Quantitative ultrastructural analysis of the human parietal cell during acid inhibition and increase of gastric potential difference by glucagon

K J IVEY¹, A TARNAWSKI², D SHERMAN, W J KRAUSE, K ACKMAN, M BURKS, AND J HEWETT

From the Departments of Medicine and Anatomy, Harry S. Truman Memorial Veterans Medical Center and the University of Missouri Medical Center, Department of Statistics, University of Missouri, Columbia, Missouri, USA

SUMMARY Glucagon inhibits gastric acid secretion and increases the negativity of gastric mucosal potential difference (PD) in man. To test the hypothesis that the increased negativity of PD after glucagon in man could be due to decreased parietal cell canalicular membrane area, a quantitative ultrastructural analysis was carried out. Four healthy volunteers with normal gastric mucosa were submitted to biopsy before and 20 minutes after intravenous injection of 2 mg glucagon (G). This time corresponded with the maximal change in PD and a decrease in gastric acid secretion. Canalicular and tubulovesicular membrane area of 80 parietal cells (40 cells before glucagon and 40 cells after glucagon) were quantified by the Loud morphometric method. After glucagon, the oxyntic cell canalicular membrane area was reduced by one-fourth ($P < 0.05$), while tubulovesicular membrane area showed an increase ($P < 0.05$) at the same time. The decrease in the area of parietal cell canalicular membrane caused by glucagon may in part be responsible for increased negativity of the gastric PD caused by this hormone.

In previous studies (Tarnawski *et al.*, 1978a,b), we have reported a significant increase in negativity of gastric mucosal potential difference (PD) in man after administration of the acid-inhibitory hormone, glucagon.

Qualitative (Vial and Orrego, 1960; Sedar and Friedman, 1961; Helander, 1964) and quantitative (Frexinos *et al.*, 1971; Helander and Hirschowitz, 1972; Helander *et al.*, 1972) ultrastructural studies have demonstrated that resting parietal cells undergo characteristic changes on stimulation to secrete acid. In piglets, Forte *et al.* (1977) reported a fall in PD (decrease in negativity) after histamine stimulation that was associated with a marked qualitative increase in the canalicular component of the parietal cell secretory surface. They postulated that the decreased negativity of PD after histamine was due to the increased canalicular membrane area which

lowered electrical resistance to ion movement. The converse of this would apply if inhibition of acid secretion was associated with decreased canalicular membrane area and raised PD.

Previous studies of antisecretory agents on parietal cell ultrastructure have been done after stimulation with a secretagogue. In these studies, the expected formation of microvilli and the increase in surface area of canaliculi was inhibited by most agents. These include qualitative studies with 2:4 dinitrophenol, an oxidative uncoupling agent (Vial and Orrego, 1963), acetazolamide, an inhibitor of carbonic anhydrase (Sedar, 1962), glycopyrrolate, an anticholinergic (Posey *et al.*, 1968), and UK-9040, an antihistaminic derivative (Hamer *et al.*, 1977). Using stereological analysis, Zalewsky and Moody (1977) showed that the H_2 receptor antagonist, cimetidine, decreased secretory membrane surface area during acid inhibition. In contrast, thiocyanate-inhibited parietal cells showed no significant decrease in secretory membrane area, although luminal acid secretion decreased (Zalewsky and Moody, 1977).

The aim of the present study was to determine if glucagon affected the ultrastructure of parietal cells

¹Address for reprint requests and correspondence: K. J. Ivey, MD, Department of Medicine, University of Missouri, Columbia, Missouri 65212, USA.

²Present address: Dr A. Tarnawski, Department of Gastroenterology, Medical Academy, Krakow, Poland.

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in man so as to test the hypothesis that increased negativity of PD after glucagon in man could be due to decreased parietal cell canalicular membrane area.

Methods

SUBJECTS

Studies were performed on five healthy subjects, aged 21 to 27 years. The study was approved by the Institutional Human Experimentation Committee, and all the subjects gave informed consent.

Studies were of two types: physiological, where gastric PD was recorded, and morphological, where gastric biopsies were taken. The physiological findings of the effect of glucagon on PD and gastric pH have been reported previously (Tarnawski *et al.*, 1978b). In both studies, 2 mg glucagon (Eli Lilly and Company, Indianapolis, Indiana) were administered in a single intravenous injection given over three minutes.

POTENTIAL DIFFERENCE

Gastric mucosal PD was measured by a well-established technique (Andersson and Grossman, 1965) using intravenous and intragastric electrodes filled with a saturated KCl solution in 3% agar, as described in previous studies (Baskin *et al.*, 1976). Continuous PD recordings were made by means of a strip chart recorder.

Fasting gastric contents were aspirated at the beginning of each study. pH was determined with a Beckman Expandomatic pH meter (Beckman Instruments, Inc., Fullerton, California). The stomach was washed with 200 ml normal saline, aspirated, a fresh 100 ml saline instilled, and PD recording begun to establish a basal control period of 30 minutes. Glucagon (2 mg) was then injected intravenously over a three minute period. The first

gastric sample after glucagon injection was taken at 15 minutes. Gastric aspiration and instillation of saline was then repeated at 30-minute intervals. Gastric aspirates were measured for pH.

GASTRIC MUCOSAL BIOPSIES

The above studies were repeated in five subjects one week after the PD recording to obtain biopsy samples. PD was not recorded during the biopsy studies.

Biopsies were performed using a Quinton Hydraulic Biopsy Tube (Quinton Instruments, Seattle, Washington) positioned under fluoroscopic control in the stomach fundus near the greater curvature (Baskin *et al.*, 1976). Biopsies (2–3 mm in diameter) were obtained for light and electron microscopy during the basal control period and 20 minutes after glucagon injection. The timing of the biopsies was chosen to coincide with maximum change in gastric PD occurring after glucagon injection in order to correlate ultrastructural and electrophysiological data (Fig. 1).

TISSUE PREPARATION

Biopsies were fixed for two hours in 3.5% purified glutaraldehyde buffered in 0.1M phosphate to pH 7.4 in the cold. Tissues were washed with buffer and post-fixed in 1% osmium tetroxide at 0°C for one hour. Specimens were dehydrated in a graded series of ethyl alcohol solutions and embedded in a mixture of five parts Epon resin (6 parts A to 4 parts B resin mixtures) (Luft, 1961) to 4 parts Spurr low-viscosity embedding resin (Spurr, 1969). Thick sections of this material, cut at 0.5 to 2 microns, were stained with toluidine blue and examined by light microscopy.

For electron microscopy, silver-grey thin sections from specimens of an approximate size of $\frac{3}{4} \times 1\frac{1}{2}$ mm

Fig. 1 Effect of glucagon injection on gastric PD and pH. Values are mean $\pm$ SEM on vertical axis. PD is expressed in terms of negative mV, the luminal gastric mucosa being negative with respect to the blood. All PD values 10 minutes after glucagon injection were significantly greater (increase in negativity of PD) than control values. The pH values at 15 and 30 minutes were significantly greater than control pH values.

were mounted on uncoated grids having parallel bars (200 line/in.) orientated so that the mucosal surface was parallel to the bars. The grid bars clearly define regions of the gastric glands in relationship to the mucosa surface. Sections were double-stained with 2% uranyl acetate (Watson, 1958) followed by lead

citrate (Reynolds, 1963) and examined in a Phillips 300 electron microscope, operated at 60 kV.

QUANTITATIVE METHODS

Cells selected for morphometric analysis were of uniform diameter, with nuclei occupying at least 3%,

Fig. 2 A parietal cell from human stomach 20 minutes after intravenous administration of glucagon. The distribution of the tubulovesicular components (arrows) and the relative size and shape of the canaliculi (C) are shown. $\times 8250$.

but no greater than 25%, of the cell area. Cells were taken evenly from five different levels of the gastric gland, since position differences were noted in another study (Frexinos *et al.*, 1971). Electron-micrographs of 20 cells were taken from each of four subjects—10 before and 10 after glucagon injection. In the fifth subject, biopsy material was technically inadequate and therefore not quantified. A total of 80 micrographs—40 before and 40 after glucagon—was available for final analysis.

Tubulovesicular and canalicular membranes were quantified according to the Loud quantitative (two-dimensional) method (Loud, 1962; Loud *et al.*, 1965). A transparent acetate grid consisting of 63 parallel lines, 180 mm long and 4 mm apart, was placed over 8×10 micrographs of parietal cells with a magnification of $\times 9300$. The area of the cell and area of the nucleus was calculated by placing a metric ruler along the grid lines and tabulating the total distance of the lines enclosed by the cell or nuclear membrane. The total cytoplasmic area of the cells was determined after subtracting the area of the nucleus. Intersections between grid lines of tubulovesicular and canalicular membranes were then counted to determine the percentage of cytoplasmic area occupied by these components. Tangential contacts also were counted. The Loud equation for membrane profile concentration (Loud, 1962; Loud *et al.*, 1965)

was then used: $\frac{\pi (C) (M)}{2000 (L)}$ where C = number of intersections; M = magnification of micrographs; L = area of cell excluding nucleus. For comparison, measurements were also made for control parietal cells (before glucagon) using the Weibel (Weibel *et al.*, 1961; Weibel and Gomez, 1962) grid method for calculating surface area of membrane. This method has been used by a number of investigators (Helander and Hirschowitz, 1972; Zalewsky and Moody, 1977).

Data of quantitative evaluation were analysed statistically by use of the one-way analysis of variance in order to detect subject differences (Snedecor and Cochran, 1967).

Results

PHYSIOLOGICAL STUDIES

The results for gastric mucosal PD and gastric pH are shown in Fig. 1. Glucagon caused a significant ($P < 0.001$) increase in negativity in gastric mucosal PD in all subjects, reaching peak values at 20 minutes and remaining significantly raised throughout the study.

Mean gastric pH in basal samples was 1.8 ± 0.2 (mean $\pm$ SE); this increased over the next 15 minutes after glucagon injection to pH 2.8 ± 0.65 ($P < 0.05$) and remained at this level throughout the study.

ULTRASTRUCTURAL STUDIES

Parietal cells from the normal human gastric gland showed a tubulovesicular and canalicular membrane system that was well developed. Canaliculi were bordered with a moderate number of microvilli. Multi-lobed nuclei in parietal cells were found occasionally in all gastric biopsies examined. The predominant shape of the parietal cells examined was roughly spherical, regardless of level in the gland. However, rectangular, triangular, oval, and irregular cells were found.

After glucagon injection parietal cells (Fig. 2) showed tubulovesicular and canalicular systems similar to those in the normal untreated parietal cell. Nuclei and mitochondria in both normal and glucagon-treated cells appeared similar in size, shape, and density. Dark staining residual bodies, presumably lysosomal in nature, were randomly distributed in similar numbers in treated and untreated cells.

The micrographs of parietal cells before administration of glucagon were analysed morphometrically by both the methods of Weibel (Weibel *et al.*, 1961; Weibel and Gomez, 1962) and Loud (1962) to determine membrane area of tubulovesicular and canalicular components. Because of the irregular cell shape and presence of multi-lobed nuclei, it was felt that the Loud method of determination in two-dimensional units (surface area) was preferable in our studies in man to that of Weibel. Weibel requires conversion to three-dimensional units (surface density—surface area per unit of cell volume) and assumes a regular cell shape. The analysis, therefore, was primarily concerned with the percentage of the tubulovesicular and canalicular membrane component per unit of cytoplasmic area.

For comparison, the Table shows data obtained for tubulovesicular and canalicular membrane areas using both the Loud and Weibel grids. No significant difference occurred between values obtained by either method. These results agreed with Weibel's findings for hepatocytes (Weibel *et al.*, 1961), and show that either method can be used with equal validity for morphometric studies of parietal cell membrane area in man.

An analysis of variance for conditions before and after glucagon demonstrated the presence of indivi-

Table Comparison of membrane area expressed as a percentage (mean $\pm$ SEM) as measured by Loud and Weibel methods

	Tubulovesicles		Canaliculi	
	Loud	Weibel	Loud	Weibel
Normal saline	100 $\pm$ 8	100 $\pm$ 9	100 $\pm$ 6	100 $\pm$ 7

There were no significant differences between vesicular or canalicular values obtained by either method.

Fig. 3 The area of the tubulovesicular (V) and canaliculi (C) systems of parietal cells before and after glucagon treatment are expressed as a percentage of the total cytoplasmic area (minus nucleus). A statistically significant decrease in canaliculi membrane area with a simultaneous significant increase in tubulovesicular area was observed 20 minutes after intravenous injection of glucagon. * $P < 0.05$. □ Control. ■ Glucagon.

dual differences in canaliculi and tubulovesicular area among the four subjects studied. Taking these individual differences into account, an analysis of variance was further used to compare the response of canaliculi and tubulovesicles after glucagon injection with those of the normal saline period. This analysis demonstrated a significant decrease in canaliculi membrane area ($P < 0.05$) and a significant increase in tubulovesicular area ($P < 0.05$) after glucagon. Figure 3 shows the canaliculi and tubulovesicular membrane area expressed as a percentage of cytoplasmic area (minus nucleus) in the four subjects before and after glucagon both individually and as a group.

Discussion

The data show that inhibition of acid secretion by glucagon is associated with a significant reduction in secretory canaliculi membrane area in parietal cells. These findings are consistent with the hypothesis of Forte *et al.* (1977) that the increase in PD caused by HCl inhibitors could be attributed to a decrease in canaliculi membrane area in the parietal cell.

Acid secretion apparently involves secretion of equal amounts of H^+ and Cl^- , superimposed on the

basal secretion of Cl^- . The basal secretion of Cl^- tends to make the PD of the luminal side of the mucosa negative (Hogben, 1955). Inhibition of acid secretion after glucagon injection involves a significant decrease in total gastric membrane area due to a decrease in canaliculi surface area. This may act to shunt the basal Cl^- secretion through a much smaller membrane area. Since the current is the same, but the resistance is much higher (resistance is inversely proportional to membrane area), the voltage has to increase by Ohm's law: E (potential difference) + I (current) $\times R$ (resistance).

The H_2 -receptor antagonist, cimetidine, like glucagon (Tarnawski *et al.*, 1978b) causes a significant rise in gastric mucosal PD (Ivey *et al.*, 1975). On the other hand, inhibition of acid secretion by thiocyanate (Schlesinger *et al.*, 1955) or anoxia (Forte *et al.*, 1975) is not followed by an increased negativity of PD. In a stereological analysis of the parietal cell during acid inhibition in dogs, cimetidine reduced secretory membrane surface area during acid inhibition, but thiocyanate did not (Zalewsky and Moody, 1977). Ultrastructurally, therefore, glucagon in this study behaved similarly to the H_2 -receptor antagonist, cimetidine, rather than to the metabolic inhibitor thiocyanate (Zalewsky and Moody, 1977).

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