VARIATIONS IN THE MORPHOLOGY AND Ca^{2+} -ATPASE ACTIVITY OF THE OVARIAN SURFACE EPITHELIUM IN RATS

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The ultrastructure and cytochemical localization of Ca^{2+} -activated adenosine triphosphatase (Ca^{2+} -ATPase) activity of the ovarian surface epithelium in immature rats and in adult rats during the estrous cycle and pregnancy were investigated at both light and electron microscopical levels. In the adult rat ovaries at all stages of the estrous cycle, the intensity of Ca^{2+} -ATPase activity and ultrastructure of the surface epithelium were considerably varied in the different regions on the same ovary. The variations in the morphology and Ca^{2+} -ATPase activity likely provide evidence for the differences in the functional activity of the surface epithelium, which are related to the topographical connection with adjacent structures of ovarian cortex, i.e., corpus luteum, preovulatory Graafian follicles, interstitium containing small follicles. In the adult rat ovaries at all reproductive stages, the cuboidal or columnar surface epithelial cells were found on the interstitium containing small follicles. In some places, the columnar surface epithelial cells, forming crypts of the epithelium, invaginated into the ovarian proper. This type of surface epithelial cell had well-developed microvilli on its apical membrane, and showed intense Ca^{2+} -ATPase activity on its apical and lateral membranes. The squamous surface epithelial cells were found on the surface of corpus luteum and preovulatory Graafian follicles. These cells had a low number of microvilli and less activity of Ca^{2+} -ATPase on the apical membrane. In the immature rat ovaries, the surface epithelial cells were in part squamous, in part cuboidal. Both types of surface epithelial cells had few microvilli. Ca^{2+} -ATPase activity on the apical membrane was considerably less on all surfaces of the ovaries. It seems that the transport function of the surface epithelium in the immature ovary is not active as compared with the mature ovary.

These results suggest that the surface epithelium may have an active function of local absorption and/or transepithelial transport between pelvic cavity and ovarian proper, and its functional activity is associated with the reproductive function of the ovary.

The ovarian surface epithelium (germinal epithelium) is continuous with the peritoneal mesothelium. It is known that the morphological and histochemical characteristics of the ovarian surface epithelium are different from other mesothelium, although the physiological significance is poorly understood. For example, the ovarian surface epithelial cells tend to be cuboidal or columnar in shape, while usual mesothelial cells are simple squamous. The morphological features of the ovarian epithelial cells are more variable than other mesothelial cells (1, 8, 11-16, 19). In par-

ticular, it is well-known that the dynamic morphological changes occur in the ovarian surface epithelium covering the preovulatory follicles during the events leading to ovulation (14, 16). Histochemically, the ovarian surface epithelial cells are positive for 17β -hydroxysteroid dehydrogenase and for alcian blue indicating presence of sulfated mucopolysaccharides, while peritoneal mesothelial cells are negative (6). In the field of gynecology, it is noteworthy that the majority of ovarian carcinomas originate from ovarian surface epithelium and the frequency is higher than mesothelioma on any other organ (20, 22). Ultrastructural observations on other mesothelium such as pelvic peritoneum, mesentery and parietal and visceral peritoneum also have been reported (4–6, 21). Among these mesothelium, the variability or heterogeneity in the morphology of the mesothelial cells in different areas of the body were noted (4, 5). On the basis of these morphological viewpoints, it can be presumed that the particular mesothelium covering a specific organ such as the ovary may be physiologically different from those of other places.

It is generally accepted that Ca^{2+} -ATPase play a role of Ca^{2+} ions transporter on the plasma membrane, which keep intracellular calcium homeostasis in most animal cells. Moreover, this enzyme activity may be involved in the transport of fluid and electrolytes (7, 9). In the field of cytochemistry, Ando *et al.* (3) devised a new one-step method for detecting Ca^{2+} -ATPase activity, and the enzyme localization was observed in the various tissues (2, 3, 7, 10, 17, 23), but not in the ovary. We assumed that the cytochemical localization of Ca^{2+} -ATPase may reveal the functional activity of the ovarian surface epithelium. Therefore, in the present study, we examined the ultrastructure and cytochemical localization of Ca^{2+} -ATPase activity in the ovarian surface epithelium in immature rats and in adult rats during normal estrous cycle and pregnancy, in an attempt to elucidate the function of the surface epithelium on the ovary during the reproductive cycle.

MATERIALS AND METHODS

Female adult Wistar rats during normal estrous cycle and pregnancy, and 10–18 days old sexually immature rats were used. Under ether anesthesia, the rats were perfused with cold 2% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing 5% sucrose for 5 min. The perfused tissues were cut into small blocks and furthermore immersed in the same fixative for 30 min at 4°C. After the fixation, the tissues were rinsed with 0.1 M cacodylate buffer, pH 7.4, containing 8% sucrose. Then, the 10–15 μm frozen sections cut with a cryostat and 40 μm non-frozen sections cut with a Microslicer (Dosaka EM, Kyoto) were prepared for light and electron microscopic histo- and cytochemistry, respectively. These sections were incubated for 30 min at room temperature according to the lead citrate method of Ando *et al.* (3). The incubation medium consisted of 250 mM glycine-KOH buffer, pH 9.0; 3 mM ATP-disodium salt; 0.1–10 mM (usually 5 mM) CaCl_2 ; 4 mM lead citrate; 2.5 mM levamisole as a potent non-specific alkaline phosphatase inhibitor. As cytochemical controls, some of the sections were incubated in substrate free media, Ca^{2+} free media or 0.1–10 mM MgCl_2 containing media instead of CaCl_2 . Some sections were pretreated with 10 mM EGTA or EDTA in 0.1 M cacodylate buffer, pH 7.4, prior to the incubation.

After the cytochemical incubation for ATPase, the sections were rinsed in the

cold buffer and colored by 2% ammonium sulfide for light microscopic observation. For electron microscopy, the tissues were postfixed with buffered 1% OsO_4 for 30 min at 4°C, dehydrated in a series of ethanols and embedded in Spurr's resin. Ultrathin sections cut with an LKB Ultratome were stained with uranyl acetate only or both uranyl acetate and lead citrate, and examined under a Hitachi H-800 electron microscope.

RESULTS

Light microscopic observation

In the adult rat ovary at all stages of the estrous cycle, ATPase activity was observed on the surface epithelium, theca cells, interstitial cells, granulosa cells and microvessels in the corpus luteum and interstitial glands (Fig. 1). The change in the localization of ATPase activity by different stages of estrous cycle was not found clearly. But the activity of ATPase in the surface epithelium was considerably varied in different regions on the same ovary (Figs. 2a-c). The surface epithelial cells overlying the interstitium, which contain small follicles, showed cuboidal or columnar in shape and intense activity of ATPase (Fig. 2a). In particular, the portion of surface epithelium forming crypts which invaded into the ovarian proper showed more intense

FIG. 1. Light micrograph of Ca^{2+} -ATPase activity in the adult rat ovary at the stage of diestrus. Ca^{2+} -ATPase activity is localized on the surface epithelium, granulosa cells (G), interstitial cells (I), and microvessels. The surface epithelium forming crypts (arrows) shows intense activity. But a portion of the surface epithelium overlying the early corpus luteum (C) show little enzyme activity. $\times 100$

FIGS. 2a-c. Variations of the surface epithelium in different regions on the same ovary at the proestrus.

- (a) The surface epithelium overlying the interstitium, which contains small follicles, is cuboidal or columnar in shape, and shows intense activity of Ca^{2+} -ATPase (arrows). The surface epithelium is becoming gradually flat near the surface of corpus luteum (C). $\times 250$
- (b) The surface epithelium covering the apex of a Graafian follicle (G) is very flattened. Ca^{2+} -ATPase activity on the surface epithelial cells cannot be seen clearly, although the theca cells and interstitial cells are positive for Ca^{2+} -ATPase activity. $\times 250$
- (c) The surface epithelium covering corpus luteum (C) is also flattened and shows little activity of Ca^{2+} -ATPase activity. $\times 250$

ATPase activity than any other places (Fig. 1). On the other hand, the surface epithelium immediately covering the preovulatory Graafin follicles was flattened and showed less activity of ATPase (Fig. 2b). The surface epithelium covering the corpus luteum was also squamous (Fig. 2c). The reaction products of ATPase activity were scarcely seen in these squamous epithelium (Figs. 2b, c)

Similar findings were also observed in the pregnant rat ovary. The surface epithelium covering the pregnant phase of corpus luteum showed little reaction products of ATPase activity. The intense reaction products of ATPase were seen only in the portion of the surface epithelium invaginating into the ovarian proper among the large pregnant corpus luteum.

In the sexually immature rat ovary, little reaction of ATPase activity was demonstrated on the surface epithelium (Fig. 3).

As cytochemical controls, no reaction product of ATPase was seen in the sections which were incubated in the substrate free media or Ca^{2+} free media (Fig. 4). No remarkable alteration of the ATPase activity in the surface epithelium could be observed when the Ca^{2+} concentration in the incubation media was changed from 0.1 mM to

FIG. 3. Light micrograph of the localization of Ca^{2+} -ATPase activity in the sexually immature rat ovary. The activity is seen on the theca cells. Little enzyme activity is observed on the surface epithelium. F: ovarian follicles. $\times 280$

FIG. 4. A cytochemical control of Ca^{2+} -ATPase activity. The section of the same ovary as Fig. 2a were incubated in the substrate-free medium. No reaction product can be seen on the ovary. $\times 250$

FIG. 5. Low-power electron micrograph of the surface epithelium forming a crypt in the adult rat ovary. The surface epithelial cells show intense activity of Ca^{2+} -ATPase on the apical membrane with well-developed microvilli. $\times 3,000$

10 mM. When 1 mM–10 mM Mg^{2+} was used instead of Ca^{2+} , similar localization of ATPase activity was detectable, but the sensitivity was lower than Ca^{2+} .

Electron microscopic observation

By morphological and cytochemical electron microscopy, two types of the surface epithelial cells from some different regions in the adult rat ovary were observed comparatively. The cuboidal or columnar cells, which were placed on the interstitium containing small follicles, had well-developed microvilli on their apical membranes. ATPase activity was demonstrated on the apical membrane with microvilli, and lateral membrane, but not basal membrane. Especially, the surface epithelial cells invaginating into the ovarian proper had a number of microvilli on their apical membranes showing intense activity of ATPase (Figs. 5, 6).

FIG. 6. Typical columnar epithelial cells from a portion of the surface epithelium forming a crypt in the adult rat ovary. The epithelial cells have a great number of microvilli showing intense Ca^{2+} -ATPase activity on their apical membranes. The lateral membrane is also positive for Ca^{2+} -ATPase activity. $\times 6,300$

FIG. 7. Typical squamous epithelial cells from a portion of the surface epithelium covering the surface of a large corpus luteum in the adult rat ovary. The epithelial cells have a low number of microvilli on the apical membrane. Reaction products of Ca^{2+} -ATPase activity are much less than that of the columnar epithelial cell. Ca^{2+} -ATPase activity on the lateral membrane (arrows) is not so different from that of the columnar epithelial cell. $\times 8,600$

FIGS. 8a, b. The surface epithelial cells from the immature rat ovary. The epithelial cells are in part squamous (a) in part cuboidal (b) on the surface of immature rat ovary. Both squamous and cuboidal cells have a very low number of microvilli on their apical membranes. The apical membrane without the microvilli shows little activity of Ca^{2+} -ATPase, though the lateral membrane (arrows) shows enzyme activity. a. $\times 6,000$ b. $\times 7,600$

The squamous cells from the portion of the surface epithelium, which were placed on the surface of preovulatory Graafian follicles or large corpus luteum, had a low number of microvilli on their apical membranes. The ATPase activity on the apical membrane was much less than that of the columnar type cell, although the activity on the lateral membrane was not so different (Fig. 7).

In the sexually immature rat ovary, the surface epithelial cells were largely squamous in shape (Fig. 8a). The cuboidal cells were also present on some places of the ovary (Fig. 8b). However, both types of the surface epithelial cells had a very low number of microvilli on their apical membranes (Figs. 8a, b). The crypts formation originating from the surface epithelium was not found in the immature ovary. Cytochemically, little activity of ATPase was observed on their apical membranes in both squamous and cuboidal cells (Figs. 8a, b).

Results of cytochemical controls were almost same as the results by the light microscopic observation.

DISCUSSION

The variations in the morphology and Ca^{2+} -ATPase activity of the rat ovarian surface epithelial cells have been demonstrated in the present study. It was previously reported that the surface epithelial cells showed changes in the morphology such as cell shapes from squamous to columnar and the number of microvilli on their apical membranes (8, 11, 12, 14–16, 19, 24). Our findings are basically in agreement with these previous reports. The significance of the dynamic changes in morphology of the cells covering the apex of Graafian follicles during the leading event of ovulation is relatively interpretable (14, 16). However, little attention has been paid to the significance of changes in the morphology of the other part of surface epithelium. From earlier electron microscopic observations, it was suggested that the surface epithelial cells had the functional activity of endocytosis, absorption, transport (1, 12, 24) and secretion (6, 18). Therefore, it can be postulated that these functional activities of the surface epithelial cells may vary, being correlated with their morphological changes.

In our observation of the mature ovary at all stages of the reproductive cycle, the simple squamous epithelial cells had few microvilli on the apical membrane. In contrast, the cuboidal or columnar epithelial cells had a number of well-developed microvilli. A number of microvilli on the apical membrane may imply that the cells have active function of absorption or transport. The columnar type of the epithelial cells with a number of microvilli showed much higher Ca^{2+} -ATPase activity on the apical membrane than that of squamous type cell. The difference in the cytochemical localization of Ca^{2+} -ATPase activity between the two types of the surface epithelial cells indicates the difference in the functional activity, since it is generally convinced that Ca^{2+} -transport ATPase on the plasma membrane is involved in the homeostasis and modulation of intracytoplasmic Ca^{2+} ions which act on many cellular metabolism as a second messenger. The cytochemical demonstration of Ca^{2+} -ATPase activity by the lead citrate method possibly represent not only Ca^{2+} -extrusion pump, but also other subtypes of Ca^{2+} -activated ATPase (Ca^{2+} , Mg^{2+} -ATPase). It is likely that a part of Ca^{2+} -ATPase activity demonstrated on the plasma membrane of the surface epithelial cells is involved in the mechanism of the transepithelial transport of fluid,

ions, and other substances (7, 9).

The distribution of the two types of surface epithelial cells had close relation to the adjacent structure of the ovarian cortex. The squamous type cells were mostly observed on the apex of preovulatory follicle and large corpus luteum. The cuboidal or columnar cells were present on the ovarian interstitium containing the immature follicles. The typical columnar cells showing high Ca^{2+} -ATPase activity were particularly found in the region where the epithelium form crypts invaginating into the ovarian proper. The crypts of surface epithelium have also been observed in the rat (11), rabbit (13) and dog (15) ovary, although the significance of this structure is controversial. Some investigators speculated that the crypts or cord-like structure of surface epithelium might represent the proliferative activity and cell cycle (15). The close relationship between these structures and interstitial glands secreting steroid hormones was also noted (13). Our cytochemical observation of Ca^{2+} -ATPase activity suggest that the surface epithelial cells forming crypts are functionally active. If these cells have active absorption or transport function, it is conceivable that the special region of the surface epithelium which invaginate into the ovarian proper containing the follicles at various stages can play a role in the local transepithelial transport of fluid, ions and/or some other substances. It appears to be convincing that the function may be involved in the modulation of the growth and arrest of the oocytes and follicles, because the growing follicles were often observed underneath a part of the surface epithelium showing intense activity of Ca^{2+} -ATPase.

In the immature rat ovary, the morphology of the surface epithelial cells was different from the adult rat ovary. The immature rat ovary also had both squamous and cuboidal epithelial cells, however, both types of the epithelial cells had few microvilli on their apical membranes. The crypts of the surface epithelium were not found in the sexually immature ovary, similar to the developing ovary at fetal period (24). Moreover, the localization of ATPase activity in the immature ovary presented a striking contrast to the ATPase localization in the mature ovary. Both types of cells showed little ATPase activity on the apical membrane. It may imply that the functional activity of the surface epithelial cells in the immature ovary is not active as compared with the mature ovary. These findings also suggest that the crypts of surface epithelium and Ca^{2+} -ATPase activity on the apical membrane of surface epithelial cells have important functions in the mature ovary.

The change in the cell shape from columnar to squamous may somewhat be owing to a progressive increase in volume of adjacent structures such as the corpus luteum and Graafian follicle. However, it can be concluded that the changes in the number of microvilli and Ca^{2+} -ATPase activity on the apical membrane, which are correlated with the change in cell shape, are not merely passive but have a physiological significance in the normal ovarian function.

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