Glucagon Immunoreactivity and Antidiabetic Action of Somatostatin in the Totally Duodeno-Pancreatectomized and Gastrectomized Human

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SUMMARY

Ten patients who had been totally duodeno-pancreatectomized and totally (N = 1) or partially gastrectomized (N = 9) for chronic pancreatitis (N = 9) or pancreatic carcinoma (N = 1) were investigated. None had a measurable basal level of either plasma C-peptide or a C-peptide response to i.v. glucagon. Immunoreactive glucagon (IRG) was present in all patients, and the mean level (69 ± 8 pg/ml) was not significantly different from the mean observed in normal subjects (81 ± 16 pg/ml). Plasma IRG was unequivocally stimulated by arginine in 2 of the 10 subjects. The effect of somatostatin on plasma glucose and IRG during an oral glucose tolerance test was studied in 5 of the 10 patients. The effects of somatostatin on spontaneous hyperglycemia, plasma growth hormone, and IRG after withdrawal of insulin treatment was studied in 4 patients. Somatostatin blunted both the hyperglycemic and paradoxical IRG responses to the glucose challenge, and reduced the spontaneous rise of blood glucose that occurred after insulin withdrawal. This latter effect was not related to clear-cut changes in plasma growth hormone or in IRG. These data confirm the existence of circulating IRG in pancreatectomized patients and demonstrate the presence of circulating IRG in a completely gastrectomized and pancreatectomized patient. The somatostatin-induced improvement in glucose tolerance in the oral glucose tolerance test seems to be related to a reduction of the paradoxical IRG response. In contrast, the inhibition by somatostatin of the rise in blood glucose which occurs after insulin withdrawal does not seem to be mediated through IRG or growth hormone. DIABETES *30*:851–856, October 1981.

Diabetes has been described as a bihormonal disorder characterized not only by insulin deficiency but by glucagon (IRG) excess as well.[1,2] As recently reviewed,[3] there is considerable experimental evidence to support this hypothesis: (1) plasma glucagon levels are relatively high in diabetes mellitus,[4–10] and are not suppressed by carbohydrates,[6–8] (2) exogenous glucagon administration in insulin-deficient subjects aggravates the metabolic abnormalities of diabetes[11,12] and, above all, (3) the improvement of diabetic control obtained with somatostatin is reversed by glucagon replacement during somatostatin infusion.[11]

Nevertheless, several objections to a major role of glucagon in diabetes mellitus have been raised. First, the studies by Barnes and Bloom in totally pancreatectomized patients have shown that the metabolic abnormalities of diabetes can occur in the absence of detectable circulating glucagon.[13–15] However, plasma glucagon measurements in pancreatectomized human subjects have yielded conflicting results: if, indeed, undetectable values were reported by Barnes and Bloom[13] and Gerich et al.,[16] other groups reported significant IRG levels[17–25] and in a few patients, a glucagon response to an arginine infusion has been described.[22,23] Thus, both the existence of an extrapancreatic source of glucagon in man and the necessary presence of glucagon for the development of hyperglycemia remain in dispute. Another objection to a crucial role of glucagon in diabetes mellitus is raised by the fact that improvement in blood glucose levels seen during infusion of somatostatin might not only result from the glucagon lowering effect of somatostatin. Its postprandial antihyperglycemic effect could also result from a decrease or delay in the intestinal absorption of glucose.[26,27] A direct suppressive effect of somatostatin on hepatic glucose release depicted in in vitro systems[28–30] has been evoked but denied in other studies.[31–33] The effect on blood glucose of the suppression of growth hormone[34–37] does not appear as an important factor in view of the effectiveness of somatostatin in hypophysectomized man.[34]

The present study was undertaken to evaluate, in totally

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pancreatectomized individuals, (1) the fasting IRG levels and the response to an arginine infusion, (2) the changes in circulating IRG levels during spontaneous and postprandial rises in blood glucose, respectively, and (3) the glucose and IRG responses to somatostatin infusion in spontaneous and postprandial hyperglycemia.

SUBJECTS AND METHODS

The 10 subjects investigated are described in Table 1. Each had given informed consent to the present study. Patients' ages ranged from 34 to 65 yr. None had insulin-dependent diabetes before pancreatectomy. All had undergone total duodeno-pancreatectomy combined with partial jejunectomy (about 15 cm), vagotomy, splenectomy, and cholecystectomy. In addition, 9 had a partial gastrectomy and 1 (case no. 10) had a total gastrectomy. The indication for surgery was severe chronic pancreatitis in 9 patients (cases nos. 2-10) and pancreatic adenocarcinoma in 1 patient (case no. 1).

The patients were investigated between 1 and 9 yr after pancreatectomy. They were all in good health and their nutritional status was good. Patients no. 5 and 10 were moderately obese.

The protocol consisted of two parts: (1) possible residual beta- and alpha-cell functions were determined by C-peptide measurements after intravenous glucagon injection and glucagon determination during arginine infusion, respectively, and (2) the effect of somatostatin (cyclic somatostatin, Clin Midy Laboratories, Montpellier, France) was studied during an oral glucose tolerance test (OGTT) or during the spontaneous hyperglycemia that occurs after insulin withdrawal.

The first series of experiments were conducted after an overnight fast; the last dose of intermediate-acting insulin (Rapitard, Novo Industries, Copenhagen, Denmark) had been administered subcutaneously about 24 h previously. Plasma immunoreactive C-peptide was measured in all 10 patients after an intravenous injection of 1 mg glucagon was administered over a 30-s period. On another day, plasma glucagon was measured in all patients during an arginine hydrochloride intravenous infusion (0.5 g/kg body wt) over a period of 30 min.

The second series of experiments was also conducted after an overnight fast, with the last dose of intermediate-acting insulin given 24 h previously. In addition, insulin was infused intravenously by an open-loop system during the overnight fast, and the blood glucose levels were maintained within the normal range by a closed-loop control ("artificial B-cell") during the last hour before administering the test. Oral glucose tolerance tests (25 g glucose/m^2 body surface area) were performed in five patients on two consecutive and randomized days with either a simultaneous somatostatin (500 μg/h for 4 h) or saline infusion. In four patients, the spontaneous rise in fasting blood glucose after insulin withdrawal was measured on two consecutive days with either saline or somatostatin infusion (500 μg/h for 4 h).

Blood glucose was continuously measured, using a modified glucose-oxidase method[38] for 2 h after the oral glucose load, and for 5 h in the insulin withdrawal study. Blood samples were withdrawn from an antecubital vein through an indwelling catheter. All tubes were immediately centrifuged and then kept at −20°C until assay. IRG was measured in all experimental protocols. Plasma growth hormone (GH) was only determined during the experiment with spontaneous rise in fasting blood glucose, and plasma immunoreactive C-peptide was measured in the intravenous glucagon test only.

Radioimmunoassays were used to measure plasma levels of IRG,[39] GH,[40] and C-peptide.[41] The IRG assay used porcine ^{125}I-labeled glucagon, Unger's 30K antiserum, and dextran-charcoal for separation of bound and free hormone. C-peptide was measured using Byk-Mallinkrodt reagents.

RESULTS

Basal levels and arginine test. None of the patients had measurable basal plasma C-peptide immunoreactivity circulating levels nor C-peptide response to i.v. glucagon (Figure 1, top). The mean basal IRG level of the pancreatectomized patients was 69 ± 8 pg/ml (Figure 1, bottom), the mean value with this assay in normal individuals being 81 ± 16 pg/ml. When all data were pooled, arginine infusion failed to elicit a significant rise in the mean IRG levels (Figure 1, bottom). However, the results were not homogenous among the whole group (Table 1), and two subgroups were identified according to patients' responses to i.v. arginine. In subgroup one (cases no. 1 and 2), there was a nor-

TABLE 1
Characteristics of the subjects studied

Case no.	Age (yr)	Sex	Height (cm)	Weight (kg)	Total duodeno-pancreatectomy For	Date	Plasma IRG responses to arginine infusion (pg/ml) −10	−5	0	10	20	25	30
1*	45	F	166	48	Adenocarcinoma	1978	81	88	85	120	131	84	81
2	48	M	167	77		1977	70	63	77	173	147	—	182
3	36	M	186	79		1977	97	86	63	80	92	75	72
4	65	M	164	55		1971	82	82	64	61	63	68	74
5	58	M	172	96	Chronic	1977	143	125	128	124	125	127	113
6	47	F	163	51	pancreatitis	1974	87	67	74	60	64	75	72
7	42	M	180	70		1978	90	79	55	28	41	36	26
8	47	M	168	71		1977	70	57	40	48	38	47	48
9	43	M	168	88		1978	47	44	42	37	32	41	48
10†	34	M	170	50		1978	72	56	58	47	81	57	61

* Patients 1–9 had partial vagotomy, proximal jejunectomy (15 cm), splenectomy, and cholecystectomy.
† Patient 10 had total gastrectomy.

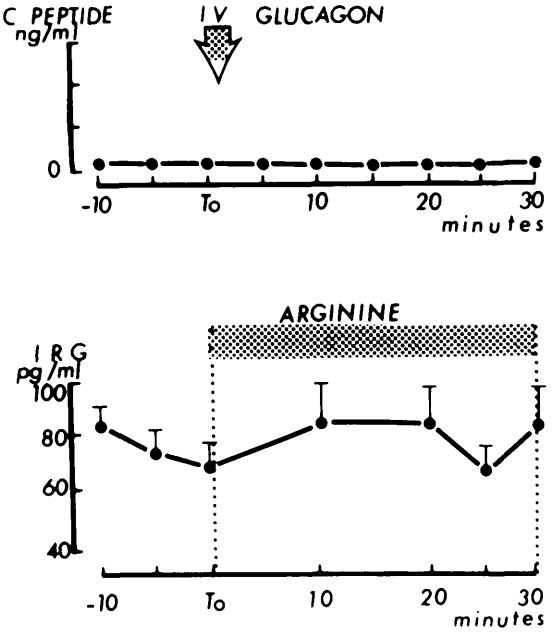


FIGURE 1. Top: lack of plasma C-peptide response to intravenous glucagon (1 mg) in the 10 pancreatectomized patients. Bottom: mean ± SEM of plasma IRG before and during intravenous infusion of arginine (see text) in 10 pancreatectomized patients.

mal fasting IRG level and a clear response to the arginine infusion. In subgroup two, there were normal or low fasting IRG levels but no reponse to arginine. This latter group included the totally gastrectomized subject.

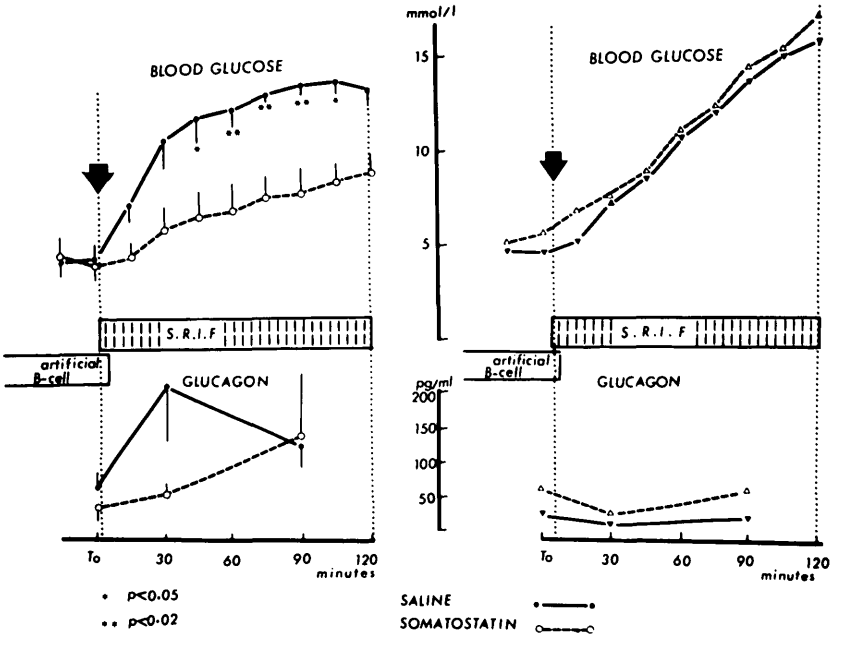
OGTT without and with somatostatin infusion. During the OGTT, four out of the five patients investigated showed a paradoxical rise in IRG levels at 30 min (Figure 2, left). In the same four subjects, simultaneous somatostatin infusion significantly reduced the blood glucose, and also reduced the paradoxical IRG response to the glucose challenge in three of the four subjects. In one individual (Figure 2, right), oral glucose did not modify IRG plasma levels and somatostatin did not alter IRG blood glucose levels. The IRG response to oral glucose seen in the single, totally gastrectomized, and totally pancreatectomized individual was particularly interesting because the glucagon level rose from 60 to 350 pg/ml within 30 min, and was blunted by somatostatin: 38 pg/ml (basal) vs. 63 pg/ml at 30 min (included in Figure 2, left panel).

Insulin withdrawal without and with somatostatin infusion. In the four patients investigated in this protocol, the spontaneous rise in fasting blood glucose observed after interrupting the i.v. insulin infusion was not accompanied by a corresponding rise in plasma IRG levels (Figure 3). Furthermore, although somatostatin infusion reduced the spontaneous rise in blood glucose in all four pancreatectomized patients, it did not lower the blood glucose level below the previous baseline level; Figure 3 illustrates the variable antihyperglycemic effects produced by the somatostatin infusion. It also shows that its efficiency was unrelated to the changes in IRG or GH circulating levels. Mean plasma IRG during saline infusion was 53 ± 6 pg/ml (32 values) vs. 51 ± 6 pg/ml (32 values) during somatostatin infusion.

DISCUSSION

The individual basal plasma IRG levels that we found in totally pancreactomized subjects were normal or low, but their mean was not different from the levels usually observed in diabetics using a similar assay system.[6,7] There was no correlation between individual values and the time elapsed since surgery. The persistence of plasma IRG several years after pancreatectomy seen in our group differs from the rapid disappearance of IRG reported by Miyata et al.[18] after pancreatectomy. Our results confirm the presence of circulating immunoreactive glucagon in pancreatectomized humans and are in agreement with numerous earlier studies using antiserum 30K[17–19,21–25] or antiserum AGS-18.[20] No detectable plasma IRG was reported in pancreatectomized subjects by Gerich et al.[16] and Barnes and coworkers.[13–15] However, the near zero values reported in the latter reports could be explained by the use of a different antiserum and the fact that the standard curve was established

FIGURE 2. Oral glucose tolerance test in pancreatectomized patients infused with saline (solid lines) or somatostatin (broken lines). On the left, mean of four patients who responded to somatostatin by a marked decrease of the blood glucose values. On the right, one patient whose glucose tolerance was not modified by somatostatin.



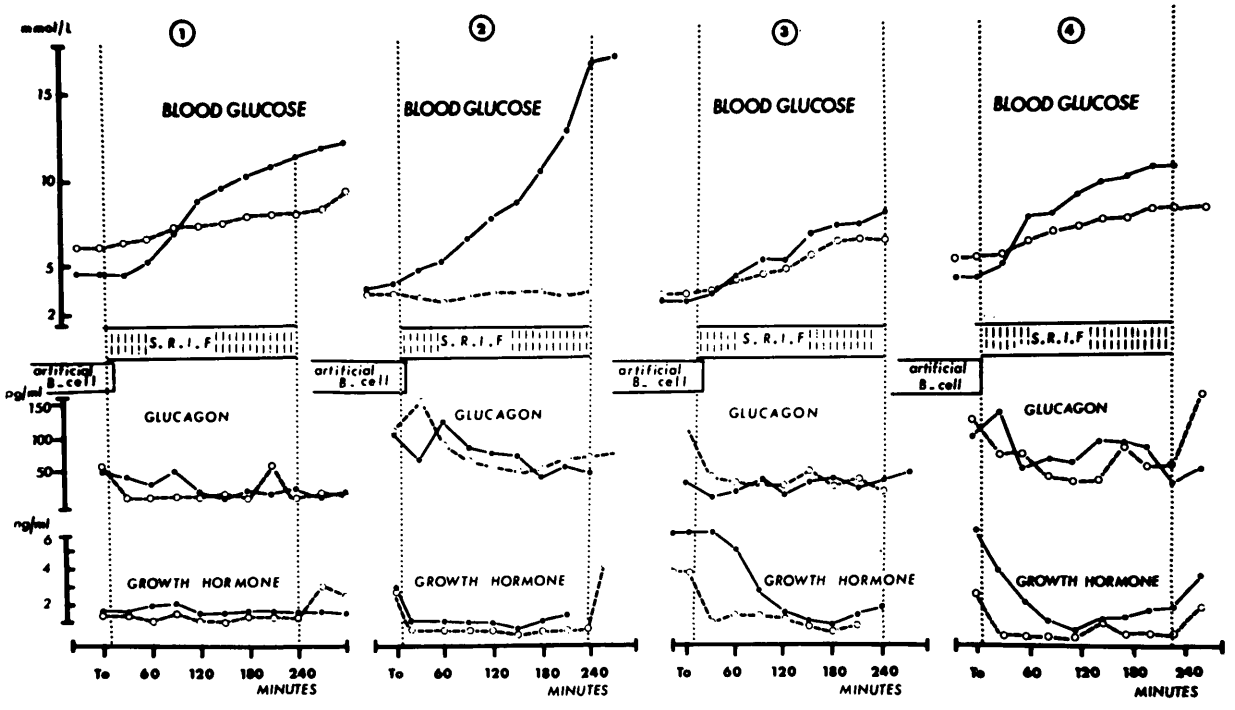


FIGURE 3. Insulin withdrawal in four pancreatectomized patients infused with saline (●——●) or somatostatin (○----○).

with a plasma stripped of glucagon by affinity chromatography.[13–15]

On the other hand, it is now well accepted that the 30K antiserum not only measures true glucagon (IRG^{3500}) but also a family of polypeptides, including a large molecular weight substance of unknown origin, BPG,[42,43] 9000–21,000-dalton polypeptides likely to represent glucagon precursors,[44–49] as well as a small 2000 mol. wt. fraction probably corresponding to a degradation product.[48] In the present study, due to the low levels of immunoreactivity detected, we did not have the opportunity to use chromatography to distinguish which species of glucagon accounted for the IRG levels. The origin of the IRG we measured is not known. The presence of gastrointestinal glucagon has been previously demonstrated in animals[50–55] and A-cells have been identified not only in the gastric mucosa of dogs[56,57] but also in duodenum[58] and gastric fundus[59] of humans. However, the measurable IRG levels found in the one duodeno-pancreatectomized and totally gastrectomized patient that we studied could not have been of stomach or duodenal origin since those organs had been completely removed. To our knowledge, this is the first report of such a patient and this observation supports the existence of an extrapancreatic and extragastro-duodenal source of IRG in man. IRG has been found in salivary glands[60,61] and recently in the canine brain with the highest concentration in the hypothalamus.[44] These two sites are possible sources of the IRG detected in our patient.

We observed a clear-cut glucagon response to arginine in 2 out of 10 pancreatectomized subjects investigated; Werner and Palmer[23] likewise reported two such patients, though one had normal levels of C-peptide, which would indicate an incomplete pancreatectomy. The other reports[13–21,24–25,62] failed to find a response to arginine of extrapancreatic glucagon in the human. A total lack of C-peptide response to exogenous glucagon in all our subjects, and the fact that all operations were performed by the same surgeon, would tend to exclude a variation in the completeness of pancreatectomy as a cause of the different responses to arginine among our patients. Also, the administration of the last dose of intermediate-acting insulin at least 24 h before the tests were performed makes a residual insulin action unlikely in explaining the lack of response in 8 out of the 10 subjects. Therefore, it seems likely that the variable glucagon responses to arginine result from differences among the subjects in their capacity to secrete extrapancreatic IRG. It has been proposed that chronic hyperstimulation of extrapancreatic residual A-cells could cause maximum IRG secretion before arginine stimulation.[46]

Plasma IRG levels rose in response to oral glucose in four of the five patients tested. Similarly, paradoxical responses in pancreatectomized humans have been reported by others.[20,23,24] Because of the low cross-reactivity of antiserum 30K with gut glucagon-like immunoreactivity (gut GLI), the apparent rise in plasma IRG after oral glucose is unlikely to be a simple assay artifact. Indeed, assuming a 3% degree of cross-reactivity of antiserum 30K with gut GLI, the glucose-induced rise in plasma IRG detected with the antiserum would correspond to a 5–10.000 pgeq/ml in circulating GLI-related peptides. However, Koranyi (personal communication) has recently demonstrated that porcine gut glucagon-like immunoreactive material infused in the anesthetized dog was transformed in vivo in biologically active IRG, reacting with a C-terminal specific antiserum. Thus, the possibility of an in vivo generation of IRG from massively released GLI has to be considered. Somatostatin-induced reduction of GLI^{4500} release might be also advocated for explaining the improved glucose tolerance. Finally, somatostatin-induced reduction of plasma glucose after oral glucose load could have resulted from the sup-

pression of the paradoxic glucagon response, although the decrease in gastrointestinal motility and glucose absorption[26,27] could be a contributory factor.

After insulin withdrawal, hyperglycemia occurred in all patients while IRG did not show any systematic change. A fall in circulating insulin with unchanged glucagon circulating levels would induce a rise in the glucagon:insulin ratio with subsequent enhanced glucose production by the liver (in review[63]). In agreement with previous studies,[15] the rise in blood glucose after insulin withdrawal was found to be slower than that seen in patients with idiopathic insulin-dependent diabetes (data not shown). This may be related to differences in plasma glucagon between such groups.

Somatostatin partially or totally prevented spontaneous hyperglycemia in pancreatectomized subjects during 4 h without systematically modifying GH levels. The antidiabetic action of somatostatin in pancreatectomized man is in agreement with the previous report of Werner and Palmer.[23] However, in our patients, somatostatin did not cause a fall in blood glucose but simply prevented hyperglycemia. The normalization and stabilization of blood glucose by a closed-loop system before our test may help to explain the difference in the effect of somatostatin. Nevertheless, the observations of Gerich et al.[16] and Botha et al.[24] failed to show a suppressive effect of somatostatin on blood glucose levels. However, in the first study,[16] the infusion of somatostatin, although it did not lower plasma glucose levels, induced an antihyperglycemic effect similar to our observations, since blood glucose remained stable during 2 h even though the last subcutaneous insulin injection had been administered 24 h previously, and control without SRIF was not shown. In the second study,[24] the large differences between the basal blood glucose level before the two tests, with and without somatostatin, respectively, and the slow rise of blood glucose during somatostatin infusion, do not allow a definite conclusion.

In the present series, the antihyperglycemic action of somatostatin was not related to unequivocal IRG suppression. Similarly, Werner and Palmer[23] found the antihyperglycemic effect of somatostatin in totally pancreatectomized subjects to be independent of glucagon suppression. They suggested that the persistent IRG after pancreatectomy could be similar to the residual somatostatin-unsuppressible IRG seen in diabetes[23,64] and that the mechanism of action of somatostatin on glucose metabolism could be due to its direct inhibitory effect on hepatic glucose release. However, several studies[31,32] have failed to demonstrate the direct inhibitory effect of somatostatin on hepatic glucose release reported by others.[28–30]

It has recently been shown[21] that the IRG^{3500} represents about 18% of the total IRG found in pancreatectomized patients. It appears possible, therefore, that IRG^{3500} could have been suppressed by somatostatin without a noticeable or significant decrease in total IRG.

However, other studies have revealed conflicting results. Barnes and Bloom,[13] Villanueva et al.,[19] and Muller et al.[25] found that duodeno-pancreatectomized humans possess no circulating 3500-dalton glucagon, although Botha et al.[24] claimed to have detected some. Tiengo et al.[62] found the glucagon immunoreactivity was precipitated by ethanol, suggesting that it was essentially related to big plasma glucagon. Biogel P30 chromatography revealed a very small peak in the region corresponding to 3500 mol wt. glucagon. In any event, the biologic activity of BPG cannot be excluded,[25] and Srikant et al.[49] have shown the effectiveness of canine BPG on hepatic glycogenolysis and adenylate cyclase activity.

Further studies are needed to clarify the mechanism of the hyperglycemic action exerted by somatostatin in pancreatectomized patients.

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