THE ROLE OF SEROTONIN IN GASTRIC ACID INHIBITION BY DUODENAL FAT

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SUMMARY

The possible role of serotonin (5-hydroxytryptamine) in the inhibition of gastric acid secretion following the infusion of fat into the duodenum was studied. Heidenhain pouches were constructed in 12 dogs and cannulae were placed in the dependent part of the pouch for the sample collection, the stomach for drainage and the duodenum for fat infusion. In all studies, histamine phosphate (0.04 mg/kg/hr) was infused during the four-hour study period. When fat (corn oil) was infused into the duodenum, gastric acid secretion gradually became inhibited ($p<0.05$ and a maximum inhibition of 59% ($p<0.001$) occurred at one hour. Following serotonin depletion by administering reserpine 24 hours prior to the study, gastric acid secretion increased ($p<0.02$) when compared to the effect of histamine infusion. Serotonin depletion by reserpine or antagonism by 1-methyl-lysergic acid butanolamide (UML491) significantly interfered ($P<0.01$) with the fat-induced inhibition of gastric acid secretion. Following serotonin depletion, replacement by 5-hydroxytryptophan restored ($p<0.02$) the inhibitory action of intraduodenal fat on gastric acid secretion. The data suggests that serotonin may be an enterogastrone which is at least partly responsible for the inhibition of gastric acid secretion following intraduodenal fat infusion.

Gastric acid secretion is inhibited following the introduction of either acid or fat into the duodenum.[7,8,12] The parenteral administration of secretin or cholecystokinin also inhibits gastric acid secretion, and, therefore, have been considered enterogastrones.[7] Johnson and Grossman however, found that the inhibition of gastric acid secretion and cholcystokinin did not equal the degree of inhibition produced by intraduodenal fat.[7] The effect of the combined administration of these hormones, however had similar stimulatory action on pancreatic secretion, as did the intraduodenal administration of fat. Johnson and Grossman, therefore, speculated that another duodenal enterogastrone may have been simultaneously released by duodenal fat and produced the inhibition that secretin and cholecystokinin could account for.[7] The gastric inhibitory polypeptide (GIP) which is also an inhibitory of gastric secretion may be this enterogastrone.[2] Debas, however, suggested that another enterogastrone may exist, since GIP is a weak inhibitor of acid secretion.[3]

Resnick and Gray, Thomson and Wise et al have suggested that serotonin may play a role in the inhibitory mechanism of duodenal acidification.[12,15,17] The parenteral administration of serotonin or its precursor, 5-hydroxytryptophan inhibits gastric acid secretion.[16] When the serotonin antagonist, 1-methyl-d-lysergic acid butanolamide (UML 491), is administered, gastric acid secretion increases.[13] Since serotonin is present in relatively large quantities in the duodenum serotinin might well be the other enterogastrone released by intraduodenal fat.[5,11] The purpose of our studies was to evaluate the possible role of serotonin in the gastric acid secretory inhibition induced by duodenal fat.

MATERIALS AND METHODS

Twelve female mongrel dogs weighing 20 to 25 Kg were provided with Heidenhain pouches and gastric and duodenal cannulae (Figure 1). The surgical procedures were performed under general anesthesia. using intravenous sodium thiopental (PentothalR). 30 mg/Kg body weight. A gastric cannula was placed in the body of the stomach in order to prevent gastric acid from entering the duodenum during the study and inhibiting gastric secretion. The duodenal cannula was located 10 cm distal to the pylorus.

Intravenous normal saline infusions (500 ml daily) were administered for two days following the operations. A liquid diet was then given for an additional two days before regular dog food was permitted. Benzathine penicillin G (Bicillin L-A, Wyeth) was given intramuscularly, 1,200,000 units daily for five days following the operations. The dogs were not used for experimentation less than two weeks following the operations since during the early post-operative period there is considerable fluctuation

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in the amount and type of gastric secretion.

Before each experiment, the dogs were fasted overnight (approximately 18 hours). Then each dog was placed in a Pavlov frame and the gastric cannula was opened and allowed to drain freely throughout the study. In addition, a No. 8 Foley catheter was threaded through the duodenal cannula until its tip just protruded into the lumen of the duodenum. The balloon, which was entirely within the lumen of the cannula, was inflated to retain the catheter in place and prevent bile and fat leakage. An intravenous cannula (Medicut "R"-Argyl) was introduced into each foreleg vein, and normal saline (0.9%) NaCl) was infused by a peristaltic pump (Holter Model 911) at a rate of 60 ml/hr through each cannula, i.e.: total volume of 120 ml was administered each hour.

Histamine phosphate, which was added to one of the saline infusions, was administered at a rate of 0.04 mg/kg/hr for the entire study of four hours. The serotonin precursor or serotonin antagonist was administered through the other cannula as the study required. Collections were made from the Heidenha in pouches at 15 minute intervals for four hours. Acid concentrations (mEq/ml) were determined by titration with 0.01 N NaOH to a pH of 7.0 by a pH meter (Corning Model 12). Acid output was expressed as mEq/15 minutes.

A. *EFFECT OF HISTAMINE*

In the control studies, histamine was infused in each of 12 dogs.

B. *EFFECT OF FAT*

In 14 experiments on 12 dogs, histamine was again infused to stimulate gastric acid secretion. During maximal acid secretion, 40 ml of fat (corn oil emulsion) was infused into the duodenum over a period of 30 minutes at a rate of 2 ml/min for 10 minutes and one ml/min for the remaining 20 minutes. With this rate of fat infusion, no wretching, vomiting, or regurgitation of fat through the gastric cannulae occured.

C. *EFFECT OF SEROTONIN ANTAGONISM*

In these studies, 8 experiments were performed on 8 dogs. The serotonin antagonist, 1-methyl-d-lysergic acid butanolamide (UML 491), was infused intravenously at 2 mg/hr, 30 minutes before fat administration.

D. *EFFECT OF SEROTONIN DEPLETION*

In 12 experiments on 9 dogs, reserpine, a serotonin depletor, was injected intramuscularly, 0.1 mg/kg. 24 hours before the infusion of fat.

E. *EFFECT OF SEROTONIN REPLACEMENT*

In these studies, 7 experiments were performed on 7 dogs. Reserpine was injected intramuscularly. 0.2 mg/kg. 24 hours before injecting intravenously 5-hydroxytryptophan (5-HTP), a serotonin precursor (20 mg/kg) simultaneously with the intraduodenal infusion of fat.

The data was analyzed by a paired t-test and statistical significance was inferred when the p-value was < 0.05

RESULTS

A. Effect of Histamine (Figure 2)

The intravenous infusion of histamine increased the gastric acid secretion from a baseline of 0.18 ± 0.03 mEq/15 minutes to a maximal secretion (peak) of 1.31 ± 0.1 mEq/15 minutes in 1½ hours which was maintained as a plateau for the remaining 2½ hours of the study.

B. *Effect of Fat* (Figure 2)

The intraduodenal administration of fat during maximal gastric acid secretion resulted in significant ($p < 0.05$) inhibition of gastric acid secretion. Maximal inhibition (59%) to 0.54 ± 0.7 mEq/15 minutes ($P < 0.001$) was reached in one hour.

C. *Effect of Serotonin Antagonist* (Figure 3)

When the intraduodenal administration of fat was preceded by intravenous UML 491 infusion, gastric acid secretion was inhibited only by 25% to 0.98 ± 0.22 mEq/15 minutes ($p < 0.2$), compared to the 59% inhibition which occurred without UML 491. The extent of interference with the inhibition was significant ($p < 0.01$)

D. *Effect of Serotonim Depletion* (Figure 4)

With serotonin depletion by reserpine, the maximal rate of gastric acid secretion increased by 31% from 1.31 ± 0.1 mEq/ 15 minutes

to 1.71±0.09 mEq/15 minutes ($p<0.05$). After the intraduodenal administration of fat, maximal gastric acid inhibition was only 19% from 1.71±0.09 mEq/15 minutes to 1.37±0.18 mEq/15 minutes; ($p<0.2$) as opposed to the 59% inhibition which occurred without reserpine.

E. *Effect of Serotonin Replacement* (Figure 5)

With serotonin depletion by reserpine, no significant inhibition of gastric acid secretion occurred after fat was introduced into the duodenum. Following the administration of 5-hydroxytriptophan (5-HPT), a serotonin precursor, there was a 40% inhibition of gastric acid secretion; from 1.71 ± 0.09 mEq/15 minutes to 1.02 ± 0.19 mEq/15 minutes ($p<0.02$).

DISCUSSION

There are four hormones, secretin, cholecystokinin, gastric inhibitory polypeptide and serotonin, which have been extracted from the duodenal mucosa and have proven to be gastric acid inhibitors.[3,7,17] Several evidences implicate serotonin in the mechanism of gastric acid inhibition. Serotonin (5-hydroxytryptamine) abounds in the alimentary tract where it is synthesized and stored in the form of granules in the enterochromaffin cells at the base of the gastric and intestinal gland.[5,13] The presence of serotonin in the enterochromaffin cells has been demonstrated with histochemical techniques and by autoradiography.[5,9] Serotonin synthesis from radioactively labelled 5-hydroxytryptophan also has been demonstrated to be confined to these enterochromaffin cells.[5]

There is good evidence which indicates that the introduction of fat into the duodenum inhibits gastric acid secretion by liberating hormones (enterogastrones) from the duodenal mucosa.[7,8] Johnson and Grossman evaluated two duodenal hormones, secretin and cholecystokinin, as mediators of gastric inhibition from Heidenhain pouches following intraduodenal fat administration. Although both secretin and cholecystokinin inhibited gastric secretion, their combined inhibitory effect was not equal to the degree of fat-induced inhibition. Accordingly, Johnson and Grossman concluded that duodenal fat might have released one or more yet unknown hormones, in addition to secretin and cholecystokinin, that could account for the additional inhibition.[7] Since GIP is only a weak inhibitor of gastric secretion, serotonin could well be the enterogastrone responsible for the remaining inhibition. Although the role of serotonin in the inhibition of gastric acid secretion by duodenal acidification has been studied, the role of serotonin on the gastric acid inhibition which follows the introduction of duodenal fat has not been previously explored.

In these experiments serotonin deficiency was created by injecting reserpine 24 hours before the study. Previous studies indicate that reserpine administration displaces serotonin granules from the enterochromaffin cells, and that 16 hours after reserpine administration the enterochromaffin cells are no longer demonstrable by histochemical techniques or by autoradiography.[1,5] This visual disappearance of serotonin granules was found to be accompanied by parallel quantitative reduction in the total and regional serotonin.[1,10] When the urinary end product of serotonin metabolism (5-hydroindoleacetoacetic acid) was measured before a reserpine administration, there was a transient significant increase following reserpine administration which corresponded to the extent and duration of serotonin depletion. When a second reserpine injection was repeated within 24 hours from the previous administration, there was no increase in urinary 5-hydroxyindoleacetoacetic acid.[14] The depletion of serotonin by reserpine was also documented by Gershon and Ross during their study of the effect of serotonin and anaphylactic shock.[4] Serotonin depletion was maximal 24 hours after reserpine administration. If rats were challenged before this time or if serotonin was replaced by injecting 5-hydroxytryptophan, the rats perished from shock.

In the present study, serotonin depletion by reserpine injection 24 hours before the study increased gastric acid secretion by 31% (p. 0.02) and almost completely abolished gastric acid inhibition when fat was introduced into the duodenum. Conversely, when the serotonin deficiency was corrected by injecting 5-hydroxytrptophan simultaneously with intraduodenal fat administration, gastric acid inhibition was restored (p. 0.02). In another set of studies, serotonin "deficiency" was produced by infusing 1-methyl-lysergic acid butanolamide (UML 491) which is a potent and specific serotonin antagonist. Gastric acid secretion increases following UML 491 administration in humans.[12] In the present study, UML491 significantly interfered with the inhibition of gastric acid secretion by intraduodenal fat (p. 0.02).

In addition to the present studies, other evidence indicates that serotonin may be an enterogastrone. The inhibition of gastric acidity during duodenal acidification has been found to be accompanied by a significant reduction in duodenal serotonin content

and by the degranulation of the enterochromaffin cells which is associated with the release of serotonin granules.[12] Following serotonin depletion by reserpine or antagonism by UML 491, the inhibition of gastric acid secretion which occurs after acidification of the duodenum is prevented.[17] Recently, Jaffe et al demonstrated that duodenal acidification elevated the level of serotonin in both peripheral and portal blood, and inhibited gastric acid secretion from gastric fisulae in dogs.[6]

Although secretin, cholecystokinin and the gastric inhibitory polypeptide have been suggested to be inhibitors of gastric secretion, these hormones require additional evidence before being labeled as the enterogastrones responsible for gastric acid inhibition by duodenal fat. Their release from the duodenum and their concentration in the portal circulation during duodenal acidification or intraduodenal fat administration should be demonstrated. The intestinal cells which synthesize these hormones have also not been identified.

The results of our study suggest that serotonin is at least partly responsible for the gastric acid inhibition by duodenal fat. Serotonin depletion has increased gastric acid secretion and has abolished gastric acid inhibition by intraduodenal fat. Serotonin antagonism by UML 491 has also significantly interferred with gastric acid inhibition.

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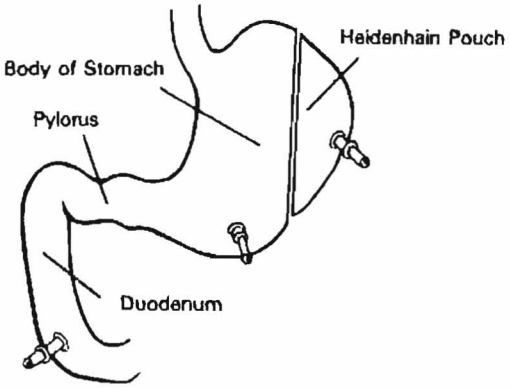

Figure 1 Experimental model

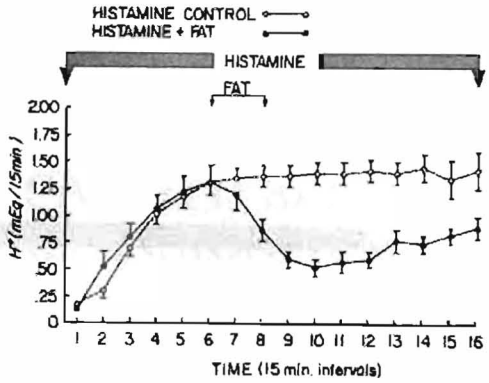

Figure 2 Effects of intraduodenal infusion of fat on histamine-stimulated gastric acid secretion.

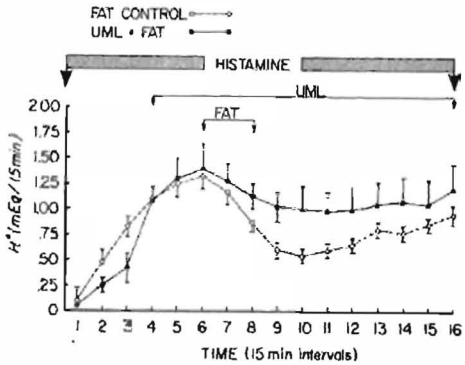

Figure 3 Effect of a serotonin antagonist (UML 491) on the inhibition of histamine-stimulated gastric acid secretion by intraduodenal fat infusion.

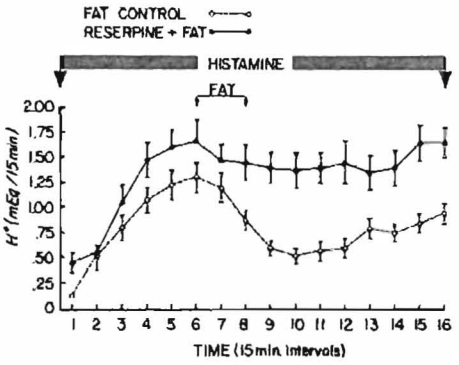

Figure 4 Effect of serotonin depletion by reserpine on the inhibition of histamine-stimulated gastric acid output by intraduodenal fat infusion.

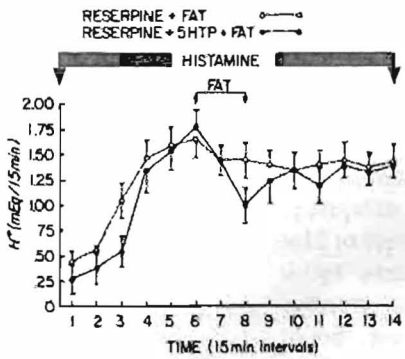

Figure 5 Effect of serotonin replacement by 5-hydroxytryptophan following serotonin depletion on the inhibition of histamine-stimulated gastric acid output by intraduodenal fat infusion.