Secretory surface area and phosphatase activity of frog gastric mucosa

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Helander, H. F., and R. P. Durbin. Secretory surface area and phosphatase activity of frog gastric mucosa. Am. J. Physiol. 232(1): E48–52, 1977—Gastric mucosa from the European frog, R. temporaria, and after 5–7 h, stimulated with histamine (10⁻⁴ M) and theophylline (5 mM). Acid secretion increased about fourfold, and this was accompanied by a threefold increase in secretory surface of the oxyntic cells, as determined in electron micrographs with conventional morphometric techniques. At the same time phosphatase activity of the secretory surface increased. Other experiments showed that the latter was due to an acid phosphatase, with pH optimum near 3. It appears that the increase in surface phosphatase with stimulation can be attributed to a diminished local pH and not to the increase in surface area demonstrated in this study.

MATERIALS AND METHODS

A number of electron microscopic studies of the isolated amphibian gastric mucosa have appeared (4, 6, 8, 11, 14, 21–26). Several of these have dealt with the changes occurring after stimulation of acid secretion, and there is a consensus that the plasma membrane of the oxyntic cell becomes more elaborate when secretion is stimulated. This should result in an increase of secretory surface, defined as the surface area of the plasma membrane facing the gland lumen, but data on this increase have not been available because no stereological investigation has previously dealt with this problem in the amphibian.

In the present study, qualitative and quantitative data are supplied on the oxyntic cells from nonstimulated and stimulated in vitro preparations of gastric mucosa from the European frog, R. temporaria. The secretory membrane of the oxyntic cell presumably harbors enzymes necessary for HCl production, and earlier studies had shown the presence of a surface phosphatase that increased in activity when secretion was stimulated (2). For this reason the morphological results are compared with enzyme data that we obtained from the same preparations.

Frogs (R. temporaria) were obtained from Robert Stein, Lausingen-Donau, Germany. They usually weighed 50–80 g and were kept in running tap water at about 12°C without food for periods up to 8 wk. Fasting did not seem to impair the response of the gastric mucosa to secretagogues. After the frog was pithed, the stomach was removed and cut open, and the mucosa freed of external muscle by combined blunt and sharp dissection under a microscope. The remaining tissue layer, referred to in the following as mucosa, was mounted between plastic chambers with an area of 1.3 cm² in cross-section. In most cases the size of the mucosa allowed it to be divided longitudinally so that the halves could be run in parallel sets of chambers, one serving as the control for the other. The chamber volume was 2 ml. The nutrient solution contained, in millimoles per liter: NaCl 79, NaHCO₃ 18, KCl 4, CaCl₂ 1.8, MgSO₄ 0.8, Na β-hydroxybutyrate 10, and glucose 11. The secretory solution was 120 mM NaCl. The nutrient solution was oxygenated with 95% O₂ + 5% CO₂, and the secretory with 100% O₂. Secretory rates were measured with the pH stat method, keeping the secretory solution at pH 7.7.

In a typical experiment, paired mucosae were allowed to rest for 5–7 h; acid secretion was then stimulated in one with histamine (0.1 mM) and theophylline (5 mM) in the nutrient solution, the other serving as control. Before and after addition of secretagogues, secretory surface p-nitrophenylphosphatase (pNPPase) was monitored (2). To begin a measurement, the secretory solution was twice exchanged rapidly with fresh secretory solution containing 0.5 mM pNPPase; the nutrient solution was also renewed. After 10–30 min, the bathing solutions were collected in test tubes containing 0.5 ml of 0.1 N NaOH, and p-nitrophenol read at 405 nm in a spectrophotometer. Usually the secretory solution was unbuffered and held at pH 7.7 by pH stat while secretory rates and pNPPase were measured. For studies of the effect of pH on pNPPase, however, secretory solution was mixed with 0.1 M acetic acid and 0.1 M tris(hydroxymethyl)aminomethane as needed, to a final buffer concentration of about 4 mM; for pH values below 3.5, pH was adjusted with 0.1 N HCl only.

After the test mucosa had been incubated with secretagogues for 90 min, both paired tissues were prepared for morphological studies. Nutrient and secretory solutions were drained and replaced by ice-cold fixative, and the chamber assembly placed in a refrigerator at 4°C. The tissue was removed after 1 h of fixation, cut in strips, rinsed well in 0.1 M cacodylate buffer (pH 7.2), dehydrated in rising concentrations of ethanol, and finally embedded in Epon.
We used the one-step fixation method described by Simionescu et al. (27), employing a mixture of formaldehyde, glutaraldehyde, OsO₄ and Pb(NO₃)₂ in 0.1 M cacodylate buffer, pH 7.4. The ingredients were mixed just before using. This fixative was chosen in connection with other experiments testing the possible use of carbohydrate molecules as tracers of extracellular space (27); it yielded good preservation.

The embedded tissue was sectioned roughly perpendicularly to the surface of the mucosa with an ultramicrotome at a feeding rate of 60 nm, the sections picked up on 100-mesh grids, contrasted with uranyl acetate and lead hydroxide, and studied in the electron microscope at 80 kV. For the stereological analyses, a square was chosen at random on each grid, and within this area all oxyntic cells that displayed apical and basal cell surfaces as well as the nucleus, were photographed. Ten oxyntic cells from either functional state in each frog stomach were thus obtained, and enlarged paper prints with a final magnification of about 14,000 times were subjected to morphometric analysis. A Weibel multipurpose grid (29) with about 400 points per 100 μm² was used to determine the surface density of the secretory membrane; the cell profile areas (limited by the plasma membrane) were also determined in this fashion. Comprehensive descriptions of the morphometric methods have been published elsewhere (29).

In order to estimate the error of the morphometric procedures, the measurements were repeated some time later in the electron micrographs of ten cells from one stimulated mucosa. In relation to the mean values, the error of the method calculated according to Fränkò (3), was 9% for the surface density and 2% for the cell profile areas.

RESULTS

Acid production. A study of the effects of stimulation on ultrastructure depends on a reliable supply of mucosae with minimal spontaneous secretion ("resting" mucosae). Kaebekar (13) has shown that overnight incubation of bullfrog gastric mucosae produced such preparations. The decline in spontaneous secretion can be correlated with a reduction in the release of endogenous histamine (19).

In a large number (> 30) of experiments, we found that acid secretion by R. temporaria mucosa was initially low, rising to a maximum several hours after isolation and then declining slowly, as shown in Fig. 1. Rehm (20), on the other hand, has reported that mucosae from European frogs (R. temporaria and R. esculenta) rest promptly after isolation. The reason for the discrepancy between his and the present results is not known. It should be noted, however, that we routinely used β-hydroxybutyrate, a substrate that augments spontaneous acid secretion in bullfrog stomach (1).

Addition of secretagogues after 6 h of incubation led to a high, sustained rate of acid secretion (Fig. 1) and to an increase in secretory surface phosphatase. In the following, we summarize first morphometric, then enzymatic data associated with stimulation of secretion.

Morphometry. The oxyntic cells were found in the lower two-thirds of the gastric mucosa. In the sections the cells varied considerably in size and shape; many of them resembled truncated pyramids surrounding the gland lumen. In the nonstimulated preparations, the cells were characterized by numerous cytoplasmic tubulovesicles and mitochondria, as shown in Fig. 2. In the basal part of the cytoplasm, there were often several lipid droplets, about 1 μm in diameter; in 1 μm sections, these droplets were stained black by Sudan black B. A few short stubby projections appeared on the apical cell surface facing the gland lumen.

In the stimulated oxyntic cells the cytoplasmic tubulovesicles were less conspicuous (Fig. 3). A radical transformation had taken place on the secretory surface, where long (2-3 μm), often curved leaflets with a thickness of some 50 nm appeared, resulting in a large increase of the secretory surface area. Most of the leaflets were in close apposition to each other, with only some 15-25 nm between them. At their bases this distance often increased somewhat.

Paracellular channels (~1 μm wide) were seen along the lateral cell borders of the oxyntic cells. Leaflets appeared also along these borders. There were no obvious changes in the structure of the paracellular channels upon stimulation of secretion.

The results of morphometric measurements in five pairs of mucosae are presented in Table 1. Some of these parameters had been previously measured in histamine-stimulated mucosae from R. pipiens (8) as listed in the last column of the table for comparison. Significant changes upon stimulation in the present study were noted only for acid secretion and the parameters derived from the measured secretory surface.
Phosphatase. As indicated by the relatively large standard deviation in Table 1, the secretory surface phosphatase varied considerably from one mucosa to another. Occasionally, large fluctuations occurred in a given experiment. It seems likely that such changes reflect variability in restricted diffusion of substrate from the bulk solution to the site of hydrolysis.

Another factor that could lead to variability in phosphatase activity is its sensitivity to pH. Normally surface phosphatase was measured with the unbuffered secretory solution held at pH 7.7 by pH stat while acid secretion was monitored. In a separate group of experiments with stimulated mucosae, we instilled lightly buffered solutions on the lumen, turning off the pH stat. Acid secretion was monitored before and after test periods. It was not grossly affected by the solutions used. Fig. 4 indicates that the surface pNPPase increased as secretory pH was lowered below neutrality, reaching a peak in the vicinity of pH 3.

The foregoing result raised the possibility that the increase in surface phosphatase with stimulation (Fig. 1 and Table 1) could be due to acid secretion diminishing the local pH near the site of pNPP hydrolysis. Accordingly we ran another series of experiments, in which surface pNPPase was measured at a secretory pH of 7.8 and 3.1, before and after the addition of secretagogues. Periods of 10 min were used, during which time the pH of the lightly buffered secretory solution remained relatively stable.

The results for six mucosae are shown in Table 2. The upper row of data demonstrates the increase in surface pNPPase occurring with stimulation at neutral pH, as
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The latter change occurs at the expense of the tubulovesicular system (9), membranes which were not quantitated in the present study. In conformity with such findings is the reciprocal relationship between tissue volumes occupied by microvilli (leaflets) and tubulovesicles, described in mouse gastric mucosa by Ito and Schofield (12). Qualitative changes in oxyntic cell morphology conforming to this pattern have been described for the gastric mucosa of piglet (7) and frog (14, 26).

Previously the secretory surface phosphatase of R. catesbeiana had been shown to increase with stimulation of acid secretion and to diminish with inhibition. It was suggested that this enzymatic activity could prove useful as a marker for secretory surface area (2). In apparent agreement, the mean surface pNPPase (like secretory area) increased about threefold in the present study.

Other results suggest that this agreement was coincidental. We found that the apparent pH optimum for the surface phosphatase was well below neutrality. Thus measured pNPPase could increase with stimulation due to the effect of gastric acid on the local pH near the site of hydrolysis, inhibition of acid secretion having the opposite effect to reduce pNPPase.

The alternative view is supported by the study of the effect of stimulation on surface pNPPase when the secretory solution had been brought previously to pH 3.1. In these circumstances stimulation did not significantly affect surface pNPPase. Because we know from other results that secretory surface must have increased several fold, we must reject the surface phosphatase as a suitable marker for change in secretory area.

This conclusion does not rule out the possibility that the surface activity is located on the oxyntic cell. A preparation of isolated gastric cells, 80% of which were oxyntic cells, exhibited a surface pNPPase that increased with the addition of secretagogues (17). Because the measurement was made at near neutrality in the bulk phase, we can now interpret that result to suggest that the cells reduced local pH by secreting acid upon stimulation.

An acid-stimulated phosphatase has been isolated from homogenates of gastric mucosa from tadpole (16) and adult (5) bullfrogs. This membrane-bound enzyme was not stimulated by external K+, nor did it require lipid for activity, factors that distinguished it from another phosphatase with a pH optimum near neutrality (5). By analogy with mouse kidney cells (18), such an acid phosphatase could be derived from lysosomes or from cell membranes. The present study suggests that one source of this acid phosphatase is the cell membrane.

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