Rapid Publication

Synthetic Pancreatic Growth Hormone-Releasing Factor (GRF-40) Stimulates the Secretion of the Endocrine Pancreas

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SUMMARY

The effects on islet hormone secretion of the synthetic replicates of two peptides with potent GH-releasing activity isolated from human pancreatic islet cell tumors (GRF-40 and GRF-44) were studied using the isolated, perfused dog pancreas. GRF-40 produced a dose-dependent stimulation of insulin, glucagon, and somatostatin secretion. The responses to GRF-40 were modified by the prevailing glucose level: higher insulin and somatostatin and lower glucagon responses were obtained at high rather than low glucose. In contrast, GRF-44 did not produce any stimulation of the endocrine pancreas. In vivo GRF-40 and GRF-44 elicited identical pronounced growth hormone responses in the rat. The findings reported here provide support that pancreatic insulin and glucagon are modulated by GRF-40 with somatostatin as its inhibitory counterpart. The question, whether GRF-40 is of physiologic relevance in the regulation of the endocrine pancreas, must await evidence that it is present and releasable from the pancreas. DIABETES 1986; 35:119–23.

Two peptides with potent GH-releasing activity, containing 40 and 44 amino acids residues, were recently found in pancreatic tumor tissue in patients with acromegaly.[1,2] These human pancreatic GH-releasing factors (hpGRF) or their synthetic replicates stimulate the pituitary specifically to secrete growth hormone.[1–6] GRF-40 and GRF-44 are of the glucagon-secretin family, a group of pancreatic-intestinal peptides that includes vasoactive intestinal peptide (VIP), glucagon, secretin, gastric inhibitory peptide (GIP), and PHI, a 27-amino acid peptide (P) with N-terminal histidine (H) and C-terminal isoleucine (I).[1,2] Since peptides of the glucagon-secretin family affect gastrointestinal and endocrine pancreatic function,[7–11] since GRF, a member of this family, was originally isolated from a pancreas tumor, and since GRF-like immunoreactivity most recently has been identified in normal pancreatic islet tissue,[12] the possibility arose that GRFs, in addition to pituitary endocrine actions, might modulate endocrine pancreas function.

The studies reported here were designed to investigate the effects of GRF-40 and GRF-44 on the release of insulin, glucagon, and somatostatin from the isolated perfused dog pancreas.

MATERIALS AND METHODS

IN VITRO

Pancreatico-duodenal preparation and perfusion media. Mongrel dogs, fasted overnight, weighing 17–28 kg, were used as pancreas donors. The technique for isolation of the pancreas and the perfusion system have previously been described in detail.[13,14] In brief, the preparation consisted of the pancreas and the proximal 10 cm of the attached duodenum. A nonrecirculating medium consisting of a Krebs-Ringer bicarbonate buffer containing 40 g/L dextran (mol wt, 75,000), 2 g/L bovine albumin, glutamate, fumarate, and pyruvate, each at a concentration of 5 mM, was pumped through the splenic and celiac arteries, and the total portal effluent was collected every minute. The ionic composition of the standard perfusion medium was as follows (meq/L): Na^+, 140.0; K^+, 4.4; Ca^{2+}, 2.6; Mg^{2+}, 1.8; Cl^-, 124.0; HCO_3^-, 24.4; SO_4^{-2}, 1.8; and $H_2PO_4^-$, 1.1.

Oxygenation of the Krebs-Ringer bicarbonate buffer was achieved by means of a rotating roller screen in an atmosphere of 94.4% O_2 and 5.6% CO_2. During the experiments, the perfusion fluid had a constant pH of 7.4 and a temperature of 37°C. The perfusion pressure was 30–40 mm Hg, and the perfusion flow was 20 ml/min.

Experimental procedure. Samples were taken every minute from the efflux. In order to prevent possible degradation of somatostatin and glucagon, 3 mg/ml EDTA was added to

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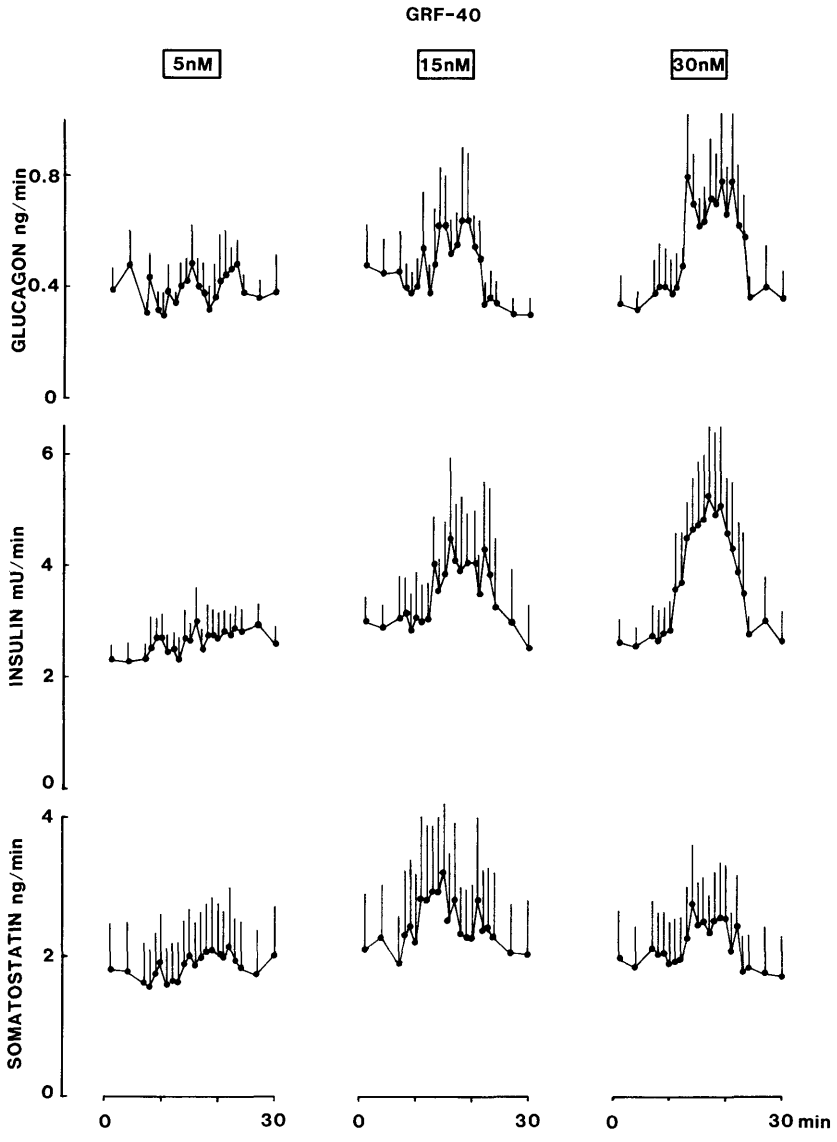

FIGURE 1. The effect of GRF-40 at concentrations of 5, 15, and 30 nM upon the secretion of glucagon, insulin, and somatostatin from the isolated dog pancreas. The glucose concentration was 5.5 nM (N = 6; mean ± SEM).

the tubes collecting the efflux. The samples were stored immediately at −18°C until assayed. The pancreas was perfused for an equilibration period of 20–30 min. Thereafter the substances to be studied were infused for 10 min, with a 20-min recovery period between each infusion period.

In the first part of the study, GRF-40 was infused for 10-min periods at three concentrations (5, 15, and 30 nM), given in random order to each of six pancreata, at a glucose concentration of 5.5 mM. In the study of the influence of glucose concentration on GRF-40-mediated (30 nM) hormone responses, concentrations of GRF-40 (1.3 and 11 mM) were administered in random order to each of five pancreata. In the last part of the in vitro study, the effects of 10-min infusions of GRF-40 and GRF-44 were compared by adding the two peptides in random order at the equimolar concentration of 30 nM to each of six pancreata in the presence of 5.5 mM glucose.

IN VIVO

Male Wistar rats weighing 200–225 g were anesthetized with sodium pentobarbitol (50 mg/kg, intraperitoneally) at time −15 min. Samples (0.3 ml) were drawn by jugular catheter at times 0, 5, 10, 20, 30, and 60 min; 1 μg of GRF-40 or GRF-44 was administered as a bolus injection at time 0. Five animals were used for each treatment.

Peptides. The peptides used in this study were GRF (1-40) (synthetic; Bachem, Bobendorf, Switzerland) and GRF (1-44) (synthetic; Bachem).

Radioimmunoassay. Somatostatin, insulin, glucagon, and growth hormone were measured by specific and sensitive RIAs as previously described.[15–17] The peptides infused did not interfere in the RIAs.

Statistical analysis. Statistical analysis of the dose-response relationship was made by Page's test,[19] which is a nonparametric paired test designed to test for the presence of trends in data. To compare the islet hormone responses at the two glucose levels, the responses above baseline at low and high glucose, respectively, were compared using Student's *t*-test for paired comparisons. The mean of the last five 1-min samples preceding stimulation indicates the baseline whereas stimulated refers to the mean of the ten 1-min values during GRF-40 infusion. In both cases a 5% limit of confidence was employed to assess the significance of the differences.

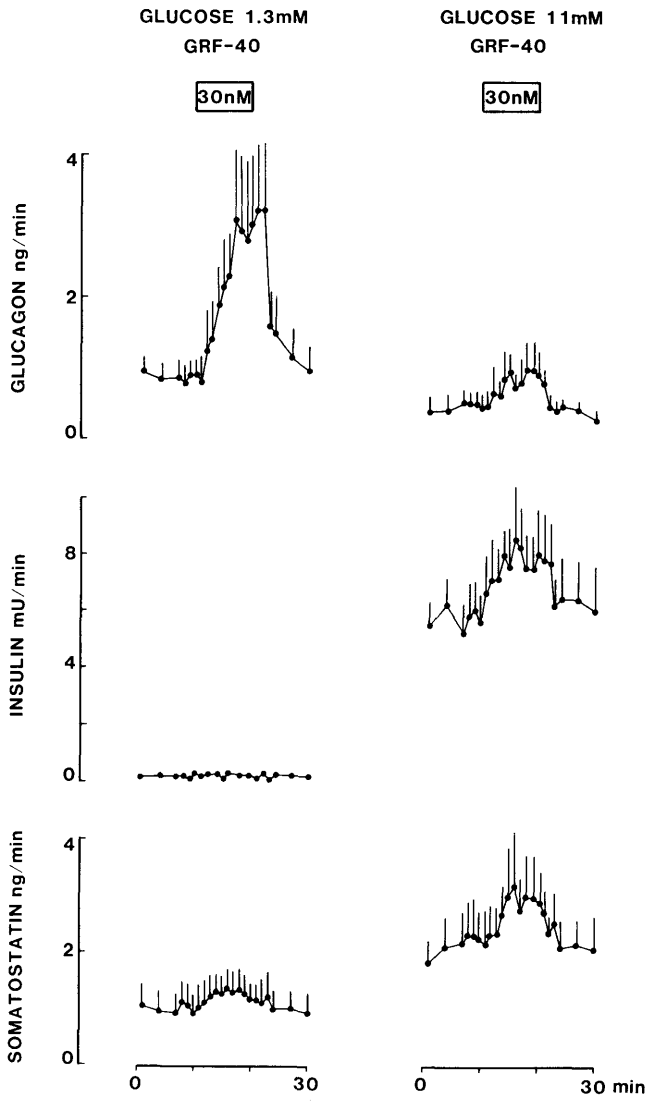

FIGURE 2. The effect of 10-min infusion of GRF-40 (30 nM) on glucagon, insulin, and somatostatin release from the isolated dog pancreas at low (1.3 mM, left panel) and high (11 mM, right panel) glucose. Values are means (± SEM) from five pancreas perfusions.

RESULTS

Effects of GRF-40. In each of six pancreas perfusions the effect of 10-min infusions of 5, 15, and 30 nM GRF-40 were studied at a constant glucose concentration of 5.5 mM. Figure 1 demonstrates that GRF-40 caused a dose-dependent stimulation of secretion of insulin ($P < 0.001$), glucagon ($P < 0.001$), and somatostatin ($P < 0.05$). The B-, A-, as well as D-cell thresholds for GRF-40 were between 5 and 15 nM.

Effects of GRF-40 at low and high glucose concentration. We then tested the effects on islet hormone secretion of 30 nM GRF-40 at low (1.3 mM) and high (11 mM) glucose (Figure 2). As expected, at 1.3 mM glucose, basal levels of somatostatin and insulin were low and those of glucagon were high. GRF-40 (30 nM) tripled glucagon, and caused a smaller increase in somatostatin ($P < 0.01$) while no measurable alteration in insulin secretion could be detected (Figure 2, left panel). When perfusate glucose concentration was 11 mM, release of insulin and somatostatin were increased and glucagon was suppressed (Figure 2, right panel). Also, at this glucose concentration, GRF-40 (30 nM) stimulated glucagon ($P < 0.01$), insulin ($P < 0.01$), and somatostatin ($P < 0.05$). The responses were modified by the prevailing glucose level; higher insulin ($P < 0.01$) and somatostatin responses ($P < 0.05$), and lower glucagon ($P < 0.05$) were obtained at high rather than at low glucose (N = 5).

Comparison of the effects of equimolar concentration of GRF-40 and GRF-44. To compare the effects of GRF-40 and GRF-44, an equimolar concentration of the peptides at 30 nM was infused in each of six pancreas perfusions in the presence of 5.5 mM glucose (Figure 3). GRF-40 caused a clear-cut stimulation of A-, B-, and D-cell secretion (Figure 3, left panel) not produced by GRF-44 (Figure 3, right panel).

rGH responses to GRF-40 and GRF-44. To compare the action on GH levels, 1 μg GRF-40 (N = 5) and GRF-44 (N = 5) was given as bolus injections to anesthetized rats (Figure 4). As seen, the two peptides caused identical, prominent increments in growth hormone levels.

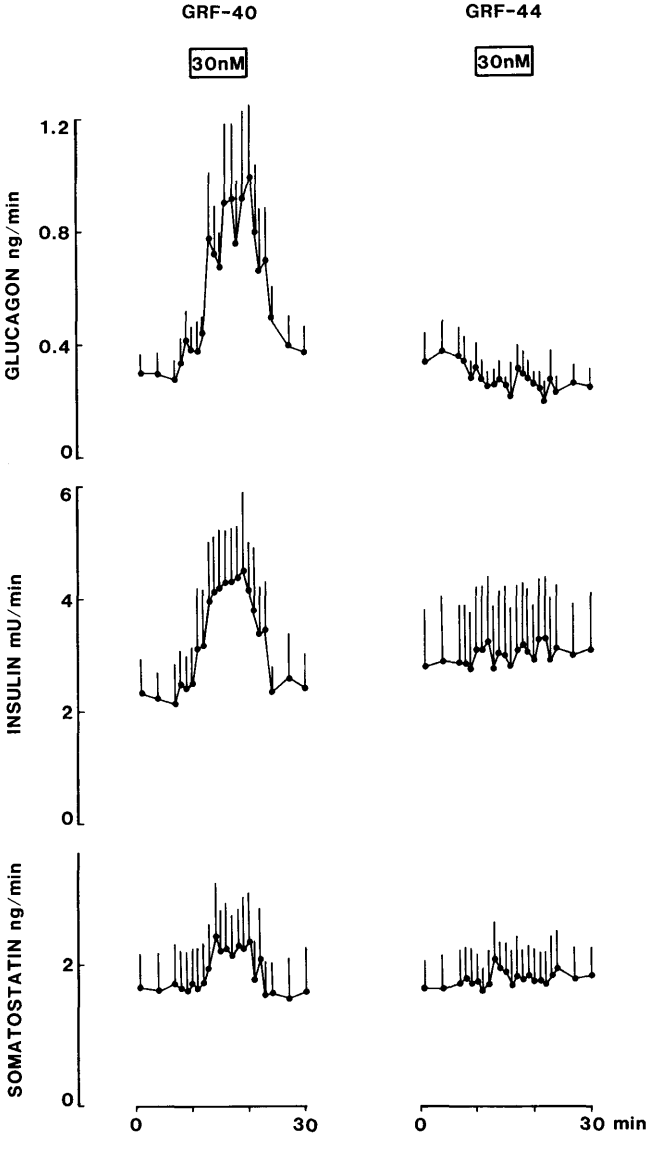

FIGURE 3. The effects on islet hormone secretion of 10-min perfusion of an equimolar concentration of 30 nM GRF-40 (left panel) or GRF-44 (right panel) were studied at a glucose concentration of 5.5 mM. The values are the means (± SEM) from six pancreas perfusions.

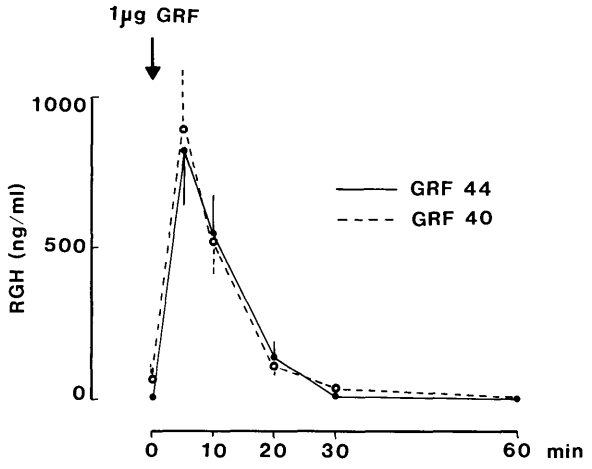

FIGURE 4. The effects upon circulating growth hormone levels in the rat of bolus injections (1 μg) of GRF-40 (N = 5, broken line) and the GRF-44 (N = 5, solid line). Values are given as means ± SEM.

DISCUSSION

The present study clearly demonstrates that synthetic GRF-40 produces a dose-dependent stimulation of the release of insulin, glucagon, and somatostatin from the isolated dog pancreas.

The neuroregulation of GH secretion is mediated in part by a stimulatory GH-releasing factor (GRF) and an inhibitory peptide, somatostatin,[18] both of which reach the adenohypophysis by the hypothalamic-hypophyseal portal system. Peptides with high GH-releasing activity were recently isolated and characterized from human pancreatic islet cell tumors.[1,2] The synthetic replicates of these peptides (GRF-40 and GRF-44) have been shown to be potent and specific stimulators of pituitary GH release[1-6] and to be indistinguishable in biologic activity from GRF present in human hypothalamus.[21] It is tempting to speculate that pancreatic insulin and glucagon secretion is also modulated by GRF and somatostatin, which respectively, stimulates and inhibits islet hormone secretion. Whether such an interrelationship of GRF and somatostatin is operating in the endocrine pancreas, as is apparently the case with respect to GH secretion from the pituitary,[20-23] remains at issue. The recent demonstration of GRF-like immunoreactivity in pancreatic PP cells[12] suggests that the pancreatic islets are a normal and not just an ectopic source of GRF.

In the present study, the effect of GRF-40 was found to be modulated by the prevailing glucose level. Thus, higher insulin and somatostatin responses and lower glucagon responses were obtained at high rather than at low glucose.

While GRF-40 caused a clear-cut stimulation of A-, B-, and D-cell secretion, islet hormone secretion was not enhanced by GRF-44. Thus, GRF with a free carboxyl terminal consisting of 40 amino acids (GRF-40), rather than the amidated form with 44 amino acids (GRF-44), appears to possess a stimulatory effect on the endocrine pancreas This diversity in action between GRF-40 and GRF-44 was also found using different batches of the two peptides (unpublished results). The demonstration of identical growth hormone responses to batches of GRF-40 and GRF-44 that caused the above-mentioned striking differential effects on the endocrine pancreas rules out the possibility that the synthetic GRF-44 used in this study is ineffective. The reason for the greater biologic activity of GRF-40 in the pancreas is not known. It should be noted that although GRF-40 and GRF-44 do have identical ability to release amylase from the exocrine pancreas and growth hormone from the pituitary,[24] it is in accordance with recent reports that various molecular forms of GRF (e.g., GRF-1-29, GRF-1-43, and GRF-44) exhibit differences in biologic activity on growth hormone release.[24,25]

In summary, GRF-40 stimulates islet hormone secretion in a dose- and glucose-dependent fashion, while GRF-44 is without any impact. The biologic effects upon the endocrine pancreas are opposite to those of its hypothalamic opponent, somatostatin. The question of whether GRF-40 is of physiologic or clinical significance for the regulation of islet hormone release must await more solid evidence that GRF-40 is present in and releasable from normal and diabetic pancreas.

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