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# The Contribution of the Distal Gastrointestinal Tract to Glucagon-like Immunoreactivity Secretion in the Rat (39464)

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Gut glucagon-like immunoreactivity (GLI) is released after large enteric loads of glucose (1-3), fats (5), and salts (4) and it has generally been assumed that its function is related to the disposal of these absorbed nutrients. Studies of its distribution in the tissues of the gastrointestinal tract, however, show that the highest concentrations are found in the distal portions of the tract (6-8). In the dog (9) and the cat[2] the release of GLI occurs from the ileum and colon, respectively, but not from the jejunum. Since it seems unlikely that a hormone involved primarily with nutrients absorbed from the proximal gut would be secreted mainly by the distal gut, it was decided that further investigation of the role of the distal intestine in GLI secretion was merited. The purpose of the study reported here was to evaluate the contribution of the ileum and colon to glucose stimulated GLI secretion in a third common laboratory species, the rat.

*Methods.* Thirty rats weighing 400-500 g were studied after an overnight fast. Anesthesia was induced by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). A laparotomy was performed. In studies in which the entire gut or only the upper gut was to be stimulated, a ligature was placed around the pylorus to prevent reflux and a 20% glucose solution was instilled over a 5-min period directly into the duodenum through a 25-gauge needle. The volume administered was adjusted to deliver 2 g of glucose/kg body weight. In studies in which only the upper gut was to be stimulated, a ligature was also placed 45 cm proximal to the cecum (the approximate mid point of the jejunum-ileum) and a drain was inserted on the proximal side. In studies in which only the lower gut was to be stimulated, the preparation was the same as for upper gut stimulation except that the glucose was instilled directly into the ileum. Blood samples were collected from a polyethylene cannula inserted in a femoral artery. Samples were collected just prior to and at 30-min intervals for 90 min after the introduction of glucose into the gut lumen. After immediately removing the plasma from each sample, the red blood cells were diluted 1:2 with normal saline and returned to the rat through a polyethylene cannula in a femoral vein. In control experiments the animal was prepared as for stimulation of the entire gut except that nothing was introduced into the lumen during sample collection. A 45-min period was allowed to pass between the time all cannulas, drains, and ligatures were placed and the start of the experiment. The animal's body temperature was maintained at 37-38° with the aid of a heating pad.

Blood samples of 1 ml volume for GLI determination were collected by syringe and placed into chilled 10 × 75-mm glass tubes containing 0.05 ml of Trasylol (FBA Pharmaceuticals, New York, New York) and 0.01 ml of sodium heparin (50 units). The samples were centrifuged immediately at 4°. The resulting plasma was stored at −20° until analyzed.

Acid-ethanol extracts of whole gut wall for assay of GLI were prepared from pooled tissues of four unfasted rats. Segments of midduodenum, midjejunum, midileum, midcolon, and pancreas were excised immediately after each rat was killed by decapitation. The tissues were rinsed in cold normal saline, blotted lightly and stored at −20° until extracted and purified by the method of Kenny (10).

The GLI concentration of samples of plasma or intestinal extracts was measured by radioimmunoassay (11) using a nonspe-

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[2] Unpublished observations.

cific glucagon antiserum raised in rabbits against beef–pork glucagon coupled to polyvinylpyrrolidone (12). Free glucagon was separated from antibody-bound glucagon with dextran coated charcoal (13). Beef–pork recrystallized glucagon (a gift of Dr. Mary Root, The Lilly Research Laboratories, Indianapolis, Indiana) was used for standards and for preparation of [$^{125}$I]glucagon by the chloramine T method of Greenwood and Hunter (14). In this assay system dilution of gut extracts and dilution of pancreatic glucagon gave parallel inhibition curves; dilution of serum, however, did not. Therefore, all serum samples were assayed at the same dilution (1:6).

The statistical significance of GLI responses to glucose stimulation was determined by Student's $t$ test applied to the difference between the response of an experimental group and that of the control group or another experimental group at designated times after the introduction of glucose. Until purified gut GLI is available for use in the radioimmunoassay, immunoassays of gut GLI using pancreatic glucagon as standard are useful only for revealing relative GLI levels, not absolute concentrations. Therefore, GLI responses here are expressed as percent of each animal's basal GLI concentration. The mean (±SEM) basal GLI concentration for all experiments was 608 ± 39 pg/ml.

*Results.* The concentrations of GLI in tissues of the gastrointestinal tract of the rat are shown in Table I. The lowest concentration was 14.4 ng/g wet weight in the duodenum. The jejunum had 77.6 ng/g. The highest gut concentrations were in the ileum, 213 ng/g, and the colon, 190 ng/g. The pancreas contained 798 ng/g.

The peripheral plasma GLI responses to the intraduodenal administration of 20% glucose, 2 g/kg, are shown in Fig. 1. When the entire gut was exposed to glucose, plasma GLI increased to a mean of 173% of basal at 30 min after glucose introduction, and remained elevated for the duration of the experiment. The response was statistically significant at all times tested ($p < 0.05$ at 30 min, $p < 0.02$ at 60 min, $p < 0.005$ at 90 min). When, however, the ileum and colon were excluded from contact with the glucose solution by a ligature and a drain placed at the midjejunum-ileum, peripheral plasma GLI increased to a mean of only 138% of basal at 30 min, remained at this level at 60 min, and then decreased slightly to 130% of basal at 90 min. The GLI response was still, however, significantly different from the control group at all times tested ($p < 0.025$ at 30 min, $p < 0.05$ at 60 min, and $p < 0.02$ at 90 min).

Since it appeared that over half of the GLI rise seen after intraduodenal glucose resulted from stimulation of the distal gut, the effect of intraluminal glucose in only the distal gut was examined. These results are shown in Fig. 2. Following the introduction into the ileum of 20% glucose, 2 g/kg, the peripheral plasma GLI concentration increased to a maximum of 163% of basal at 60 min. The response was statistically significant at all times tested ($p < 0.05$ at 30, $p < 0.01$ at 60, and $p < 0.01$ at 90 min). The GLI response to glucose in only the upper gut shown in Fig. 1 is repeated in Fig. 2 to facilitate comparison with the response to

TABLE I. CONCENTRATION OF GLI IN GASTROINTESTINAL TISSUES OF THE RAT.[a]

| Tissue | [GLI] ng/g wet weight |
|---|---|
| duodenum | 14.4 |
| jejunum | 77.6 |
| ileum | 213 |
| colon | 190 |
| pancreas | 798 |

[a] Each sample analyzed was an acid ethanol extract of 1 g of tissue obtained by pooling 0.25 g pieces of tissue from four rats. Mean of four determinations on each sample.

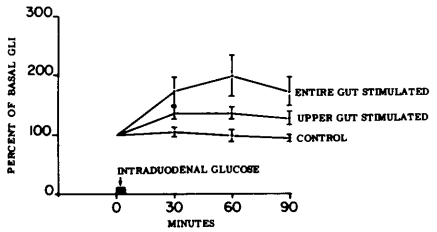


FIG. 1. Mean responses of peripheral plasma GLI concentrations, expressed as percent of basal concentration, to no stimulation (control, $N = 4$), to stimulation of only the upper gut ($N = 10$), and to stimulation of the entire gut ($N = 8$) by the intraduodenal instillation of 2 g/kg body weight of 20% glucose. The vertical lines indicate 1 SEM.

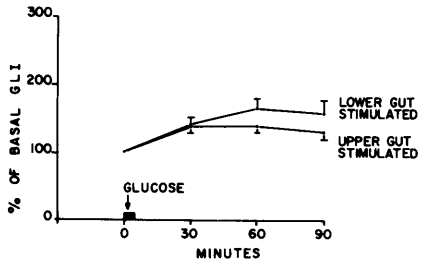


FIG. 2. Mean responses of peripheral plasma GLI concentration, expressed as percent of basal concentration, to stimulation of only the upper gut ($N = 10$) and only the lower gut ($N = 8$) by the intraluminal instillation of 2 g/kg body weight of 20% glucose. The vertical lines indicate 1 SEM.

glucose in only the lower gut. Glucose in the lower bowel resulted in a slightly greater GLI release at 60 and 90 min than did glucose in the upper bowel but the difference was not statistically significant.

*Discussion.* This study shows that GLI is present throughout the intestinal tract of the rat with the highest concentrations occurring in the distal portion of it. A similar distribution has been reported by Unger *et al.* (15) and Schopman *et al.* (8) in man, by Buchanan *et al.* (17) in the dog, by Bloom and Bryant (7) in the rat and baboon, and by Bataille *et al.* (16) in the pig. Immunofluorescent staining techniques show that enteroglucagon producing cells exist primarily in the ileum of man (6) although the colon was not examined in this study. The rat, therefore, seems to be a typical species with reference to its pattern of distribution of GLI in tissue.

This study further shows that in the rat, as in man (1, 2) and the dog (3, 17), enteric glucose stimulates the secretion of GLI *in vivo*. This finding supports the results of Luyckx and Lefebvre (18) who found release of GLI from rat jejunum incubated with 20% glucose *in vitro*. Zandomeneghi and Buchanan (19) also demonstrated GLI release from rat small intestine incubated with glucose solutions *in vitro*. The results reported here indicate that both the proximal and distal intestine are involved in glucose stimulated GLI secretion, with each contributing approximately equal portions of the total response when large glucose loads are introduced into the duodenum.

When glucose was placed directly into the ileum so that only the ileum and colon were exposed to it the observed GLI response was essentially the same as that seen when only the duodenum and jejunum were stimulated by intraluminal glucose. The distal and proximal gut of the rat therefore release equal amounts of GLI when both are exposed to the same glucose load. This observation is somewhat surprising in view of the fact that greater concentrations of GLI are extracted from the ileum and colon than from the duodenum or jejunum. Matsuyama and Foa (9) have shown that, in the dog, stimulation of ileal loops with 5% glucose solutions elicited a marked rise in GLI in the venous effluent of the loop, whereas identical stimulation of jejunal loops produced no significant change in GLI concentration. In the cat it has been shown that the colon secretes GLI in response to intraluminal glucose but the jejunum and ilem do not.[2]

In three different species, therefore, GLI secretion from the distal intestinal tract equals or exceeds that from the proximal tract when both parts are equivalently stimulated. If a similar situation exists in man, it could explain the hypersecretion of gut GLI following oral glucose reported in gastrectomized persons (20–22). It could also explain the excessive GLI responses to alimentary glucose described in patients with dumping syndrome (23, 24). In both types of subjects a larger portion of an ingested glucose load would be expected to reach the distal intestine than would reach it in normal persons. Bloom (25) in fact has hypothesized that gut GLI release from the ileum may explain the period of hypomotility which follows the initial rapid transport of substances through the small bowel in subjects with dumping syndrome. That gut GLI may cause hypomotility was suggested by the pronounced stasis of the large and small bowel seen in a patient with an enteroglucagon secreting tumor (26, 27). The stasis remitted when the tumor was removed. The physiological role of gut GLI is purely speculative at this point. GLI's presence in and secretion from the distal gut, however, are factors which should be considered in future research on this problem.

*Summary.* Radioimmunoassay of acid ethanol extracts of the rat intestinal tract showed the presence of glucagon-like immunoreactivity (GLI) from the duodenum to the colon with maximal concentrations in the ileum and colon. Twenty percent glucose instilled in the duodenum at a dose of 2 g/kg body weight stimulated a twofold increase in peripheral plasma GLI concentration. When the instilled glucose load was restricted to only the duodenum and jejunum, or to only the ileum and colon, the increase in peripheral plasma GLI was approximately half that seen when the entire gut was exposed to the glucose. It is concluded that the distal gut as well as the proximal gut releases GLI in the rat.

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