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Pancreatic immunoreactive somatostatin release

(insulin/glucagon/arginine/D-cells/perfused pancreas)

G. S. PATTON*, E. IPP*, R. E. DOBBS*, L. ORCI†, W. VALE‡, AND R. H. UNGER* §

* VA Hospital, Dallas, Texas 75216, and The University of Texas Southwestern Medical School, Dallas, Texas 75216, Department of Internal Medicine; † Institute of Histology and Embryology, Geneva, Switzerland; and ‡ The Salk Institute, La Jolla, California 92112

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ABSTRACT **The location of the somatostatin-containing D-cells of the pancreatic islets between the A- and B-cells suggests that their function might be to inhibit insulin and/or glucagon secretion by these neighboring cells. To determine if insulin and/or glucagon, in concentrations that might be present in the extracellular space surrounding the D-cells, stimulate immunoreactive somatostatin (IRS) release, we perfused 10 μg of glucagon or 10 milliunits of insulin per ml in 11 isolated dog pancreases, for 40 min in seven experiments and for 100 min in four experiments. In eight of the nine experiments in which glucagon was perfused, a prompt and significant rise in mean IRS release, ranging from 71 to 128% above the control level, was observed. In the eight experiments in which insulin was perfused, IRS did not increase during the first 40 min; in the two 100-min insulin experiments, it did rise during the final 50 min, however. To determine the effect of an A- and B-cell secretogogue on IRS release, we perfused 20 mM arginine for 60 min in six experiments. In all, IRS rose within 3 min and reached a level 71–465% above the control, remaining significantly elevated throughout the perfusion, while glucagon and insulin rose to peak levels at 2 min and then declined somewhat despite continuing arginine perfusion. The results indicate that perfusion of the normal dog pancreas with high doses of glucagon or arginine is accompanied by a prompt increase in IRS release and are compatible with a local feedback circuit involving A- and D-cells. Insulin appears not to augment IRS release, at least not promptly, but IRS stimulated by local endogenous glucagon could inhibit the B-cell response to locally secreted glucagon and thereby influence the composition of the insulin/glucagon secretion mixture.**

Somatostatin immunoreactivity has been demonstrated by immunocytochemical technics (1, 2), by radioimmunoassay (3), and by bioassay (4) to be present in the islets of Langerhans and more recently has been identified in the secretion granules of D-cells (5–7). The fact that D-cells are situated between A- and B-cells, cells whose secretory function somatostatin so profoundly inhibits (8–10), has suggested the possibility of local hormone–cell interactions between the secretory product of the D-cells and one or both of the neighboring cell types, interactions that might influence glucagon and/or insulin secretion (11).

To test this hypothesis, we measured immunoreactive somatostatin (IRS) in the effluent of the isolated dog pancreas during perfusion with high concentrations of glucagon and insulin intended to simulate those which might be present in the extracellular space surrounding the D-cells. In additional experiments, arginine, which stimulates endogenous glucagon and insulin secretion, was also perfused.

MATERIALS AND METHODS

Pancreases were isolated from fasting 17- to 20-kg mongrel male dogs by the technic of Iversen and Miles (12). Lymph nodes in the pancreatic region were ligated and removed. Blood vessels and organ attachments were left intact until 1–3 min before perfusion was initiatiated. The effluent cannula, secured in the portal vein immediately proximal to the point of bifurcation, drained the entire organ. The duodenal lumen was intubated and drained by gravity flow.

The perfusion buffer was Krebs–Ringer bicarbonate solution as modified by Iversen and Miles (12), except that the glucose concentration was 1 g/liter, fumarate, glutamate, and pyruvate were not added, and the albumin concentration was 0.6% (wt/vol). Liter aliquots of perfusion fluid with a 95% O_2/5% CO_2 mixture bubbling constantly at a rate of 3 liters/min were maintained in a water bath at 37°, and perfused by means of a Harvard peristaltic pump at a constant flow of 17 ml/min. In the first three experiments, collections were made for 30–45 sec and in all subsequent experiments for 1-min periods. Samples were collected in chilled tubes maintained in an ice bath and then stored at −20° until assayed.

Insulin and glucagon were perfused at a concentration of 10 milliunits (mU)/ml and 10 μg/ml, respectively. In other experiments arginine was perfused at a 20 mM concentration. An experiment was considered technically unacceptable if the appearance of the pancreas changed during the experiment as a consequence of edema, hemorrhage, or cyanosis, if leaks developed in the perfusion system, if the flow rate declined, if levels of all hormones were unmeasurable throughout an experiment, and if the viability test of insulin or glucagon response to 10 mM arginine after perfusion was impaired.

IRS was measured by a modification of the method of Patel and Reichlin as described by Patel and Weir (13) and subsequently modified (4) using antiserum kindly provided by A. Arimura for assay of the six experiments in which glucagon and insulin were perfused in alternating sequence for 40 min. In all other experiments the antiserum generously supplied by Y. Patel was used. The maximum sensitivity of the assay was 20 pg/ml. IRS in the pancreatic effluent gave dilution slopes that paralleled those of purified somatostatin. Exogenous somatostatin perfused through the pancreas could be recovered by assay of the pancreatic effluent, and recovery was not improved by an inhibitor of proteolysis. Neither the perfusion fluid nor any of the substances perfused, insulin, glucagon, and arginine, influenced the radioimmunoassay for somatostatin.

Insulin and glucagon were assayed in every third sample by previously described methods (14–16). Because of interference of the perfusing buffer in the insulin and glucagon assays, the buffer was added to the assay standards.

The one-tailed Mann and Whitney modification (17) of the Wilcoxon test (18) was used for statistical analyses.

RESULTS

Perfusion of buffer alone

To determine if spontaneous variations in the release of IRS occurred, we perfused buffer alone for 140 min in five exper-

Abbreviations: IRS, immunoreactive somatostatin; U, units.

§ To whom reprint requests should be addressed.

Table 1. IRS concentration (pg/ml, mean ± SEM*) in effluent of isolated dog pancreas during 40-min insulin and glucagon perfusions and 20-min perfusions with hormone-free buffer

Exp. no.	Perfusate: Buffer	Hormone I†	Buffer	Hormone II†	Buffer
A1	56 ± 16	117 ± 10‡	135 ± 14	54 ± 5	93 ± 10‡
2	183 ± 13	389 ± 13‡	364 ± 31	281 ± 10	370 ± 19‡
3	138 ± 4	147 ± 6	127 ± 8	111 ± 4	107 ± 5
4	77 ± 8	144 ± 9‡			
B5	316 ± 4	351 ± 18	347 ± 11	413 ± 16‡	384 ± 16
6	64 ± 6	66 ± 3	135 ± 20‡	276 ± 14‡	220 ± 36
7	56 ± 6	76 ± 6	70 ± 7	120 ± 10‡	116 ± 14

* Mean ± SEM of the number of samples examined in each perfusion period (for buffer, $n = 20$; for hormone perfusion, $n = 40$).
† In series A: hormone I = glucagon (10 μg/ml), hormone II = insulin (10 mU/ml). In series B: hormone I = insulin (10 mUml), hormone II = glucagon (10 μg/ml).
‡ Value statistically greater than that of preceding period ($P < 0.01$).

iments. Each experiment was divided into 20- and 40-min periods to correspond with the experimental design of the glucagon and insulin perfusion experiments. No significant difference in the IRS level of any two perfusion periods was observed in any experiment.

Perfusion of insulin

There were eight experiments in which insulin was perfused. In six experiments, insulin was perfused for 40 min either before or after perfusion with glucagon (Table 1). In two others (Table 2), insulin was perfused for 100 min. In all experiments, insulin in a concentration of 10 mU/ml failed to increase immunoreactive somatostatin significantly above the preceding period of hormone-free buffer perfusion during the first 40 min of perfusion. A typical result is shown in Fig. 1.

In the two experiments in which insulin was perfused continuously at 10 mU/ml for 100 min (Table 2A), IRS concentrations remained unchanged for the first 50 min, but during the last 50 min a statistically significant rise in somatostatin above the control period was observed.

Perfusion of glucagon

There were nine experiments in which glucagon in a concentration of 10 μg/ml was perfused. In six experiments, glucagon and insulin were each perfused in alternating sequence for 40 min, and in a seventh the experiment was terminated immediately after 40 min of glucagon perfusion (Table 1A). In two additional experiments (Table 2B), glucagon was perfused continuously for 100 min. All but one of the nine experiments were characterized by a statistically significant increase in IRS concentration above that of the preceding buffer perfusion period during the period of glucagon perfusion (Tables 1 and 2B). A typical experiment is shown in Fig. 1. In both experiments in which glucagon perfusion was continued for 100 min (Table 2B), the statistically significant rise in immunoreactive somatostatin which appeared within the first 10 min of the glucagon perfusion period persisted throughout.

Table 2. IRS concentrations (pg/ml, mean ± SEM*) during perfusion of dog pancreas with insulin (A) or glucagon (B) for 100-min and with buffer for 20-min

Exp. no.	Perfusate: Buffer	Hormone: 1–50 min	Hormone: 51–101 min
A 8	135 ± 12	161 ± 8	326 ± 17†
9	107 ± 7	108 ± 5	147 ± 7†
B10	213 ± 13	336 ± 13†	397 ± 11†
11	196 ± 6	419 ± 19†	755 ± 32†

* Mean ± SEM of the number of samples examined in each perfusion period (for buffer, $n = 20$; for hormone perfusion, $n = 50$).
† Significantly greater than during perfusion with hormone-free buffer ($P < 0.01$).

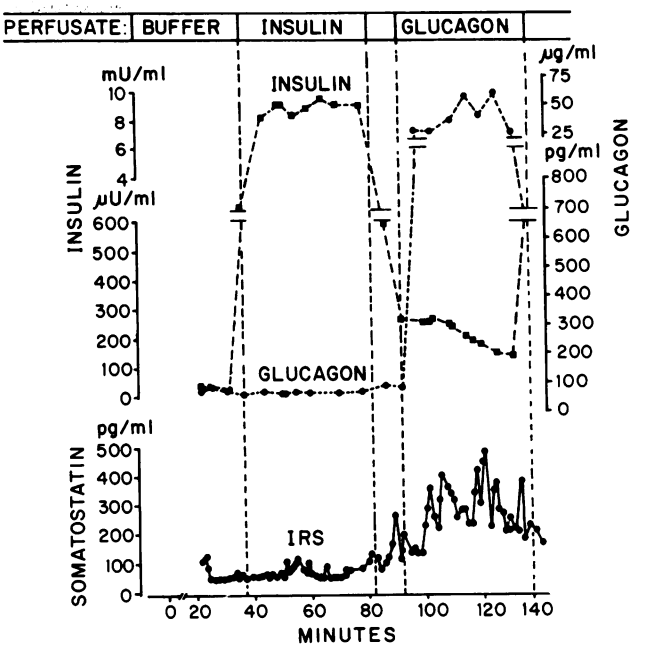

FIG. 1. Concentrations of IRS in the venous effluent of an isolated dog pancreas during perfusion with hormone-free buffer, insulin (10 mU/ml), and glucagon (10 μg/ml).

Perfusion of arginine

To determine if stimulation of endogenous glucagon would similarly increase IRS, we perfused 20 mM arginine for 60 min in six experiments, one of which is shown in Fig. 2. In all six, IRS increased within the first 3 min of the arginine perfusion and rose to levels averaging two to five times the mean value of the preceding buffer control period (Table 3). In all experiments, glucagon rose to a peak within 2 min, the first specimen assayed, and declined thereafter. Insulin also was at a peak at this time and declined thereafter.

DISCUSSION

The perfusion of glucagon in the high concentration that might be present in the intercellular space between A- and D-cells during stimulated glucagon secretion elicited an increase in mean IRS concentration ranging from 71 to 128% of the control level, in the effluent of the perfused pancreas of normal dogs. Inasmuch as somatostatin is a powerful inhibitor of glucagon release (8–10), these results are compatible with a feedback relationship between the A- and D-cell in which glucagon secretion stimulates the release of somatostatin, which in turn reduces glucagon secretion and perhaps glucagon-mediated insulin secretion. Perfusion with arginine, a powerful stimulus of endogenous glucagon secretion (19, 20), was also accompa-

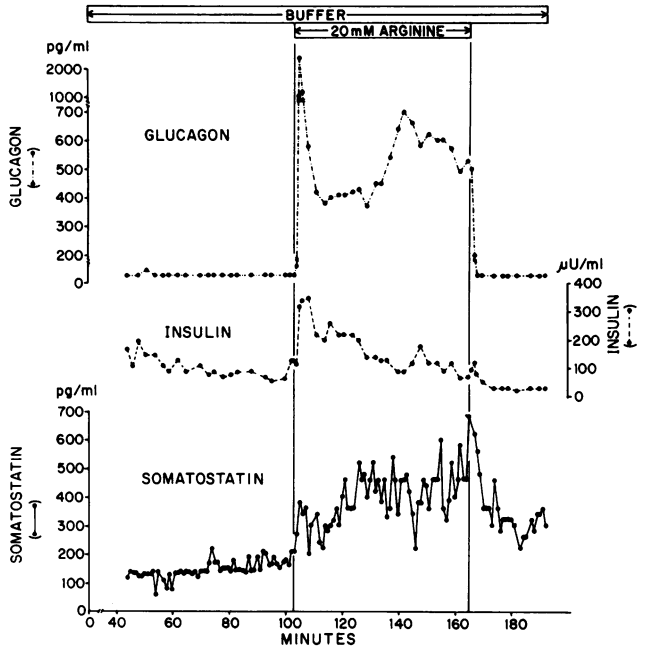

FIG. 2. Concentration of glucagon, insulin, and IRS in the venous effluent of an isolated dog pancreas during perfusion with 20 mM arginine.

nied by a striking rise in mean IRS concentration, ranging from 71 to 465% of the control level. Both glucagon and insulin concentrations declined from their initial peak despite continued perfusion of arginine, while somatostatin remained high throughout the arginine perfusion, a pattern compatible with somatostatin-mediated inhibition of the response of the A- and B-cells during continuous arginine perfusion. It is, of course, not clear whether arginine stimulates somatostatin release directly, and/or via glucagon mediation.

Under these experimental conditions, perfusion of high concentrations of insulin failed in any of eight experiments to enhance significantly the release of IRS during 40 min of perfusion; however, in two experiments in which insulin was perfused for 100 min, IRS increased significantly during the final 50 min of perfusion. Whether or not insulin influences D-cell activity, somatostatin-mediated restraint of the insulin response to secreted glucagon provides a teleologically attractive explanation for the relatively weak insulin response when glucagon secretion is enhanced by certain stimuli. In fact, the D-cell could well serve to regulate concentrations of insulin and glucagon emerging from the pancreatic islets.

Although 3′:5′-cyclic AMP (21) and glucose (22) have recently been demonstrated to increase the release of IRS in isolated rat pancreatic islets, these studies, confirming previous preliminary reports (23, 24), demonstrate somatostatin release from the whole pancreas.¶ They support the suggestion that the D-cells may be a functionally active component of the islets of Langerhans and may be involved in a "paracrine" relationship with A-cells, a high percentage of which are in direct contact with D-cells and B-cells (7, 11). In view of the abnormalities of pancreatic D-cells and in IRS content reported in the islets of diabetic humans (7) and animals (7, 13, 25), disturbances in the relationships between A- and D-cells could play a pathophysiologic role in the relatively unrestrained glucagon secretion that characterizes that disease (26).

¶ Since submission of this manuscript, the following report has been brought to our attention: Weir, G. C., Samols, E., Ramseur, R., Day, J. A. & Patel, Y. C. (1977) "Influence of glucose and glucagon upon SRIF secretion from the isolated perfused canine pancreas," *Clin. Res.*, in press.

Table 3. IRS, glucagon, and insulin concentrations (mean ± SEM*) during 1-hr perfusion of arginine

Exp. no.	Perfusate (60 min)	IRS (pg/ml)	Insulin (μU/ml)	Glucagon (pg/ml)
12	Buffer	60 ± 5	31 ± 3	30 ± <1
	Arginine	339 ± 9†	138 ± 31	143 ± 36†
13	Buffer	79 ± 5	5 ± <1	30 ± <1
	Arginine	181 ± 7†	21 ± 4†	214 ± 7†
14	Buffer	89 ± 10	34 ± 4	30 ± <1
	Arginine	165 ± 7†	58 ± 6†	90 ± 13†
15	Buffer	146 ± 4	107 ± 8	30 ± <1
	Arginine	400 ± 12†	208 ± 49‡	571 ± 88†
16	Buffer	75 ± 5	65 ± 4	30 ± <1
	Arginine	128 ± 4†	150 ± 43	336 ± 69†
17	Buffer	46 ± 6	46 ± 1	30 ± 1
	Arginine	237 ± 32†	131 ± 11‡	123 ± 34

* Mean ± SEM of the number of samples examined in each perfusion period (n = 60 for buffer + arginine perfusion).
† Value statistically greater than that during perfusion with hormone-free buffer ($P < 0.01$).
‡ Value statistically greater than that during perfusion with hormone-free buffer ($P < 0.05$).

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