The Effect of Glucagon on Enterokinase Secretion from Brunner's Gland Pouches in Dogs[1] (35941)

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(Introduced by A. L. Jones)

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Stening and Grossman (1) have demonstrated that both natural and synthetic secretin stimulate secretion from Brunner's gland pouches in dogs and cats. Secretin's other actions on the gastrointestinal tract include inhibition of gastric acid secretion (2), inhibition of gastric motility (3), and stimulation of bile flow (4). Glucagon has been shown to share these actions on the gastrointestinal tract and to inhibit secretin stimulated pancreatic secretion as well (5–8). Secretin has 27 amino acid residues and a molecular weight of 3056 (9), while glucagon has 29 amino acid residues and a molecular weight of 3482 (10). The structures of secretin and glucagon are similar having identical amino acid residues at 14 positions.

Jones and Hall (8) have recently shown that glucagon stimulates volume secretion in canine Brunner's gland pouches. The present study was carried out to determine the effect of glucagon on the secretion of enterokinase [enteropeptidase (EC 3.4.4.8)] by Brunner's gland pouches, an action that has not previously been studied.

Methods. Three mongrel dogs, weighing 14.5 to 18.2 kg, were prepared with duodenal pouches (Fig. 1) by constructing a double mucosal septum at the pylorus. The duodenum was transected proximal to the entry of the common bile duct and the proximal end was brought through an abdominal stab wound and sutured to the skin. The distal cut end was joined to the stomach by a side-to-end gastroduodenostomy.

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Experiments were begun 3 weeks following surgery. After an 18 hr fast the dogs were placed in sling harnesses and 0.15 *M* NaCl was infused through a polyethylene tube (PE 50) in a leg vein at 66 ml/hr with a calibrated peristaltic pump, (Harvard Apparatus Co., Millis, Mass.). After NaCl has been infused for four 15 min periods, glucagon (Eli Lilly, Indianapolis, Ind.) was added to the NaCl and infused at 64 μg/kg hr for eight 15 min periods. Glucagon was followed by four 15 min periods of NaCl infusion. Pouch secretion was collected in calibrated centrifuge tubes through plastic funnels strapped to the ventral surfaces of the dog abdomen. Care was taken to avoid contact between the funnel and the pouch mucosa. Volumes of secretion were measured to the nearest 0.1 ml. Specimens with volume less than 0.5 ml were diluted with known volume of distilled water. All specimens were then centrifuged at 27,000*g* at 4° for 20 min and the supernatants were frozen until assayed.

Enterokinase was assayed by a modification of the method of Kunitz (11). One half milliliter samples were incubated at 25° with 0.1 ml of bovine trypsinogen (1 mg/ml in 0.001 *N* HCl), and 3.8 ml of buffer (0.01 *M* Tris maleate, pH 5.8). After 15 min incubation, a 0.1 ml aliquot was taken and immediately assayed for tryptic activity using the method of Hummel (12). The aliquot was rapidly added to a spectrophotometric cuvette containing 0.3 ml of substrate (0.01 *M* *p*-toluene-sulfonyl-L-methyl ester) and 2.7 ml of buffer (0.046 *M* Tris maleate, pH 8.1, containing 0.0115 *M* $CaCl_2$). The rate of change in absorbance was recorded on a Gilford recording spectrophotometer at 240 mμ. Enterokinase activity was expressed as 1 unit

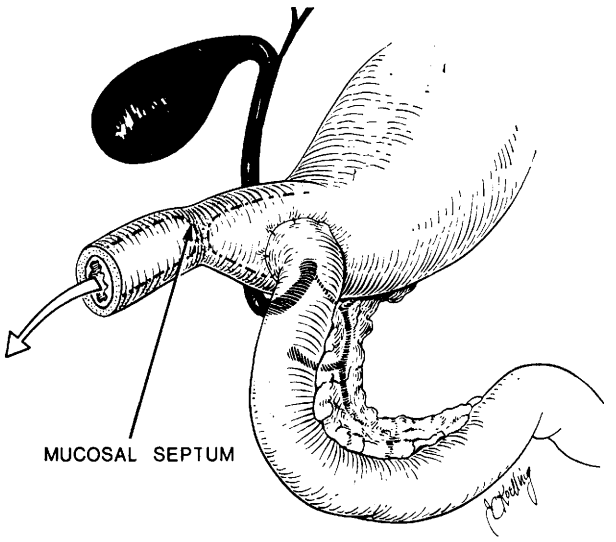

FIG. 1. A schematic drawing of duodenal pouches with double mucosal septum in dogs.

equal to the activity converting 1 μg of trypsinogen to trypsin/min at 25°. Protein was assayed with the method of Lowry *et al.* (13). A linear relationship was observed between the increasing amount of enterokinase and the rate of change of absorbance when duodenal juice was used as a source of the enzyme. No free trypsin activity could be detected in any of the samples studied.

Results. During NaCl infusion, pouch secretion averaged 0.2 ml/15 min (Fig. 2). The volume rose during the first 15 min of glucagon infusion and remained elevated during the 2 hr of glucagon infusion. The mean volume during glucagon infusion was 1.2 ml/15 min. During NaCl infusion following glucagon, volume returned to the preglucagon level.

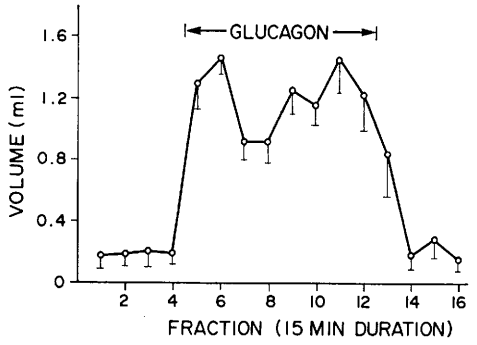

FIG. 2. Volume response of Brunner's gland pouches to glucagon infusion. Each point in Figs. 2–5 represents the mean of eight experiments in 3 dogs; the vertical bars are the standard errors of the mean.

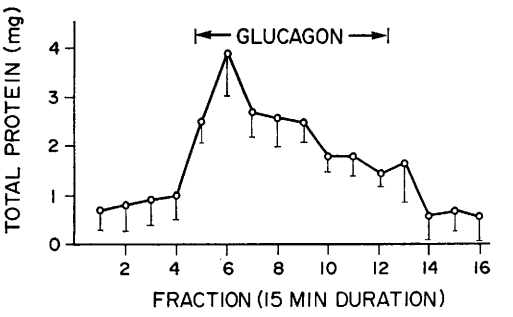

FIG. 3. Total protein output in Brunner's gland pouch secretion during glucagon infusion.

Protein output before glucagon infusion averaged 0.9 mg/15 min. During glucagon infusion, protein output rose to a peak of 3.9 mg/15 min, 30 min after starting glucagon (Fig. 3). Total protein output remained elevated above the NaCl control level throughout glucagon infusion, although later values were significantly less than the peak value. Protein output returned to control level during postglucagon NaCl infusion.

Mean enterokinase output was 0.9 units/15 min during NaCl infusion (Fig. 4). During the first 15 min of glucagon infusion enterokinase output rose to a peak value of

TABLE I. Comparison of Total Volume Output and Concentrations of Protein and Enterokinase in Brunner's Gland Pouch Secretion During the Periods of Glucagon Administration and of Pre- and Postglucagon Administration.

	Mean ± SE (15 min fractions)		
	Preglucagon	Glucagon	Postglucagon
Vol (ml)	0.2 ± 0.0	1.3 ± 0.1	0.4 ± 0.1
Total enterokinase (units)	0.9 ± 0.3	11.6 ± 1.5	0.8 ± 0.3
protein (mg)	0.9 ± 0.2	2.4 ± 0.2	0.9 ± 0.3
Enterokinase sp act (units/mg)	2.3 ± 0.2	8.3 ± 1.1	1.8 ± 0.3
conc (units/ml)	6.1 ± 1.6	8.6 ± 1.1	2.1 ± 0.4
Protein conc (mg/ml)	3.9 ± 0.3	2.1 ± 0.2	2.4 ± 0.3

21.6 units/15 min. A second, though lesser peak, occurred in fraction 11. Mean output during glucagon infusion was 11.6 units/15 min. Output returned to control level during postglucagon NaCl infusion. During NaCl infusion, mean enterokinase specific activity was 2.3 units/mg of protein. Specific activity reached its initial peak in the first 15 min of glucagon infusion, then rose to a second larger peak in fraction 11 (Fig. 5). Mean specific activity during glucagon infusion was 8.3 units/mg of protein. Specific activity returned to the control level during the NaCl infusion following glucagon. These results are summarized in Table I.

Discussion. Duodenal secretion is influenced by various stimuli. Wright and his coworkers (14) increased duodenal secretion in decerebrate and decapitated cats by stimulating the vagus nerve. They also observed that atropine abolished the secretory response to vagal stimulation. Ponomarew (15) first observed that feeding stimulated duodenal secretion and produced increased duodenal secretion by acidification of the duodenum. Florey and Harding (16) further demonstrated that a humoral agent, possibly secretin, was involved in Brunner's gland secretion by showing that feeding stimulated both denervated and transplanted cat duodenal pouches. Recently Stening and Grossman (1) have stimulated secretion from isolated duodenal pouches with histamine, gastrin II, cholecystokinin-pancreozymin, and cerulein, as well as with secretin. The results of the present experiment confirms our previous report that glucagon is also a stimulant of duodenal secretion (8). However, it cannot be stated from this study whether endogenously released glucagon will stimulate duodenal secretion or whether the response observed in this study is a pharmacologic effect. The dose of glucagon used in this experiment, 64 μg/kg hr, was chosen because a previous study (8) showed that it was the maximal

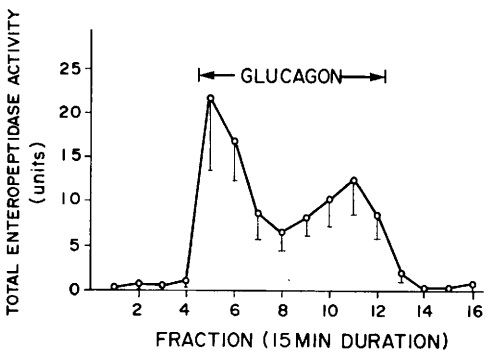

FIG. 4. Total enterokinase output in Brunner's gland pouch secretion during glucagon infusion.

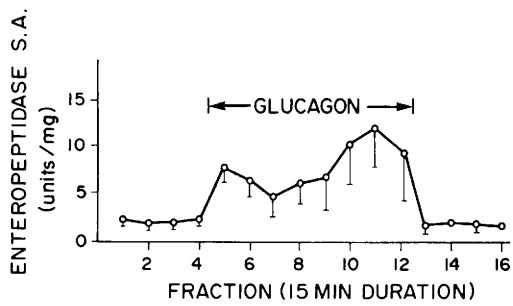

FIG. 5. Specific activity (sp act) of enterokinase in Brunner's gland pouch secretion during glucagon infusion.

dose for Brunner's gland secretion in dogs.

Ponomarew (15) found protease, lipase, amylase, sucrase, and enterokinase activities in duodenal secretion. Wright *et al.* (14) proposed that only amylase and enterokinase were secreted, while the other enzymes were present from autolyzed and desquamated cells. Recently enterokinase activity was reported to be localized in the surface epithelial cells but not in the crypt cells or in the cells of the Brunner's gland in the rat (17). Furthermore, it has also been reported that a significant amount of enterokinase activity was present in the purified brush border membranes of the guinea pig small intestinal mucosa (18). It has yet to be established, however, that enterokinase is exclusively localized in the brush border membrane. The increased enterokinase activity in the duodenal pouch secretion following glucagon administration observed in this study may be due to either the direct release by glucagon of membrane bound enterokinase into the lumen or the increased volume output induced by glucagon washing out the enzyme from the membrane or from the surface mucus coatings. The pattern of glucagon stimulated output of enterokinase, a double-peaked response (Fig. 4), resembles the duodenal output of pepsin as measured by Cooke and Grossman (19).

Conclusion. The effects of glucagon on Brunner's gland secretion were studied in three mongrel dogs with pouches of the proximal duodenum. A continuous intravenous injection of glucagon given in a dose that produced maximal volume output caused sustained increases in volume, total protein, and enteropeptidase secretion from the pouches.

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