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Research Article

The nature and extent of growth hormone-release inhibiting hormone (GH-RIH, somatostatin)-induced inhibition of pancreatic secretion of bicarbonate and protein, an index of enzyme secretion, were studied by administration of exogenous secretin or cholecystokinin (CCK) and of a number of stimulants for endogenous release of these hormones in fasted pancreatic fistula dogs with and without an infusion of GH-RIH. The results of this study show that GH-RIH inhibits the pancreatic fluid and bicarbonate secretion induced by duodenal acidification and exogenous secretion. The kinetic analysis shows that the interaction between GH-RIH and secretin affecting pancreatic bicarbonate secretion possesses the characteristics of competitive inhibition. GH-RIH does not change the pancreatic protein response to exogenous CCK, but profoundly inhibits pancreatic response to a variety of the endogenous stimulants of CCK release, including duodenal perfusion of sodium oleate, amino acid mixture, or feeding of a peptone meal. We conclude that GH-RIH is a very potent inhibitor of the endogenous release of CCK from the intestinal mucosa and inhibits competitively the action of secretin but not CCK on the exocrine pancreatic secretion.

Effect of Growth Hormone-Release Inhibiting Hormone on Hormones Stimulating Exocrine Pancreatic Secretion

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ABSTRACT The nature and extent of growth hormone-release inhibiting hormone (GH-RIH, somatostatin)-induced inhibition of pancreatic secretion of bicarbonate and protein, an index of enzyme secretion, were studied by administration of exogenous secretin or cholecystokinin (CCK) and of a number of stimulants for endogenous release of these hormones in fasted pancreatic fistula dogs with and without an infusion of GH-RIH.

The results of this study show that GH-RIH inhibits the pancreatic fluid and bicarbonate secretion induced by both duodenal acidification and exogenous secretin. The kinetic analysis shows that the interaction between GH-RIH and secretin affecting pancreatic bicarbonate secretion possesses the characteristics of competitive inhibition. GH-RIH does not change the pancreatic protein response to exogenous CCK, but profoundly inhibits pancreatic response to a variety of the endogenous stimulants of CCK release, including duodenal perfusion of sodium oleate, amino acid mixture, or feeding of a peptone meal. We conclude that GH-RIH is a very potent inhibitor of the endogenous release of CCK from the intestinal mucosa and inhibits competitively the action of secretin but not CCK on the exocrine pancreatic secretion.

INTRODUCTION

Growth hormone-release inhibiting hormone (GH-RIH)[1] inhibits the release of several anterior pituitary and pancreatic hormones (1–4). It has also been shown to suppress the release of gastrin (5) and inhibits gastric acid and pepsin secretion (6).

In the present investigation we have studied the effect of GH-RIH on pancreatic secretion in response to both endogenously released and exogenously administered secretin and cholecystokinin (CCK) in conscious dogs provided with chronic gastric and pancreatic fistulas.

METHODS

Four mongrel dogs, weighing 20–24 kg, were prepared with a gastric fistula drained by a Thomas cannula (7), and a pancreatic fistula was constructed according to a modification of the method of Herrera and associates (8). The cannula of the pancreatic fistula was placed in the duodenum about 15 cm distal to the pylorus. The secretory studies were started 2 mo after surgery. The dogs were deprived of food but not water for at least 18 h before each test.

Pancreatic secretion was collected in 15-min samples. The experiments were not started unless there were at least two consecutive 15-min periods with basal secretion less than 1.5 ml each. During each test two infusions of 0.15 M NaCl were given at 60 ml/h by a peristaltic pump through polyethylene tubes (PE 50), one inserted into the duodenum through the hollow obturator of the pancreatic cannula and another into a leg vein.

Several series of tests were performed on each dog to study the effect of both exogenous and endogenous stimulants of pancreatic electrolyte and enzyme secretion. Pancreatic secretion was stimulated either by intravenously given exogenous secretin and synthetic octapeptide of cholecystokinin (OP-CCK) or by intraduodenal perfusion of a hydrochloric acid, sodium oleate, and amino acid mixture, and a meal as described below. Secretin was infused intravenously in doses ranging from 0.5 to 4.0 U/kg·h. OP-CCK was given in a constant dose of 0.5 μg/kg·h, shown previously to elicit near-maximal stimulation of pancreatic enzyme secretion (9).

The test solutions for release of endogenous hormones were infused into the duodenum through the intestinal limb of the pancreatic cannula at a constant rate shown previously (9) to provoke near-maximal pancreatic response. The liver extract meal consisted of a 10% aqueous solution

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[1] *Abbreviations used in this paper:* CCK, cholecystokinin; CMR, calculated maximal response; D_{50}, half-maximal response; GH-RIH, growth hormone release-inhibiting hormone; OP-CCK, synthetic octapeptide of CCK.

of liver concentrate powder (Armour Pharmaceutical Co., Chicago, Ill.). About 300 ml of the meal solution was adjusted to pH 5.0 by adding 4 N HCl and then introduced into the stomach where, by intragastric titration (10, 11), it was held at this same pH for 210 min. For comparison, a meal of 500 g of cooked beef liver was fed.

Afer 90 min for pancreatic secretion to reach a steady state, GH-RIH was added to the intravenous infusion for 60 min. Finally, the secretory stimulant alone was continued for an additional 60 min. The dose of GH-RIH used in this study ranged from 0.62 to 20 μg/kg·h. In most of the experiments, a standard dose of 2.5 μg/kg·h was used, found in separate studies to be the lowest dose that caused maximum inhibition of secretin-induced pancreatic bicarbonate secretion. The interaction of GH-RIH with secretin was determined in the following manner: Secretin was infused intravenously for 210 min on any given test day in one of the following doses: 0.5, 1.0, 2.0, or 4.0 U/kg·h. 90 min after the start of secretin infusion, when the rates of secretion had become relatively constant, GH-RIH was infused in a constant dose of 0.62 μg/kg·h for 60 min. Finally, secretin alone was continued for an additional 60 min. In control experiments the animals received secretin alone for the duration of the tests. All tests were performed in duplicate on each of four dogs. The mean bicarbonate output obtained in the last two 15-min periods during GH-RIH administration or comparable periods when secretin was infused alone were used to determine the kinetics of the inhibition by GH-RIH of secretin-induced bicarbonate secretion. The GH-RIH was synthesized by a solid-phase method in the cyclic (native) form, as previously described by Coy et al. (12), and used in the pure form.

During each test except those with a test meal, the gastric fistula was kept open throughout the experiment to prevent acid from entering the intestine and releasing endogenous secretin.

Pancreatic juice was collected continuously and separated into 15-min samples. The volume was measured to the nearest 0.1 ml. Bicarbonate and total protein outputs were determined and expressed as described before (13).

Results are presented as mean values±SE of the means from eight experiments in four dogs. The responses to each stimulant in the different stages were compared by the *t* test for paired values. Differences were regarded as significant if $P < 0.05$.

RESULTS

An intravenous infusion of 2.0 U/kg·h secretin produced near maximal bicarbonate stimulation in chronic pancreatic fistula dogs. In control tests with secretin alone, pancreatic bicarbonate output reached a peak at the end of 1st h of secretin infusion and remained at a well-sustained plateau for most of the experiment. GH-RIH, infused in a standard dose of 2.5 μg/kg·h, caused about 70% inhibition of secretin-induced pancreatic bicarbonate secretion (Fig. 1). Pancreatic protein output in response to secretin was only slightly above the basal level and remained unaffected by GH-RIH.

Secretin infused intravenously in constant background doses ranging from 0.5 to 4.0 U/kg·h produced a well-sustained plateau of bicarbonate secretion at each dose level. The addition of GH-RIH in a dose of 0.62 μg/kg·h to intravenous infusion of secretin caused a significant inhibition of pancreatic volume and bicarbonate response to secretin at all dose levels. Pancreatic bicarbonate output was inhibited by about 80% at the secretin dose of 0.5 U/kg·h, 70% at 1.0 U/kg·h, 50% at 2.0 U/kg·h, and 30% at 4 U/kg·h. Thus, while the percentage of inhibition decreased with increasing doses of secretin, the absolute amount of inhibition remained essentially constant except for the lowest dose of secretin. These responses, (except those to the lowest dose of secretin, were analyzed by an analogy of dose-response data to Michaelis-Menten kinetics for enzyme action (14, 15). The fit of the secretory data to a linear transformation of the dose-response expression is shown in Fig. 2. Calculated maximal response (CMR) is given by the intercept on the axis and the dose required for half-maximal response (D_{50}) by the linear slope. For secretin alone, mean (±SE) CMR was 4.81±1.05 meq of bicarbonate/15 min, and mean ±SE D_{50} was 1.92± 0.38 U secretin/kg·h. For the combination of GH-RIH with different doses of secretin, the CMR was 4.89±1.25 meq of bicarbonate/15 min and the D_{50} was 4.58±0.63 U of secretin/kg·h. The difference in D_{50} was significant while the difference in CMR was not.

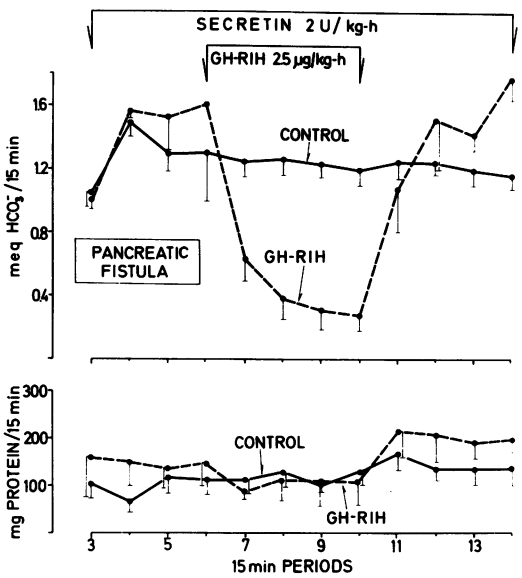

FIGURE 1 Effect of GH-RIH (2.5 μg/kg·h) on pancreatic bicarbonate and protein secretion in response to secretin (2 U/kg·h). In this and subsequent figures, each point on the line represents the mean value from eight experiments on four dogs with chronic pancreatic fistulas. Vertical bars are SEM.

Duodenal instillation of hydrochloric acid at a rate of 8 meq/h to cause endogenous release of secretin evoked pancreatic secretion, reaching a peak at the beginning of the 2nd h of the experiment. The bicarbo-

nate output was relatively well sustained throughout the duodenal acidification and corresponded to that evoked by exogenous secretin at a dose of 2 U/kg·h. GH-RIH in a standard dose of 2.5 μg/kg·h caused a profound inhibition of pancreatic response, amounting to about 90% of the control level. Pancreatic protein response to duodenal acidification was almost twice that evoked by exogenous secretin, and GH-RIH almost completely abolished this response (Fig. 3).

Pancreatic response to OP-CCK was characterized by a high protein content, reaching a peak value usually during the 1st h of OP-CCK infusion and then remaining at a relatively well-sustained plateau for the duration of the study. GH-RIH given in a standard dose of 2.5 μg/kg·h during a stable rate of protein response to OP-CCK had no effect on protein secretion. Pancreatic bicarbonate response to OP-CCK was negligible and also remained unaffected by GH-RIH (Fig. 4).

Liver extract meal adjusted to pH 5.0 and kept at this pH in the main stomach by intragastric titration produced a high protein and a relatively low bicarbonate secretion. Protein output in response to this artificial peptone meal was almost as high as that reached with OP-CCK, whereas bicarbonate output was higher than with OP-CCK and reached about 10% of the maximal response to exogenous secretin. GH-RIH produced a dose-dependent inhibition of both protein and bicarbonate response to a meal (Fig. 5).

With duodenal perfusion of L-tryptophan and L-phenylalanine mixture, the patterns of pancreatic secretion were similar to that obtained with OP-CCK, i.e., high in protein and low in bicarbonate content. GH-RIH resulted in profound inhibition of protein secretion and, to a lesser degree, of bicarbonate output (Fig. 6).

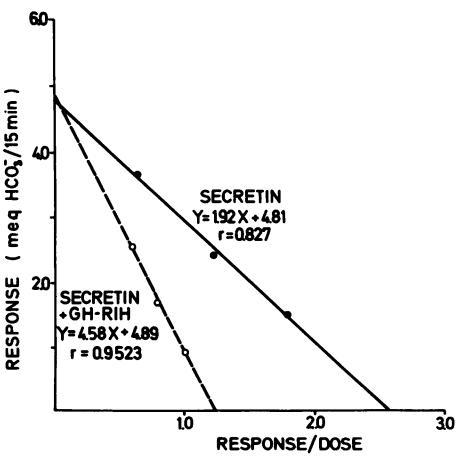

FIGURE 2 Relationship between pancreatic bicarbonate response and the response/dose in tests with secretin alone and secretin combined with GH-RIH. Equations for lines and coefficients of correlations are shown.

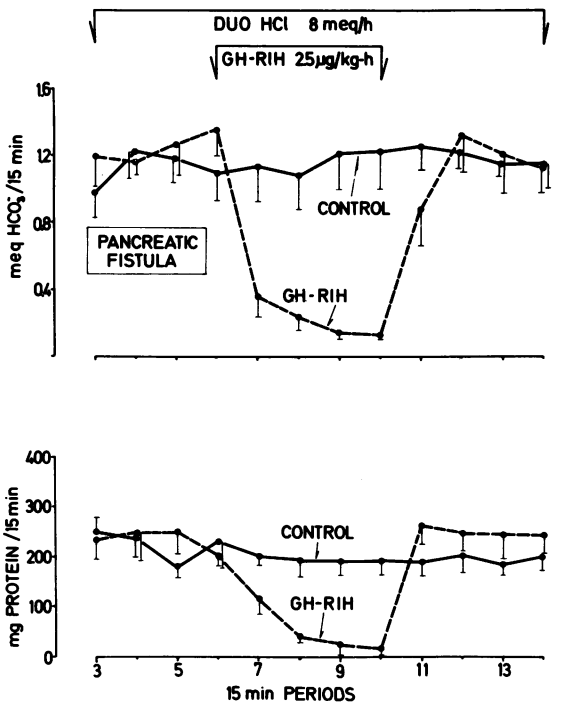

FIGURE 3 Effect of GH-RIH (2.5 μg/kg·h) on pancreatic bicarbonate and protein secretion in response to duodenal acidification (8 meq/h).

The characteristic high protein output was also seen with duodenal perfusion of sodium oleate. By contrast to other stimulants of endogenous CCK, the bicarbonate output seen during duodenal perfusion of sodium oleate was significantly higher than that during infusion of

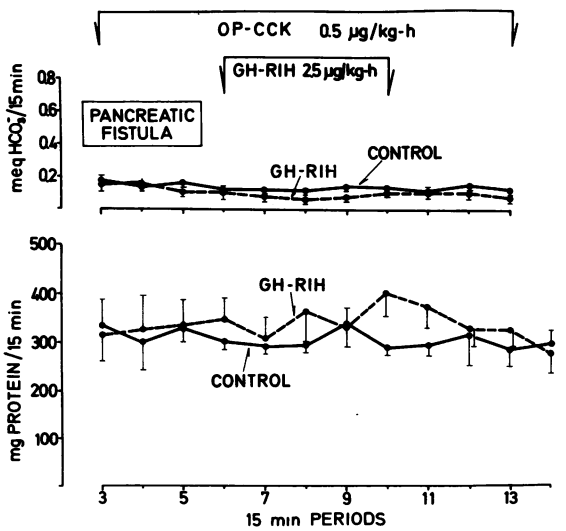

FIGURE 4 Effect of GH-RIH (2.5 μg/kg·h) on pancreatic bicarbonate and protein secretion in response to OP-CCK (0.5 μg/kg·h).

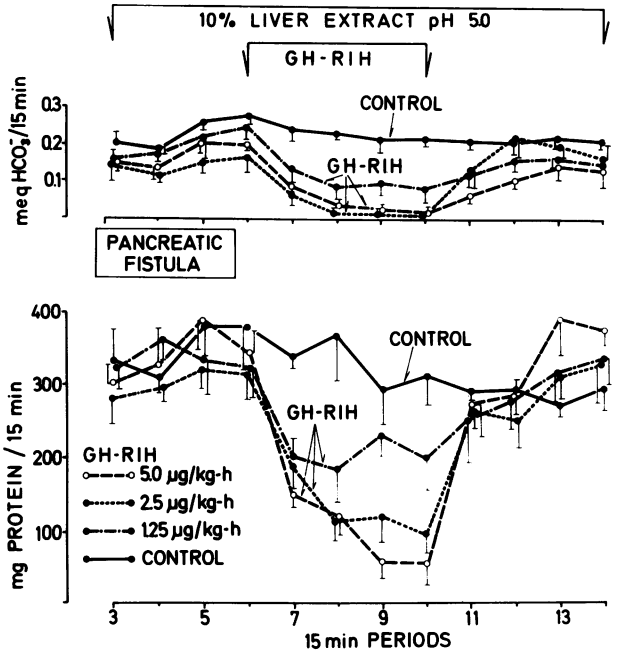

FIGURE 5 Effect of various doses of GH-RIH (1.25–5.0 µg/kg·h) on pancreatic bicarbonate and protein secretion in response to liver extract meal kept in the stomach at pH 5.0.

OP-CCK. GH-RIH inhibited both protein and bicarbonate response to sodium oleate in the duodenum (Fig. 7).

Feeding a liver meal produced a pancreatic protein secretion almost identical to that attained with an artificial liver extract meal introduced into the stomach at pH 5.0. Bicarbonate output in response to feeding reached a peak amounting to about 65% of the maximal response to exogenous secretion. GH-RIH inhibited strongly both the pancreatic protein and bicarbonate secretion induced by feeding (Fig. 8).

DISCUSSION

The reduction by GH-RIH of gastric acid and pepsin response to exogenous stimuli such as pentagastrin or histamine and the suppression of serum gastrin level under basal and stimulated conditions were reported previously (5, 6), and indicated that GH-RIH can inhibit both the exocrine and endocrine functions of the stomach. Because a substance that cross-reacts with an antibody to GH-RIH has been found in large quantities not only in the stomach but also in the duodenum and pancreas (16, 17), it was expected that it may also affect the release and action of intestinal hormones stimulating pancreatic secretion.

The results of our present study demonstrate that GH-RIH does not inhibit the action of CCK on the pancreas but does inhibit the actions of CCK releasers, suggesting that these effects are due to inhibition of CCK release. However, in the case of secretin release, no such statement can be made. Since GH-RIH strongly inhibits the action of secretin on the pancreas, it is impossible to determine whether or not it inhibits the release of secretin from the experiments presented in this report. The issue of course could be resolved by immunoassay of the effect of GH-RIH on serum secretin level. All of the secretory effects reported here can be accounted for by inhibition of CCK release plus inhibition of action of secretin on the pancreas. Acid in the intestine releases both secretin and CCK (18), and these two hormones act synergistically to stimulate pancreatic bicarbonate secretion. Inhibition by GH-RIH of pancreatic bicarbonate secretion in response to acid in the intestine can be fully accounted for by inhibition of CCK release plus inhibition of the action of secretin on the pancreas, leaving unanswered the question of whether GH-RIH also suppresses secretin release. Whether the very slightly greater bicarbonate response seen with an intragastric peptone meal titrated to pH 5.0 and with intestinally perfused oleate is due to secretin release or to some other mechanism is unknown. Even if it is assumed to be due to secretin release, inhibition by GH-RIH can as readily be accounted for by inhibition of the action of secretin as by inhibition of release of secretin. A re-

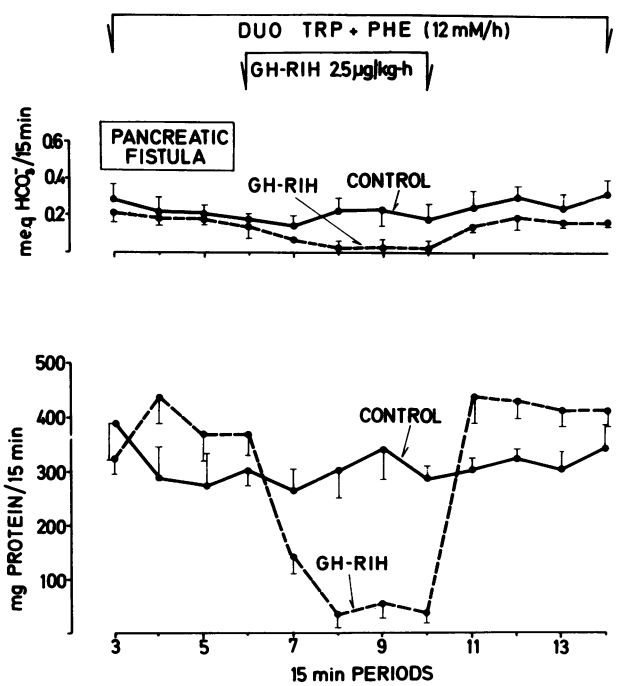

FIGURE 6 Effect of GH-RIH (2.5 µg/kg·h) on pancreatic bicarbonate and protein secretion in response to duodenal perfusion of the mixture of L-tryptophan (50 mM) and L-phenylalanine (100 mM) at a rate of 80 ml/h.

cent report of Boden et al. (19), with radioimmunoassay to determine the serum level of secretin, indicated that somatostatin is an effective inhibitor of secretin release, but the dose used to elicit this effect was probably pharmacological.

This study shows that GH-RIH inhibits pancreatic secretion stimulated by feeding, a most potent stimulant of both bicarbonate and enzyme secretion (13). The inhibition by GH-RIH of pancreatic bicarbonate secretion in response to a meal can be accounted for by its known effect on gastric secretion (5), once again leaving unanswered the question of whether GH-RIH does or does not inhibit release of secretin.

The kinetic analysis shows that the interaction between GH-RIH and secretin on pancreatic secretion of fluid and bicarbonate possesses characteristics of competitive inhibition: decreased D_{50} and unchanged CMR. With competitive inhibition, GH-RIH becomes proportionally less effective as the secretory rate is increased. Although simple competition between GH-RIH and secretin for a receptor site might be implicated in the interaction of GH-RIH and secretin, competitive kinetics is a necessary but not sufficient condition for action of two agents at a single site. The inhibition by GH-RIH of pancreatic bicarbonate secretion could be attributed to some other, as yet unknown, actions of GH-RIH, including the effects on the destruction of secretin or its delivery to the pancreas. Recent findings that secretin-induced pancreatic secretion is mediated by cyclic AMP (20), whereas CCK-induced secretion depends on cyclic GMP (21), suggest that GH-RIH may act on the enzymatic processes involving cyclic AMP.

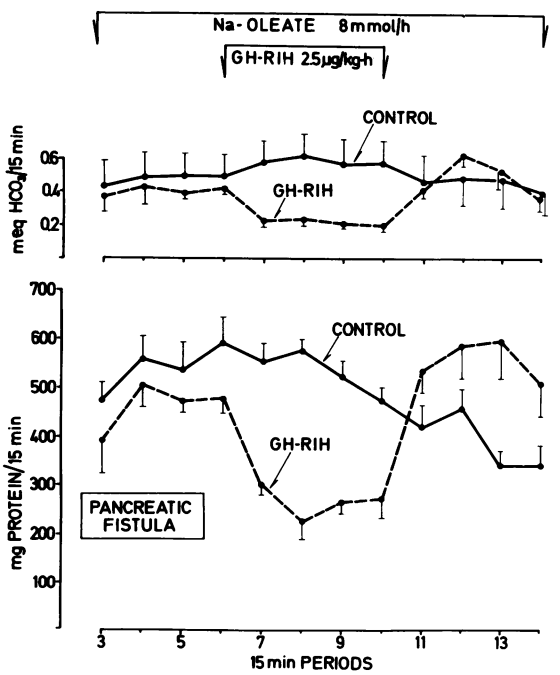

FIGURE 7 Effect of GH-RIH (2.5 µg/kg·h) on pancreatic bicarbonate and protein secretion in response to duodenal perfusion of sodium oleate (50 mM) at a rate of 160 ml/h.

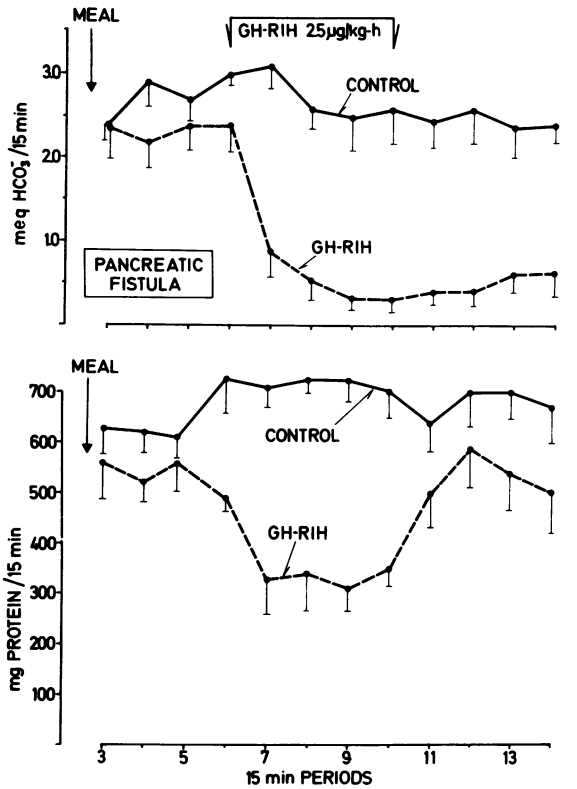

FIGURE 8 Effect of GH-RIH (2.5 µg/kg·h) on pancreatic bicarbonate and protein response to feeding a liver meal (25 g/kg).

Exogenous secretin caused a negligible protein-stimulating action not changed by GH-RIH. Pancreatic protein secretion induced by exogenous CCK was very high but also remained unaffected by GH-RIH. By contrast, pancreatic protein response to endogenous stimulants of CCK, such as sodium oleate, amino acid mixture, peptone meal (liver extract meal kept in the stomach at pH 5.0), or feeding a meal of liver, was comparable to that reached with exogenous CCK but almost completely inhibited by GH-RIH. The observations that GH-RIH inhibits strongly the stimulatory action of CCK releasers on pancreatic proteins but does not affect the action of exogenous CCK can be explained by assuming that GH-RIH inhibits the release but not the action of CCK on the pancreas. If cyclic AMP is involved in the release of CCK from the intestinal endocrine cells, as it is in the release of gastrin (22), the inhibition of the release of this hormone by GH-RIH could be explained by the blockade of cellular mechanisms common to these dif-

ferent cells, namely the adenyl cyclase-cyclic AMP system.

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