MORPHOLOGICAL AND LECTIN-HISTOCHEMICAL STUDIES ON THE TESTIS, ESPECIALLY ON THE SERTOLI CELL, IN SOME MAMMALS

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Introduction

The Sertoli cells play an important role in spermatogenesis. Although a number of studies using many mammals have been made on their morphological characteristics by light (LM) and transmission electron microscopy (TEM), the three-dimensional (3D) configuration was not accurately described, probably due to their complicated morphological features as well as their close attachment to spermatogenic cells. The scanning electron microscopy (SEM) is of great advantage to grasp the 3D architecture of the cell. In the present study, we tried to visualize the 3D structure of the Sertoli cell by SEM. Similarly, to understand the 3D structure of cell organelles is important to elucidate their functional significance. We also tried to visualize the 3D structure of some intracellular organelles of the Sertoli trunk using Osmium –Dimethyl sulfoxide-Osmium (O-D-O method) [1]. On the other hand, glycoconjugates have been noticed in various tissues containing testes, since they play an essential role in cell differentiation and cell-to-cell interaction. Lectins have been used to detect the distribution of glycoconjugates, due to their specific binding affinity for the sugar residues of glycoconjugates. Although some lectin-histochemical studies on testicular tissues have been carried out, most of them were restricted to humans [2-4] and rodents [5, 6]. In this study, we picked up goats (domestic animal; Order Artiodactyla) , musk shrews (Order Insectivora) and common tree shrews (Order Scandentia), and the distribution of glycoconjugates in these mammals was examined by lectin-histochemistry.

Methods and Materials

First, in order to observe the 3D structure of the Sertoli cell, the HCl digestion method was adopted. The testes from adult goats were fixed in 2.5% glutaraldehyde/0.1M phosphate buffer (PB),

after perfusing with the same fixative through the testicular artery. They were cut into smaller pieces, washed in 0.1M PB containing 8% sucrose and rinsed in 8N HCl at 60°C for 20-30 min. The specimens were washed with an ultrasonic oscillator for 10 min. They were postfixed in 1% osmium tetroxide (OsO4), dehydrated in graded ethanol and dried with liquid CO2 at the critical point. Finally, they were coated with gold by sputtering in a vacuum evaporator and observed by SEM. Secondly, in order to observe the 3D structure of some intracellular organelles of the Sertoli trunk, the O-D-O method was adopted. The surgically excised testes obtained from cotton rats under ether anesthesia were cut into smaller pieces and fixed in 1% OsO4 at 4°C without perfusion. The specimens were freeze-cracked using dimethyl sulfoxide and washed overnight in 0.1M PB. They were immersed in 0.1% OsO4 for 3-4 days to macerate the cytoplasm, followed by further fixation in 1% OsO4 and dehydrated in graded ethanol. Thereafter, through the same procedure mentioned above, they were observed by SEM. Thirdly, in order to observe the distribution of some glycoconjugates in testes, lectin-histochemistry was adopted. The testes of some mammals (goats, musk shrews and common tree shrews) were perfused through the testicular artery or right ventricle with 5% glutaraldehyde in 0.05M cacodylate buffer and cut into smaller pieces. Lectins used here were *Canavalia ensiformis* agglutinin (ConA), wheat germ agglutinin (WGA), *Ricinus communis* agglutinin I (RCA-I), soybean agglutinin (SBA), peanut agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA), *Griffonia simplicifolia* agglutinin I (GS-I), *Griffonia simplicifolia* agglutinin II (GS-II) and *Ulex europeus* agglutinin I (UEA-I). For LM, the specimens were dehydrated in graded ethanol and embedded in paraffin. The sections were cut at 4μm, deparaffinized and rehydrated. They were treated with 1% bovine serum albumin (BSA) in 10mM phosphate-buffered saline (PBS) and incubated with biotinyl lectins (25μg/ml) in 0.1% BSA-PBS for 30 min. They were washed again with PBS, immersed in 3, 3'-diaminobenzidine (DAB, 0.2 mg/ml)-H202 (0.005%) for 10 min, rinsed in distilled water, dehydrated, stained with hematoxylin and observed by LM. For TEM, the specimens were washed with PBS for 2 hr, dehydrated in dimethyl-formamide and embedded in Lowicryl K4M by exposure to UV irradiation at –20°C for 1 hr. Thin sections were cut, treated with 1% BSA-PBS for 10 min and incubated with biotinyl lectins (12.5-25μg/ml) in 0.1% BSA-PBS for 1 hr. After washing briefly with PBS, they were incubated with streptavidin-gold (10nm in diameter) conjugates in 0.1% BSA-PBS for 30 min, washed again with PBS, stained with uranyl acetate and lead citrate and observed by TEM.

Results and Discussion

First, the 3D structure of the Sertoli cell was examined by SEM. The Sertoli cell, extending from the basement membrane to the lumen, stood like a tree with some branches. For convenience, the

seminiferous epithelium was divided into three portions;basal, middle and apical portions. In the basal portion, spermatogonia were located in compartments enclosed by neighboring Sertoli cells. Only a few Sertoli cell processes were present in this region. In the middle portion, early round spermatids halfway embedded in the Sertoli cell were recognized. The exposed surfaces of these spermatids were wrapped with ramifying processes derived from the Sertoli trunk. Sertoli processes were abundant from the middle to apical portions. In the apical portion, only the heads of maturing spermatids invaded into the Sertoli cell. As the spermatid matured, the apical Sertoli process varied in range to finally release the spermatid head. It seems likely that the maturing spermatids gradually leave the apical Sertoli process and ultimately separate from the seminiferous epithelium. Secondly, the 3D structure of some Sertoli cell organelles was observed by SEM. Mitochondria of the Sertoli trunk in the SEM observation appeared much longer and more densely distributed than those in the TEM observation and had a different shape from oval mitochondria in the basal portion. These rod-shaped mitochondria, 2-3μm in length, accumulated closely among the ER network. Smooth ER in the Sertoli trunk was composed of continuous small bulges like strings of beads and were connected with each other to form a net-arranged network. Thirdly, lectin-binding patterns in the testes of some mammals were examined by LM and TEM. SBA, GS-II, PNA and BPA showed a positive reaction in the acrosomal region of developing spermatids in mammals examined here. However, the period of appearance/disappearance of these lectins were somewhat different among species. The PNA reaction was observed from Golgi- to maturation-phase spermatids in goats, while it was detected from Golgi- to acrosome-phase spermatids in musk shrews and common tree shrews. ConA revealed a wide affinity for germ cells and non-germ cells, as reported in other mammalian species. Although the reactions of PNA, BPA and RCA-I appeared intensely in the Sertoli cell cytoplasm of musk shrews, lectins used here showed no reaction in the Sertoli cell of other mammals. Judging from the present results and other literatures, it seems that the reaction of some lectins in the Sertoli cell is specific to musk shrews.

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