Effect of Truncated Glucagon-Like Peptide-1 [Proglucagon-(78–107) amide] on Endocrine Secretion from Pig Pancreas, Antrum, and Nonantral Stomach

C. ØRSKOV, J. J. HOLST, AND O. V. NIELSEN

Institute of Medical Physiology C, The Panum Institute (C.Ø., J.J.H.), DK-2200 Copenhagen; and the Department of Surgery C, Rigshospitalet (O.V.N.), DK-2100 Copenhagen, Denmark

ABSTRACT. We studied the effect of truncated glucagon-like peptide-1 [naturally occurring GLP-1; proglucagon-(78–107) amide], a potent insulinotropic peptide from the pig ileum, on endocrine and exocrine secretion of potential gastrointestinal target organs using isolated perfused preparations of the porcine pancreas, antrum, and nonantral part of the stomach. Truncated GLP-1 significantly increased somatostatin secretion from the pancreas at 10^{-10} mol/liter and more than doubled the secretion at 10^{-9} mol/liter, but had no effect on either somatostatin or gastrin secretion from the antrum or on somatostatin secretion from the nonantral stomach in concentrations up to 10^{-8} mol/

liter. Insulin secretion from the pancreas (with 7 mmol/liter glucose in the perfusate) increased 2-fold with truncated GLP-1 at 10^{-10} mol/liter and almost 5-fold at 10^{-9} mol/liter. Pancreatic glucagon secretion was inhibited by 50% at 10^{-10} mol/liter and by 70–80% at 10^{-9} mol/liter. Full-length GLP-1 [proglucagon-(72–107)] and GLP-2 [proglucagon-(126–159)] had no effect on hormone secretion from any of the perfused organs. It is concluded that truncated GLP-1 may participate in an entero-insular control of pancreatic endocrine secretion. (*Endocrinology* **123:** 2009–2013, 1988)

M AMMALIAN proglucagon has been shown to contain two glucagon-like sequences [glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 [GLP-2)] in addition to glucagon itself (1). We recently isolated from pig small intestine and partially sequenced a naturally occurring GLP-1 (2). This peptide was found to correspond to proglucagon-(78–107) and thus represents a truncated form of GLP-1 [which corresponds to proglucagon-(72–107 or 108)] (1). Natural GLP-1 is highly homologous to glucagon, with 14 identically positioned amino acids. In preliminary studies, truncated GLP-1 had strong insulinotropic effects on perfused preparations of pig and rat pancreas (2, 3).

Many of the members of the glucagon family of peptides have been shown to stimulate both insulin and somatostatin secretion from the pancreas and to increase somatostatin release from the stomach (4). We, therefore, studied the effects of truncated GLP-1 as well as full-length GLP-1 and GLP-2 on the endocrine secretions of the pig pancreas and stomach using three isolated tissue preparations perfused *in vitro*: the pancreas, the antrum plus pancreas, and the nonantral (antrectomized) stomach.

Materials and Methods

Twenty pigs of the strain LYY, weighing 12–18 kg, served as donors. Anaesthesia was induced with 2.5% halothane and maintained with chloralose (no. 2420, Merck, Darmstadt, West Germany; 100 mg/kg) after intubation and artificial ventilation. Details on the preparation of the pancreas and the antrum for perfusion have been described (5, 6). The perfused antrum consists of the antrum plus the pancreas, because these structures to a great extent share the same vascular supply. Effluent derived solely from the antrum was obtained by catheterization of the right gastroepiploic vein (6), while the effluent collected simultaneously from a catheter placed in the portal vein is derived from the antrum as well as the pancreas.

Isolation and perfusion of nonantral porcine stomach were performed as described previously (7). In short, the stomach was isolated together with the spleen; in the pig it is impossible to separate the vascular supply of the two organs. The antrum was excised, leaving the gastroepiploic vascular arcades intact. The stoma was closed by sutures in two layers. The remaining stomach (together with the spleen) was placed in a thermostated bath containing Ringer solution and perfused through the coeliac trunk. A draining tube was inserted into the stomach lumen, which was perfused at a rate of 8 ml/min with preheated saline. The acid secretion of the preparation was determined by titration of the luminal effluent with 0.1 mol/liter sodium bicarbonate (7). The medium for vascular perfusion was a Krebs-Ringer bicarbonate solution supplemented with washed bovine erythrocytes (20%, vol/vol), 1 g/liter human serum albumin (Behringwerke, Marburg, West Germany), 5% dextran

T-70 (Pharmacia, Uppsala, Sweden), 7 mmol/liter glucose, a total of 5 mmol/liter of a mixture of amino acids (Amodex Asa, Pharmacia), aprotinin (100,000 kallikrein inhibitor units/liter, Trasylol, Bayer, Leberkusen, West Germany), and indomethacin (5 mg/liter; Confortid, Dumex, Copenhagen, Denmark) to prevent the formation of prostaglandins from the red blood cells. (The same perfusion medium, but containing only 15% red blood cells, was used for the pancreas and antrum perfusions.) The perfusion medium was equilibrated with a mixture of 95% O_2 and 5% CO_2. The perfusion flow was kept constant at 0.3 ml/min·g. The flow of effluent from the right gastroepiploic vein varied between 2–5 ml/min. The portal venous outflow varied between 24 and 42 ml/min. Oxygen consumption, determined as described previously (7), averaged 5.7 μl O_2/min·g tissue. The venous effluent was collected every minute, chilled on ice, centrifuged within 20 min, and stored at −20 C until analysis for somatostatin, glucagon, gastrin, and insulin.

Synthetic truncated GLP-1 [proglucagon-(78–107) amide] was obtained from Peninsula Laboratories (Merseyside, England) by custom synthesis (lot no. 008802; peptide purity by amino acid analysis, 72%). Before it was used in physiological studies the peptide was purified to homogeneity by isocratic reverse phase HPLC on an 8 × 250-mm Nucleosil C-18 column, employing LKB pumps and detectors (LKB, Bromma, Sweden). Gas phase sequence analysis of the synthetic peptide confirmed that the peptide was proglucagon-(78–107) amide (2). Synthetic full-length GLP-1 [proglucagon-(72–107) amide] and GLP-2 [proglucagon-(126–159)] were obtained from Peninsula Laboratories (Belmont, CA; catalog no. 7166 and 7167); synthetic vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP) were also from Peninsula (code no. 7161 and 7188). the peptides were dissolved in perfusate, previously equilibrated with N_2 to prevent oxidation, and infused into the arterial line, resulting in final perfusate concentrations between 10^{-11}–10^{-8} mol/liter. Carbachol was obtained from Danmarks Apotekerforenings Kontrollaboratorium A/S (Copenhagen, Denmark).

Somatostatin-like immunoreactivity was measured using antiserum 1758, directed against the 5–10 sequence, synthetic somatostatin-(1–14) (Ferring, Malmo, Sweden) and [^{125}I]Tyr$_1$-somatostatin (Novo Research Center, Bagsværd, Denmark), as described previously (8). This assay measures with equal efficiency all molecular forms of somatostatin (9). Gastrin-like immunoreactivity was measured using antiserum 2604, directed against the C-terminus of gastrin-17, synthetic gastrin, and [^{125}I]gastrin, as described previously (10). Insulin and glucagon concentrations were measured by RIAs, as described previously (5). For all RIAs the sensitivity and experimental detection limit were below 2 pmol/liter. None of the assays showed cross-reactions with any known pancreatic or gastric peptide apart from those that contain the antigenic determinants against which the antisera were raised (5, 8–10). Specifically, the somatostatin antiserum did not bind calcitonin gene-related peptide (our unpublished studies), and the glucagon antisera did not cross-react with any of the GLPs derived from the C-terminal part of proglucagon (11). For statistical evaluation of results, average hormone outputs obtained during the 10-min

stimulation periods were compared with the average basal outputs in the preceding 5-min period, using the t test for paired data. For evaluation of the effects of time the Friedman analysis of variance with multiple comparisons was used where applicable (12).

Results

The results of this study are shown in Figs. 1–4, where the mean hormone outputs (±SEM) from the organs are plotted against time. We found that truncated GLP-1 significantly increased somatostatin output from the pancreas in a dose-dependent manner, when measured in both venous effluent from the completely isolated pancreas (Fig. 1) and effluent from the portal vein (as opposed to the right gastroepiploic vein) from the preparation that consisted of the pancreas plus the antrum (Fig. 2A). The average somatostatin output measured in the portal vein in six perfusion experiments increased from 16.6 ± 0.2 to 20.3 ± 0.5 pmol/min ($P < 0.001$) at a concentration of truncated GLP-1 of 10^{-10} mol/liter and from 15.7 ± 0.4 to an average output of 30.1 ± 1.2 pmol/
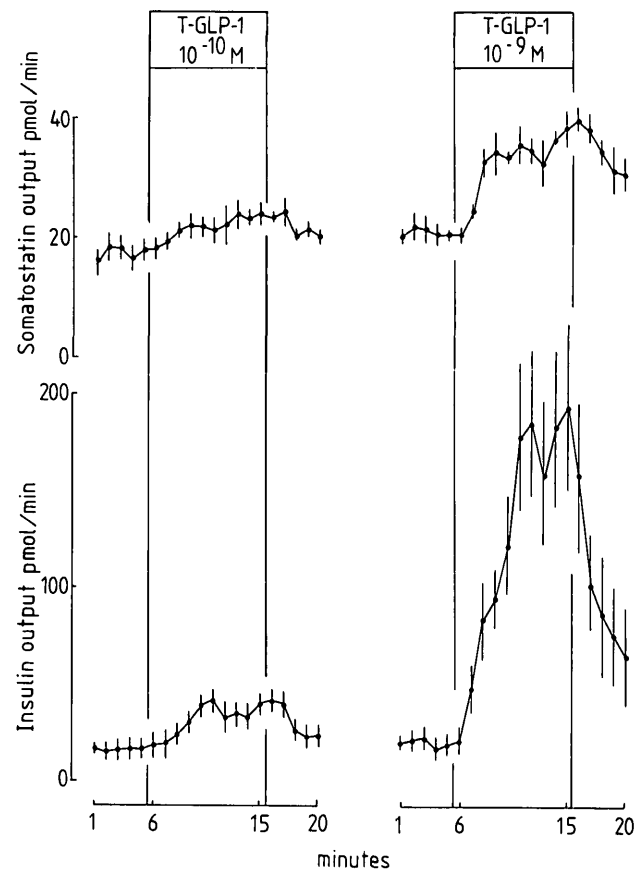

FIG. 1. Effect of truncated GLP-1 (T-GLP-1) at 10^{-10} (*left*) and 10^{-9} mol/liter (*right*) on somatostatin secretion (*top panel*) and insulin secretion (*bottom panel*) from the isolated perfused pancreas (n = 3). Hormone outputs (picomoles per min; mean ± SEM) are plotted against time (minutes). The average hormone outputs during infusion of truncated GLP-1 at both 10^{-10} and 10^{-9} mol/liter increased significantly above the mean baseline output ($P < 0.05$).
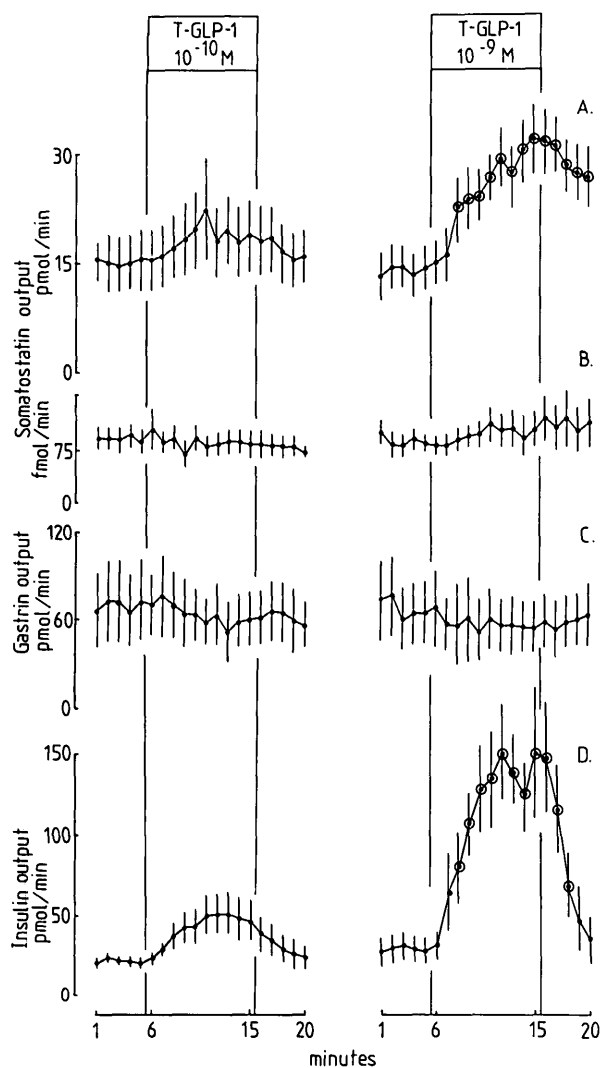
FIG. 2. Effects of truncated GLP-1 (T-GLP-1) at 10^{-10} (*left*) and 10^{-9} mol/liter (*right*) on somatostatin secretion, as measured in the portal vein (A) and the gastroepiploic vein (B), on gastrin secretion (C), and on insulin secretion (D); measured in the portal vein effluent) from the isolated perfused antrum (n = 6). Hormone outputs (picomoles per min or femtomoles per min; mean ± SEM) are plotted against time (minutes). The mean somatostatin and insulin outputs in portal vein (A and D, *left*) during infusion of truncated GLP-1 at 10^{-10} mol/liter increased significantly above basal levels (*P* < 0.001). ⊙, Significant change from baseline hormone secretion in response to infusion of truncated GLP-1 at 10^{-9} mol/liter (identified after analysis of variance).

min at 10^{-9} mol/liter. Truncated GLP-1 at 10^{-10} and 10^{-9} mol/liter (n = 6) had no effect on the gastrin or somatostatin output from the antrum (as determined in effluent from the right gastroepiploic vein; Fig. 2, B and C). Neither were there any effects on antral somatostatin output in two experiments with truncated GLP-1 at 10^{-8} mol/liter, although insulin and portal venous somatostatin outputs exceeded those obtained with GLP-1 at 10^{-9} mol/liter. In the same six perfusion experiments, infusion of GRP to a final concentration of 10^{-9} mol/liter for 10 min increased the average antral venous
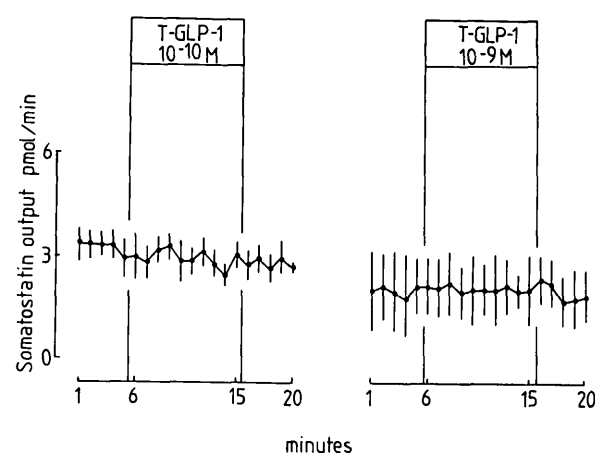

FIG. 3. Effect of truncated GLP-1 (T-GLP-1) at 10^{-10} (*left*) and 10^{-9} mol/liter (*right*) on somatostatin secretion from the isolated nonantral perfused stomach (n = 3). Somatostatin output (picomoles per min; mean ± SEM) is plotted against time (minutes).
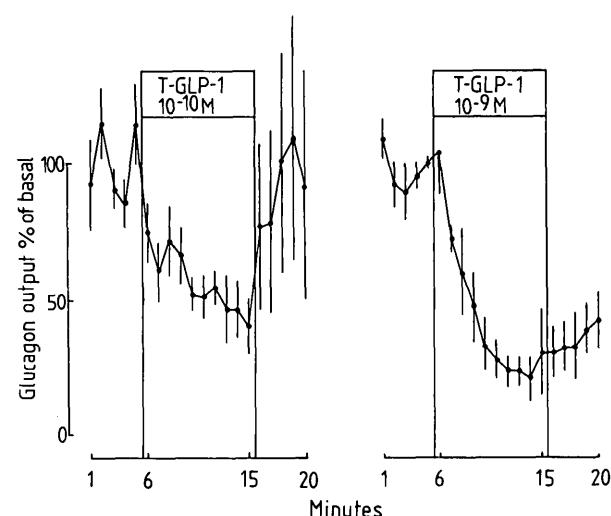

FIG. 4. Effect of truncated GLP-1 (T-GLP-1) at 10^{-10} (*left*) and 10^{-9} mol/liter (*right*) on glucagon secretion from the pancreas or pancreas plus antrum (n = 5). Hormone output, expressed as a percentage of the mean prestimulatory hormone output ± SEM, is plotted against time.

somatostatin output from 0.125 ± 0.02 to 0.23 ± 0.02 pmol/min (*P* < 0.001). Truncated GLP-1 at 10^{-10} mol/liter increased insulin secretion (as measured in the portal vein) from 24 ± 0.54 to an average of 51.2 ± 2.9 pmol/min (*P* < 0.001); it increased insulin secretion from 34.5 ± 0.7 to 144 ± 11 pmol/min at 10^{-9} mol/liter. In the pancreatic perfusions (Fig. 1) truncated GLP-1 at 10^{-9} mol/liter increased insulin secretion more than 6-fold. In general, glucagon outputs from both preparations were low (as a consequence of the 7 mmol/liter glucose in the perfusate). Thus, a consistent glucagon output was measurable in only five perfused preparations (two completely isolated pancreas preparations and three preparations of pancreas plus antrum). The prestimulatory basal output averaged 5.5 ± 4.0 pmol/min before 10^{-10} mol/liter GLP-

1) and 2.03 ± 0.98 pmol/liter (before 10^{-9} mol/liter). The responses to truncated GLP-1 infusion ar shown in Fig. 4 (presented as fractional changes from basal levels because of large differences in absolute levels). Truncated GLP-1 caused a highly significant inhibition of glucagon output to less than 50% at 10^{-10} mol/liter and to as little as 20% of basal output at 10^{-9} mol/liter.

As shown in Fig. 3, there was no effect of truncated GLP-1 on somatostatin secretion from the isolated antrectomized nonantral stomach. In these experiments an intraarterial infusion of VIP to a final perfusate concentration of 10^{-9} mol/liter for 10 min increased somatostatin secretion from a basal value of 1.08 ± 0.5 to an average value of 3.29 ± 1.1 pmol/min ($P < 0.001$). Hydrochloric acid secretion from the antrectomized stomach, which averaged 0.037 ± 0.012 mmol/min in the present experiments remained unaffected by truncated GLP-1 regardless of dose, but increased to 0.15 ± 0.01 mmol/liter in response to a 10-min infusion of carbachol at 10^{-6} mol/liter.

In all four pancreas perfusions and five nonantral stomach perfusions full-length GLP-1 and GLP-2 in concentrations ranging from 10^{-11}–10^{-8} mol/liter were without effect on somatostatin secretion or any other measured parameter. At a concentration of 10^{-11} mol/liter in the perfusate none of the GLPs caused significant changes in hormone output from any of the preparations (not shown).

Discussion

Recent results from our own and other laboratories suggest that truncated GLP-1, a 30-amino acid fragment of proglucagon, which is produced and secreted by the lower intestinal mucosa (2, 3, 14), is an incretin that may participate in an entero-insular control of the endocrine pancreas and, thus, in the postprandial regulation of blood sugar. Somatostatin has also been suggested to play a role in maintaining blood sugar homeostasis (4, 14, 15). It was, therefore, natural to investigate the effect on somatostatin secretion from the pancreas and stomach of this new member of the glucagon-secretin family of peptides, many of which have been shown to enhance somatostatin secretion (16–20. We have previously found concentrations of immunoreactive GLP-1 in peripheral human venous plasma around 100 pmol/liter in the fasting state and increases in response to mixed meals up to 150 pmol/liter (11). In further studies (21) we found that the majority of the GLP-1 immunoreactivity in fasting plasma was due to a large mol wt molecule, presumably the large proglucagon fragment that is secreted from the pancreas synchronously with glucagon (21). After the meal, however, the majority of GLP-1 immunoreactivity in plasma corresponded in size to the intestinal form of GLP-1 (presumably truncated GLP-1) (22). The physiologically relevant concentration interval of GLP-1 in plasma would, therefore, be expected to be 10^{-11}–10^{-9} mol/liter, which was the range investigated in this study. In a few instances we also studied the effects of higher concentrations so as not to overlook eventual pharmacological effects, but such concentrations would not be expected to occur under physiological circumstances.

In agreement with our preliminary studies (2) and recent studies of perfused rat pancreases (3), truncated GLP-1 very potently stimulated insulin release from the pancreas at slight hyperglycemia. In addition, it stimulated somatostatin secretion from the pancreas with almost the same potency. In striking contrast, there was no effect on somatostatin secretion from two preparations of the stomach, an organ believed to contribute significantly to the concentrations of somatostatin measured in peripheral venous blood (9, 23, 24). It is unlikely that the lack of effect on the stomach should reflect impaired responsiveness of these preparations, since, in agreement with previous experience (25), GRP strongly stimulated somatostatin secretion from the antrum, and VIP increased somatostatin secretion from the antrectomized stomach, as has been reported for rat stomach (18, 20). The lack of effect of truncated GLP-1 is, therefore, more likely to reflect differences in the regulation of the gastric and pancreatic somatostatin cells. That the pancreatic and gastric somatostatin cells are different is also illustrated by the fact that the common precursor, prosomatostatin, is processed differently in the two tissues. In the pancreas the predominating product is somatostatin-14, whereas in the stomach significant amounts of somatostatin-28 and other larger forms are produced (9, 26). The viability of the perfused antrectomized stomach is further attested by the preserved secretory responses of hydrochloric acid (and pepsin) (7) to carbachol stimulation; in this case, however, somatostatin secretion is strongly inhibited.

Truncated GLP-1 strongly inhibited pancreatic glucagon secretion. This interesting effect could be due to interaction of truncated GLP-1 with specific receptors associated with an inhibitory second messenger system in the glucagon cells. Specific receptors for truncated GLP-1 (but not full-length GLP-1) have recently been demonstrated on insulin-producing rat insulinoma cells in culture (27), but so far nobody has identified receptors for truncated GLP-1 on glucagon cells. Another possibility is a paracrine effect, exerted via the increased secretion of somatostatin in the pancreatic islets. Such increases have previously been reported to inhibit glucagon secretion by a local paracrine pathway (28).

Full-length GLP-1 [proglucagon-(72–107) amide] and GLP-2 [proglucagon-(126–159(] were without effect on the endocrine or exocrine secretion of the three perfused

organ preparations. For GLP-1 this shows that the biological activity of the natural (truncated) GLP-1, as for glcuagon, requires an intact N-terminus (29). If full-length GLP-1 exists in mammalian tissues (30), it is, therefore, likely to be inactive. As previously shown, the amino acid sequence of porcine GLP-2 differs at four positions from the human sequence; in addition, the porcine form lacks the C-terminal basic amino acid residue of synthetic human GLP-2 (31). Our inability to detect any effects of synthetic human GLP-2 may, therefore, simply reflect the fact that we studied a peptide which is inactive because of differences between species. Porcine GLP-2 is not available for experimental studies at present.

In summary, our data suggest that truncated GLP-1 may participate in the control of pancreatic insulin, somatostatin, and glucagon secretion. In addition, our data show that the pancreatic somatostatin-producing cells respond to different stimuli and, therefore, probably express other receptors than the gastric somatostatin cells.

References

1. Bell GI, Sanchez-Pescador R, Laybourn PJ, Narajian RC 1983 Exon duplication and divergence in the human preproglucagon gene. Nature 304:368
2. Holst JJ, Ørskov C, Nielsen OV, Schwartz TW 1987 Truncated glucagonlike peptide-1, an insulin releasing hormone from the distal gut. FEBS Lett 211:169
3. Mojsov S, Weir G, Habener JF 1987 Insulinotropin: glucagon-like peptide 1 (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79:616
4. McIntosh CHS 1985 Minireview. Gastrointestinal somatostatin: distrition, secretion and physiological significance. Life Sci 37:2043
5. Lindkær Jensen S, Fahrenkrug J, Holst JJ, Kuhl C, Vagn Nielsen O, Schafflitzky de Muckadell OB 1978 Secretory effects of secretin on isolated perfused porcine pancreas. Am J Physiol 235:E381
6. Holst JJ, Jensen SL, Knuhtsen S, Nielsen OV, Rehfeld JF 1983 Effect of vagus, gastric inhibitory polypeptide, and HCl on gastrin and somatostatin release from perfused pig antrum. Am J Physiol 244:G515
7. Skak-Nielsen T, Holst JJ, Vagn Nielsen O, Role of Gastrin-releasing peptide in the neural control of pepsinogen secretion from the pig stomach. Gastroenterology, in press
8. Hilsted L, Holst JJ 1982 On the accuracy of radioimmunological determination of somatostatin in plasma. Regul Peptides 4:13
9. Baldissera FGA, Holst JJ, Jensen SL, Krarup TH 1985 Distribution and molecular forms of peptides containing somatostatin immunodeterminants in extracts from the entire gastrointestinal tract of man and pig. Biochim Biophys Acta 838:132
10. Stadil F, Rehfeldt JF 1973 Determination of gastrin in serum. Scand J Gastroenterol 8:101
11. Ørskov C, Holst JJ (1987). Radioimmunoassays for glucagon-like peptides 1 and 2 (GLP-1 and GLP-2) Scand J Clin Lab Invest 47:165
12. Conover WJ 1980 Practical Nonparametric Statistics, ed 2. Wiley and Sons, New York, p 299
13. Kreymann B, Williams G, Ghatei MA, Bloom SR 1987 Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet 2:1300
14. Koerker DK, Ruch W, Chidechel E, Palmer J, Goodner CJ, Ensinck J, Gale CC 1984 Hypothalamic inhibitor of the endocrine pancreas. Science 184:482
15. Colturi TJ, Unger RH, Feldman M 1984 Role of circulating somatostatin in regulation of gastric acid secretion, gastrin release and islet cell function. Studies in healthy subjects and duodenal ulcer patients. J Clin Invest 74:417
16. Ipp E, Dobbs RE, Harris V, Arimura A, Vale W, Unger RH 1977 The effects of gastrin, gastric inhibitory polypeptide, secretin and the octapeptide of CCK upon immunoreactive somatostatin release by the perfused canine pancreas. J Clin Invest 60:1216
17. Patton GS, Dobbs R, Orci L, Vale W, Unger RH 1977 Stimulation of pancreatic immunoreactive somatostatin (IRS) release by glucagon. Metabolism [Suppl 1] 25:1499
18. Saffouri B, DuVal JW, Arimura A, Makhlouf GM 1984 Effects of vasoactive intestinal peptide and secretin on somatostatin secretion in the perfused rat stomach. Gastroenterology 86:839
19. Rouiller D, Schuzdiarra V, Harris V, Unger RH 1980 Release of pancreatic and gastric somatostatin-like immunoreactivity in response to the octapeptide of cholecystokinin, secretin, gastric inhibitory polypeptide and gastrin 17 in dogs. Endocrinology 107:524
20. Chiba T, Taminato T, Kadowaki S, Inoue Y, Mori K, Seino Y, Abe H, Chihara K, Matsukura S, Fujita T, Goto Y 1980 Effects of various gastrointestinal peptides on gastric somatostatin release. Endocrinology 106:145
21. Ørskov C, Holst JJ, Knuhtsen S, Baldissera FGA, Poulsen SS, Nielsen OV 1986 Glucagon-like peptides GLP-1 and GLP-2, predicted products on the glucagon gene, are secreted separately from the pig small intestine but not pancreas. Endocrinology 119:1467
22. Ørskov C, Holst JJ, Seier Poulsen S, Kirkegaard P 1987 Pancreatic and intestinal processing of proglucagon in man. Diabetologia 30:874
23. Taborsky Jr GJ, Ensinck JW 1984 Contribution of the pancreas to circulating somatostatin-like immunoreactivity in the normal dog. J Clin Invest 73:216
24. Baldissera FGA, Nielsen OV, Holst JJ 1985 The intestinal mucosa preferentially releases somatostatin-28 in pigs. Regul Peptides 11:251
25. Holst JJ, Knuhtsen S, Ørskov C, Skak-Nielsen T, Poulsen SS, Nielsen OV 1987 GRP-producing nerves control antral somatostatin and gastrin secretion in pigs. Am J Physiol 253:G767
26. Skak-Nielsen T, Holst JJ, Baldissera FGA, Seier Poulsen S 1987 Localization in the gastrointestinal tract of immunoreactive prosomatostatin. Regul Peptides 19:183
27. Ørskov C, Høirilis Nielsen J 1988. Truncated glucagon like peptide-1 (proglucagon 78–107 amide), an intestinal insulin-releasing peptide, has specific receptors on rat insulinoma cells (RIN 5AH). FEBS Lett 229:175
28. Barden N, Lavoie M, Dupont A, Cote J, Cote J-P 1977 Stimulation of glucagon release by addition of antisomatostatin serum to islets of Langerhans in vitro. Endocrinology 101:635
29. Holst JJ 1983 Radioreceptor assays for glucagon. Handb Exp Pharmacol 66/I:245
30. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF 1986 Preproglucagon gene expression in pancreas and intestine diversifies at the level of posttranslational processing. J Biol Chem 261:11880
31. Buhl T, Thim L, Kofod H, Ørskov C, Harling H, Holst JJ 1988 Naturally occurring products of proglucagon 111–160 in the porcine and human small intestine. J Biol Chem 263:862