Release of Immunoreactive Somatostatin from the Pancreas in Response to Glucose, Amino Acids, Pancreozymin-Cholecystokinin, and Tolbutamide

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ABSTRACT The effects of glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide upon the release of immunoreactive somatostatin (IRS) from the isolated perfused pancreas were studied. In seven experiments in which glucose was perfused either at a concentration of 100 or 350 mg/dl or at 25 mg/dl, IRS levels were significantly greater at the higher glucose concentrations. In three dose-response experiments in which the perfusing glucose concentration was increased at 30-min intervals from an initial concentration of 25 mg/dl to a final concentration of 300 mg/dl, progressive increases in IRS release were noted at glucose concentrations of 100 mg/dl and above. Perfusion of a 20 mM mixture of 10 amino acids also elicited a prompt and significant biphasic IRS rise in each of six experiments. In five experiments, 20 mM leucine evoked a similar response in mean IRS. Perfusion with 0.075 Ivy U/ml of pancreozymin-cholecystokinin, with or without the presence of a 1 mM 10-amino acid mixture, elicited a prompt rise in IRS with a pattern resembling that of insulin in a total of six experiments. Tolbutamide (0.75 mg/min) also stimulated IRS release in five of six challenges. The IRS responses to nutrients and to pancreozymin and their similarity to the insulin responses raise the possibility that, like insulin, pancreatic somatostatin may have an endocrine role related to nutrient homeostasis.

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

Received for publication 6 April 1977 and in revised form 20 June 1977.

INTRODUCTION

The physiologic role of the somatostatin-containing D-cells of the endocrine pancreas (1–5) is unknown. Recent studies of the perfused canine pancreas have shown that immunoreactive somatostatin (IRS)[1] is released from the pancreas during perfusion with high concentrations of arginine (6, 7) and that glucose stimulates IRS release (8–10). This raises the possibility that, like insulin and glucagon, somatostatin may have a regulatory role in nutrient homeostasis (11). The present studies were, therefore, designed to explore further the response of insular IRS to nutrients and other substances known to influence islet cell secretion.

METHODS

Pancreases from fasting 17–25-kg male mongrel dogs were isolated and perfused according to the technic of Iversen and Miles (12), as previously modified (7). The preparation included a 12-cm segment of duodenum. The celiac artery and portal vein were catheterized, and the preparation was placed into a tissue flotation bath kept at 37°C and perfused without recirculation with a Krebs-Ringer bicarbonate solution with 4% dextran T 70 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N.J.), 0.6% bovine serum albumin, and 5.5 mM glucose, maintained at 38°C, and bubbled constantly with a 95% O_2, 5% CO_2 mixture. A constant flow rate of 19–21 ml/min and a perfusion pressure of 25–50 mm Hg were maintained by means of a Harvard peristaltic pump (Harvard Apparatus Co., Millis, Mass.). Test substances were added to the perfusate by means of a three-way stopcock with a delay of about 20 s before reaching the pancreas. The venous effluent was

[1] *Abbreviations used in this paper*: IRS, immunoreactive somatostatin; PZ-CCK, pancreozymin-cholecystokinin.

collected in chilled tubes and divided into 1-min samples, and aliquots were frozen within 30 min and stored at −20°C until the time of assay.

IRS was assayed in every sample by a double-antibody modification of the method of Arimura et al. (13, 14) using rabbit antiserum #101. The standard dilution curve was set up with perfusate as a diluent. The lower limit of sensitivity of the assay was 40 pg/ml. The coefficient of variation was 15.5% between assays and 12% within the assay. Unknown samples gave proportional dilution slopes that paralleled those of synthetic somatostatin. Insulin and glucagon were measured in every third sample, i.e. every 3rd min, by previously described methods (15, 16). None of the agents perfused in this study influenced any of the assays.

Nutrients and other agents were perfused for 10–50 min. In five previous control experiments, buffer alone perfused for 140 min caused no significant change in the mean effluent concentration of any of the three hormones measured (7).

Statistical analysis of the results was performed by using either the Mann and Whitney test (17) or the one-sided Wilcoxon signed rank test for paired data (18).

RESULTS

Effect of varying glucose concentrations upon pancreatic IRS release. Glucose was perfused at 25 mg/dl (1.35 mM) for 20 min and at 350 mg/dl (19.4 mM) for 50 min in five experiments (Fig. 1). IRS levels were significantly greater ($P < 0.05$) at 12 of the 19 points measured during perfusion with the higher glucose concentration. In two other experiments, glucose was first perfused at 100 mg/dl (5.5 mM) for 30 min, and then at 25 mg/dl (1.35 mM) for 40 min (Table I). In these two experiments, as in all others analyzed individually, IRS release was significantly greater ($P < 0.001$) at the higher glucose concentration (Table I).

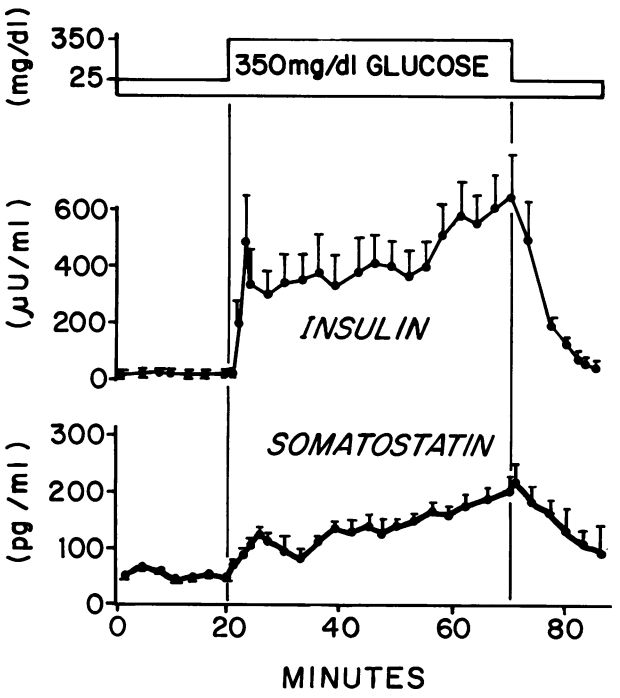

FIGURE 1 The effect of change in perfusing glucose concentration from 25 to 350 mg/dl upon mean (±SEM) IRS and insulin concentrations in the pancreatic effluent ($n = 5$). At the high glucose concentration, IRS was significantly greater than at the low glucose concentration ($P < 0.05$) from 24–27 min and from 39–70 min.

TABLE I

Effect of Decreasing Glucose Concentrations upon IRS and Insulin Concentrations in the Effluent of the Perfused Dog Pancreas*

	IRS (pg/ml)		Insulin (μU/ml)	
Perfusion period, *min*	30	40	30	40
Glucose concentration, *mg/dl*	100	25	100	25
Experiment 1	89±2.2	68±2.7‡	30±0.9	7±1.8
2	102±3.0	84±3.2‡	38±2.9	6±1.0

* Mean±SEM of all samples measured within a perfusion period, e.g., 30 IRS measurements and 10 insulin measurements during a 30-min perfusion period; see Methods.
‡ $P < 0.001$ vs. perfusion with preceding glucose concentration.

In three other experiments, glucose concentration was increased progressively at 30-min intervals from 25 to 300 mg/dl (Fig. 2). In all three experiments, IRS levels were above the sensitivity limits of the assay at a glucose concentration of 50 or 100 mg/dl; progressive and highly significant increases ($P < 0.01$) in IRS were observed with each further increase in glucose concentration (Table II).

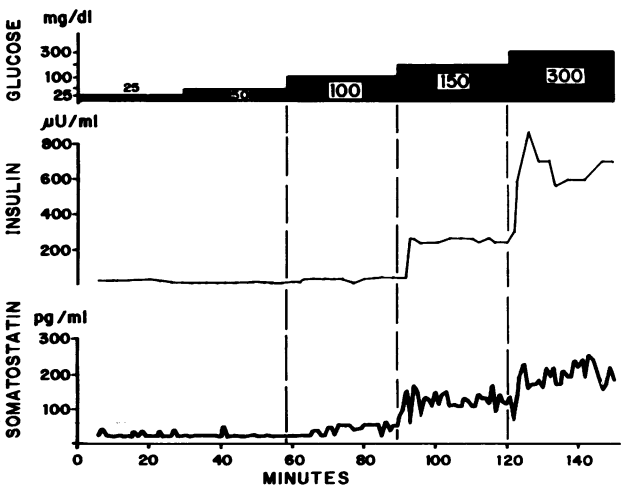

FIGURE 2 The effect of progressive increases in glucose concentration upon release of IRS and insulin by the isolated perfused dog pancreas.

TABLE II

Effect of Progressively Increasing Glucose Concentration at 30-min Intervals on IRS and Insulin Release in the Effluent of the Perfused Dog Pancreas*

	Glucose Concentration, *mg/dl*				
	25	50	100	150	300
Experiment 1					
IRS, *pg/ml*	<40	<40	46±2	117±5‡	191±8.9‡
Insulin, *μU/ml*	16±1	9±0.4	30±3	234±17	632±46
Experiment 2					
IRS, *pg/ml*	63±3.5	86±5	171±5‡	298±9.6‡	361±7.2‡
Insulin, *μU/ml*	15±0.6	9±0.6	7±0.3	74±9	247±20
Experiment 3					
IRS, *pg/ml*	<40	54±1.8	109±4.5‡	186±3.8‡	239±4.6‡
Insulin, *μU/ml*	15±0.7	16±0.5	167±25	951±99	1882±93

* Mean±SEM of all samples measured within a perfusion period.
‡ $P < 0.01$ vs. preceding perfusion period.

Effects of amino acids and pancreozymin-cholecystokinin (PZ-CCK)[2] *on pancreatic IRS release.* The 10-amino acid mixture of Floyd et al. (19) was perfused at a concentration of 20 mM for 45 min in six experiments. IRS increased significantly ($P < 0.05$) above the baseline at 34 of the 45 points measured during the amino acid perfusion (Fig. 3). In three of the six experiments, the amino acid mixture was perfused subsequently at a 1 mM concentration after a 30-min interval of perfusion with buffer. In all three, a slight rise in IRS was observed. This was greatly potentiated by superinfusion with PZ-CCK at a concentration of 0.075 Ivy U/ml (1.5 Ivy U/min) (Table IIIB), which elicited a biphasic increase of IRS, insulin, and glucagon. In three other experiments in which PZ-CCK was perfused alone at this concentration (Table IIIA), highly significant increases in IRS release which paralleled the insulin response were observed. A representative experiment is shown in Fig. 4.

Effects of leucine on pancreatic IRS release. 20 mM leucine was perfused for 45-min periods in five experiments. During this period, the IRS level rose significantly above the baseline in 36 of the 45 points measured ($P < 0.05$) in a biphasic response that paralleled the insulin response (Fig. 5).

Effects of tolbutamide on pancreatic IRS release. Tolbutamide was perfused at a concentration of 37.5 μg/ml (0.75 mg/min) for six 10–15-min periods in three experiments. The tolbutamide perfusion periods were separated by a 30-min buffer perfusion period.

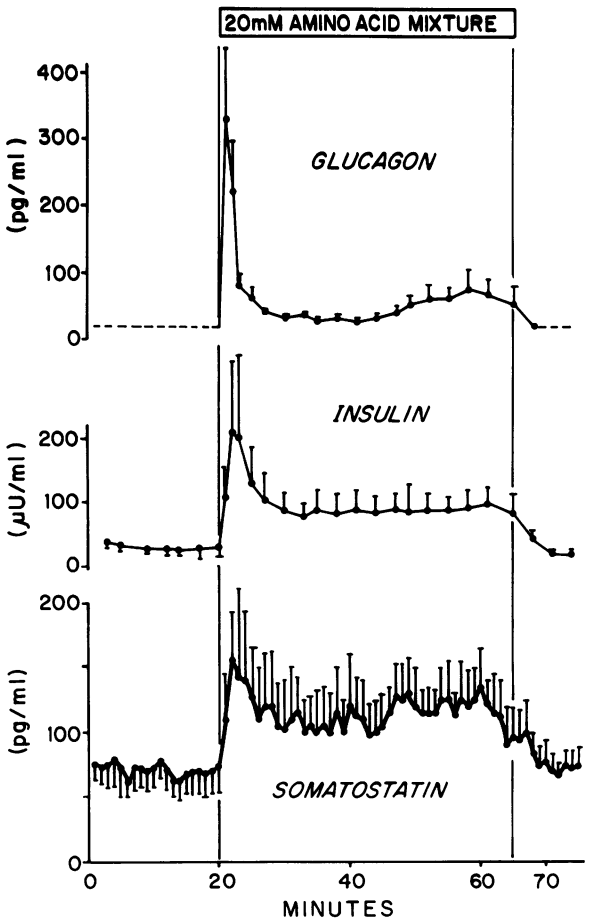

FIGURE 3 The effect of perfusion of a 20 mM mixture of 10 amino acids upon mean (±SEM) IRS, insulin, and glucagon concentrations in the pancreatic effluent ($n = 6$). During amino acid perfusion, IRS was significantly greater than the baseline ($P < 0.05$) at 34 of the 45 measurements.

[2] Lot number 27561, kindly supplied by Professor Viktor Mutt, Karolinska Institutet, Stockholm, Sweden. Recent lots contain less than 2% immunoreactive gastric inhibitory polypeptide (personal communication from Dr. John C. Brown, Vancouver, B.C.).

TABLE III

Effects of Pancreozymin-Cholecystokinin on IRS, Insulin, and Glucagon Concentrations in the Effluent of the Perfused Dog Pancreas*

	Perfusion Period, *min*	IRS, *pg/ml*			Insulin, *μU/ml*			Glucagon, *pg/ml*		
		30	15	15	30	15	15	30	15	15
	Perfusate	Buffer	PZ-CCK‡	Buffer	Buffer	PZ-CCK‡	Buffer	Buffer	PZ-CCK‡	Buffer
A	Experiment 1	66±2.6	182±17.5§	92±13.1	77±4.6	1,581±193	627±249	<20	40±3.4	<20
	2	121±4.5	160±7.6§	125±3.4	84±7.0	704±86	355±115	<20	24±1.5	<20
	3	215±7.8	590±26.0§	325±21.8	33±2.6	1,790±345	527±31	<20	29±1.6	<20
B‖	Experiment 4	178±6.2	304±22.0§	96±25.0	114±5.0	1,831±284	1,093±104	<20	33±4.0	<20
	5	193±10	422±14.0§	—	51±2.0	1,088±123	—	<20	95±2.0	—
	6	98±4.2	122±6.0¶	72±6.0	60±9.0	465±139	225±55	<20	71±15	<20

* Mean±SEM of all samples measured within a perfusion period.

‡ 1.5 Ivy U/min.

§ $P < 0.01$ vs. preceding buffer perfusion period.

‖ In this group of experiments, the buffer and stimulated period contain a mixture of 10 amino acids (1 mM).

¶ $P < 0.05$ vs. preceding buffer perfusion period.

IRS rose significantly ($P < 0.01$) in five of the six perfusions, but in all six experiments IRS increased during the first 3 min of tolbutamide perfusion (Table IV). A representative experiment is shown in Fig. 6.

DISCUSSION

These studies in the isolated dog pancreas confirm an enhancement of IRS release by glucose at perfusing concentrations of 100 mg/dl and above. Others have also reported the stimulatory action of glucose upon

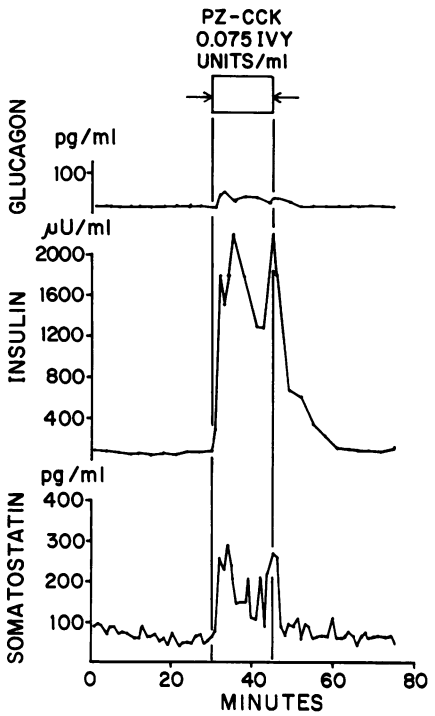

FIGURE 4 The effect of perfusion with PZ-CCK upon IRS, insulin, and glucagon concentrations in the pancreatic effluent. This single experiment is representative of those in Table III.

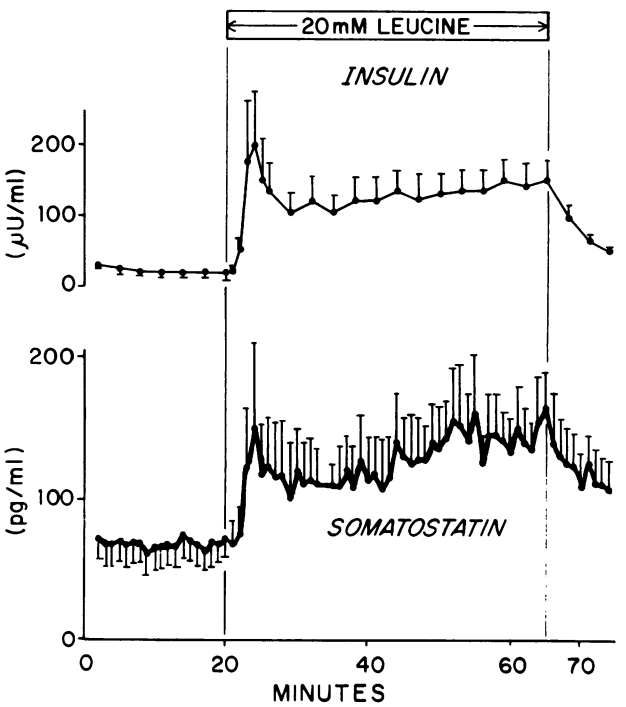

FIGURE 5 The effect of perfusion with 20 mM leucine upon mean (±SEM) IRS, insulin, and glucagon concentrations in the pancreatic effluent ($n = 5$). IRS was significantly greater than the baseline in 36 of the 45 measurements.

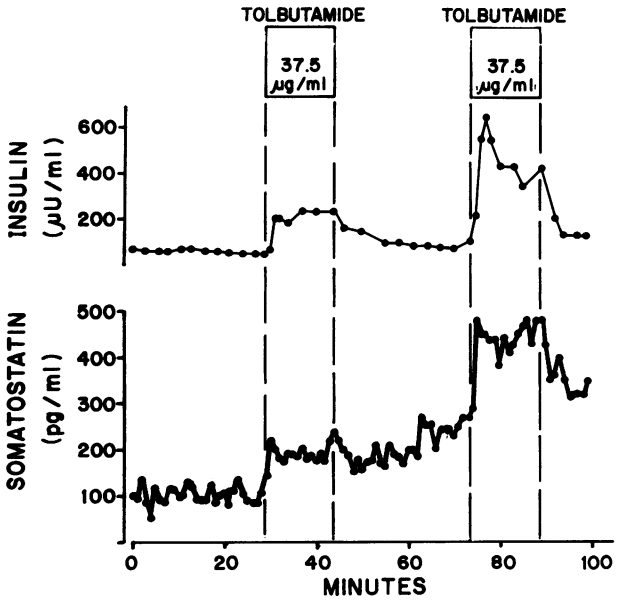

FIGURE 6 The effect of tolbutamide (37.5 μg/ml) perfused at a rate of 0.75 mg/min upon the release of IRS and insulin by the isolated dog pancreas.

IRS release by the perfused canine pancreas (9), and in isolated rat islets (8), although Barden et al. failed to observe an IRS response to glucose (20). In addition, these studies demonstrate for the first time increased IRS release in response to 20 mM solution of a 10-amino acid mixture, 20 mM leucine, and to the alimentary hormone PZ-CCK, all of which stimulate insulin secretion. It had been reported previously that arginine and glucagon, both stimulators of insulin secretion, also stimulate IRS, whereas insulin itself does not (7).

The fact that high nutrient concentrations and the alimentary hormone PZ-CCK appear to elicit an IRS secretory response, together with the similarity of IRS and insulin responses, is compatible with a role of the D-cell in the regulation of nutrient homeostasis (11). The nature of this role remains to be established, but there are lines of evidence pointing to the gastrointestinal tract and digestive organs as possible targets. In pharmacologic doses, somatostatin inhibits gastric emptying (21), HCl secretion (22), gall bladder contraction (23), pancreatic exocrine function (23–25), and release of gastrin (22), motilin (21), and secretin (25), and retards absorption of glucose, xylose (26), and triglycerides (27, 28), suggesting its biological capability to retard the entry of ingested nutrients into the circulation. Indeed, the intraportal administration of exogenous somatostatin at a physiologic rate also retards the appearance of ingested xylose and triglycerides (29). Thus, the gastrointestinal tract and the digestive organs could be a physiologic target of pancreatic somatostatin secreted in response both to alimentary hormones and to high nutrient concentrations (11). If so, the A-, B-, and D-cells of the islets of Langerhans would have an influence over all routes through which fuels move into and out of the extracellular space. In particular, coordinated responses of pancreatic somatostatin and insulin secretion would permit coordination of the rates at which exogenous nutrients enter and leave the extracellular space and thereby enable the islets to regulate their turnover and concentrations (11).

The demonstration of a tolbutamide-induced rise in IRS release raises the possibility that the antihyperglycemic action of this drug may, at least in part, involve somatostatin-mediated retardation of nutrient entry from the gut.

TABLE IV

Effect of Tolbutamide upon IRS and Insulin Concentrations in the Effluent of the Perfused Dog Pancreas*

Perfusion period, *min*	IRS, *pg/ml*			Insulin, *μU/ml*		
	15	10–15	10	15	10–15	10
Perfusate	Buffer	Tolbutamide‡	Buffer	Buffer	Tolbutamide‡	Buffer
Experiment 1a	102±5.0	193±5.0§	183±7.2	55±2.3	192±55.6	142±24.9
1b	243±8.1	447±7.6§	355±15.4	84±4.8	451±48.0	144±18.8
2a	59±4.1	110±5.3§	90±10.4	6±0.3	43±4.2	26±2.4
2b	95±7.5	96±7.2	54±3.8	18±0.8	175±18.4	40±9.1
3a	73±8.9	107±4.0§	57±8.0	27±3.5	68±6.2	61±1.0
3b	67±5.6	131±5.9§	114±9.0	55±2.6	91±5.3	55±8.0

* Mean±SEM of all samples measured within a perfusion period.
‡ 0.75 mg/min.
§ $P < 0.01$ vs. preceding buffer perfusion period.

ACKNOWLEDGMENTS

The authors wish to thank the following people for their help in the various phases of this work: Kay McCorkle, Danny Sandlin, Robert Goegel, and Joan Reisch.

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, Ciba-Geigy Corp., Ardsley, N. Y., and Eli Lilly and Company, Indianapolis, Ind.

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