at 4°C. They were rinsed in the same buffer and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hr at 4°C. After rinsing in the same buffer, the specimens were dehydrated in a graded ethanol series and embedded in Epon 812. Thin sections were cut on an ultramicrotome, stained with uranyl acetate and lead citrate and observed with an H-7000 KU transmission electron microscope (TEM) at 75 kV.

For morphometry, more than 20 cross-sections of the endothelial layer of the deep circumflex iliac and caudal superficial epigastric arteries and the mammary gland capillaries were obtained from different rats and tissue
blocks. Electron micrographs of the endothelium were obtained at a magnification of × 10,000 and enlarged to yield working prints with magnification of × 25,000. The area of the endothelial cytoplasm excluding the nucleus and the length of the marginal folds and microvillous processes were measured by an image analyzer (Nikon Cosmozone Is). Pinocytic vesicles (PV) and mitochondria were counted on enlarged photographs. The density of PV and of mitochondria was calculated as the number per μm² of endothelial cytoplasm. The data were statistically analyzed by Student's t-test.

RESULTS

TEM observation: The endothelial cells lined the deep circumflex iliac and caudal superficial epigastric arteries supplying the abdomino-inguinal mammary glands, formed a continuous sheet without any apparent intercellular gaps
and rested on both the internal elastic lamina and a fine network of collagen and elastic fibers. The internal elastic lamina was thickest in the rats at the virgin and post-weaning stages and was thinnest during lactation (Fig. 1). The subendothelial space in the junctional area was broad at the virgin and post-weaning stages but narrower at the pregnancy and lactation stages (Figs. 1, 2). In some instances, the endothelial cells of the arteries projected cytoplasmic processes through fenestrations into the internal elastic lamina.

The cytoplasm of the endothelial cells of the arteries and capillaries contained well-defined mitochondria, strands of endoplasmic reticulum, ribosomes, Golgi complex and numerous PV. Although the PV were scattered throughout the cytoplasm, the mitochondria were frequently concentrated near or around the nucleus of the cell. Marginal folds and microvillous processes projected from the luminal surfaces of the cells in many places (Figs. 1, 2).

The least number of PV and mitochondria for both the arteries and the capillaries was found at the virgin stage (Figs. 1–3). At this stage, the capillary endothelium showed a lesser density of these two structures than did the deep circumflex iliac or caudal superficial epigastric artery. The marginal folds and microvillous processes at the virgin stage were comparatively shorter in the caudal superficial epigastric artery compared to the deep circumflex iliac artery or the capillaries. The number of PV and mitochondria, as well as the length of marginal folds and microvillous processes, increased during pregnancy and reached maximum values during lactation, then decreased following weaning. The pattern of change of PV and mitochondria was more prominent in the mammary gland capillaries compared to the two arteries. The length of the marginal folds and microvillous processes in the capillaries also showed greater fluctuation than that in the arteries.

**Morphometry**. The density of PV in the deep circumflex iliac and caudal superficial epigastric arteries and the capillaries, respectively, was 21.71 ± 0.50 (mean ± SEM), 21.26 ± 0.64 and 14.82 ± 0.38 at the virgin stage, 34.28 ± 0.77, 28.88 ± 0.89 and 28.32 ± 0.76, during pregnancy, 50.05 ± 0.95, 41.97 ± 0.87 and 55.70 ± 1.03 at lactation and 29.91 ± 0.59, 24.54 ± 0.45 and 24.11 ± 0.79 at the post-weaning stage (Fig. 4). The density of mitochondria in the capillaries increased significantly during lactation, but showed no significant change in the deep circumflex iliac or caudal superficial epigastric artery (Fig. 5). Although the length of the marginal folds and microvillous processes

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Figs. 1–3. Electron micrographs of the endothelial cells at virgin (a), pregnant (b), lactating (c) and post-weaning stage (d), × 25,000.

Fig. 1. The deep circumflex iliac artery. The number of PV is low at the virgin stage, significantly increases during pregnancy, reaches its peak in lactation, and decreases at the post-weaning stage. The marginal folds show their maximum length in lactation. The subendothelial space is broader at the virgin and post-weaning stages, while it is narrower in the lactating period. The internal elastic lamina in lactation shown here is thinned compared to that at the virgin stage.

Fig. 2. The caudal superficial epigastric artery. The ultrastructural changes of the artery parallel those of the deep circumflex iliac artery.

Fig. 3. Mammary gland capillaries. The pattern of change of the density of PV is similar to that seen in the deep circumflex iliac artery. E: Epithelial cell.
DISCUSSION

As it has also been described by many other authors [3, 4, 8, 12, 18], in our study, numerous small vesicles of the endothelial cells of both the arteries and the mammary gland capillaries were observed at the luminal, lateral and basal surface scattered throughout the cytoplasm. Ross [24] reported that the permeability of the arterial endothelium is not only an important factor for transport of nutrients to the intimal and medial smooth muscle cells but also in the close relationship observed between abnormal increases in endothelial permeability and atherogenesis. According to O’Donnell and Vargas [16], the transport properties of the arterial endothelium are similar to those of continuous capillary endothelium, but there are morphological differences in terms of the endothelial thickness, intercellular junctional system, and vesicular density [10, 25]. The present results accord well with that description [16].

Although many hypotheses have been presented in the literature regarding the functions of PV at the cellular level [2, 4, 9, 12, 17, 19, 26], it is commonly agreed that these vesicles are involved in the active transport of fluid and nutrients across the cell. Recently, physiological and morphological studies have revealed that the endothelium constitutes a dynamic system which influences the physiology of both the blood vessel wall and the surrounding tissues [5].

The morphometrical analysis in the present study clearly revealed that, in the arteries and capillaries, the density of PV increased significantly in pregnancy reached its maximum during lactation, and decreased at the post-weaning stage. Ehrenbrand [7] described the active transport by PV in the endothelial cells of mammary gland capillaries during lactation. Matsumoto et al. [12] have observed that large numbers of PV are present during lactation in the capillaries of the mouse mammary gland. The present findings are very similar to that observation.

Matsumoto et al. [12] reported that the marginal folds and microvillous processes of the endothelial cells of mammary gland capillaries increase in length from the late
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stage of pregnancy to the middle stage of lactation and suggested the possibility of a participation of these structures with the uptake of fluid and particles or as a barrier acting to attenuate the blood velocity. The increased length of the marginal folds and microvillus processes in pregnant and lactating periods observed in the present study is direct evidence supportive of this hypothesis.

It has been reported that sex steroid hormones regulate the blood flow through a direct effect on uterine arterial walls [23]. We have suggested that the diameters of the blood vessels supplying the mammary gland, the number of PV, and the length of the marginal folds and microvillus processes of mammary gland capillaries are regulated by estrogen together with progesterone [11]. We speculate that the density of PV and length of the marginal folds and microvillus processes in the rat as observed in the present study may be partly regulated directly or indirectly by estrogen, progesterone and prolactin.

In our previous study [1], we noted that both the diameter and thickness of the arterial wall supplying the abdomino-inguinal mammary glands increased significantly during pregnancy, reached their maximum values in lactation and declined at the post-weaning stage. It seems clear that the arteries supplying the mammary glands and the capillaries play an increased functional role in responding to augmented circulatory and physiological demand during that period. As muscular arteries supply the target organs by contraction and relaxation phenomenon due to the increased circulatory demand of the glands, the vessel wall is undoubtedly more active during pregnancy and lactation than the virgin and post-weaning stages. The requirement by the vessel wall for nutrients and energy to work smoothly is increased in these periods. It is assumed that the nutrients are absorbed from the lumen of the vessel by the PV and subsequently utilized by the vessel wall, and that the energy utilized by the cells is generated by mitochondria.

In conclusion, the increased density of the PV and mitochondria and the increased length of the marginal folds and microvillus processes during pregnancy and lactation are exclusively dependent upon the functional circulatory demand of the vessels supplying blood to the abdomino-inguinal mammary glands. The decrease of the density and length of these structures observed at the post-weaning stage is assumed to be due to decreased flow of blood and the comparatively reduced functional role of the vessels, as apparently a lesser amount of blood is utilized by the involuted mammary glands.

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