DEFINITION OF THE EXTRACELLULAR SPACE IN SECRETING AND NONSECRETING OXYTIC CELLS

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INTRODUCTION

Oxytic, or acid-secreting, cells of the gastric epithelium contain a system of abundant smooth-surfaced tubular membranes within the cell apices. It has been proposed that this tubular membranous system may communicate with the apical plasma membrane, and thereby contribute to the elaborate ultrastructural changes which are associated with secretory activity (1-3). Direct evidence that such intercommunications can occur has recently been provided by Sedar, who applied horseradish peroxidase to the mucosal solution of bullfrog stomachs and traced its incorporation into oxytic cells after exposures of 0.5-1.5 hr (4). He found that the large molecular weight tracer substance (40,000 mol wt) penetrated the tubular membrane system even to the level of the nucleus, and that all peroxidase activity was confined to the inner aspects of the tubular membranes. Sedar’s experiment shows that the luminal extracellular space can be continuous with the smooth-surfaced endoplasmic tubules of oxytic cells. However, as he pointed out, it leaves open one important aspect for the mode of penetration, that is to say, whether penetration of tracer occurred by simple diffusion into pre-existing and permanent channels, or whether the route of access was directly dependent upon secretory activity, e.g., via active membrane transformations which occur with HCl secretion.

Our experiments were designed to distinguish between the above possibilities. We exposed glutaraldehyde-fixed gastric tissue to lanthanum nitrate, which is often used for the determination of extracellular space at the ultrastructural level (5, 6), and determined its distribution in and around the oxytic cells. Moreover, we assessed the distribution of the extracellular tracer in resting gastric tissue and in counterparts stimulated to maximum HCl secretion.

MATERIALS AND METHODS

Bullfrogs (Rana catesbeiana) were used for the experiments. Pieces of gastric mucosa were rapidly dissected from two freshly pithed bullfrogs and subjected to the fixation procedures. In one case a portion of the gastric mucosa was prepared for the measurement of acid secretion according to the method given in an earlier publication (7). This piece of tissue was fixed after 35 min in the in vitro chamber. In two experiments in which a comparison between resting and secreting oxytic cells was intended to be found, paired halves of the same mucosa were incubated overnight in HCO3-/CO2-buffered Ringer’s solution in order to provide an initial basal secretory state (8). After establishing that the rate of H+ secretion was very low for both mucosal halves (less than 0.1 μeq H+/cm² per hr), one-half representing the resting state was fixed in glutaraldehyde containing lanthanum. To the other mucosal half, we added 10⁻⁵ M histamine and monitored the stimulation to H+ secretion. After reaching a steady state of secretion (usually 20-40 min after histamine administration), the tissues were fixed for subsequent histological preparation. Tissue was minced into small pieces (less than 1 mm³) and fixed for 90 min in 3.5% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.4, and containing 1.5% lanthanum nitrate (6). Postfixation was carried out in 1% OsO₄ in 0.1 M s-collidine buffer, pH 7.4, containing 1.5% lanthanum nitrate. Tissue was embedded in Epon (Shell Chemical Co., New York), and sections were examined with or without heavy metal staining in a Hitachi HU-11 electron microscope.

RESULTS AND DISCUSSION

The electron micrograph in Fig. 1, of gastric mucosa fixed immediately after excision, shows that lanthanum completely outlines the extracellular regions of oxytic cells and their neighboring mucous-secreting cells. Lanthanum can be traced along the lateral borders between adjacent cells, but it is excluded from the tight junctional region; tortuous intercellular interdigitations are very prominent. On the basal surface, lanthanum is localized between the basement membrane and the cell membrane, and penetrates into deep basal infoldings. Such infoldings are often very tortuous and frequently extend almost to the nucleus. Lanthanum also diffuses into the gastric gland lumen, and is found between the cytoplasmic extensions and the microvilli located at the apical surfaces of oxytic cells. However, lanthanum...
FIGURE 1 An electron micrograph showing a portion of a gastric gland which was fixed and treated with lanthanum immediately after excision of the tissue. Lanthanum completely outlines the oxyntic cells as well as an adjacent mucous neck cell (MNC), except for tight junctional regions (TJ). The heavy metal tracer can be seen in the gland lumen (L), between the apical cytoplasmic extensions, along the basal border, and in the basal infoldings (BF). Stained with uranyl and lead salts. X 14,000.
does not penetrate the membranous systems within the cells. Thus it would appear that under these conditions of fixation the tubular system of oxyntic cells is not directly continuous with the free surface. In very rare cases (three cells out of many examined) an intense darkening of the ground substance of the cytoplasm occurred, but the tubular lumina remained free of the tracer. These cells were undoubtedly damaged, and became freely accessible to lanthanum in a manner similar to that described by Revel and Karnovsky (6), and Ritch and Philpott (9).

We have frequently found a greater cytological distortion in gastric preparations incubated by the overnight technique, i.e., many vesicular profiles in the apical portion of the cell appear distended in comparison to fresh or short-term in vitro preparations. However, cellular integrity is maintained after overnight incubation, since the tissue is physiologically responsive. Furthermore, lanthanum does not freely permeate through the plasmalemma into the cytoplasm of the cells. The luminal surfaces of oxyntic cells from non-secreting stomach preparations show relatively restricted surface elaborations, thus confirming earlier observations (2, 3). It is quite clear from Fig. 2 that, in the resting oxyntic cell, lanthanum is confined to the immediate region of the apical plasmalemma and does not penetrate the vesicular and tubular elements in subadjacent regions.

A somewhat different pattern of accessibility is seen for oxyntic cells stimulated to secrete H⁺ (Fig. 3). The apical cell surface appears much more structurally elaborate, with long cytoplasmic extensions and complex membrane infoldings at the secretory lumen. Lanthanum readily penetrates this complex network of membrane continuities. However, most of the membranous units of the tubular system within the apical cytoplasm are devoid of the heavy metal tracer. This would suggest that the bulk of the tubular membranous system of oxyntic cells is not continuous with the plasmalemma after glutaraldehyde fixation. The inaccessibility of the oxyntic cell tubules to the extracellular tracer is in direct contrast to results obtained on the somewhat analogous smooth membranous system of the Cl⁻-secreting cells of teleost fishes. For the latter system, Ritch and Philpott (9) have shown that lanthanum readily penetrates the tubules and have concluded that this membranous system is contiguous with, and hence a part of, the extracellular space.

In the case of the actively secreting gastric oxyntic cell, the apparent "incomplete penetration" of lanthanum is consistent with several proposals for membrane interactions and transformation which accompany the secretory process (1–3, 10). Moreover, when the present results are considered in conjunction with Sedar's demonstration of a more extensive in vivo tracer incorporation into the tubular membranes (4), an hypothesis of secretion-dependent membrane turnover becomes more tenable. Such a scheme would involve the smooth tubular system as an immediate source for apical surface membrane elaboration, and the evidence further suggests that the membrane interconnections are transient and dependent upon secretory activity.

This work was supported in part by United States Public Health Service Grant number AM-10141 and HE-12710.

Received for publication 4 May 1970, and in revised form 17 June 1970.

**Figure 2** Lanthanum-accessible space in a section from a resting stomach preparation. The lumen (L) of the gastric gland is surrounded by several oxyntic cells whose apical cytoplasmic extensions (MV) are short and clublike. Lanthanum can be seen at the luminal surfaces of the cells and in shallow, surface-connected invaginations (arrows). Section has not been counterstained with uranyl or lead salts. × 16,000.

**Figure 3** Section from an actively secreting stomach preparation which has been exposed to lanthanum. Histamine has been added to stimulate HCl secretion in this preparation, which is the paired mucosal half to that shown in Fig. 2. Just prior to fixation, the rate of secretion was 3.3 µeq H⁺/cm² per hr. The apical surfaces of the oxyntic cells show extensive membrane elaborations and infoldings. Lanthanum penetrates these luminal clefts (L) and can be found within membranous vesicular profiles (arrows) at least to the level of the desmosome of the junctional complex (JC). Stained with uranyl and lead salts × 18,000.
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