

The Effects of Gastrin, Gastric Inhibitory Polypeptide, Secretin, and the Octapeptide of Cholecystokinin upon Immunoreactive Somatostatin Release by the Perfused Canine Pancreas

E. IPP, R. E. DOBBS, V. HARRIS, A. ARIMURA, W. VALE, and R. H. UNGER,
*Veterans Administration Hospital and The University of Texas
Southwestern Medical School, Department of Internal Medicine,
Dallas, Texas 75235; Veterans Administration Hospital, New Orleans,
Louisiana 70146 and Tulane University, Department of Medicine,
New Orleans, Louisiana 70112; and The Salk Institute, La Jolla,
California 92037*

ABSTRACT The effects of gastrin, gastric inhibitory polypeptide, secretin, and the octapeptide of pancreozymin-cholecystokinin on immunoreactive somatostatin release were studied in the isolated perfused dog pancreas. Gastrin at a concentration of 65 ng/ml and the octapeptide of pancreozymin-cholecystokinin at a concentration of 25 ng/ml produced a prompt, but transient statistically significant, twofold rise in mean somatostatin concentration. Secretin at a concentration of 0.3 U/ml and gastric inhibitory polypeptide concentration of 58 ng/ml produced a prompt two- to threefold rise in mean somatostatin release, which persisted throughout the perfusion period. With all four polypeptides the pattern of the somatostatin response resembled that of insulin. It appears that pancreatic somatostatin release is stimulated by gastrointestinal hormones that influence the secretion of insulin and glucagon.

INTRODUCTION

The demonstration of somatostatin-containing D cells (1, 2) within the islets of Langerhans has raised the possibility that their physiological role or roles, like that of their neighboring cells, may be related to nutrient homeostasis (3). If this were true, the secretory

activity of the D cell might then be affected by nutrients and hormones that influence the A and B cells. Indeed, it has already been reported that arginine (4), glucagon (5), glucose (6–8), leucine (8), an amino-acid mixture (8), and pancreozymin-cholecystokinin (8), all stimulators of insulin secretion, also enhance the release of somatostatin.

The present study was designed to investigate further the effects of gastrointestinal hormones upon pancreatic somatostatin release.

METHODS

The preparation used was the isolated perfused dog pancreas (9), as previously modified (5). The isolated pancreas with the attached segment of duodenum was perfused with a synthetic medium (9) without recirculation. Flow rate was constant at 19–21 ml/min, and pressure was maintained between 25–50 mm Hg throughout the experiment. In all perfusions, glucose concentration was 100 mg/dl. In the gastrin experiments, a 1 mM mixture of 10 amino acids (10) was added to the perfusate to simulate the hyperaminoacidemia of a protein meal. The pancreatic effluent was collected continuously from a cannula in the portal vein and divided into 1-min aliquots in chilled tubes containing an EDTA-Benzamidinium mixture (0.003M/0.03M). These were stored at -20°C until assay. Assay of immunoreactive somatostatin was performed by the method of Arimura et al. (11) using Arimura antiserum 101. Insulin and glucagon assays were performed as previously described (12, 13). None of the hormones perfused interfered in any of the three assays.

Each hormone was perfused for two or three 8–10-min periods per experiment in three or four experiments. Only one hormone was perfused in each experiment. Hormone-free

This work was presented in part at the 37th Annual Meeting of the American Diabetes Association, St. Louis, Mo., 6 June 1977.

Received for publication 11 July 1977 and in revised form 15 August 1977.

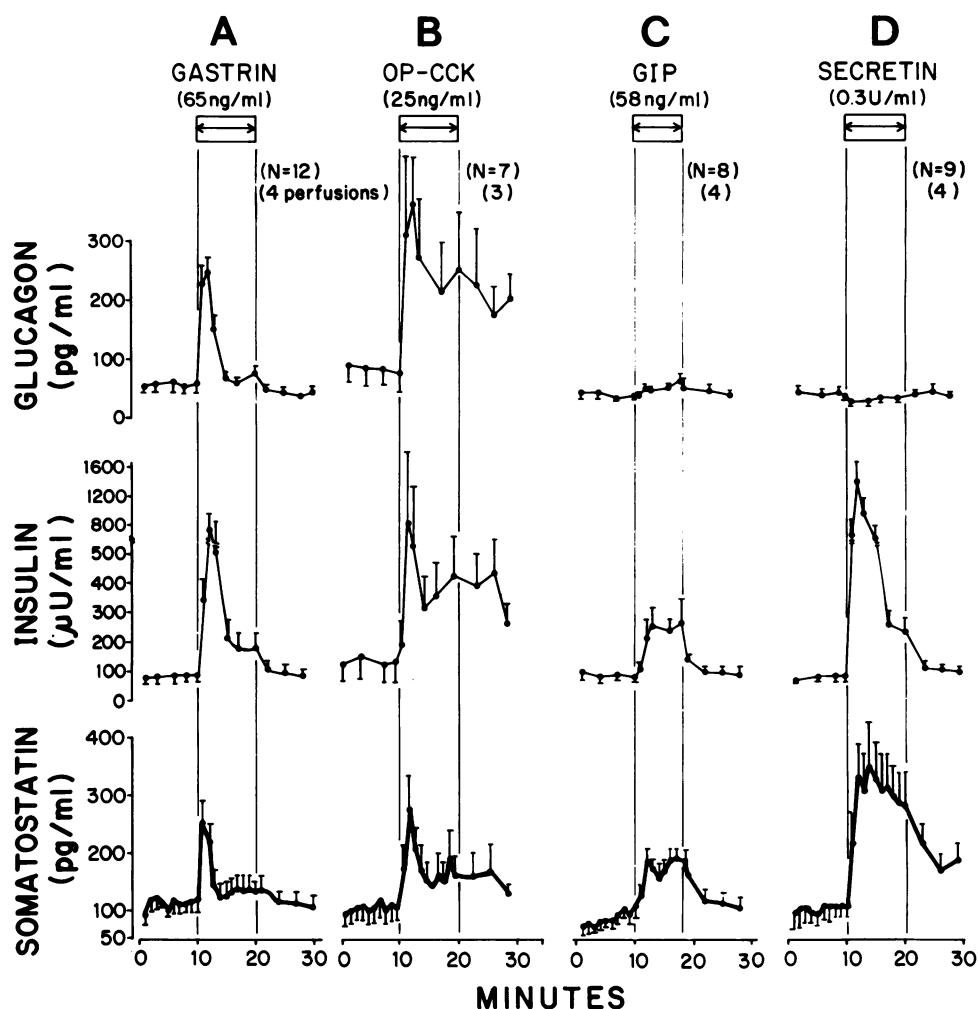


FIGURE 1 The effects of (A) gastrin, (B) the octapeptide of cholecystokinin (OP-CCK), (C) GIP, and (D) secretin on immunoreactive somatostatin, insulin, and glucagon release from the isolated perfused dog pancreas (mean \pm SEM). All hormones were perfused for a period of 10 min, except GIP which was perfused for 8 min. N = number of challenges with a polypeptide, and the figure in parentheses indicates the number of experiments, i.e., the number of pancreases perfused.

buffer was perfused for 30 min between each challenge with a polypeptide. 17-amino acid hog gastrin, generously supplied by Drs. Morton Grossman and John Walsh, porcine gastric inhibitory polypeptide (GIP),¹ lot EGIII, by Dr. John Brown, pure porcine secretin, lot 17561, by Professor Viktor Mutt, and the octapeptide of cholecystokinin, lot UTA-860-H/TJ-5, by E. R. Squibb and Sons, Inc., Princeton, N. J. Secretin was solubilized in normal saline and bovine serum albumin and other polypeptides in distilled water in 0.2% bovine serum albumin.

The results were analyzed statistically using the Wilcoxon signed rank test for paired data, and the Mann-Whitney test for analysis of individual experiments. Data is expressed as the mean \pm SEM.

¹ Abbreviation used in this paper: GIP, gastric inhibitory polypeptide.

RESULTS

Effect of gastrin. Gastrin was perfused at a concentration of 65 ng/ml on 12 occasions in four pancreases. On each occasion, this resulted in a simultaneous rise of immunoreactive somatostatin, insulin, and glucagon, all three of which reached a peak within 2 min (Fig. 1A). Mean (\pm SEM) somatostatin levels rose from the base-line level of 117 ± 18 pg/ml to a peak of 252 ± 41 pg/ml at 1 min ($P < 0.01$) and receded rapidly thereafter. A slight increase above base line persisted during the latter part of gastrin perfusion, but this did not differ significantly from the pre-perfusion values. The patterns of glucagon and insulin response were similar to that of somatostatin.

Effects of the octapeptide of pancreozymin-cholecystokinin. Although incompletely purified pancreozymin-cholecystokinin has been shown to stimulate somatostatin release (8), the effects of the synthetic octapeptide, free of other gut hormones such as GIP, had not been studied. The octapeptide in a concentration of 25 ng/ml was perfused on seven occasions in three pancreases. Mean somatostatin rose from the base-line value of 106 ± 21 pg/ml to a peak of 276 ± 62 pg/ml at 2 min ($P < 0.02$), the biphasic release pattern again resembling that of insulin and glucagon release (Fig. 1B). The second phase of somatostatin release was not consistently elevated, however. The mean of the values after the 3rd min in each experiment was significantly above the respective base-line value in five of the seven challenges ($P < 0.01$), but the mean increase in somatostatin for the entire group was not significant during this period. This is in contrast to the more sustained response previously reported with perfusion of partially purified full-sequence pancreozymin-cholecystokinin, which is said to contain about 2% GIP (8).

Effects of GIP. GIP at a concentration of 58 ng/ml was perfused on eight occasions in four pancreases. On each occasion the somatostatin level rose promptly. The mean value increased from 104 ± 15 pg/ml to a peak of 181 ± 22 pg/ml at 2 min ($P < 0.01$) and remained significantly elevated throughout the period of perfusion reaching another peak of 186 ± 13 pg/ml at 7 min ($P < 0.01$) (Fig. 1C). Again, the pattern of release of somatostatin was remarkably similar to that of insulin. A small but nonsignificant rise in glucagon release was also observed.

Effects of secretin. Secretin at a concentration of 0.3 U/ml was perfused on nine occasions in four pancreases. On each occasion a prompt rise in somatostatin concentration occurred. The mean level rose from 106 ± 20 pg/ml to a peak of 347 ± 74 pg/ml at 4 min ($P < 0.01$) (Fig. 1D) and remained above 281 ± 61 pg/ml throughout the perfusion. Again, the pattern resembled that of the insulin response. Slight suppression of glucagon release occurred (NS).

DISCUSSION

These studies reveal that somatostatin release by the canine pancreas is stimulated during infusion with pharmacologic concentrations of gastrin, GIP, secretin, and the octapeptide of pancreozymin-cholecystokinin. It was previously reported (8) that 33-amino acid pancreozymin-cholecystokinin increases somatostatin release. Thus, the D cell appears to be a possible target of at least four of the alimentary hormones that influence the B and A cells (14–21). With each hormone the pattern of the somatostatin response was remarkably similar to that of insulin. Although these

studies do not permit assessment of the contribution of somatostatin by the duodenal remnant, measurements of tissue concentration of somatostatin in the dogs suggest that the pancreas contains approximately 10 times as much somatostatin as the duodenum (22).

The physiologic significance of the response of somatostatin release during perfusion of gastrointestinal hormones remains to be established. It may be relevant that, in pharmacological amounts, somatostatin inhibits the release of gastrin (23), secretin (24), and GIP (25), and perhaps of pancreozymin-cholecystokinin (26), and may also interfere with their effects on gall bladder contraction (27) and on pancreatic (24,27) and gastric (23) exocrine function. The doses used were at least 20 times the maximum estimated rate of pancreatic release from the pancreas (28). If such inhibitory effects upon digestive function also occur at physiologic concentrations of somatostatin, as has recently been suggested (28), a physiologic feedback system may be considered in which somatostatin secretion, stimulated by nutrients and gastrointestinal hormones, reduces the release of the gut hormones and, in this way and (or) by direct action, controls the rate of the digestive functions that they stimulate. If so, this might permit the D cell, by varying somatostatin release, to influence the rate at which ingested nutrients enter the circulation (3). The responses of pancreatic somatostatin secretion to all nutrients and gut hormones thus far tested, together with the diverse effects of somatostatin upon the gastrointestinal tract and on insulin and glucagon secretion (29, 30), make a role of the D cell in regulation of nutrient homeostasis a plausible possibility.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Roger Guillemin for his interest and advice, Dr. Yogesh Patel for his generous gift of antiserum employed in the development of the somatostatin assay in this laboratory, Kay McCorkle and Daniel Sandlin for their invaluable technical assistance during these studies, and Billie Godfrey and Susan Freeman for secretarial assistance.

This work was supported by Veterans Administration Institutional Research Support grant 549-8000-01, grants AM 02700-16 and AN 09094-11 from the National Institutes of Health, The Salk Institute-Texas Research Foundation, Eli Lilly and Company, Indianapolis, Ind., The Upjohn Company, Kalamazoo, Mich., Ciba-Geigy Corporation, Ardsley, N. Y., and Bristol-Myers Company, New York.

REFERENCES

1. Luft, R., S. Efendic, T. Hökfelt, O. Johansson, and A. Arimura. 1974. Immunohistochemical evidence for the localization of somatostatin-like immunoreactivity in a cell population of the pancreatic islets. *Med. Biol. (Helsinki)*. **52**: 428–430.
2. Dubois, M. P. 1975. Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas. *Proc. Nat. Acad. Sci. U.S.A.* **72**: 1340–1343.

3. Unger, R. H., E. Ipp, V. Schusdziarra, and L. Orci. 1977. Hypothesis: physiologic role of pancreatic somatostatin and the contribution of D-cell disorders to diabetes mellitus. *Life Sci.* **20**: 2081-2086.
4. Patton, G. S., E. Ipp, R. E. Dobbs, W. Vale, L. Orci, and R. H. Unger. 1976. Response of pancreatic immunoreactive somatostatin to arginine. *Life Sci.* **19**: 1957-1960.
5. Patton, G. S., E. Ipp, R. E. Dobbs, L. Orci, W. Vale, and R. H. Unger. 1977. Pancreatic immunoreactive somatostatin release. *Proc. Nat. Acad. Sci. U.S.A.* **74**: 2140-2143.
6. Schauder, P., C. McIntosh, G. Arends, R. Arnold, H. Frerichs, and W. Creutzfeldt. 1976. Somatostatin and insulin release from isolated rat pancreatic islets stimulated by glucose. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **68**: 225-227.
7. Weir, G. C., E. Samols, R. Ramseur, J. A. Day, Jr., and Y. C. Patel. 1977. Influence of glucose and glucagon upon somatostatin secretion from the isolated perfused canine pancreas. *Clin. Res.* **25**: 403A. (Abstr.)
8. Ipp, E., R. Dobbs, A. Arimura, W. Vale, V. Harris, and R. H. Unger. 1977. Release of immunoreactive somatostatin from the pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide. *J. Clin. Invest.* **60**: 760-765.
9. Iversen, J., and D. W. Miles. 1971. Evidence for feedback inhibition of insulin on insulin secretion in the isolated, perfused canine pancreas. *Diabetes.* **20**: 1-9.
10. Floyd, J. C., Jr., S. S. Fajans, J. W. Conn, R. F. Knopf, and J. Rall. 1966. Stimulation of insulin secretion by amino acids. *J. Clin. Invest.* **45**: 1487-1502.
11. Arimura, A., S. Hanuko, D. H. Coy, and A. V. Schally. Radioimmunoassay for GH-release inhibiting hormone. *Proc. Soc. Exp. Biol. Med.* **148**: 784-789.
12. Yalow, R. S., and S. A. Berson. 1960. Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39**: 1157-1175.
13. Faloona, G. R., and R. H. Unger. 1974. Glucagon. In *Methods of Hormone Radioimmunoassay*. B. M. Jaffe and H. R. Behrman, editors. Academic Press, Inc. New York. 317-330.
14. Dupre, J. 1964. An intestinal hormone affecting glucose disposal in man. *Lancet.* **II**: 672-673.
15. Pfeiffer, E. F., M. Telib, J. Ammon, F. Melani, and H. Ditschuneit. 1965. Direkte Stimulierung der Insulinsekretion in Vitro durch Sekretin. *Dtsch. Med. Wochenschr.* **90**: 1663-1669.
16. Meade, R. C., H. A. Kneubuhler, W. J. Schulte, and J. J. Barboriak. 1967. Stimulation of insulin secretion by pancreozymin. *Diabetes.* **16**: 141-144.
17. Unger, R. H., H. Ketterer, J. Dupre, and A. M. Eisentraut. 1967. The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. *J. Clin. Invest.* **46**: 630-645.
18. Kaneto, A., Y. Tasaka, K. Kosaka, and K. Nakao. 1969. Stimulation of insulin secretion by the C-terminal tetrapeptide amide of gastrin. *Endocrinology.* **84**: 1098-1106.
19. Frame, C. M., M. B. Davidson, and R. A. L. Sturdevant. 1975. Effects of the octapeptide of cholecystokinin on insulin and glucagon secretion in the dog. *Endocrinology.* **97**: 549-553.
20. Iversen, J. 1971. Secretion of glucagon from the isolated perfused canine pancreas. *J. Clin. Invest.* **50**: 2123-2136.
21. Dupre, J., S. A. Ross, D. Watson, and J. C. Brown. 1973. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J. Clin. Endocrinol. Metab.* **37**: 826-828.
22. McIntosh, C., R. Arnold, J. Kobberlung, H. Becker, and W. Creutzfeldt. 1977. Immunoreactive somatostatin in the gastrointestinal tract of dog and human. *Eur. J. Clin. Invest.* **7**: 252. (Abstr.)
23. Bloom, S. R., C. H. Mortimer, M. O. Thorne, G. M. Besser, R. Hall, A. Gomez-Pan, V. M. Roy, R. C. G. Russell, D. H. Coy, A. J. Kastin, and A. V. Schally. 1974. Inhibition of gastrin and gastric acid secretion by growth hormone release inhibiting hormone. *Lancet.* **II**: 1106-1109.
24. Boden, G., M. C. Sivitz, O. E. Owen, N. Essa-Koumar, and J. H. Landor. 1975. Somatostatin suppresses secretion and pancreatic exocrine secretion. *Science (Wash. D. C.)*. **190**: 163-165.
25. Peterson, R. A., J. R. Dryburgh, and J. C. Brown. 1975. The effect of somatostatin on release and insulinotropic action of gastric inhibitory polypeptide. *Can. J. Physiol. Pharmacol.* **53**: 1200-1205.
26. Konturek, S. J., J. Tasler, W. Obtulowicz, D. H. Coy, and A. V. Schally. 1976. Effect of growth hormone-release inhibiting hormone on hormones stimulating exocrine pancreatic secretion. *J. Clin. Invest.* **58**: 1-6.
27. Creutzfeldt, W., P. G. Lankisch, and U. R. Folsch. 1975. Hemmung der Sekretin und Cholezystokinin-Pankreozymin-induzierten Saft- und Enzymsekretion des Pankreas und der Gallenblasenkontraktion beim Menschen durch Somatostatin. *Dtsch. Med. Wochenschr.* **100**: 1135-1138.
28. Schusdziarra, V., E. Ipp, and R. H. Unger. 1977. Somatostatin, a physiologic regulator of nutrient influx. *Diabetes.* **26** (Suppl. 1): 359. (Abstr.)
29. Alberti, K. G. M. M., N. J. Christensen, S. E. Christensen, A. P. Hansen, J. Iversen, K. Lundbaek, K. Seyer-Hansen, and H. Orskov. 1973. Inhibition of insulin secretion by somatostatin. *Lancet.* **II**: 1299-1301.
30. Koerker, D. J., W. Ruch, E. Chideckel, J. Palmer, C. J. Goodner, J. Ensink, and C. C. Gale. 1974. Somatostatin: hypothalamic inhibitor of the endocrine pancreas. *Science (Wash. D. C.)*. **184**: 482-483.