Proinsulin-Like Component of Circulating Insulin in the Basal State and in Patients and Hamsters with Islet Cell Tumors

PHILLIP GORDEN, BARRY SHERMAN, and JESSE ROTH

From the Diabetes Section, Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT The proinsulin-like component comprised approximately 20% of total circulating basal immunoreactive insulin in 15 patients without islet cell tumors. 15 min after oral glucose, the concentration of the proinsulin-like component was unchanged and its percentage of the total immunoreactive insulin decreased with the acute release of the insulin component. By 2 hr after oral glucose, the concentration of the proinsulin-like component increased and the insulin component concentration decreased so that the percentage of the proinsulin-like component was essentially the same as in the basal state.

In five patients with islet cell tumors and fasting hypoglycemia, basal proinsulin-like component ranged from 26 to 79% of the total immunoreactive insulin. While basal proinsulin-like component was higher in the islet cell tumor patients, the fluctuations after stimulation were qualitatively similar to the nontumor patients. Acute stimulation with glucose, tolbutamide, leucine, and streptozotocin mainly released the insulin component resulting in a fall in the per cent proinsulin-like component with a subsequent increase in percentage of this component as the total insulin concentration returns towards basal levels. Three islet-cell tumor patients with less than 46% proinsulin-like component had favorable therapeutic responses to diazoxide whereas one patient with over 80% proinsulin-like component was completely refractory.

Syrian hamsters bearing islet cell tumors provided an excellent model for islet cell tumors in man. These animals have a high proportion of a proinsulin-like component in plasma; stimulation of tumor slices in vitro with tolbutamide and glucagon releases mainly the insulin component similar to the observations in man.

These studies suggest that the mechanisms regulating the release of the proinsulin-like and of the insulin components are different.

A portion of this work has appeared in abstract form (1).
Received for publication 18 December 1970.

INTRODUCTION

Following the description of proinsulin in a human islet cell tumor (2) and in normal rat pancreas (3), we described a higher molecular weight, immunoreactive component of plasma insulin (4). This higher molecular weight component of circulating insulin found in unextracted plasma by gel filtration (5, 6), appears to be indistinguishable from the higher molecular weight component in acid-ethanol extracts of plasma (7–9).

Specific characterization of the circulating immunoreactive insulin components is limited by the small quantity of material present in plasma. Proinsulin, a single-chain polypeptide in which the A- and B-chains of insulin are linked by a connecting segment, has distinctive gel filtration, immunologic, and biologic properties (10). In addition to single chain proinsulin, double-chain proinsulin intermediate components have been found in vitro in extracts of bovine (11) and porcine pancreas (12). The higher molecular weight plasma component has been characterized in terms of the known properties of proinsulin.

In nontumor subjects, the higher molecular weight plasma component has the same gel filtration properties, biological activity, and response to tryptic digestion as proinsulin (6). Immunoreactivity with anti-insulin serum (6), anti-proinsulin serum (6), and anti-C-peptide serum (9) is also similar to proinsulin.

Recent evidence indicates that in patients with islet cell tumors, the higher molecular weight circulating component can be distinguished from proinsulin and from the higher molecular weight plasma component

found in nontumor subjects. These differences are found in gel filtration properties (13, 14), immunoreactivity (14), and biologic activity (13, 14). The higher molecular weight circulating component in the islet-cell tumor patient is converted from a gel filtration migration similar to proinsulin to that of insulin upon exposure to small concentrations of trypsin (13–15); the larger plasma component also reacts with anti-C-peptide serum (9, 15). It is as yet unclear whether these components are related to the in vitro pancreatic proinsulin intermediates.

While there is heterogeneity in the higher molecular weight, circulating, immunoreactive insulin components in different disease states, these components all appear to be related to proinsulin. These higher molecular weight circulating components can, therefore, be referred to as proinsulin-like components rather than the more general term "big" insulin.

We have previously reported fluctuations in proinsulin-like component after the administration of glucose and other stimuli (16). By technical refinement and the study of a group of patients with high unstimulated insulin concentrations, we have now systematically studied the circulating components in the basal state and have related them to values obtained after stimulation in normal individuals, patients with abnormal glucose tolerance, and patients with islet cell tumors.

The proinsulin-like component has been reported to comprise a high percentage of the total insulin in patients with islet cell tumors. We find that the percentage is high in the basal state but fluctuates in the same qualitative way to stimuli of insulin secretion as nontumor subjects. These findings have been corroborated by studies in hamsters bearing malignant β-cell tumors.

METHODS

Subjects. 3 healthy volunteers and 20 selected patients were studied as inpatients on a metabolic unit (Table I). 5 of the patient group had islet cell tumors and the remaining 15 were selected because in the basal state, their total plasma insulin concentration exceeded 20 μU/ml.

Tests. Drugs were discontinued at least 48 hr before the studies except where noted. In the nontumor subjects, basal insulin is defined as a specimen drawn in the morning after an overnight fast. Basal insulin in the islet-cell tumor subjects represents the longest period of fasting possible and was usually taken when the patient was hypoglycemic. Both the basal samples and the samples obtained after the ingestion of 100 g of glucose were collected after a normal diet that contained 300 g carbohydrate that had been given daily for at least 3 days. Sodium tolbutamide was diluted in normal saline and infused over a 2 min period. L-Leucine (150 mg/kg) was mixed with water and given orally. Streptozotocin provided as a sterile dry powder was dissolved in isotonic saline and administered intravenously. Venous blood was drawn into heparin through an indwelling. No. 19 gauge needle connected to a polyethylene cannula, and plasma was stored at −20°C until use.

TABLE I
Clinical Profile of Patients

Subject	Age/Sex	Ideal weight	Comments
		%	
Bro	20 F	92	Normal volunteer
Kin	20 M	104	Normal volunteer
McC	19 M	91	Normal volunteer
Abr	37 M	292	Obese
Bel	16 F	180	Obese
Lev	18 M	149	Obese
Miq	14 F	117	Obese
Sch	24 M	131	Obese
Lew	30 F	118	Obese, Cushing's Disease
Mel	42 F	150	Obese, Cushing's Syndrome
Len	38 F	134	Obese, Myotonic Dystrophy
Cas	27 M	103	Acromegaly, growth hormone = 27 mμg/ml
Hol	41 F	114	Acromegaly, growth hormone = 40 mμg/ml
Mck	34 M	125	Acromegaly, growth hormone = 150 mμg/ml
Cru	12 F	70	Hypokalemia
Joh	18 M	74	Hypokalemia
McD	40 M	100	Hypokalemia
Dre	47 F	100	Islet cell carcinoma, severe fasting hypoglycemia
Han	24 M	89	Islet cell carcinoma, severe fasting hypoglycemia
Mot	49 M	100	Islet cell carcinoma, severe fasting hypoglycemia
Fol	56 F	95	Islet cell carcinoma, severe fasting hypoglycemia
Pri	56 F	117	Islet cell adenoma, severe fasting hypoglycemia

Gel filtration and assay procedure. Blood glucose and total plasma insulin were determined as previously described (4). In most instances, 2 ml of plasma or less were applied to columns (1 × 50 cm) of G-50 Sephadex for separation of the insulin components. The columns were eluted with the diluent for the radioimmunoassay and the concentration and per cent of the proinsulin-like component determined (16). For the purpose of calculation, the term proinsulin-like component is used in the same context as "big" insulin (16) and insulin component in the same context as "little" insulin (16). Total insulin refers to the total immunoreactive insulin (16). In instances where the total insulin concentrations were less than 20 μU/ml, 4–5 ml of plasma was filtered on 2.5 × 45 cm columns and eluted with 0.05 M $(NH_4)_2CO_3$. The resultant fractions were lyophilized to dryness, dissolved in the assay diluent, and the insulin concentration measured by radioimmunoassay. In plasma samples where the proinsulin-like component represented a very high proportion of total insulin, the components were better discriminated using taller columns (1.5 × 90 cm). The accuracy and reproducibility of the procedure is largely a function of how well the component peaks are resolved on the column; samples with poorly resolved peaks were always refiltered. By the nature of the gel filtration process, there is always some contamination of one component by the other. To evaluate the significance of this cross contamination, the components, from two samples, one with a high and the other with a low percentage of the proinsulin-like component, were separated free of each other by gel filtration. The individual components were then refiltered. The percentage of the proinsulin-like component determined in this way was essentially the same as determined by the standard gel filtration procedure. This is analogous to the results of filtration of proinsulin and insulin on a Sephadex G-50 column (5). To ascertain the reproducibility of gel filtration determinations of the per cent proinsulin-like component, two plasma samples were rerun 18 months after their first determination; initial values were 53 and 55% and the later values were 49 and 50% respectively. In an obese subject whose basal total insulin on three separate occasions was 1.3, 1.1, and 1.2 mμg/ml, the proinsulin-like component was 17, 20, and 18% respectively. All radioimmunoassay results were obtained with the same anti-insulin serum and expressed in terms of a porcine insulin standard (Lilly, lot No. PJ5589) (6, 16).

Hamster studies. Syrian hamster islet-cell tumors were a gift of Dr. H. A. Kirkman. The tumors were maintained in young hamsters by serial subcutaneous transplantation. Plasma was obtained from tumor-bearing and normal hamsters through retroorbital puncture and processed in an identical manner to the human plasma samples.

200-mg slices of hamster islet-cell adenoma were incubated at 37°C for 120 min in complete Hanks media (17) containing 20 mg/liter of each of the 18 naturally occurring amino acids similar to the method used by Steiner and Oyer (2) for human adenoma. To individual flasks were added glucose 1 mg/ml, glucose 3 mg/ml, tolbutamide 100 μg/ml, and glucagon 5 μg/ml; after incubation 1-ml portions were removed from each flask and filtered on G-50 Sephadex (16) to determine the proportions of each insulin component.

RESULTS

In the basal state, the proinsulin-like component usually comprised about 20% of the total plasma insulin (Fig. 1). The exceptions were one obese patient with recurrent hypokalemia in whom basal percentage proinsulin-like component exceeded 40% on all four occasions tested and patients with islet cell tumors; the former will be discussed in detail elsewhere,[1] the latter will be discussed below. Thus in 17 adults, the proinsulin-like component was above 10% and below 30% regardless of other variables including glucose tolerance, insulin or growth hormone concentrations, or degree of obesity.

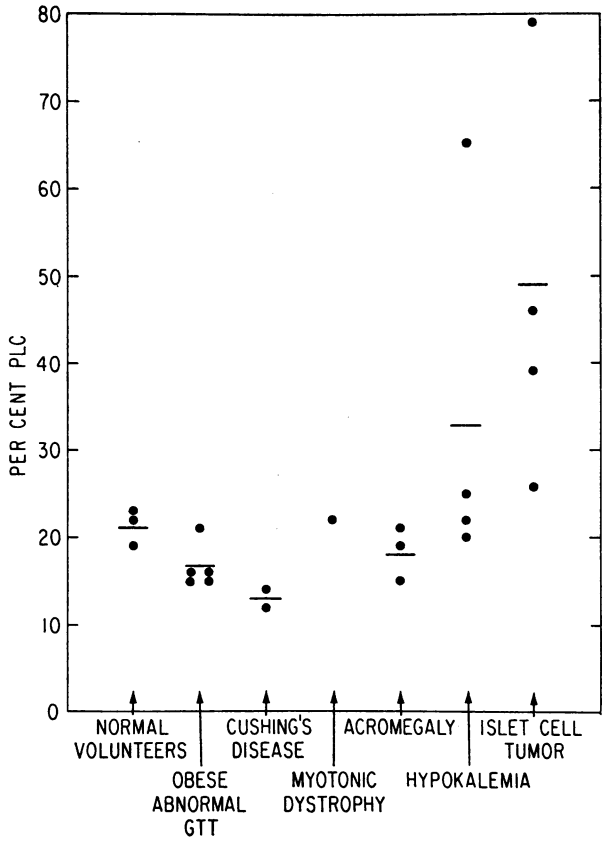

FIGURE 1 Per cent basal proinsulin-like component (PLC). Each point represents the basal per cent proinsulin-like component on individual patients shown in Table I, and the bar indicates the mean for each group. The hypokalemic patients had low serum potassium values at the time of the study.

The relationship between basal and the early and late glucose stimulated values was studied in 14 subjects (Fig. 2). At 15 min after the administration of glucose, the total plasma insulin was elevated. This increase was accounted for almost entirely by an increase in the concentration of the insulin component. The concentration of the proinsulin-like component was virtually unchanged, resulting in a fall in the percentage of this component (Fig. 2, left).

[1] Manuscript in preparation.

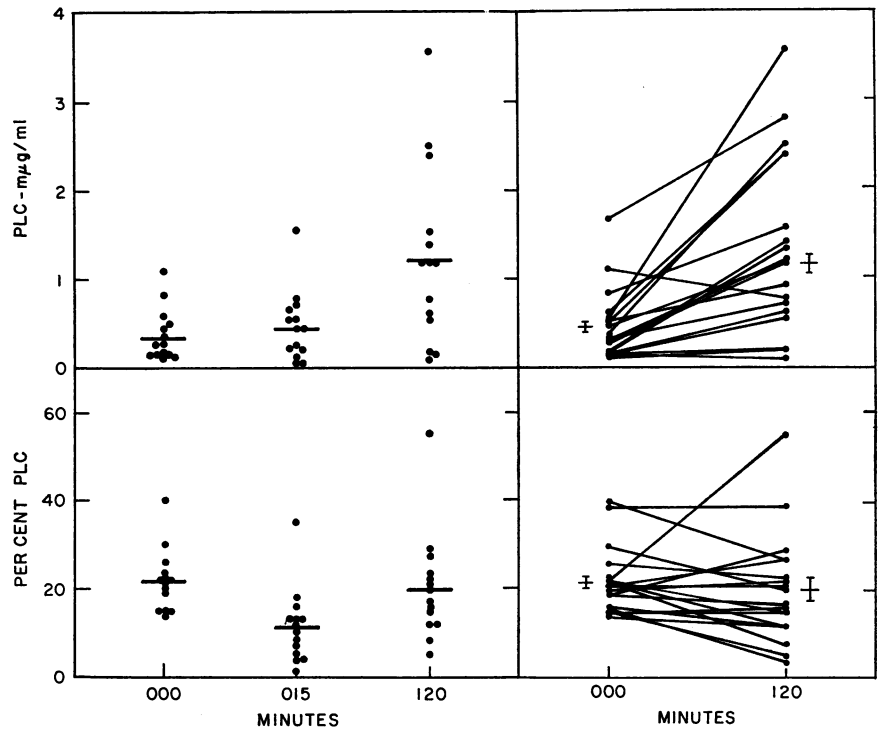

FIGURE 2 (Left), Proinsulin-like component (PLC) in 14 patients (Table I) during a 100 g oral glucose tolerance test. Each point represents an individual patient and the bar indicates the mean for the group. (Right), Basal vs. 100 g oral glucose stimulated PLC in 19 patients. Each point represents an individual subject before (000) and after 100 g of oral glucose (120). (‡) represents mean ±SEM.

By 120 min, the concentration of the proinsulin-like component had risen and the mean per cent proinsulin-like component returned to levels observed in the basal state (Fig. 2, left). In paired values from 19 individual subjects, the concentration of the proinsulin-like component rose in 15, was unchanged in 3, and fell slightly in 1 (Fig. 2, right).

Islet cell tumors. The presence of circulating insulin during fasting hypoglycemia is the cardinal feature of islet cell tumors (18). Abnormal glucose tolerance is common and the plasma insulin response to glucose stimulation is often subnormal whereas the responses to tolbutamide or leucine stimulation are frequently exaggerated (19). The basal per cent proinsulin-like component is higher than most other patients and ranges from 26 to 79% of the total insulin (Fig. 1). A wide spectrum of response is seen after stimulation with glucose and other agents.

In patient PRI, before removal of the adenoma, glucose tolerance was abnormal and the patient demonstrated abnormal insulin responses to tolbutamide and leucine (Fig. 3). The dynamics of the proinsulin-like component after glucose stimulation (Fig. 3) are similar to the nontumor group (Fig. 2). After surgery, which involved a hemipancreatectomy with removal of the adenoma, total insulin secretion was decreased but the concentration and per cent of the proinsulin-like component after glucose, leucine, and tolbutamide were not greatly different from preoperative determinations (Fig. 3).

We have previously demonstrated an arteriovenous difference for both insulin components across the pancreas of the dog (4); a similar arteriovenous difference across the pancreas in this patient with an islet cell adenoma was found at laporatomy in samples obtained simultaneously from a pancreatic vein and peripheral artery.[2]

Patients DRE and HAN with islet cell carcinomas demonstrated a high basal percentage of the proinsulin-like component (38–46%) but fluctuated similarly to the nontumor patients after stimulation of insulin secretion (Fig. 4). When total insulin concentrations were low, the per cent proinsulin-like component was high (Figs. 3 and 4). However, when insulin was acutely released with concentrations of greater than 375 μU/ml after stimulation with tolbutamide (Figs. 3 and 4) or streptozotocin (Fig. 5), the per cent proinsulin-like component

[2] Plasma samples obtained by Dr. William Hammond.

fell to low levels; the rise in total insulin concentration was almost entirely due to the release of fully converted insulin. The outpouring of insulin after the administration of streptozotocin in patient DRE (Fig. 5) was similar to what has been observed in the mouse (20) and rat (21).

Patient MOT had an undifferentiated carcinoma of the head of the pancreas discovered at least 2 months before the onset of hypoglycemia. His basal plasma insulin concentartaion was 12 mμg/ml (300 μU/ml) and there was no further rise after oral glucose administration (Fig. 6). The proinsulin-like component constituted 78% of the total insulin in the basal state, i.e., 4 hr of fasting with blood glucose 65 mg/100 ml. There was no change in total insulin during the period of high blood glucose and the proportions of the insulin components did not change throughout the glucose-stimulated period. (Fig. 6). The intravenous administration of 0.5 g of sodium tolbutamide produced an 83% increase in total insulin concentration. The concentration of the proinsulin-like component increased by only 36%, resulting in a reduction in the percentage of this component to 61%. Similarly, patient FOL with 53% proinsulin-like component showed essentially no fluctuation with further hyperglycemia, but had a significant increase in the concentration of the insulin component with tolbutamide stimulation and a resultant fall in the percentage of the proinsulin-like component (Table II). Thus the rise in total insulin after tolbutamide in patients MOT, FOL, PRI, and HAN is due almost entirely to an increase in the insulin component. After 10 days of treatment with 800 mg of oral diazoxide per day in patient MOT, there was no change in the fasting hypoglycemia or in total insulin secretion. The proinsulin-like component constituted 82% of the total plasma insulin.

Hamster studies. Studies of changes in plasma concentration of the insulin components in man do not distinguish events involving insulin secretion from changes occurring in the peripheral circulation. Syrian hamsters bearing islet cell tumors provide an excellent model for a more detailed study of insulin secretion.

When a tumor was homogenized and extracted by the classic acid-ethanol technique, the proinsulin-like component constituted 32% of the total extracted insulin.

The nontumor bearing hamster has a similar proportion of the insulin components in plasma as the nontumor patient whereas the tumor-bearing animal has a high proportion of the proinsulin-like component similar to patients with islet cell tumors (Fig. 7).

When slices of the hamster tumor are studied, the

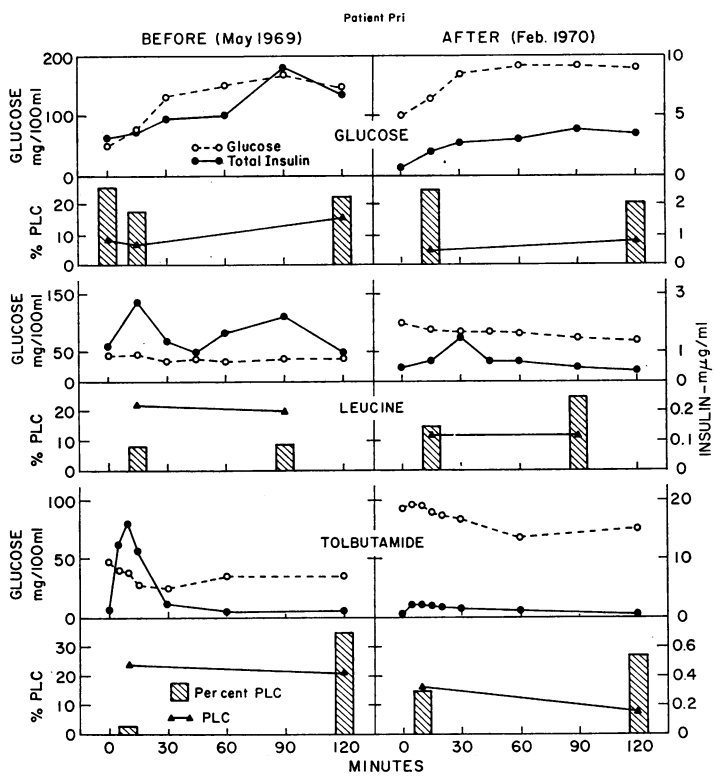

FIGURE 3 Patient PRI studied by glucose tolerance test, leucine tolerance test, and tolbutamide tolerance test before and 6 months after the removal of an islet cell adenoma.

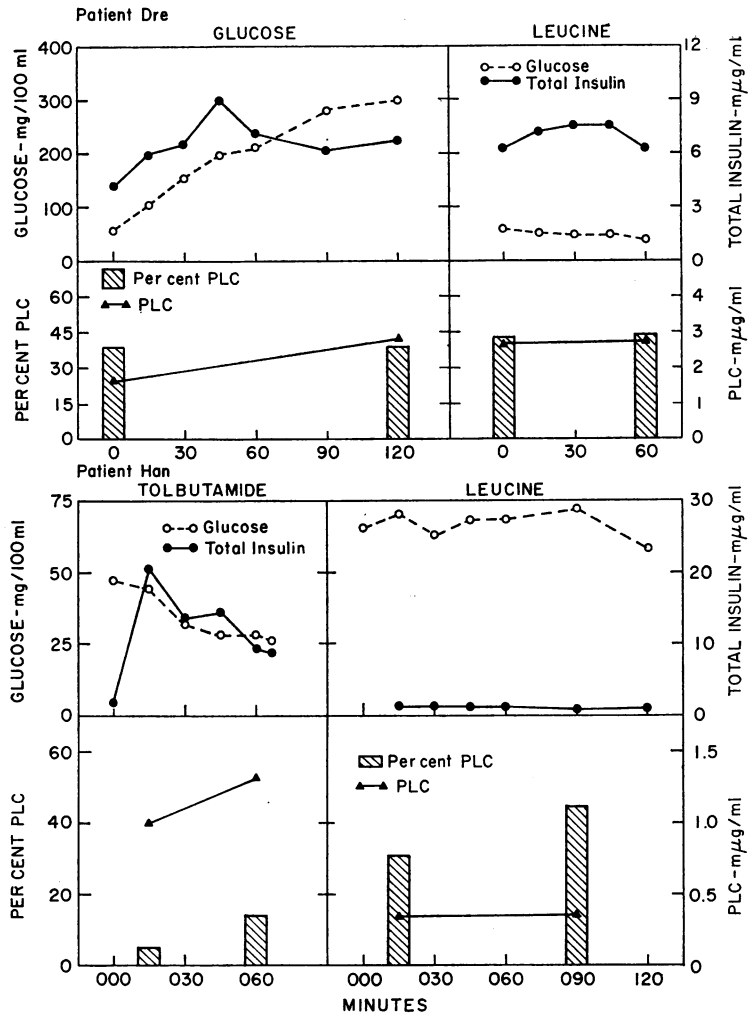

FIGURE 4 (Upper), Islet cell carcinoma patient (DRE) studied by glucose and leucine tolerance test. (Lower), Islet cell carcinoma patient (HAN) studied by tolbutamide and leucine tolerance test.

highest proportion of the proinsulin-like component is seen in the control media containing 1 mg/ml glucose; further stimulation with glucose results in an increase in both insulin components (Fig. 8). This finding is compatible with the known effects of glucose in stimulating both the synthesis and release of insulin (3, 22). Tolbutamide and glucagon mainly stimulate the release of the insulin component and decrease the percentage of the proinsulin-like component (Fig. 8). These effects are similar to those observed in islet cell tumors in man.

DISCUSSION

In the nontumor subject, the proinsulin-like component comprised approximately 20% of the basal circulating insulin. The rise in total insulin 15 min after oral glucose stimulation is accounted for entirely by the insulin component since the concentration of the proinsulin-like component is unchanged; the percentage of the proinsulin-like component at 15 min is accordingly decreased. By 120 min after glucose stimulation, the percentage of the proinsulin-like component increased to that seen in the basal state and resulted from an increase in the concentration of this component and a fall in the concentration of the insulin component. Similarly, the administration of tolbutamide results in the acute release of the insulin component with an increase in the per cent proinsulin-like component in the 2nd hr as the total plasma insulin concentration returns to the basal state (16).

Melani, Rubenstein, and Steiner, found from 2 to 18% of the proinsulin-like component in the basal state in extracts of plasma from seven subjects (8) based on measurements against an insulin standard; measure-

ments against a human proinsulin standard yield higher percentages but cannot be compared with other results in the literature. A rise in concentration of the proinsulin-like component occurred in each of their patients after glucose stimulation and the proinsulin-like component comprised 2–29% of the total insulin, 1–4 hr after glucose administration (8). Goldsmith, Yalow, and Berson did not measure the proinsulin-like component in the basal state, but found from 0–20% in samples taken from 30 min to 3 hr after glucose stimulation (23). In most of their patients the per cent proinsulin-like component was higher in the 2nd and 3rd hr after oral glucose than at 30 min after the glucose load.

The half-time of disappearance and space of distribution of human proinsulin in plasma has not been precisely determined. If the half-time of disappearance and space of distribution of human proinsulin are approximately that of human insulin and if proinsulin represents only about 1% (11) to 10% (12) of the total pancreatic insulin, proinsulin, to account for 20% or more of the circulating insulin, must be preferentially released. If, however, the half-time of disappearance for proinsulin is much longer than insulin and the space of distribution less, the high plasma to pancreas ratio for proinsulin could be explained by the secretion of a constant ratio of proinsulin to insulin. It remains unexplained why an insulin precursor of low biologic activity should represent an appreciable amount of circulating insulin.

The mean and range of per cent proinsulin-like com-

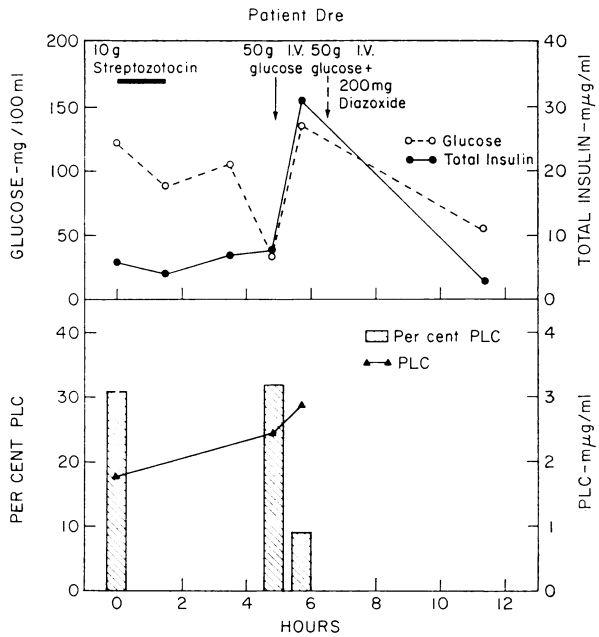

FIGURE 5 10 g of streptozotocin as a therapeutic dose were infused over 90 min. 10% glucose in water was infused at a constant rate throughout the entire course of the study and the augmented glucose infusions were given to prevent severe hypoglycemia. At 7 hr after the streptozotocin administration, 200 mg of diazoxide was given by mouth. Note this dose of streptozotocin produced severe toxicity and was only given after three smaller doses failed to produce any therapeutic benefit.

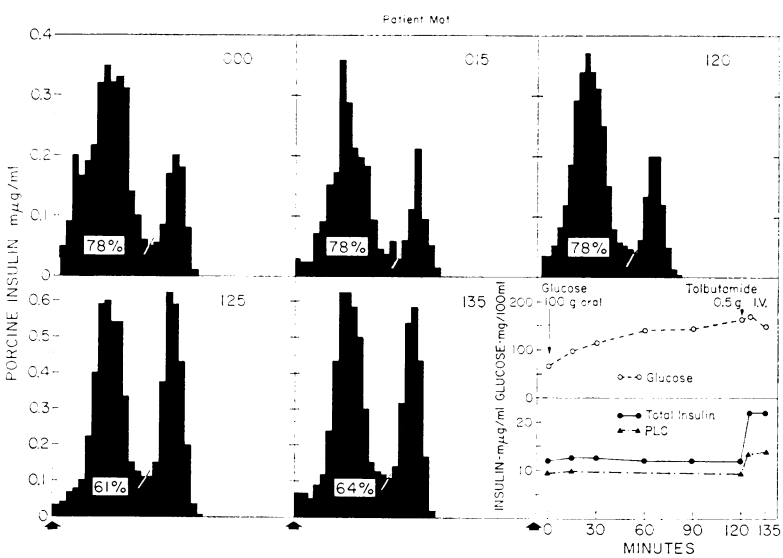

FIGURE 6 Islet-cell carcinoma patient (MOT). G-50 Sephadex gel filtration patterns of plasma taken during 100 g oral glucose tolerance test followed by the intravenous administration of 0.5 g of sodium tolbutamide. The arrows denote albumin-^{125}I and ^{125}I markers and the percentages represent the proportion of the proinsulin-like component (PLC) at each time period.

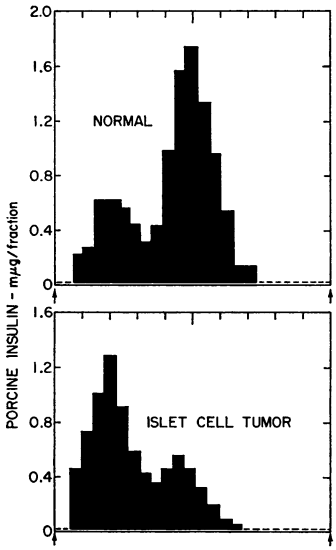

FIGURE 7 Sephadex gel filtration pattern of plasma from Syrian hamsters. Plasma was obtained in the basal state in a normal hamster and a hamster bearing an islet cell tumor.

ponent is higher in islet-cell tumor patients than in most other patients studied, comprising from 26 to 79% of the total insulin. In individual cases a range of from 3 to 67% of basal and stimulated proinsulin-like component has been reported (1, 15, 16, 23–25).

Patients with islet cell tumors differ from nontumor patients in exhibiting an exaggerated insulin release to various stimuli and inappropriate insulin release with hypoglycemia. These patients, however, demonstrate the same qualitative dynamics with regard to the insulin components as the nontumor subjects. If a similar ratio for proinsulin exists from pancreas to plasma as in the nontumor patient, then only a small percentage increase of pancreatic proinsulin could account for a predominance of the proinsulin-like component in the plasma of the islet-cell tumor patient. Evidence for an increased tumor content of proinsulin in some patients with islet cell tumors has been obtained (15).[3] After the administration of stimuli of insulin release our patients either had very little response in total insulin concentration or a marked rise. With small changes in the total insulin concentration the per cent proinsulin-like component remained high as in the basal state but with large increases in total insulin there was a concomitant fall in the per cent proinsulin-like component.

Similar observations were made in Syrian hamsters bearing islet cell tumors. These tumors make large quantities of proinsulin-like material under basal conditions. When stimulated acutely with glucose, glucagon, or tolbutamide they release predominantly the insulin component into the incubation media in a manner which closely resembles the release of the insulin component into the circulation in the islet-cell tumor patients.

The case reported by Melani, Ryan, Rubenstein, and Steiner (15) is the only other systematic study of basal and stimulated proinsulin-like components. The basal proinsulin-like component was 42% of the total (measured against an insulin standard), falling to 36% as insulin was acutely released by tolbutamide and increased to a high of 67% in the 2nd and 3rd hr as the total insulin concentration returned to basal levels (15). Goldsmith, Yalow, and Berson found total insulin 110 μU/ml at 15 min and 70 μU/ml at 30 min after the intravenous administration of tolbutamide to a patient with an islet cell tumor. The proinsulin-like component constituted 32 and 55% of the total respectively (23). Lazarus, Tanese, Gutman, and Recant reported greater than 50% proinsulin-like component 15 min after tolbutamide stimulation but other time periods are not given for comparison (24). Blackard, Garcia, and Brown found 37, 32, and 53% proinsulin-like component in an untreated patient with islet cell carcinoma at 1, 2, and 3 hr after oral glucose; the percentage tended to increase as the total insulin concentration decreased toward basal levels (25).

The percentage of the proinsulin-like component in the plasma of both tumor and nontumor subjects at any given time is determined largely by how close the total plasma insulin concentration approaches concentrations in the basal state. The quantitatively higher proportion of the proinsulin-like component in islet-cell tumor patients can be accounted for by the same mechanism as in nontumor patients. It is not necessary to postulate that the tumor patients have a deficiency in the proinsulin to insulin converting mechanism. In fact, the acute release of the insulin component by tolbutamide and streptozotocin suggests that the converting mechanism is operative.

It is reasonable to speculate that in the presence of hypoglycemia, insulin release from the pancreas is maximally inhibited by a variety of physiologic processes. Furthermore, pharmacologic agents like diazoxide are potent inhibitors of insulin release. Diazoxide inhibits insulin secretion from the mature beta granule (26). Since the mature beta granule is largely devoid of proinsulin (10, 27), maximal inhibition of this process of insulin release may have little therapeutic effect in a patient who secretes predominantly the proinsulin-like component. Diazoxide was therapeutically effective in three islet-cell tumor patients (PRI, DRE, and HAN) with 26–46% proinsulin-like component but had no effect in patient MOT. This patient's total plasma insulin was comprised almost entirely of the proinsulin-like component.

[3] In the tumor extract from patient DRE, the proinsulin-like component represented 16% of the total insulin in an analysis carried out by Dr. Ronald E. Chance.

TABLE II

Islet Cell Carcinoma (Patient FOL)

	Time	Glucose	Total insulin	Insulin component	PLC‡	PLC
	min	*mg/100 ml*		*mμg/ml*		*%*
Glucose, 100 g oral →	000	27	3.0	1.4	1.6	53
	015	32	3.6	1.7	1.9	53
	120	94	4.4	2.1	2.3	53
Glucose,* 20 g IV →						
	135	202	4.4			
Tolbutamide, 1 g IV →						
	140	181	9.0	5.8	3.2	36
	145	156	8.5	5.4	3.1	36

* Additional glucose given because patient appeared symptomatically hypoglycemic.

‡ Proinsulin-like component.

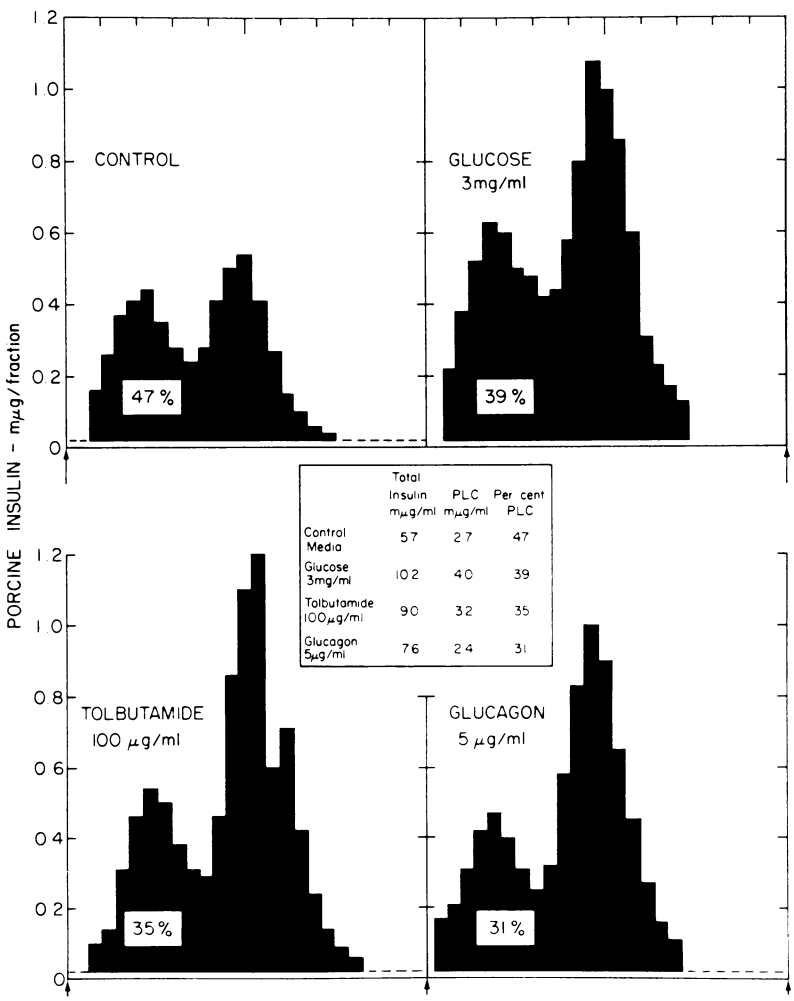

FIGURE 8 Sephadex gel filtration pattern from incubation media of 200 mg slices of hamster islet-cell tumor. The control media contained glucose 1 mg/ml and each of the other studies shown contained the control media plus total glucose of 3 mg/ml, tolbutamide 100 μg/ml, and glucagon 5 μg/ml.

Whether the failure to respond to diazoxide was a unique feature of this patient or a general phenomenon seen in patients who secrete predominantly proinsulin-like material, remains to be determined.

The extrapolation of events measured in the circulation to events occurring in the islet cell in man is speculative at this time but further studies of the insulin components should lead to a better understanding of the mechanisms regulating the formation and secretion of insulin.

ACKNOWLEDGMENTS

We are grateful to C. M. Hendricks for superior technical assistance; to the nurses and physicians on Ward 8 West, Clinical Center; and Drs. William Hammond and Samuel Wells of the Surgical Branch for aid in the care of the patients; to Drs. Howard Nankin, Wesley M. Oler, Henry Scruggs, G. Bertling Smith, and Vincent Devita for referral of patients with islet cell tumors; and to Dr. Saul Rosen for critical review of the manuscript.

REFERENCES

1. Gorden, P., B. Sherman, and J. Roth. 1970. "Big" insulin (circulating proinsulin-like components): a high proportion of the basal insulin. *Diabetes.* **19** (Suppl. 1): 360. (Abstr.)
2. Steiner, D. F., and P. E. Oyer. 1967. The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. *Proc. Nat. Acad. Sci. U. S. A.* **57**: 473.
3. Steiner, D. F., D. Cunningham, L. Spigelman, and B. Aten. 1967. Insulin biosynthesis: evidence for a precursor. *Science (Washington).* **157**: 697.
4. Roth, J., P. Gorden, and I. Pastan. 1968. "Big insulin": a new component of plasma insulin detected by immunoassay. *Proc. Nat. Acad. Sci. U. S. A.* **61**: 138.
5. Gorden, P., and J. Roth. 1969. Circulating insulins: "big" and "little." *Arch. Intern. Med.* **123**: 237.
6. Sherman, B. M., P. Gorden, J. Roth, and P. Freychet. 1971. Circulating insulin: the proinsulin-like properties of "big" insulin in patients without islet cell tumors. *J. Clin. Invest.* **50**: 849.
7. Rubenstein, A. H., S. Cho, and D. F. Steiner. 1968. Evidence for proinsulin in human urine and serum. *Lancet.* **1**: 1353.
8. Melani, F., A. H. Rubenstein, and D. F. Steiner. 1970. Human serum proinsulin. *J. Clin. Invest.* **49**: 497.
9. Melani, F., A. H. Rubenstein, P. E. Oyer, and D. F. Steiner. 1970. Identification of proinsulin and C-peptide in human serum by a specific immunoassay. *Proc. Nat. Acad. Sci. U. S. A.* **67**: 148.
10. Steiner, D. F., J. L. Clark, C. Nolan, A. H. Rubenstein, E. Margoliash, B. Aten, and P. E. Oyer. 1969. Proinsulin and the biosynthesis of insulin. *Recent Progr. Hormone Res.* **25**: 207.
11. Steiner, D. F., O. Hallund, A. Rubenstein, S. Cho, and C. Bayliss. 1968. Isolation and properties of proinsulin, intermediate forms, and other minor components from crystalline bovine insulin. *Diabetes.* **17**: 725.
12. Chance, R. E. 1971. Chemical, physical, biological, and immunological studies on porcine proinsulin and related polypeptides. Proceeding 7th International Congress on Diabetes. Buenos Aires. In press.
13. Gutman, R. A., N. R. Lazarus, J. C. Penhos, L. Recant, and S. S. Fajans. 1970. Proinsulin (PI) and proinsulin-like material (PI-LM) in serum of patients with islet cell tumors. *Diabetes.* **19**: 360. (Abstr.)
14. Gorden, P., P. Freychet, and H. Nankin. 1971. New form of circulating insulin in islet cell carcinoma. *Clin. Res.* **19**: 560. (Abstr.)
15. Melani, F., W. G. Ryan, A. H. Rubenstein, and D. F. Steiner. 1970. Proinsulin secretion by a pancreatic beta-cell adenoma. *N. Engl. J. Med.* **283**: 713.
16. Gorden, P., and J. Roth. 1969. Plasma insulin: fluctuations in the "big" insulin component in man after glucose and other stimuli. *J. Clin. Invest.* **48**: 2225.
17. Hanks, J. H., and R. E. Wallace. 1949. Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proc. Soc. Exp. Biol. Med.* **71**: 196.
18. Yalow, R. S., and S. A. Berson. 1965. Dynamics of insulin secretion in hypoglycemia. *Diabetes.* **14**: 341.
19. Floyd, J. C., Jr., S. S. Fajans, R. F. Knopf, and J. W. Conn. 1964. Plasma insulin in organic hyperinsulinism: comparative effects of tolbutamide, leucine and glucose. *J. Clin. Endocrinol. Metab.* **24**: 747.
20. Schein, P. S., and R. W. Bates, 1968. Plasma glucose levels in normal and adrenalectomized mice treated with streptozotocin and nicotinamide. *Diabetes.* **17**: 760.
21. Junod, A., A. E. Lambert, L. Orci, R. Pictet, A. E. Gonet, and A. E. Renold. 1967. Studies of the diabetogenic action of streptozotocin. *Proc. Soc. Exp. Biol. Med.* **126**: 201.
22. Howell, S. L., and K. W. Taylor. 1967. The secretion of newly synthesized insulin *in vitro*. *Biochem. J.* **102**: 922.
23. Goldsmith, S. J., R. S. Yalow, and S. A. Berson. 1969. Significance of human plasma insulin Sephadex fractions. *Diabetes.* **18**: 834.
24. Lazarus, N. R., T. Tanese, R. Gutman, and L. Recant. 1970. Synthesis and release of proinsulin and insulin by human insulinoma tissue. *J. Clin. Endocrinol. Metab.* **30**: 273.
25. Blackard, W. G., A. R. Garcia, and C. L. Brown, Jr. 1970. Effect of streptozotocin on qualitative aspects of plasma insulin in a patient with a malignant islet cell tumor. *J. Clin. Endocrinol. Metab.* **31**: 215.
26. Creutzfeldt, W., C. Creutzfeldt, H. Frerichs, E. Perings, and K. Sickinger. 1969. The morphological substrate of the inhibition of insulin secretion by diazoxide. *Horm. Metab. Res.* **1**: 53.
27. Sorenson, R. L., M. W. Steffes, and A. W. Lindall. 1970. Subcellular localization of proinsulin to insulin conversion in isolated rat islets. *Endocrinology.* **86**: 88.