ENZYME CYTOCHEMISTRY OF GASTRIC PARIETAL CELLS AT A FINE STRUCTURE LEVEL

Cytochemical Separation of the Endoplasmic Reticulum from the "Tubulovesicles"

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Gastric parietal cells contain prominent smooth-surface tubules and/or vesicles ("tubulovesicles," 12, 13, 22), the morphology of which is probably a function of fixation (6, 7, 12, 13, 26, 27). These organelles have been thought to represent a smooth endoplasmic reticulum (6-10, 17, 24); some recent studies, however, have suggested a closer morphologic resemblance to the plasmalemma (4, 12, 13, 22, 27, 28). In the present study the tubulovesicles of mammalian parietal cells are cytochemically differentiated from the endoplasmic reticulum.

METHODS

Small pieces of glandular gastric mucosa, obtained from adult, male Sprague-Dawley rats and from normal human subjects by previously described methods (21) were fixed for 3 hr at 0-4°C in 2.5% glutaraldehyde in 0.1 M, pH 7.4 cacodylate buffer (23). Approximately 50-µ slices, prepared by the method of Smith and Farquhar (30), were incubated at 37°C for 15-60 min in specific substrate for demonstrating the following enzymes by the respective methods: inosine diphosphatase and thiamine pyrophosphatase by the methods of Novikoff and Goldfischer (16); alkaline and acid phosphatases by the methods of Gomori (2); and adenosine triphosphatase (ATPase) by the method of Wachstein and Meisel (35). Control slices were incubated in media lacking the specific substrates. The slices were postfixed for 2 hr at 0-4°C in 1% osmium tetroxide in 0.1 M, pH 7.4 cacodylate buffer, were dehydrated in graded alcohols and propylene oxide, and were embedded in Epon 812 (14). Both unstained and lead-stained (32) thin sections were examined with a Philips EM 200 or 300 electron microscope at 40 and 60 kv and at original magnifications of 4,000-50,000.

RESULTS

The tubulovesicles in both species were distributed primarily along the luminal and canalicular surfaces (Figs. 1 and 2; references 3, 9, 11, 20, 22). Classical profiles of endoplasmic reticulum were distributed throughout the cytoplasm (Figs. 1 and 2), but predominantly in the basal portions. Most profiles had attached ribosomes, but agranular profiles were also observed; individual profiles sometimes exhibited both rough and smooth portions (Figs. 1 and 2). Several small Golgi complexes were often observed within the same cell (Fig. 1), usually in the basal cytoplasm.

Inosine diphosphatase activity was localized to the classical profiles of endoplasmic reticulum, the nuclear envelope, and Golgi lamellae (Figs. 3-5). The amount of precipitate varied somewhat from cell to cell, but the reaction was readily demonstrable. Neither the tubulovesicles nor the plasmalemma exhibited activity (Figs. 3-5). Thiamine pyrophosphatase activity had a similar distribution, but the precipitate was much lighter and more variable; some cells contained no apparent deposition, while others exhibited precipitate only in Golgi lamellae. Alkaline phosphatase and adenosine triphosphatase activities were not detected, whereas acid phosphatase activity was restricted to the dense bodies and occasionally and irregularly to Golgi lamellae.

DISCUSSION

This study cytochemically differentiates the tubulovesicles from the endoplasmic reticulum in mammalian parietal cells, primarily by the localization of inosine diphosphatase and thiamine pyrophosphatase activity to classical profiles of endoplasmic reticulum, nuclear envelope, and Golgi lamellae. This distribution of these enzymes is similar to that previously observed in other types of cells (1, 15).

The results of this study support earlier suggestions that the tubulovesicles may be more closely related, both structurally and functionally, to the plasmalemma than to the endoplasmic reticulum (4, 5, 12, 13, 22, 27, 28, 33, 34). The tubulovesicles are located primarily near the luminal and ca-
FIGURE 1 The tubulovesicles in this human parietal cell appear as vesicles or vacuoles and are located predominantly near the glandular lumen (L) and intracellular canaliculus (C). The small particles (arrows at P) within some of them may be artifactual (>). Note the classical profiles of endoplasmic reticulum distributed throughout the cytoplasm of the same cell. Several of these profiles exhibit both granular and agranular (unlabeled arrows) portions. Golgi complexes (G) may be observed at the lower left, and an argentaffin cell (A) at the lower right. × 15,000. Scale line on all figures = 1μ.
FIGURE 2. This rat parietal cell had been incubated in an "inosine diphosphate medium" which lacked specific substrate. Note the absence of reaction product and the structural similarity to the human parietal cell. The tubulovesicles (V) are located predominantly about the intracellular canaliculi (C). Note the many classical profiles of endoplasmic reticulum; some agranular portions are denoted by arrows. X 28,000.

Nalicular surfaces (3, 9, 11, 20, 22), and their membrane has a width which is similar to that of the plasmalemma, but greater than that of the endoplasmic reticulum (4, 12, 13, 22). Both the tubulovesicle membrane and the membrane covering the microvilli have been shown to stain with phosphotungstic acid, presumably owing to a polysaccharide coat (28). When parietal cells are stimulated to secrete acid, their apical and canalicular surfaces seem to increase, whereas the tubulovesicles become less prominent (3, 19, 20, 25, 27, 29, 31, 33, 34); when acid secretion is inhibited (27, 34), the apical and canalicular surfaces appear to decrease and the tubulovesicles to increase. These phenomena have been attributed to an eversion or fusion of the tubulovesicle membrane with the plasmalemma during acid secretion, and to the reformation of the tubulovesicles by pinocytosis or inversion of the plasmalemma during secretory inhibition (4, 19, 27, 29, 31, 33, 34).
The tubulovesicles of parietal cells may actually represent a system of interconnecting smooth-membrane tubules, the true morphology of which is difficult to preserve (6-8, 26, 27). The same cells, however, also contain a second system of membrane-enclosed cisternae, the structure and cytochemistry of which more closely resemble those of an "endoplasmic reticulum." The tubulovesicle membrane is probably more similar to the plasmalemma; it possibly represents an anastomosing tubular invagination of the cell surface, as postulated earlier (6) and as observed in teleost chloride cells (18).

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REFERENCES

Figure 4  Inosine diphosphatase activity in this unstained rat parietal cell is restricted to the nuclear envelope (arrow at N) and to classical profiles of endoplasmic reticulum. The numerous tubulovesicles (predominantly vesicles) distributed about the lumen (L) and canaliculi (C) are unreactive. X 17,000.
This lead-stained rat parietal cell exhibits inosine diphosphatase activity in the nuclear envelope (arrows at N) and in the endoplasmic reticulum (unlabeled arrows). The tubulovesicles (V) are unreactive. X 42,000.


