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Effect of Pentagastrin on Potential Difference in Rat Stomach[1] (40740)

ANDRZEJ TARNAWSKI AND KEVIN J. IVEY

Divisions of Gastroenterology, The Harry S. Truman Memorial Veterans Hospital and the University of Missouri, Columbia, Missouri 65201, and the Medical Academy, Krakow, Poland

In previous studies we have shown that parenteral administration of pentagastrin causes a significant reduction in gastric mucosal potential difference (PD) in rat and in man (1, 2). Infusion of human gastrin I-17 produced this reduction in PD, with blood levels of gastrin well within the physiological range (3). Pentagastrin, however, is a potent stimulator of acid secretion. Mixture of secreted acid and basal gastric contents could result in liquid-junction potentials at the interface of these fluids (4, 5). The effect of pentagastrin or gastrin on PD could be due to formation of these liquid-junction potentials, and not a separate mucosal effect of this hormone.

To test this hypothesis, we studied the effect of pentagastrin in both acid-secreting and non-acid-secreting parts of the rat stomach. In addition, in order to minimize liquid-junction potentials, the effect on PD of acid stimulation by pentagastrin in the presence of exogenous intragastric acid was tested.

Materials and methods. Animals. Fifty Sprague–Dawley male rats, average weight 240 g, after fasting for 24 hr, were anesthetized by intraperitoneal injection of 50 mg Nembutal per kilogram of body weight.

Preparation of stomach (6). The abdomen was opened and the stomach gently delivered from the wound. The gastric artery and vein were freed from the esophagus before it was ligated just above the cardiac end. The duodenal artery and vein were dissected free of the duodenum and a tie was passed between these vessels and duodenum. The stomach was washed three times with isotonic saline (total amount, 9 ml) through a polyethylene catheter introduced through a small incision in the duodenum before it was ligated 0.2 cm distal to the pylorus. Intragastric solutions of normal saline or HCl solution were administered via this catheter in 3-ml volumes. For recording of PD in the forestomach, a forestomach "pouch" was formed by tying the upper part of the forestomach around the electrode tip in order to prevent acid reflux into this part.

PD recording (6). For PD recording, electrodes were connected through beakers of saturated KCl to standard calomel reference electrodes which provided the input to a self-balancing Keithley 610 B electrometer (Keithley Instruments, Inc., Cleveland, Ohio) having a 100-mV sensitivity full-scale and less than a 0.5-mV error. The electrometer was connected to a strip chart recorder (Fisher Recordall 5000, Houston Instrument Co., Auston, Tex.).

Electrode placement (6). An electrode consisting of a polyethylene tube filled with a saturated KCl solution in 3% agar was placed in the spleen as the reference electrode (Fig. 1). As mentioned above, a similar electrode was introduced through an incision in the duodenum prior to its ligation, and placed in (1) glandular mucosa of the fundus, or (2) the forestomach (rumen).

Studies. Study 1. In 20 rats, gastric PD was recorded in (a) the gastric fundus (acid-secreting mucosa) and (b) the forestomach pouch (non-acid-secreting mucosa). To allow exact comparisons, PD was recorded *simultaneously* in both areas. For this purpose, two exploring electrodes were introduced through a small duodenal incision. One was placed in fundic mucosa on the anterior wall near the greater curvature, 2 cm below the border of the glandular mu-

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cosa and the forestomach. The second exploring electrode was introduced into the pouch formed by gentle ligation of the forestomach wall from the external side around the electrode tip. For detection of reflux, 0.1 ml of phenol red (phenolsulfonephthalein, Fisher Scientific Co., Chicago, Illinois, MW 354, 50 mg/liter) was added to test solutions introduced into the lumen of the stomach fundus. Only experiments without signs of gastric reflux (no fall in pH and absence of phenol red visually and colorimetrically) into forestomach pouch contents at the end of the study were included. Two electrometers, one for each set of exploring and reference electrodes, were connected to a strip chart double recorder which enabled simultaneous recordings of PD from both sites on the same chart (Fig. 1).

At the beginning of the study, 3 ml normal saline was placed into the main stomach and less than 0.5 ml into the forestomach pouch. After PD had reached a steady state for at least 20 min, pentagastrin (Peptavlon Ayerst Lab, Inc.), 100 μg/kg body wt, was injected intravenously. PD was recorded continuously throughout.

Study 2. In another group of 10 rats, the profile of gastric acid output was studied in 3-min periods prior to and for 15 min after intravenous pentagastrin administration using the perfusion technique described by Struve and Hantschmann (7). The stomach was perfused with normal saline by means of a Harvard infusion pump at a rate of 2.5 ml per 3 min. Samples obtained from each 3-min period were collected separately and titrated with 0.01 N NaOH, to pH 7.0.

Study 3. The effect on PD of intragastric instillation of 3 ml of 100 mM HCl made isotonic with 0.9% NaCl was studied in another group of 10 rats. In these rats, PD was recorded from the corpus area. No forestomach pouch was formed. A steady baseline PD recording with isotonic saline solution in the stomach was obtained for at least 20 min. The saline solution was then aspirated and HCl instilled. Pentagastrin, 100 μg/kg body wt, was given intravenously 15 min after HCl instillation and PD recorded for an additional 45 min.

PD data before and after pentagastrin injection or HCl administration were analyzed statistically by use of Student's paired t test, as was acid output in Study 2.

Results. Values of gastric PD recorded simultaneously in the *in vivo* rat corpus and forestomach pouch were almost identical: mean ± SE −39.8 ± 0.5 and −39.6 ± 0.8 mV, respectively (Fig. 2). Pentagastrin injection caused the same ($P < 0.01$) drop in PD (decrease in the mucosal electronegativity) of 18.2 ± 0.5 and 18.2 ± 0.9 mV in the corpus and forestomach pouch, respectively (Fig. 2). In both sites, this drop began a mean of 24 sec after pentagastrin injection and reached its lowest level at a mean of 1.8 ± 0.1 min. In the corpus, mean PD returned to baseline in 12 min and sub-

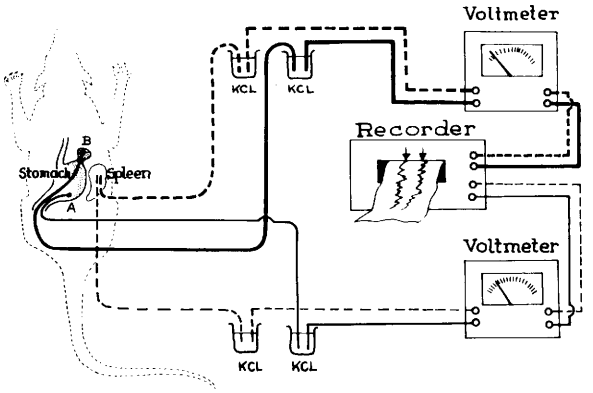

FIG. 1. Diagram of simultaneous PD recordings in *in vivo* rat stomach in corpus (A) and forestomach pouch (B) by means of two exploring electrodes, two electrometers (voltmeters), and one double chart recorder. Spleen was used as the site for reference electrodes. See text for details.

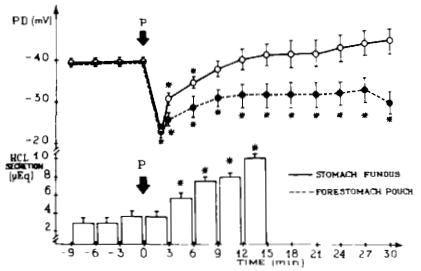

FIG. 2. The effect of iv pentagastrin (P) 100 μg/kg body wt on gastric PD in stomach fundus (corpus) and forestomach pouch. Bar graphs represent HCl output in 3-min periods prior to and after pentagastrin injection. Values are mean ± SEM; $^*P < 0.05$ for values compared with baseline. On the upper vertical axis, PD is expressed in terms of negative mV, the luminal gastric mucosa being negative with respect to blood (spleen).

sequently rose a little above baseline. In the forestomach pouch, PD remained significantly lower than baseline for 30 min (Fig. 2).

Gastric acid output did not increase during the first 3 min after pentagastrin (Fig. 2). Significant increase in gastric acid secretion began in the second 3-min period and continued with time (Fig. 2).

Intragastric instillation of 100 mM HCl caused a significant increase in PD (increase in electronegative charge of mucosa) of −12.5 ± 1 mV within 15 min in the corpus (Fig. 3). Pentagastrin injected 15 min after intragastric HCl instillation caused a sharp drop in PD of the corpus, mean 23 ± 2 mV, $P < 0.01$ (Fig. 3). This change was a

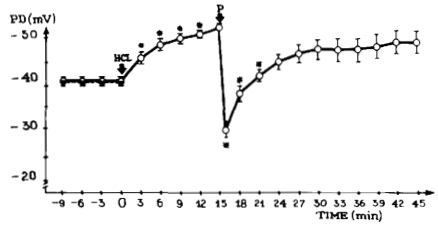

FIG. 3. The effect of intragastric instillation of 100 mM HCl on gastric (corpus) PD. Fifteen minutes after HCl instillation, pentagastrin (P) was injected intravenously in a dose of 100 μg/kg body wt $^*P < 0.05$ for PD values compared with baseline after HCl administration and for PD values compared with post-HCl after pentagastrin (P) injection.

mean of 5 mV *greater* than the effect produced by pentagastrin with normal saline in the lumen. As was the case without intragastric acid, the drop in PD started 24 sec after injection and reached its lowest level in 1.6 min.

Discussion. The current studies confirmed the high resting PD in the rat forestomach made up of squamous epithelium with no parietal cells (6). This is not surprising when one considers that PD across squamous epithelium in the rabbit esophagus (−26 to −29 mV) is considerably higher than across the rabbit stomach (−12 mV) (8, 9). Powell *et al.* (8) attributed the PD across rabbit esophagus to active, electrogenic transport of sodium ions from lumen to blood. It was speculated (8, 9) that the origin of transesophageal mucosal PD in other species is the same.

The fact that pentagastrin caused a significant fall in PD across the squamous epithelium of the rat forestomach, which has no parietal cells, is evidence against this effect being directly dependent on acid secretion. This is in agreement with the studies of Cummins and Vaughan (10, 11), who showed in *in vitro* preparations of rat gastric mucosa that secretion of HCl was not related to PD. In their preparation, substitution of choline for sodium in the fluid bathing the mucosa eliminated PD, but the amount of HCl formed was not affected. Since the fall in PD in both squamous and acid-secreting mucosa in our studies was identical quantitatively and qualitatively, it would seem that the same mechanism is responsible in both sites. A possible explanation for the rapid drop in PD is a decrease in transepithelial resistance. This was not studied, however.

Second, pentagastrin injection produced the same significant fall in PD (actually quantitatively greater) in the presence of exogenous acid in the lumen of the acid-secreting mucosa as in its absence. The speed of onset and time to peak fall were again almost identical. Therefore, it is unlikely that the major cause of the fall in PD after pentagastrin was the increase in liquid-junction potential between secreted acid and the fluid in the lumen. Since publication in preliminary form of our results (1),

Read and Fordtran (5) used flowing electrodes to study the role of intraluminal junction potentials in the generation of gastric PD in man after pentagastrin. Even after subtracting junction PD from measured gastric PD, pentagastrin still caused a significant reduction in transepithelial PD (5). These findings support our own conclusions.

Third, the effect of adding acid to the gastric lumen was to raise (not lower) PD across rat gastric mucosa. The same effect (elevation of gastric PD) occurs when exogenous acid is added to the gastric lumen in man (12). Recovery of gastric PD after pentagastrin injection occurred in the acid-secreting rat corpus and seemed to coincide with secretion of acid into the lumen (Fig. 2). PD remained significantly lower than baseline in the non-acid-secreting forestomach. It is thus tempting to relate failure of PD recovery in the forestomach to absence of acid secretion. Recovery of PD is unlikely to be solely a liquid-junction effect, as some recovery did occur in the forestomach in the absence of acid, and second, the same pattern of PD recovery in the corpus occurred in the presence of exogenous acid as occurred in its absence.

The peak fall in PD preceded detection of gastric acid in the stomach lumen. This also suggests that the establishment of liquid-junction potential is not the main factor responsible for the fall in PD following pentagastrin injection. It may be argued that our system was not accurate enough to detect pH changes occurring in the depths of the gastric gland; nevertheless, our experience is not unique. Forte, *et al.* (13), using an *in vitro* preparation of piglet gastric mucosa, found a definitive change in PD within 30 sec after histamine administration, while increased HCl secretion was first detected 4–5 min after this secretagogue.

It should be pointed out that when the stomach is secreting HCl, the potential difference recorded appears to be the sum of several components; (1) whatever causes the transmembrane potential in the first place ie the active transport of sodium and/or hydrogen and chloride; (2) the diffusion potential for hydrogen and/or sodium as a result of the asymmetry concentration of these ions (due to the marked concentration of HCl in the lumen); (3) the oppositely directed junction potential between the HCl and the lumen and the electrode.

In conclusion, pentagastrin causes a significant fall in PD in the rat forestomach, which contains no acid-secreting cells. Addition of acid to the gastric lumen did not prevent the fall in PD after pentagastrin. Thus, liquid-junction potential between secreted acid and luminal contents is not the major factor in the fall in PD after pentagastrin administration.

Summary. We studied gastric mucosal potential difference (PD) in *in vivo* rat gastric acid-secreting mucosa (corpus) and rat forestomach pouches containing squamous epithelial mucosa. Gastric PD was almost identical in the rat gastric corpus and forestomach pouch. Pentagastrin (100 μg/kg body wt, iv) produced a similar reduction in PD in both sites. In the gastric corpus the drop in PD preceded detectable secretion of gastric acid, but recovery of PD coincided with the onset of acid secretion. Recovery of PD did not occur in the forestomach pouch separated from gastric acid. Intragastric instillation of HCl into the nonstimulated gastric corpus was associated with a steady rise in PD. Injection of pentagastrin now caused the same significant fall in PD as in the absence of intragastric acid. These findings indicate that liquid-junction potential between secreted acid and luminal contents is not the major factor in the fall in PD after pentagastrin.

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