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### Research Article

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To determine if stimulation of GLI release by substances other than calcium would lower serum calcium, glucose was administered intraduodenally. Despite a marked increase in GLI, plasma calcium fell only 9%, a decline which could be entirely accounted for by hemodilution.

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# The Effect of Calcium and Other Salts upon the Release of Glucagon-Like Immunoreactivity from the Gut

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**ABSTRACT** It has been suggested that glucagon-like immunoreactivity (GLI) of gastrointestinal tissues might, like pancreatic glucagon, have calcium-lowering activity. Studies were designed, therefore, to determine if calcium absorption was associated with GLI release from the gut. The intraduodenal administration of 4.5 mmoles of calcium chloride per kg of body weight to conscious dogs was associated with a prompt rise in plasma GLI from a base line of 2.2 ng/ml ( $\text{SEM} \pm 0.2$ ) to a peak of 4.3 ng/ml ( $\text{SEM} \pm 0.3$ ) at 45 and 60 min, in association with a rise of plasma calcium from 8.6 to 10.4 mg/100 ml. Neither pancreatic glucagon, insulin, nor glucose changed. Smaller calcium loads had progressively diminishing effects on GLI release. Calcium lactate also appeared to stimulate effectively GLI release. Both magnesium chloride and sodium chloride given intraduodenally were associated with a significant though modest increase in GLI.

To determine if stimulation of GLI release by substances other than calcium would lower serum calcium, glucose was administered intraduodenally. Despite a marked increase in GLI, plasma calcium fell only 9%, a decline which could be entirely accounted for by hemodilution.

Although the physiologic significance of this demonstration that the absorption of calcium salts is associated with GLI release is open to serious question, the findings are not incompatible with the concept that glucagon-like polypeptides are released from the gut during the absorption of certain salts, possibly to alert appropriate homeostatic regulators so as to avoid major changes in electrolyte concentration after the ingestion of large salt loads.

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## INTRODUCTION

Extracts of the upper gastrointestinal tract contain polypeptides which react to a varying degree with antisera against pancreatic glucagon (1-3). Because they appear to differ in certain respects from pancreatic glucagon (4-6) these substances have been referred to as "glucagon-like immunoreactivity" (GLI)<sup>1</sup> (4) or "enteroglucagon" (7) so as to differentiate them from true pancreatic glucagon. Although physiologic functions of GLI have not been identified as yet, it has generally been assumed that any such role would be in the sphere of carbohydrate metabolism.

Not until the demonstration by Paloyan, Paloyan, and Harper of the hypocalcemic activity of pancreatic glucagon (8), which, according to Birge and Avioli (9), is the result of calcitonin release, was it suggested by both Potts and Deftos<sup>2</sup> and by Grey and Munson<sup>2</sup> that GLI might be involved in calcium homeostasis, perhaps as a factor in the thyrocalcitonin response to ingested calcium. For these reasons, studies were designed to determine if the intestinal absorption of calcium is accompanied by an increase in the release of GLI.

## METHODS

Healthy mongrel dogs weighing between 13 and 28 kg were employed in these studies. 2 days or more before an experiment a polyethylene catheter was inserted through the jugular vein into the inferior vena cava between the heart and liver, and another was passed through a midline incision into the duodenum. After full recovery of the dog from the surgery, the experiment was performed. Dogs were fully conscious. Salt solutions, adjusted to pH 8, were instilled into the duodenum over a 15 min period; 25% of the dose was given rapidly and the remaining volume was administered at a constant rate over the remainder of the

<sup>1</sup> Abbreviation used in this paper: GLI, glucagon-like immunoreactivity.

<sup>2</sup> Personal communication.

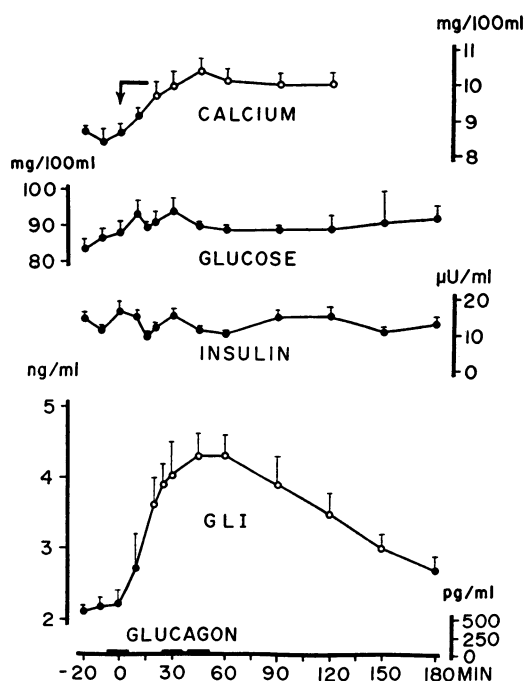


FIGURE 1 The effect of intraduodenal administration of calcium chloride (4.5 mmol/kg) on plasma GLI, glucagon, insulin, and glucose. Open circles represent statistically significant changes from the base line values.

period. 5-ml blood samples for glucose, glucagon, and insulin determinations were obtained from the inferior vena cava in heparinized plastic syringes and transferred into chilled tubes containing 5000 U of Trasylol.<sup>8</sup> Specimens were immediately centrifuged at 4°C. Plasma glucose was determined immediately by the Hoffman method (10) using the Technicon Autoanalyzer.<sup>4</sup> Serum calcium was determined by atomic absorption spectrophotometry. The remainder of the plasma was stored at -20°C for later GLI, glucagon, and insulin determinations.

Plasma GLI was measured by means of a previously described (11) radioimmunoassay using antiserum 78J, which cross-reacts strongly with GLI. Pancreatic glucagon was measured by the same technique employing antiserum 30K, which is highly specific for pancreatic glucagon. Insulin was measured by the radioimmunoassay method of Yalow and Berson (12) as modified by Herbert, Lau, Gottlieb, and Bleicher (13).

## RESULTS

*Effect of intraduodenal calcium chloride upon GLI release.* To determine the effect of calcium absorption on GLI release, 4.5 mmol of calcium chloride per kg of body weight, estimated to be about 39% more than the daily calcium requirements for adult dogs (14), was administered as a 0.15 M solution intraduodenally to six dogs.

<sup>8</sup> FBA Pharmaceuticals, Inc., New York.

<sup>4</sup> Technicon Co., Inc., Tarrytown, N. Y.

As depicted in Fig. 1, mean plasma calcium rose from a baseline value of 8.6 mg/100 ml ( $SEM \pm 0.2$ ) to a peak of 10.4 mg/100 ml ( $SEM \pm 0.4$ ) at 45 min. This was accompanied by a prompt rise in the plasma GLI concentration from a base line of 2.2 ng/ml ( $SEM \pm 0.2$ ) to a peak of 4.3 ng/ml ( $SEM \pm 0.3$ ) at 45 and 60 min. These increments were statistically highly significant ( $P < 0.01$ ) between 20 and 120 min. Neither pancreatic glucagon, insulin, nor glucose changed from base line values.

To determine the effect of smaller calcium loads upon GLI release, additional experiments were conducted using 2.25 mmol of calcium chloride per kg of body weight, 31% less than the daily calcium requirements, and 0.45 mmol per kg of body weight, which is only 14% of the daily requirements. As shown in Fig. 2, in a group of six dogs the 2.25 mmol/kg dose was associated with a rise in mean serum calcium from 8.6 mg/100 ml ( $SEM \pm 0.1$ ) to a peak of 9.2 mg/100 ml ( $SEM \pm 0.2$ ) at 30 min. GLI rose from a base line level of 2.0 ng/ml ( $SEM \pm 0.5$ ) to a peak of 3.2 ng/ml at 60 min, but this was not statistically significant. However, the increase was statistically significant at 75 min ( $P < 0.02$ ) and at 90 min ( $P < 0.05$ ). Again, neither glucose, insulin, nor pancreatic glucagon changed.

The administration of the 0.45 mmol/kg dose to a group of seven dogs was not accompanied by a change in the mean levels of serum calcium, GLI, insulin, or glucose.

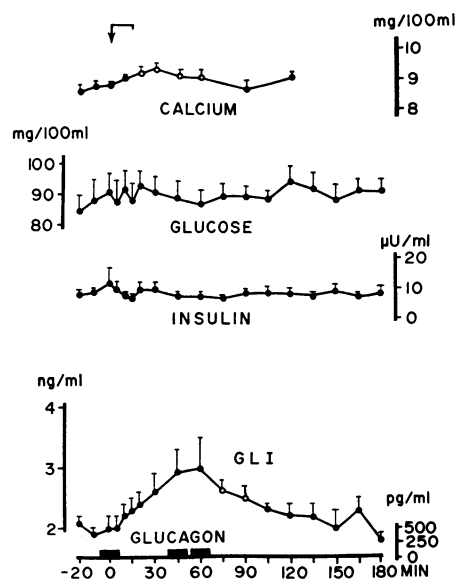


FIGURE 2 The effect of intraduodenal administration of calcium chloride (2.25 mmol/kg) on plasma GLI, glucagon, insulin, and glucose. Open circles represent statistically significant changes from the base line values.

**Effect of organic calcium salt on GLI release.** To determine if an organic calcium salt would similarly effect GLI release, 4.5 mmoles of calcium lactate were administered to a group of three dogs. Plasma calcium rose from 8.7 mg/100 ml ( $\text{SEM} \pm 0.2$ ) to a peak of 11.3 mg/100 ml ( $\text{SEM} \pm 0.2$ ) at 120 min. GLI increased from 1.4 ng/ml to a peak of 2.6 ng/ml at 90 min. The increase was statistically significant at 60 min ( $P < 0.05$ ), 90 min ( $P < 0.02$ ), and at 120 min ( $P < 0.05$ ). Plasma insulin, glucose and pancreatic glucagon did not change. The results are depicted in Fig. 3.

**Effect of magnesium chloride on GLI release.** To determine the effect of another divalent cation upon GLI release, magnesium chloride was administered intraduodenally in a dose of 4.5 mmoles/kg in a group of six dogs as a 0.15 M solution. As shown in Fig. 4 GLI rose from a base line level of 1.8 ng/ml ( $\text{SEM} \pm 0.2$ ) to a peak of 2.8 ng/ml ( $\text{SEM} \pm 0.1$ ) at 90 min and remained elevated throughout the experiment. These changes were significant between 10 and 135 min ( $P < 0.02$ ). Plasma glucose rose significantly from a base line of 93 mg/100 ml ( $\text{SEM} \pm 4$ ) to 102 ( $\text{SEM} \pm 5$ ) at 20 min ( $P < 0.05$ ) and 100 mg/100 ml ( $\text{SEM} \pm 4$ ) at 30 min ( $P < 0.001$ ). Neither insulin nor pancreatic glucagon changed.

**Effect of sodium chloride on GLI release.** To determine the effect of other salts upon GLI release, 4.5 mmoles/kg of sodium chloride as a 0.15 M solution was administered intraduodenally to a group of six dogs.

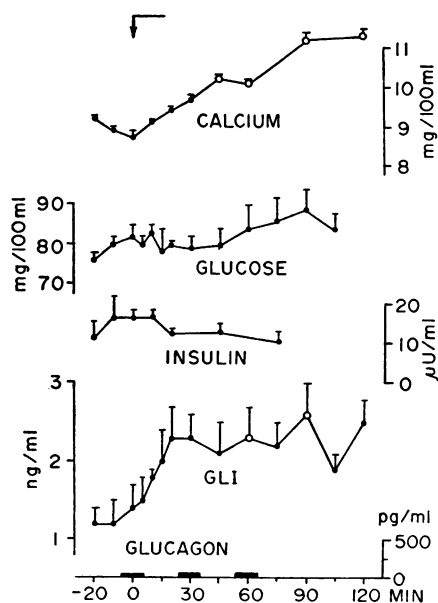


FIGURE 3 The effect of intraduodenal administration of calcium lactate (4.5 mmoles/kg) on plasma GLI, glucagon, insulin, and glucose. Open circles represent statistically significant changes from the base line values.

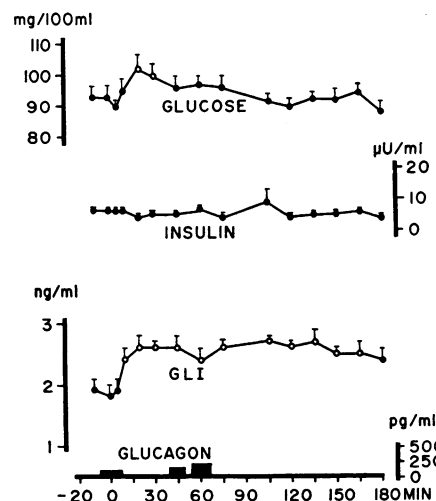


FIGURE 4 The effect of intraduodenal administration of magnesium chloride (4.5 mmoles/kg) on plasma GLI, glucagon, insulin, and glucose. Open circles represent statistically significant changes from the base line values.

GLI increased significantly from a base line of 1.9 ng/ml ( $\text{SEM} \pm 0.1$ ) to peaks of 2.6 ng/ml ( $\text{SEM} \pm 0.2$ ) at 20 min ( $P < 0.005$ ) and 2.6 ng/ml ( $\text{SEM} \pm 0.3$ ) at 30 min ( $P < 0.05$ ). Neither insulin nor pancreatic glucagon changed (Fig. 5A). A dose of 9.0 mmoles/kg of sodium chloride given as a hypertonic 0.3 M solution to a group of four dogs had a still greater effect on GLI (Fig. 5B). GLI rose from a base line value of 2.8 ng/ml ( $\text{SEM} \pm 0.4$ ) to a peak of 4.4 ng/ml ( $\text{SEM} \pm 0.7$ ) at 15 min and remained above base line throughout the experiment. Neither insulin, glucose, nor pancreatic glucagon changed.

**Effect of hypotonic calcium chloride on GLI release.** The fact that a rise in plasma GLI was observed after the administration of three different salt solutions suggested that the water or the volume of fluid introduced into the gut might have induced the response. For this reason, the effect upon GLI release of the intraduodenal administration of an identical volume of hypotonic  $\text{CaCl}_2$ , 30 ml/kg as a 0.015 M solution, was studied in seven dogs. No change in GLI concentration was observed in any dog.

**Effect of amino acid solution on GLI release.** Because the intraduodenal administration of every salt and sugar (15) solution thus far tested was associated with a rise in GLI, it seemed possible that the effect was the nonspecific consequence of solute absorption. For this reason the 10 amino acid solution of Floyd, Fajans, Conn, Knopf, and Rull (16) was administered to a group of eight dogs. No change in GLI was noted.

**Effect of glucose-induced GLI release upon plasma calcium levels.** If GLI release is of physiologic importance in calcium homeostasis, it seemed possible that

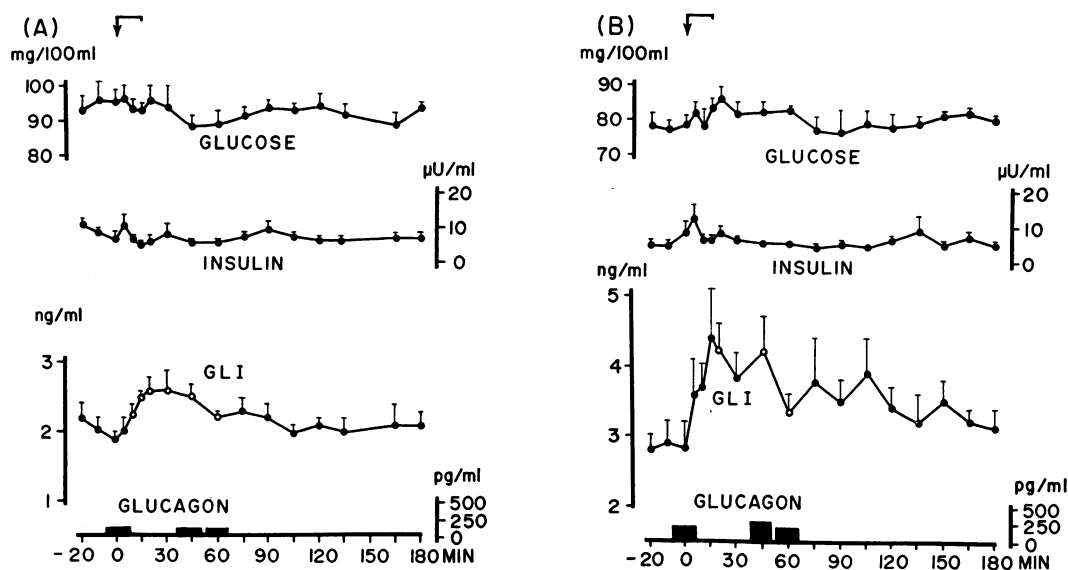


FIGURE 5 The effect of intraduodenal administration of 4.5 mmoles/kg of sodium chloride (A) and 9 mmoles/kg (B) on plasma GLI, glucagon, insulin, and glucose. Open circles represent statistically significant changes from the base line values.

high levels of endogenous GLI might lower serum calcium. Since glucose is the most potent known stimulus of GLI release (15), serum calcium was measured before and after the intraduodenal administration of 2

g/kg of glucose as a 5% solution in a group of six dogs. As shown in Fig. 6, as plasma glucose rose from 92 mg/100 ml to 196 mg/100 ml, plasma GLI rose from a base line level of 2.8 ng/ml ( $\text{SEM} \pm 0.3$ ) to a peak of 10.7 ng/ml ( $\text{SEM} \pm 2$ ) at 60 and 90 min. Plasma calcium decreased 9% from 7.2 mg/100 ml ( $\text{SEM} \pm 0.1$ ) to a nadir of 6.5 mg/100 ml ( $\text{SEM} \pm 0.3$ ) at 75 min. However, plasma protein, measured to monitor the effect of hemodilution, changed in parallel with the calcium and showed a maximal decrease of 9% at 75 min, suggesting that the observed decrease in plasma calcium can be attributed to hemodilution rather than to an effect of increased GLI levels upon calcium homeostasis.

## DISCUSSION

These studies indicate that the intraduodenal administration of both calcium chloride and calcium lactate in extremely large doses is associated with a rise in GLI which coincides in general with the rise in calcium. These findings would be compatible with the notion that a gastrointestinal polypeptide resembling pancreatic glucagon with respect to both immunologic and thyrocalcitonin-stimulating activities, is released during calcium absorption to alert the parafollicular cells of an impending influx of calcium so that they can respond early enough to prevent a major perturbation of calcium homeostasis. The concept of a "gastroentero-parafollicular axis" is not an unattractive one and recently Cooper, Schwesinger, Mahgoub, and Ontjes (17) have suggested that gastrin may participate in such a system. Equally plausible, however, is the possibility

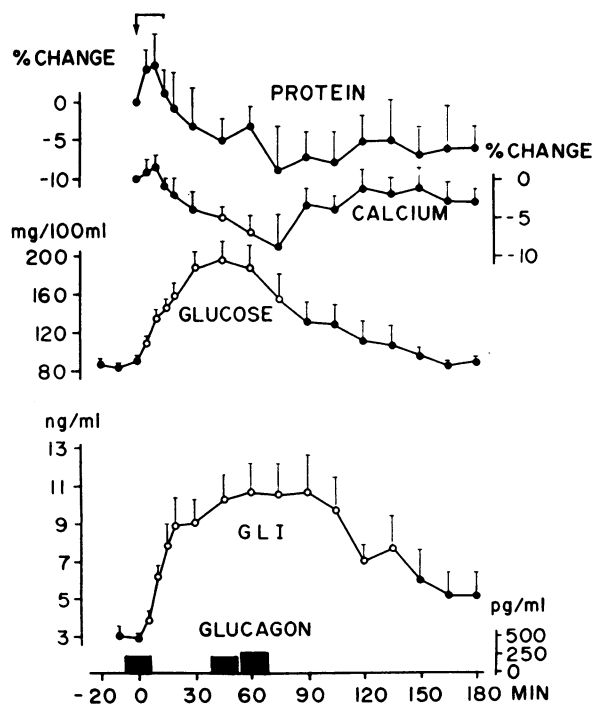


FIGURE 6 The effect on serum calcium of increased plasma levels of GLI stimulated by the intraduodenal administration of glucose (2 g/kg). Open circles represent statistically significant changes from the base line values.

that GLI might modify calcium homeostasis by suppressing parathormone secretion, reducing intestinal absorption of calcium, or increasing its excretion.

However, the specificity of calcium-induced release of GLI in physiologic terms is open to challenge. Calcium is well-known to influence the release of many hormones in a manner which cannot be regarded as physiologically significant. Moreover, the fact that every one of five absorbable monosaccharides tested, glucose, galactose, fructose, xylose, and mannose (17), and at least two salts other than calcium, magnesium chloride and sodium chloride, also produced apparent small increases in GLI release, raises serious doubt concerning the significance and meaning of a rise in plasma GLI during intestinal absorption. And the failure of increased levels of GLI stimulated by glucose absorption to cause a clear-cut reduction in serum calcium mitigates against the participation of GLI in calcium homeostasis, at least on the short-term basis of the glucose experiments.

On the other hand, these doubts do not necessarily exclude the possibility that increments in plasma GLI reflect a physiologically important part of the complex endocrine function of the gut, the largest and perhaps most versatile endocrine organ of the body. The endocrine functions of the gut can be divided on teleologic grounds into at least two categories: first, hormonal influence over components of the digestive organs themselves so as to promote optimal digestion and absorption of ingested substances; and, second, hormonal influence over the remote regulators of the homeostasis of these substances after they have entered circulation. According to the latter concept, endocrine cells within the gastrointestinal tract would have responsibility for reducing the likelihood of major homeostatic perturbation as a consequence of a massive influx of an ingested substance by providing early warning to the appropriate endocrine regulator of that substance as to the magnitude of the incoming load. A specific gut hormone would be released by a given substance in proportion to the amount of the substance ingested and elicit a prompt and appropriate discharge of the hormone which regulates that substance's concentration, and thus prevent an undesirably large increase in its extracellular concentration.

In the case of a protein load, it has been proposed that pancreozymin provides an early stimulus to insulin and glucagon secretion (18) to minimize hyperaminoacidemia. For glucose, secretin has been suggested as the signal which elicits an early release of insulin (19) and suppression of glucagon (20), and thereby limits the magnitude of the hyperglycemia after a large glucose load.

It is teleologically reasonable to consider that this type of system might extend to substances other than nutrients, such as calcium, sodium, potassium, and perhaps other cations; gastrointestinal modification of the secretion of the appropriate homeostatic regulators might provide a valuable safeguard against major changes in electrolyte concentration resulting from the ingestion of a large quantity of a salt. And the apparent lack of specificity of the GLI response may not be real; inasmuch as eight GLI fractions have been reported by Moody, Markussem, Schaich Fries, Steenstrup, and Sundby (6) to be present in gut extracts, one could postulate that each cation and nutrient elicits a different GLI which cannot be differentiated in plasma by the methods used in this study. This would also explain the failure of glucose-stimulated GLI to lower serum calcium.

## ACKNOWLEDGMENTS

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