Immunoreactive Glucagon Responses to Intravenous Tolbutamide in Chronic Pancreatitis

W. J. Kalk, M.B.Ch.B., M.R.C.P., A. I. Vinik, M.D., F.C.P., M. Paul, P. Keller, Ph.D., and W. P. U. Jackson, M.A., M.D., F.R.C.P., Cape Town, South Africa

SUMMARY

The effects of tolbutamide infusion (1 gm. over forty minutes) on plasma pancreatic glucagon-like immunoreactivity (PGLI), serum insulin, and blood glucose were studied in six patients with chronic pancreatitis and six matched controls. Basal PGLI levels were significantly higher in the patients, despite higher fasting glucose concentrations. Tolbutamide infusion had no significant effect on mean PGLI levels in controls but was associated with significant elevation in pancreatitis patients, despite higher circulating glucose levels in the latter. The data suggest that chronic calcific pancreatitis patients hypersecrete immunoreactive glucagon, possibly from a nonpancreatic source and that this immunoreactive material may be stimulated by sulfonylureas. DIABETES *24*:851-55, September, 1975.

Acute administration of a sulfonylurea lowers blood glucose primarily by stimulating the release and/or enhancing the actions of insulin. Its effects on release of immunoreactive glucagon are controversial. The "glucagon-lowering" effects found by Samols et al.[1] in ducks, in normal healthy volunteers, in patients with glucagon-secreting tumors, and in isolated rat islets have not been confirmed in later studies. In vitro studies with mouse islets failed to demonstrate changes in glucagon secretion.[2] Perfusion of rat[3] pancreas with tolbutamide led to inhibition of glucagon secretion, and perfusion of the isolated dog pancreas with tolbutamide inhibited arginine-stimulated glucagon release but failed to inhibit the secretion of glucagon induced by hypoglycemia.[4] Tolbutamide injected intravenously in man[5] had no effect on glucagon levels but prolonged the hyperglucagonemia when administered simultaneously with amino acids. Similarly, Aguilar-Parada et al.[6] found no reduction in glucagon levels after a single injection of glibenclamide in dogs, but in four of the seven dogs the levels in the pancreaticoduodenal vein rose significantly thirty minutes later. Intravenous infusion of tolbutamide in dogs has had no effect on PGLI levels.[7] Since we[8] found excessive glucagon responses to arginine infusion in patients with chronic pancreatitis, we decided to examine the effects of tolbutamide on pancreatic glucagon-like immunoreactivity in the same patients.

PATIENTS AND METHODS

Six patients who had had one or more attacks of pancreatitis associated with long-standing overindulgence in alcohol were studied. All had grossly reduced responses of pancreatic bicarbonate and enzymes to secretin and pancreozymin stimulation[9] (i.e. a mean bicarbonate concentration less than 60 mEq. per liter and amylase less than 5 U. per milliliter after Boots secretin and pancreozymin, respectively). Three of these six had had clinical and radiologic evidence of pseudo-pancreatic-cysts in the past. Four had radiologically-diagnosed pancreatic calcification, and in the remaining two patients the diagnosis of pancreatitis was confirmed at laparotomy. The patients were all males, thirty-five to forty-eight years old and 51 to 86.4 kg. in weight. Each was within 12 per cent of desirable body weight.* None was taking, or had taken, oral antidiabetic agents, and none had a family history of diabetes. There was no clinical or biochemical evidence of malabsorption or chronic liver disease. A 50-gm. oral glucose tolerance test was performed on all patients as a screening procedure. Three patients (Ja, Mo, Co) had diabetic glucose tolerance tests by the criteria of Jackson and Vinik,[10] and three had normal tolerance to glucose. The six control subjects studied were thirty-eight to forty-seven years old and

From the Endocrine and Diabetes Research Group, Department of Medicine, University of Cape Town and Groote Schuur Hospital.

Accepted for publication June 9, 1975.

*Documenta Geigy Scientific tables

weighed 59 to 86.5 kg., which was also within 12 per cent of body weight.* All were social drinkers and none had a family history of diabetes. Glucose tolerance tests and routine liver function tests were normal. The socioeconomic background of these patients and the control subjects was such that the antecedent diet was predominantly carbohydrate. After an overnight fast, polyethylene cannulae were placed in both antecubital veins and three basal blood samples were drawn at fifteen-minute intervals. Sodium tolbutamide (Hoechst) in 20 ml. of the supplied diluent was administered intravenously as an infusion of 1 gm. for forty minutes. Further blood samples were drawn at five and ten minutes and then at ten-minute intervals for 100 minutes. The blood was divided into three aliquots. One was placed in a heparinized tube containing 500 K.I.U. aprotinin (Trasylol—Bayer), and centrifuged immediately, and the plasma was deep-frozen for glucagon assay. Serum was used for insulin assay and venous whole blood for glucose analysis.

Pancreatic glucagon was measured on unextracted plasma samples[10] with an antiserum that does not cross-react with 100 μg. of a crude gut glucagon-like extract (MUC 101†), which contains 560 ng. equivalents of gut glucagon-like immunoreactivity (GGLI) per milligram dry weight with a cross-reacting antiserum K36, which gives values of 200 to 800 pg.Eq. per milliliter in normal fasting subjects. Somatostatin does not cross-react, and secretin and cholecystokinin-pancreozymin have less than 0.001 per cent cross-reaction. The major locus of pancreatic glucagon recognized by this antiserum is the 18-21 amino-acid sequence, but both C-terminal and N-terminal peptides were capable of displacing labeled pancreatic glucagon from the antiserum. Hence, because the antiserum does recognize glucagon fragments and may thus detect peptides containing these sequences, which are not true pancreatic glucagon, results are expressed as picogram equivalents of a pancreatic glucagon standard. The least detectable glucagon was 10 pg. per milliliter plasma. Plasma from an insulin-treated, pancreatectomized patient had undetectable glucagon immunoreactivity, and standards assayed in buffer and plasma gave similar results. Insulin was measured with Amersham kits and glucose on the Technicon AutoAnalyzer by the ferricyanide method of Hoffman.[11]

The mean fasting values for PGLI, insulin, and glucose were taken as the means for the -30, -15, and 0 minute values for each study. Maximum level and nadir represent the highest or lowest values obtained at any time during or after infusion, and maximum increment or decrement the difference between this value and the mean fasting level in each patient.

The results were analyzed statistically by use of the two-tailed Student's t test for significance of differences between means.

RESULTS

The mean fasting PGLI concentration of all samples drawn at -30, -15, and 0 minutes in pancreatitis patients was 163 ± 20.5 pg.Eq. per milliliter, which was significantly ($p<0.001$) higher than 55.4 ± 10.8 pg.Eq. per milliliter in the controls. These elevated PGLI levels were found despite a significantly ($p<0.005$) higher mean fasting glucose concentration of 113.6 ± 6.5 in patients with pancreatitis as against 86.6 ± 1.6 mg. per 100 ml. in controls (table 1).

With the tolbutamide infusion, despite considerable fluctuation, there was a progressive increase in mean PGLI concentration in pancreatitis patients, with a peak at sixty minutes (figure 1) significantly ($p<0.05$) higher than the basal value, whereas in the controls there was no significant rise and values remained within the region of the basal concentration throughout the infusion and postinfusion period. Mean PGLI values at all times were significantly ($p<0.05$ or less) higher in the pancreatitis patients than in the controls (figure 1). The mean maximum PGLI concentration of 317.5 ± 58.96 was significantly ($p<0.05$) higher than the mean fasting value of 163.3 ± 20.5, whereas the mean maximum value in controls of 89.2 ± 24.3 was not statistically higher than the basal level of 55.4 ± 10.8 pg.Eq./ml.

Both controls and pancreatitis patients had significant elevation of plasma insulin and depression of blood glucose levels caused by the tolbutamide infusion (table 1). However, neither the glucose decrement nor the insulin increment differed significantly between patients and controls. The fasting molar insulin/PGLI ratios in pancreatitis patients and controls were similar and failed to change with tolbutamide infusion in the patients but rose significantly ($p<0.01$) in controls. The apparent absence of a rise in pancreatitis patients is due to the rise in PGLI despite an increase in insulin levels (table 2).

DISCUSSION

We have previously shown that some patients with

*Documenta Geigy Scientific tables

†Kindly supplied by Lise Heding, Novo Institute, Denmark.

TABLE 1

Fasting (F) pancreatic glucagon-like immunoreactivity (PGLI), insulin, and glucose and peak (P) or nadir (N) concentrations with tolbutamide infusion in control subjects and chronic pancreatitis patients

Subjects	Age	Sex	% Ideal Body wt.*	PGLI pg.Eq./ml. F.	PGLI pg.Eq./ml. P.	Insulin μU./ml. F.	Insulin μU./ml. P.	Glucose mg./100 ml. F.	Glucose mg./100 ml. N.
Controls:									
Vi	46	M	98.2	117	170 (20)	4	33 (10)	88.2	42 (40)
Tr	38	M	100.0	18	21.5 (40)	10	25 (10)	83.3	55 (60)
Ho	47	M	101.4	90	100 (20)	9	23 (10)	87.0	58 (40)
Man	38	M	92.5	13	19 (5)	6	20 (20)	78.3	66 (50)
Bo	40	M	93.7	55	125 (30)	12	37 (30)	86.3	67 (40)
Bu	39	M	96.0	46	100 (20)	9	38 (30)	95.4	67 (40)
Mean	41.3		97.1	55.4	89.2	8.3	29.3	86.6	59.2
S.E.M.	1.7		1.5	10.8	4.3	1.2	3.1	1.6	±4.02
Pancreatitic Patients:									
Fi	48	M	95.0	223	250 (70)	19	35 (10)	94.0	49 (50)
Tu	35	M	96.4	68	175 (20)	25	89 (10)	89.7	50 (40)
Ja	38	M	101.0	123	240 (60)	11	18 (5)	139.0	74 (70)
Mo	45	M	97.8	118	240 (50)	10	31 (5)	128.7	73 (60)
Co	45	M	90.0	100	500 (50)	19	23 (5)	146.7	135 (1)
Sw	43	M	102.0	290	500 (20)	16	40 (30)	83.7	54 (60)
Mean	42.3		97.1	163.3	317.5	16.6	39.3	113.6	72.2
S.E.M.	2.00		1.8	20.5	58.9	2.9	10.5	6.5	13.04

*Documenta Geigy Scientific tables.

Figures in parentheses are the time from start of tolbutamide infusion.

Significant differences between patients and controls occur for fasting PGLI and insulin ($p<0.001$), fasting glucose ($p<0.005$), and peak PGLI ($p<0.05$).

Control subjects have a significant fall in glucose ($p<0.001$) and rise in insulin ($p<0.001$) but an insignificant change in PGLI level.

Pancreatitis patients have a significant fall in glucose ($p<0.005$) and rise in insulin ($p<0.05$) and PGLI ($p<0.025$) concentrations.

pancreatitis have relative hypersecretion of immunoreactive glucagon in response to arginine infusion[8] and postulated[12] that the pancreatic alpha cell became "blind" to the suppressive effects of glucose. The present data tend to support these suggestions in that these pancreatitis patients have raised basal PGLI levels which are inappropriately high for the circulating glucose concentration. The higher PGLI concentrations occurred at levels of blood glucose ranging between a mean of 115 mg./100 ml. before and 72 mg./100 ml. during the tolbutamide infusion and thus suggest failure of suppression of the alpha cell by glucose within this range of glucose levels. This may not necessarily mean that the alpha cell is defective, but in pancreatitis patients it may be under constant stimulation by excessive release of cholecystokinin-pancreozymin, for example, which could possibly prevent the suppressive effects of glucose.

Whether or not tolbutamide causes changes in pancreatic glucagon secretion is controversial. The cross-reaction of GGLI with pancreatic glucagon could have produced some erroneous observations,[1] while the blood glucose-lowering and insulin-releasing properties of this sulfonylurea could secondarily influence glucagon release.[13,14] Since it has been shown[14] that pancreatitis patients hyposecrete PGLI in response to insulin-induced hypoglycemia, any differing result from tolbutamide administration is likely to indicate a primary effect of tolbutamide and not an effect of the hypoglycemia, or the rise in insulin levels. Indeed, in patients with pancreatitis there was a significant rise in plasma PGLI concentration during or after tolbutamide infusion in contrast to the lack of significant

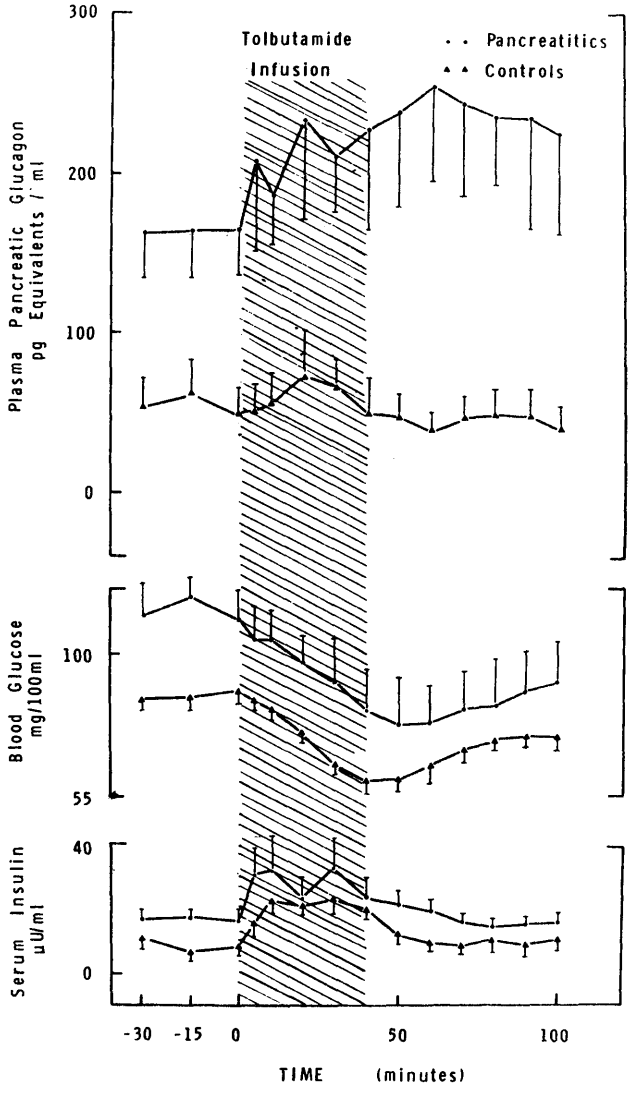

FIG. 1. The effects of 1 gm. sodium tolbutamide infusion from zero to forty minutes on (Mean ± S.E.M.) plasma PGLI, insulin and blood glucose in controls (n=6) (solid triangles) and patients with pancreatitis (solid circles). The mean PGLI values at all times are significantly ($p<0.05$ or less) higher in pancreatitis patients than in controls.

change in controls, as shown in the present studies and in those of Pek et al.[5]

Although the rise in PGLI levels preceded the fall in glucose concentration, the persistent elevation may in part have been due to the hypoglycemia.[5] However, control patients who had a quantitatively similar fall in glucose concentration had no late rise in PGLI concentration.

The rise in PGLI concentration could be attributed to (1) increased release of glucagon from pancreatic alpha cells; (2) increased release of glucagon from extra-pancreatic alpha cells; (3) an effect of tolbutamide on glucagon inactivation or removal.

TABLE 2

Insulin/glucagon molar ratios, before and with tolbutamide infusion in controls and pancreatitis patients

Subjects	Fasting	Peak
Controls:		
Vi	0.79	4.53
Tr	12.96	27.13
Ho	2.33	5.36
Man	11.11	25.92
Bo	5.07	6.92
Bu	4.57	8.97
Mean	6.14	13.14
S.E.M.	1.98	4.28
Pancreatitis Patients:		
Fi	1.99	3.27
Tu	8.58	11.84
Ja	2.08	1.75
Mo	1.98	3.02
Co	4.48	1.07
Sw	1.29	1.87
Mean	3.40	3.81
S.E.M.	1.13	1.64

Significant differences in I/G ratios are as follows:

Controls vs. Pancreatitis patients	Fasting	p=N.S.
	Peak	$p<0.05$
Controls Fasting vs. Peak		$p<0.01$
Pancreatitis pts. Fasting vs. Peak		p=N.S.

Loubatières et al.[15] have recently made observations similar to our own from studies in dogs in vivo and in isolated perfused rat pancreas preparations which show that at low glucose concentrations, tolbutamide stimulates rather than inhibits the release of glucagon. It would thus appear that when the alpha cells become resistant to the suppressive effect of glucose, as in chronic pancreatitis, tolbutamide may be able to stimulate the release of glucagon.

Nevertheless, we cannot exclude with certainty the possiblility that the measured PGLI derives from alpha cells in the proximal gastrointestinal tract since recent animal studies in dogs[16,17] have shown increases in PGLI forty-eight hours after pancreatectomy if insulin is withheld. On this basis one might have expected inappropriately high PGLI responses in insulinopenic or diabetic subjects only, but the three diabetics (Jo, Mo, Co) had PGLI responses no greater than nondiabetic pancreatitics. Furthermore, the glucagon antiserum used in this study does not cross-react with extracts of porcine or human duodenum but reacts with several gut peptides after polyacrylamide disc gel electrophoresis of the gut extract, as has been shown[18] with antiserum K814 (Lise Heding, Novo Institute, Denmark), which is said to be pancreatic glucagon-specific. While it seems likely, therefore, that the measured PGLI is of pancreatic origin,

further studies of tolbutamide action in totally pancreatectomized human subjects on and off insulin are necessary to resolve this question.

The possible association of liver disease with alcohol ingestion might contribute to the raised PGLI values in these patients since cirrhotics do have elevated PGLI[19] and tolbutamide may interfere with glucagon inactivation. In none of our patients was there biochemical evidence of liver disease or high secretin/CCK-PZ induced pancreatic secretion characteristic of liver disease. Other studies in these same patients on the effects of somatostatin on PGLI levels also indicate a normal half-life time for PGLI. It seems, therefore, that release rather than impaired inactivation is responsible for the raised PGLI levels in these patients.

The higher fasting glucose concentrations in pancreatitis patients parallel the raised PGLI levels and suggest that the measured PGLI is indeed biologically active. However, the equally good decline in blood glucose concentration in pancreatitics and controls despite a rise in PGLI in the pancreatitics suggests that the PGLI may be inactive or that there was a concomitantly greater insulin secretion, since it has been suggested that it is the insulin/glucagon ratio[20,21] which ultimately determines glucose turnover and not the hormones individually. The lower peripheral peak insulin/PGLI values in pancreatitics suggests insulin sensitivity or glucagon resistance. In the absence of measurements of portal insulin/PGLI ratios and blood glucose responses to exogenous pancreatic glucagon infusion, this question remains to be answered.

ACKNOWLEDGMENT

The work was supported by a grant from the South African Medical Research Council.

REFERENCES

[1]Samols, E., Tyler, J., and Miahle, P.: Suppression of pancreatic glucagon release by the hypoglycaemic sulphonylureas. Lancet *1*:174-76, 1969.

[2]Chesney, T. McC., and Schofield, J.G.: Studies on the secretion of pancreatic glucagon. Diabetes *18*:627-32, 1969.

[3]Laube, H., Fussgänger, R., Goberna, R., Schroeder, K., Straub, K., Sussman, K., and Pfeiffer, E.F.: Effects of tolbutamide on insulin and glucagon secretion of the isolated perfused rat pancreas. Horm. Metab. Res. *3*:238-42, 1971.

[4]Iversen, J.: Secretion of immunoreactive insulin and glucagon from the isolated perfused canine pancreas following stimulation with glucose, pancreozymin, arginine and tolbutamide. Excerpta Medica Intl. Cong. Ser. *209*:11, 1970.

[5]Pek, S., Fajans, S.S., Floyd, J.C. Jr., Knopf, R.F., and Conn, J.W.: Failure of sulfonylureas to suppress glucagon in man. Diabetes *21*:216-23, 1972.

[6]Aguilar-Parada, E., Eisentraut, A.M., and Unger, R.H.: Effect of HB419-induced hypoglycemia on pancreatic glucagon secretion. Horm. Metab. Res. (Suppl.) *1*:48-50, 1969.

[7]Kajinuma, H., Kuzuya, T., and Ide, T.: Effects of hypoglycemic sulfonamides on glucagon and insulin secretion in ducks and dogs. Diabetes *23*:412-17, 1974.

[8]Kalk, W.J., Vinik, A.I., Bank, S., Buchanan, K.D., Keller, P., and Jackson, W.P.U.: Glucagon responses to arginine in chronic pancreatitis (possible pathogenic significance in diabetes). Diabetes *23*:257-63, 1974.

[9]Marks, I.N., Bank, S., and Louw, J.H.: The diagnosis and management of pancreatitis. *In* Progress in Gastroenterology, Glass, G.B.J., Ed. Grune and Stratton, 1968, pp. 412-17.

[10]Vinik, A.I., and Hardcastle, A.: Structure/antigenicity of glucagon and related peptides. Studies using radioimmunoassay. Horm. Metab. Res. *6*:396-99, 1974.

[11]Hoffman, W.S.: A rapid photoelectric method for the determination of glucose in blood and urine. J. Biol. Chem. *120*:51, 1937.

[12]Vinik, A.I., Kalk, W.J., and Jackson, W.P.U.: A unifying hypothesis for hereditary and acquired diabetes. Lancet *1*:485-87, 1974.

[13]Müller, W.A., Faloona, G.R., and Unger, R.H.: The effect of experimental insulin deficiency on glucagon secretion. J. Clin. Invest. *50*:1992-99, 1971.

[14]Persson, I., Gyntelberg, F., Heding, L.G., and Ross Nielsen, J.: Pancreatic glucagon-like immunoreactivity after intravenous insulin in normal and chronic pancreatitis patients. Acta Endocrinol. (Kbh) *67*:401-04, 1971.

[15]Loubatières, A.L., Loubatières-Mariani, M.M., Alric, R., and Ribes, G.: Tolbutamide and glucagon secretion. Diabetologia *10*:271-76, 1974.

[16]Vranic, M., Pek, S., and Kawamori, R.: Increased "glucagon immunoreactivity" in plasma of totally depancreatized dogs. Diabetes *23*:905-12, 1974.

[17]Matsuyama, T., and Foa, P.P.: Plasma glucose, insulin, pancreatic and enteroglucagon levels in normal and depancreatized dogs. Proc. Soc. Exp. Biol. Med. *147*:197-202, 1974.

[18]Vinik, A.I., and Jackson, W.P.U.: Glucagon in pathogenesis of diabetes. Lancet *1*:694-95, 1975.

[19]Marco, J., Diego, J., Villanaesa, M., Diaz-Fierros, M., Valverde, I., and Segovia, J.M.: Elevated plasma glucagon level in cirrhosis of the liver. N. Engl. J. Med. *289*:1107-11, 1973.

[20]Unger, R.H., and Orci, L.: The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet *1*:14-16, 1975.

[21]Parrilla, R., Goodman, M.N., and Toews, C.J.: Effect of glucagon:insulin ratios on hepatic metabolism. Diabetes *23*:725-31, 1974.