Ultrastructural and Cytochemical Studies of the Subcommissural Organ in the Domestic Chicken with Special Reference to the Apical Secretion

By

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Summary: Ultrastructural and cytochemical studies were undertaken with special reference to the apical secretion in the ependymal cells of the subcommissural organ in the domestic chicken. Glucose-6-phosphatase (G6Pase) and acid phosphatase (AcPase) were used as cytochemical markers for the endoplasmic reticulum (ER) and lysosomes, respectively. Periodic acid-thiocarbohydrate-protein (PA-TCH-SP) cytochemistry was employed to detect glycoconjugates. Most dense bodies were negative with AcPase staining. Dilated cisternal of ER were reacted with G6Pase cytochemistry. Golgi saccules and several kinds of spherical bodies were stained with PA-TCH-SP cytochemistry. Our results suggest that: 1) most dense bodies in the apical cytoplasm belong to the secretory granules discharged into the ventricular cavity, 2) dilated cisternae of the ER serve as the storage site of the substance which eventually becomes the secretory product, however, contents of the ER are not directly released into the ventricular cavity. Findings of this studies lead us to speculate that there are two intracellular transportation via different compartments: one is a transport via small vesicles from the perinuclear ER to the Golgi apparatus and the other is a transport bypassing the Golgi apparatus via cisternae of ER from the perinuclear ER to the supra-Golgi region. The substances in these two structures seem to be intermingled with each other and matured into secretory granules. This granules may be discharged into the ventricular cavity by exocytosis.

The subcommissural organ (SCO) is one of circumventricular organs found in the almost all vertebrates (Tsuneki 1986). The well-known characteristics of this organ are synthesis and discharge of the secretory substance into the ventricular cavity. These secreted materials are thought to form the Reissner's fiber (Leonhardt 1980; Rodriguez et al. 1987). Electron microscopic studies have demonstrated that the ependymal cells of the SCO contained numerous dilated cisternal structures and dense bodies in their cytoplasm. However, significant of these cisternae and the dense bodies have appeared to be conflicting. Previous investigations of the SCO in the several vertebrates designated dilated cisternal structures as light secretory sacs (Papacharalampous et al. 1968; Herrlinger 1970; Biosca and Azcoitia 1989 a,b), light secretion (Hofer et al. 1980), and secretory vacuoles (Stanka et al. 1964). Some researchers implied that these cistern-like structures are equivalent to the cisternae of the endoplasmic reticulum (ER) (Diederen 1970; Chen et al. 1973). Furthermore, Marcinkiewicz and Bouchaud (1983) assumed that these were the secretory granules discharged into the ventricular cavity, however, Chen et al. (1973) and Biosca and Azcoitia (1989a) insisted that these were the lysosome dense bodies.

In the present study, we examined intracellular structures in the ependymal cells of the SCO in the domestic chicken. We utilized glucose-6-phosphatase (G6Pase) and acid phosphatase cytochemistry (AcPase) to determine the topographical distribution patterns of the ER and the lysosome, respectively. We also employed periodic acid-thiocarbohydrate-protein to visualize glycoconjugates. To reveal the roles of ER and dense bodies involved in the synthesis, processing, storage of the secretory substances and their release into the ventricular cavity in the ependymal cells of the SCO in the domestic chicken, special attention was paid to the distribution and characteristics of intracellular structures.
Materials and Methods

Adult male White Leghorn chickens (1,500 ~ 2,200g BW) were used. The chickens were sacrificed by decapitation under sodium pentobarbital anesthesia. For conventional TEM study, the diencephalic regions including the SCO were immersed in the cacodylate-buffered fixative solution (a mixture of 1.2% glutaraldehyde, 0.8% osmium tetroxide and 4.5% sucrose) at 4°C for two hours. Tissues were then dehydrated with graded ethanol series and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and a lead salt complex.

For observations of enzyme activities of AcPase and G6Pase, diencephalic regions including SCO were immersed in buffered fixative solution containing 2% glutaraldehyde for 40 minutes. For the AcPase and G6Pase cytochemistry, 1mM β-glycerophosphate and 1mM glucose-6-phosphate were added to the fixative, respectively. After fixation, block tissues were rinsed in the appropriate buffer, and cut into 75 μm thick sections using a tissue sectioner. The sections were collected in the buffer solution and maintained at 4°C before incubation. The sections were incubated in the cacodylate-buffered solution containing 1mM β-glycerophosphate or 1mM glucose-6-phosphate and 2mM CeCl3 (Robinson and Karnovsky 1983). The reactions were carried out at 37°C. The medium was once replaced with freshly prepared medium after 30 minutes. Following the cytochemical reaction, sections were washed two or three times in the appropriate buffer and refixed with the cacodylate-buffered (pH 7.2) glutaraldehyde solution for one hour. Thereafter, sections were rinsed and post-fixed with 1% osmium tetroxide for 1.5 hours, dehydrated with graded ethanol series, and embedded in Epon 812.

For PA-TCH-SP cytochemistry, the diencephalic regions inclusive of the SCO were immersed in the phosphate buffered (pH 7.4) fixative (containing 1.5% glutaraldehyde and 1.4% paraformaldehyde) for two hours. After rinsing with phosphate buffered solution, specimens were dehydrated with graded ethanol series and embedded in Epon 812. Staining procedure for PA-TCH-SP cytochemistry was done following the method of Thiéry (1969).

Results

Ultrastructure

The cytoplasm of the columnar ependymal cells of the SCO was filled with several kinds of well-developed intracellular structures (Fig. 1). Some light and small vesicles were observed just beneath the apical cell membrane as well as in the apical cytoplasm (Fig. 1a, b). In the apical cytoplasm, many cisternae of ER containing flocculent materials in their lumina and many dense bodies were accumulated. Most of these dense bodies were homogeneous and round in shape, while small part of them were heterogeneous and varied in shape (Fig. 1a, c).

Well-developed Golgi apparatus occupied the perinuclear region (Fig. 1d, e). Small vesicles contained moderately electron dense material distributed close to the trans face of the Golgi apparatus. In these region, such vesicles were occasionally associated with the cisternae of the ER (Fig. 1d). Many cisternae of the ER also observed in the perinuclear region (Fig. 1e). Many of them were short and dilated cisternae and studded with a few ribosomes. On the other hand, several cisternae of the ER were elongated and densely studded with ribosomes. The tips of these cisternae often dilated and contained flocculent material in their lumina. Occasionally, their cisternae were attenuated (Fig. 1e). Many small (transport?) vesicles were observed between the long flattened ER and the Golgi area.

G6Pase

The most intensive positive reactions were observed in the lumina of ER (Fig. 2). Occasionally, positive stained cisternae represented a reticular structure which surrounded the Golgi apparatus in the perinuclear region (Fig. 2c). Many small, moderately stained, vesicles were observed between the perinuclear cisternae of the ER and Golgi apparatus. The nuclear envelope seem to be continuous with the perinuclear ER both morphologically and cytochemically. Reacted cisternae of the ER were also dispersed in the apical cytoplasm (Fig. 2a). Light vesicles beneath the apical cell membrane were not stained (Fig. 2a). In the Golgi apparatus, positive reaction was always seen at the most cis saccule. Small vesicles were associated with stained cisternae of the ER in the supra-Golgi region (Fig. 2b). Accumulated spherical bodies in the apical cytoplasm did not react to G6Pase staining.

AcPase

Small number of dense bodies in the apical cytoplasm and Golgi area reacted positively to AcPase (Fig. 3a, b). These bodies were varied in shape. Beneath the apical cell membrane, reacted dense bodies were scarcely observed. In the Golgi region, some reacted dense bodies were observed. Small particles were observed in such dense bodies (Fig. 3b). Obviously, the most trans saccules reacted positively with AcPase staining (Fig. 3c).

PA-TCH-SP

PA-TCH-SP reaction was observed in several intracellular structures (such as ER, Golgi apparatus, and...
several kinds of spherical bodies). We classified these spherical bodies into three types based on their PA-TCH-SP stainings (Fig. 4). First type was a spherical body exhibiting homogeneous and intensive reaction (Fig. 4a, d). These bodies were widely distributed in the apical cytoplasm from the supra-Golgi region to the apical cell border. Second type was a spherical body with a reacted rim and a central lucent content (Fig. 4b). These bodies were mainly distributed near the apical border. The last type had a heterogeneous content (Fig. 4b, d). Some of this type obviously contained strongly reacted particles. These bodies mainly observed in the Golgi region.

The saccules of the Golgi apparatus reacted with PA-TCH-SP (Fig. 4c). The intensity of the reacted material increased gradually from cis to trans saccules. The cisternae of the ER represented weak reactions with PA-TCH-SP in the apical and perinuclear cytoplasm (Fig. 4a, d).

Discussion

The intracellular localization of ER and its topographical relationship to other organelles are essential for understanding of the roles of ER in the secretory activity in the ependymal cells of the SCO. Cytochemistry of G6Pase, as a maker enzyme of ER, represents the localization of ER in the ependymal cells of the SCO. Recently, Robinson and Karnovsky (1983) introduced new techniques using cerium for detecting G6Pase. Browdwell and his co-workers (1983, 1987) also demonstrated G6Pase activity in ER of nerve cells in the mouse. The abundant ER in the ependymal cells of the SCO were reacted with G6Pase in this study. G6Pase enzyme-cytochemistry clearly showed that the ER continuous reticular structure in the perinuclear region. This figure resembles to the ER of hepatocytes in configuration (Leskes et al. 1971).

The numerous cisternae of the ER are observed in the cytoplasm of the ependymal cells. Although several elongated cisternae of the granular ER were located only in the perinuclear region, dilated cisternae of the ER were dispersed in the perinuclear region as well as in the apical cytoplasm. Our results indicated the topographical close relationship between the ER and dense granules in the apical cytoplasm. Previously, such dilated cistern-like structures were reported as light secretory sacs (Papacharalampous et al. 1968; Herringer 1970; Biosca and Azcoitia 1989a, b), light secretion (Hofer et al. 1980), secretory vacuoles (Stanka et al. 1964), light secretory materials (Oksche 1969), and secretory canals (Stanka 1967) in the ependymal cells of the SCO. Chen et al. (1973) suggested that flocculent materials in the dilated cisternae were discharged into the ventricular cavity in the mouse SCO (also see Sterba 1967, Rodriguez 1970). Biosca and Azcoitia (1989a) supported the idea of Chen et al. (1973), however, Oksche (1961) and Hofer et al. (1980) insisted that this light materials were not discharged into the third ventricle, therefore, they did not contribute to formation of the Reissener's fiber. We did not get any evidence of the discharge of the contents in the cisternae of ER into the ventricular cavity.

The comparison of the intracellular structures between conventional and three cytochemical observations are summarized in Table 1. According to our results, dense bodies in the conventional observation are classified into two type: one is a homogeneous dense body and the other is a heterogeneous one. The former type is predominant in number and most of them did not react with AcPase. On the basis of this results, this type of dense bodies dose not seem to belong the category of lysosome. The evidence for determing the nature of these dense bodies appears to be conflicting. Previously, Chen et al. (1973) reported that most of these dense bodies were lysosomal in nature using

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<td>ER</td>
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<td>Secretory granules</td>
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<td>Lysosomes</td>
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<td>Recycling vesicles</td>
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<td>Golgi apparatus</td>
<td>well-developed (cis saccules)</td>
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AcPase cytochemistry. Stanka et al. (1964) and Bisoca and Azocita (1989a, b) demonstrated these dense bodies possessed lysosomal characteristics with conventional electron microscopy. However, Murakami et al. (1972) insisted that electron-dense granules were accumulated beneath the ventricular cell membrane and the content of these secretory granules was released into the third ventricle. Hofer et al. (1980) confirmed the results of the Murakami’s findings by conventional electron microscopy. Other investigations showed that dense granules were abundantly present in the apical cytoplasm of the ependymal cells and involved in the secretion into the ventricle (Papacharalampous et al. 1968; Wakahara 1974; Marcinkiewicz and Bouchaud 1983). However, these studies did not deal with the cytochemical characteristics of such granules.

PA-TCH-SP cytochemistry represented the homogeneous and intensive reactivity in the spherical bodies of the apical cytoplasm. Many of them were distributed beneath the apical cell membrane. The shape and distribution of these bodies correspond to the homogeneous dense bodies observed with conventional electron microscopy. In the electron microscopic level, Krstič (1973) reported the dense granules near the apical plasma membrane containing mucousubstance visualized by ruthenium red staining. Wakahara (1974) demonstrated complex carbohydrates in the dense granules by the periodic acid-chromic acid-silver methenamine staining. Our study of PA-TCH-SP cytochemistry confirmed both results. Taking these results into consideration, we suggest that these spherical bodies are the secretory granules which possess glycoprotein discharged into the ventricular cavity and the light coated vesicles beneath the apical cell membrane may contribute to recycle the membrane after secretion by exocytosis.

The other type of dense bodies located in the apical cytoplasm and Golgi area were the lysosomal in nature. They appeared heterogeneous characteristic and showed positive reactivity with the AcPase cytochemistry. The presence of the lysosomal dense bodies in the ependymal cells of the SCO were reported by several investigators. Chen et al. (1973) demonstrated that dense bodies in the Golgi region were lysosomal in nature by AcPase cytochemistry. Other studies suggested the presence of lysosomes in the apical cytoplasm by conventional electron microscopy (Diederen 1970; Biosca and Azocita 1989a). According to our results, lysosome having heterogeneous contents seemed to be located mainly in the Golgi region. The distribution of these lysosomal dense bodies were usually close to the Golgi apparatus. This results is the same as Chen and his coworkers reported (1973).

There are some discrepancies regarding secretory processes in the ependymal cells of the SCO. Rodríguez (1970) doubted the involvement of the Golgi apparatus in chemical modification for the secretory substance in the ependymal cells of the SCO. Papacharalampous et al. (1968) postulated that the secretory granules were originated in the rough ER. However, they also supposed that secretory granules required the involvement of the Golgi apparatus in the granule maturation. Sterba et al. (1968) assumed that the secretory material was in the cisternae of the ER and subsequently was transported to the Golgi apparatus, then chemically modified in the Golgi stacks. Herrlinger (1970) suggested that the protein and carbohydrate components of the secretory substance were originated in the rough ER and smooth ER, respectively, and that these substances were intermingled with each other somewhere in the cytoplasm.

On the basis of the present morphological and cytochemical findings, we speculate possible secretory pathways in the ependymal cells of the SCO in the domestic chicken. Proteins are synthesized in the rough ER and these proteins become high mannose type glycoconjugates in the cisternae of the ER. These glycoconjugates seem to be segregated into two parts. For these glycoconjugates, there might be two intracellular transportation manner via different compartments. One is a pathway via transport vesicles to the Golgi apparatus. This is a well-known intracellular transport route in the acinar cells of pancreas (Palade 1975). Transported glycoconjugates are chemically modified in the Golgi apparatus to form complex type glycoconjugates and subsequently pinched off as small vesicles (Palade 1975; Farquhar and Palade 1981). The other is a transport bypassing the Golgi apparatus via cisternae of the ER from the perinuclear ER to the supra-Golgi region. The synthesized high mannose type glycoconjugates are packed and pinched off as the cisternae of the ER which migrate to the supra-Golgi region without any chemical modification in the Golgi apparatus. The present study showed many cisternae of the ER in the every parts of cytoplasm and G6Pase cytochemistry particularly showed the presence of the cistern of the ER around the Golgi apparatus. These cisternae might contribute to store the high mannose type glycoconjugate and transport them to the supra-Golgi region bypassing the step of chemical modification in the Golgi apparatus. Conventional electron microscopy and G6Pase cytochemistry reveal the presence of transport vesicles from the perinuclear ER to the Golgi apparatus and Golgi vesicles originated in the most trans saccule of the Golgi apparatus. Some studies suggested that the secretory substances were processed chemically by the Golgi apparatus with conventional electron microscopy (Papacharalampous et al. 1968; Sterba et al. 1968) and the others suggested that the secretory substances were processed without the participation of the Golgi apparatus by conventional electron microscopy (Stanka et al. 1964; Oksche 1969;
Rodríguez 1970; Wakahara 1974). Chen et al. (1973) supported the latter idea with enzyme cytochemistry. Recently, Lösecke et al. (1984) suggested that there were two different mechanisms of preparation with immunohistochemistry. One is a mechanism involving the roles of Golgi apparatus and the other is a mechanism bypassing the Golgi apparatus. Our idea may confirm the hypothesis of the Lösecke and his co-worker (1984).

Interestingly, the association of the cisternae of the ER with Golgi vesicles in the apical cytoplasm were observed in this study. These results might indicate that the substances in these two structures are intermingled with each other in the supra-Golgi region. Subsequently, mixed substances are possibly matured into the dense secretory granules. As described above, Herrlinger (1970) assumed that carbohydrate and protein were coupled together in the cytoplasm. Papacharalampous et al. (1968) and Chen et al. (1973) reported the fusion of the Golgi vesicles with the cisternae of the ER within the Golgi region. Even though Chen et al. (1973) and Wakahara (1974) doubted the involvement of the Golgi apparatus in chemical modification of the secretory substances, they postulated that the synthesized protein was matured into the secretory products by association of its proteinaceous component (Wakahara 1974) with carbohydrate somewhere in the cytoplasm. In the present study, the conventional and G6Pase cytochemical observations showed the profiles suggesting the fusion of Golgi vesicles with the cisternae of the ER. These results might indicate the role of the Golgi apparatus for addition of certain components, possibly some sugar moieties. Furthermore, topographical close relationships between the dense granules and cisternae of the ER may support this idea. Further studies using an autoradiographic tracing method in the electron microscopic level are required to verify this hypothesis.

References

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Explanations of Figures

Plate I

Fig. 1. Conventional electron micrographs of the ependymal cells of the SCO. a-c: Apical cytoplasm of the ependymal cells. Many dense bodies (DB) and cisternae of the ER are observed. Cells having many dense bodies contained small number of light cisternae in the apical cytoplasm, while cells having many light cisternae contained small number of dense bodies. Light vesicles (LV) are usually observed beneath the apical cell membrane. He: Heterogeneous dense body, Ho: Homogeneous dense bodies. d-e: Perinuclear cytoplasm. Well-developed Golgi apparatus (GA) and numerous ER are observed. Tips of perinuclear ER are attenuated (Double arrows). Vesicles originated from Golgi apparatus are occasionally associated with the cisternae of the ER (Arrow). N: nucleus. a: $\times 3,800$, b: $\times 11,000$, c: $\times 21,300$, d: $\times 50,700$, e: $\times 32,000$. 
Fig. 2. a-c: Micrographs of G6Pase cytochemistry. a: Negative reacted light vesicles (LV) are observed beneath the apical cell membrane. b: Negatively reacted vesicles (Arrows) from the Golgi apparatus (GA) are associated with ER. c: In the Perinuclear region, ER occasionally exhibit reticular structure. Golgi apparatus (GA) is surrounded by ER. N: nucleus. a: ×27,700, b: ×63,600, c: ×40,000.
Fig. 3. a-c: Micrographs of the AcPase cytochemistry. a: Apical cytoplasm. Only a few lysosomal dense bodies (Ly) are observed near apical surface. Many light vesicles (LV) are observed. b: Supra-Golgi region. Heterogeneous bodies are varied in shape and reactivity. c: In the perinuclear region, trans saccules of the Golgi apparatus (GA) are strongly stained. Negatively stained vesicles are originated from the Golgi apparatus.

a: x 16,000, b: x 30,000, c: x 46,700.
Plate IV

Fig. 4. a-d: Micrographs of PA-TCH-SP cytochemistry. a: The apical plasma membrane exhibits topographical close relationship between homogeneous spherical body (Ho) and cisternae of the ER. b: Heterogeneous spherical bodies (He) and homogeneous spherical bodies reacted only on the rim are observed in the apical cytoplasm. c: The intensity of reacted material increased from cis (lower) to trans (upper) face in the Golgi apparatus (GA). d: In the perinuclear region, many spherical bodies are observed. There are homogeneous bodies and heterogeneous bodies.

a: ×30,000, b: ×32,000, c: ×68,000, d: ×40,000.
Fig. 5. Schematic diagram of the ependymal cells of the SCO. The diagram shows possible secretory pathways in the ependymal cells of the SCO in the domestic chicken. Proteins are synthesized on the rough ER and segregated into two parts. One is a transport via small vesicles to the Golgi apparatus (GA). The other is a transport bypassing the Golgi apparatus via cisternae of the ER to the supra-Golgi region. The substances in these two structures may be intermingled with each other in the supra-Golgi region (Asterisks). Subsequently, mixed substances are possibly matured as the dense secretory granules (SG) and discharged into the ventricular cavity. Small arrow: transportation via small vesicles. Large arrow: transportation via cisternae of ER. ER: endoplasmic reticulum, Ly: lysosome, N: nucleus, RV: recycling vesicle.