Insulin and Proinsulin Content of Pancreases from Diabetic and Nondiabetic Subjects

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SUMMARY

The content of immunoreactive insulin (IRI) extractable with acid ethanol from the head, body and tail of the pancreas was studied in thirty-two nondiabetics, eleven maturity-onset diabetics and four juvenile diabetics. In nondiabetics IRI content ranged from 0.1 to 4.8 U. per gram wet pancreas. The tail had the highest insulin content, but the body of the pancreas could be taken to represent the entire pancreas. The insulin content of pancreases from eleven maturity-onset diabetics ranged from 0.1 to 6.5 U. per gram wet pancreas. It showed a progressive decline with the duration of diabetes. The IRI content of pancreases from four juvenile diabetics was low. The proportion of proinsulinlike component, estimated by gel filtration in the pancreatic extracts, ranged from 0.2 to 5 per cent of the insulin peak and was not different in diabetics. DIABETES *22*:804-07, November, 1973.

Experimental and human diabetes mellitus results from an absolute or relative deficiency of insulin. Extrapancreatic factors might be operative in early stages, but ultimately islet cell function is invariably disordered. Assessment of the state of islet cells in healthy persons, prediabetics and diabetics could throw light on the pathogenesis of diabetes in man.[1] The extractable insulin content of the pancreas, with reference to the duration, type, treatment and course of diabetes, is of obvious interest, particularly since a large body of literature has accumulated regarding the microanatomy of the islets and circulating insulin levels in health and disease. Roy, O'Brien and their coworkers[2-4] have illustrated that the biologic activity of insulin in the pancreas differs in diabetics and nondiabetics.

Extraction of insulin from the entire pancreas is laborious and wasteful, as only a minute quantity of the extract is required for an assay. Since islet concentration varies in different parts of the pancreas,[5,6] the content of immunoreactive insulin (IRI) extractable with acid ethanol has been studied in different parts of the pancreas. The proportion of proinsulinlike component (PI) in pancreatic extracts from diabetic and nondiabetic subjects has also been quantitated.

MATERIALS AND METHODS

Fresh human pancreases were obtained at autopsy within six hours after death from nondiabetic and diabetic subjects. After the organs were cleared of fat and fibrous tissue, a small piece from the head, body and tail was stored at —20° C. Immunoreactive insulin was extracted from the tissue with acid ethanol as detailed in an earlier communication.[7] The amount of acid ethanol was 20 ml. per gram pancreatic tissue. Lyophilized extracts in three different dilutions were assayed for IRI by the double antibody technic.[8] IRI concentration of the extract multiplied by 20 gave the IRI in units per gram wet pancreas. More than 95 per cent of I-125-insulin was recovered in the extract when added before homogenizing the tissue. Analysis of four pancreases obtained within two hours after death demonstrated that there was no loss of extractable IRI upon storing the pancreatic tissue at 4° C. up to six hours. Hartroft and Wrenshall did not detect loss of extractable insulin upon storage of pancreatic tissue at 4° C. for up to eight days.[9]

Separation of PI from insulin was effected by gel filtration on Sephadex G-50 fine (1 x 50 cm. column) equilibrated and eluted with 1 M. acetic acid. Lyophil-

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Accepted for publication March 27, 1973.

ized aliquots of extracts (500 to 1,000 μU.) were dissolved in 0.5 ml. of 1 M. acetic acid and charged on the column; 1 ml. fractions were collected. Lyophilized fractions dissolved in assay buffer were individually assayed for IRI content. The column was characterized for insulin and proinsulin positions by 125-I-labeled bovine proinsulin and insulin. The recovery of tracers and IRI from the column ranged from 79 to 90 per cent. The proportion of IRI in PI peak was expressed as per cent of insulin peak.[10]

RESULTS

In thirty-two nondiabetics, the insulin content of the head, body and tail of the pancreases ranged from 0.1 to 4.8 U. per gram wet pancreas. Table 1 lists clinical details regarding the subjects, cause of death, and insulin content of different parts. All the nondiabetics were of average weight. The IRI content of the tail was highest according to mean figures but not in each instance. In the nondiabetics the IRI content of the tail, 1.62 ± 0.2 (mean ± S.E.M.) was significantly higher ($p < 0.01$) than that of the head (1.39 ± 0.19) but not significantly different ($p > 0.05$) from that of the body of the pancreas (1.53 ± 0.2).

IRI content of different parts of the pancreases from diabetics showed a similar pattern. Table 2 provides data regarding eleven maturity-onset and four juvenile diabetics. One obese patient who died of myocardial infarction six months after diabetes was diagnosed and who had taken 1 gm. tolbutamide per day irregularly had pancreatic insulin content of 6.5 U. per gram. The extractable insulin content of the pancreases from diabetics decreased progressively with increasing duration of diabetes. No insulin could be extracted from the pancreas of a juvenile diabetic who had the disease for four years. In the other three young patients, only small quantities could be extracted.

Proinsulinlike component comprised 0.2 to 5 per cent of the IRI in insulin peak. There was no significant difference between PI component of the pancreatic extracts from diabetics ($n = 12$) and nondiabetics ($n = 11$).

TABLE 1

Clinical parameters, causes of death and IRI content of different parts of the pancreas from thirty-two nondiabetic subjects

No.	Patient	Age (Yrs.)	Sex	Cause of death	Pancreatic insulin content U./gm.		
					Head	Body	Tail
1.	P.S.	28	M	Chronic renal failure	0.3	0.5	0.8
2.	T.P.K.	32	M	Amebic liver abscess	0.2	0.4	0.5
3.	A.D.	23	F	Rheumatic heart disease, C.C.F.*	0.6	0.5	0.7
4.	S.S.	48	M	Myocardial infarction	2.0	2.3	2.8
5.	T.K.	59	F	Ischemic heart disease, C.C.F.	0.5	0.7	0.6
6.	B.D.	41	F	Amebic liver abscess	1.3	1.7	1.5
7.	K.K.	62	M	Pneumonia and septicemia	0.7	0.4	0.5
8.	T.L.	68	M	Ischemic heart disease	0.8	0.6	0.4
9.	G.K.	18	F	Chronic nephritis	0.6	0.8	0.8
10.	V.S.	17	M	Viral hepatitis, hepatic coma	1.2	1.0	1.4
11.	S.M.	47	M	Cor pulmonale	2.3	2.7	2.8
12.	M.D.	67	F	Myocardial infarction	1.2	1.9	2.3
13.	P.S.	17	M	Encephalitis	3.1	3.0	3.0
14.	J.K.	54	M	Cerebral infarction	1.4	2.0	2.0
15.	B.B.M.	70	M	Cor pulmonale	1.5	1.5	1.4
16.	S.P.K.	65	M	Myocardial infarction	1.0	1.7	1.8
17.	K.B.	17	F	Rheumatic heart disease, C.C.F.	2.0	2.3	2.5
18.	C.K.	29	M	Chronic renal failure	1.6	1.2	1.0
19.	P.S.P.	57	M	Hypertension, C.C.F.	2.0	2.1	2.0
20.	N.S.	37	M	Subarachnoid hemorrhage	0.9	1.2	1.0
21.	J.B.	28	F	Cerebral venous thrombosis	2.6	2.0	2.0
22.	I.S.	52	M	Myocardial infarction	4.4	4.0	4.4
23.	N.N.	28	M	Rheumatic heart disease, C.C.F.	0.4	0.7	0.4
24.	C.S.	73	M	Cor pumonale	2.4	2.8	2.8
25.	A.P.	14	F	Congenital heart disease	0.3	0.6	0.3
26.	R.D.	21	M	Chronic pyelonephritis	0.4	0.3	0.4
27.	R.S.	35	F	Rheumatic heart disease, C.C.F.	0.1	0.2	0.4
28.	Y.P.	57	M	Cirrhosis of the liver	2.3	1.6	2.8
29.	L.D.	34	F	Hypertension, C.C.F.	1.1	1.8	2.6
30.	D.S.	48	M	Bronchial carcinoma	3.8	4.8	4.0
31.	F.C.	77	M	Hypertension, cerebral infarction	2.6	2.5	2.8
32.	G.K.	49	F	Cervical carcinoma	0.3	0.3	0.3

*C.C.F. = Congestive cardiac failure

TABLE 2

Clinical parameters and mean pancreatic insulin content in eleven maturity-onset and four juvenile diabetics

No.	Patient	Age	Sex	Build	Known duration of diabetes in years	Treatment	Cause of death	Mean pancreatic insulin content U./gm.
MATURITY-ONSET DIABETICS								
1.	N.D.	52	M	Obese	0.5	Tolbutamide*	Myocardial infarction	6.5
2.	P.S.	71	M	Obese	1.2	Diet	Cerebral infarction	2.5
3.	G.K.	38	F	Average	1.5	Tolbutamide*	Chronic renal failure	2.0
4.	P.K.I.	42	M	Obese	2.0	Chlorpropamide	Ischemic heart disease	1.3
5.	S.S.P.	52	M	Average	3.0	Tolbutamide*	Myocardial infarction	1.6
6.	T.S.G.	49	M	Average	4.0	Diet/Insulin	Cirrhosis of liver	1.3
7.	M.K.	58	F	Thin	4.5	Chlorpropamide	Hypertension, C.C.F.	0.8
8.	R.D.P.	60	F	Average	7.0	Tolbutamide*	Cerebral infarction	0.1
9.	N.N.	47	M	Average	9.0	Diet/Insulin*	Diabetic nephropathy	0.15
10.	Y.P.	65	F	Average	10.0	Tolbutamide*	Hypertension, C.C.F.	0.14
11.	H.S.	69	M	Average	12.0	Tolbutamide*	Diabetic nephropathy	0.1
JUVENILE DIABETICS								
1.	G.K.	18	M	Thin	4.0	Insulin*	Meningitis, ketosis	0
2.	T.L.	16	M	Thin	0.4	Nil	Ketosis	0.2
3.	P.K.	13	F	Thin	0.5	Insulin*	Infection, ketosis	0.05
4.	L.S.	15	M	Thin	0.6	Insulin*	Infection, ketosis	0.12

* Taken irregularly.

DISCUSSION

Though the tail of the pancreas is described to have the highest concentration of the islets,[5,6] its extractable IRI content was not invariably highest in our study. Stating the mean IRI content of the head as 1, the ratio of IRI content was 1.1 for the body of the pancreas and 1.16 for the tail. The figures do not correlate with the recently described percentage area of islets in the head, body and tail as 1, 1.33 and 1.7, respectively.[12] In this small series, although the tail of the pancreas had the highest mean IRI content, the insulin content of the body of the pancreas could be taken to represent the whole pancreas, in agreement with the suggestion of Wrenshall et al.[13]

Whereas levels of circulating insulin in maturity-onset diabetics are high, most reports describe the insulin content of the diabetic pancreas as 40 per cent or less than that of the nondiabetic pancreas.[12-15] The discrepancy has not been explained. In the first five years or so after the diagnosis of maturity-onset diabetes, the pancreatic insulin content is comparable to that in nondiabetics. The insulin content of the pancreas declines with increasing duration of diabetes. Whether insulin secretion and levels of circulating insulin also decrease in maturity-onset diabetics with increasing duration is not yet determined. A study of a large number of pancreases from diabetics is indicated.

The IRI content of pancreases from juvenile diabetics was low and in one instance, where duration of diabetes was four years, no IRI could be extracted. This finding is consistent with available information on circulating insulin levels in juvenile diabetics and with histologic data. The number of beta cells in patients with acute juvenile diabetes is as a rule less than 10 per cent in early stages and in later stages, only alpha cells and atrophic tissue devoid of beta cells are seen.[16]

It should be pointed out that in view of the marked variation in the pancreatic insulin content, which no doubt is influenced by the mode of death and preceding therapy, the pancreatic insulin content as determined post mortem may not be a good index of the insulin secretory capacity during life.

Considering the recent reports that the biologic activity of insulin is different in diabetic and nondiabetic pancreases,[2-4] it was in order to quantitate the proinsulinlike component in the pancreatic extracts. Proinsulin is weaker than insulin in biologic effectiveness[10,17] and does appear in the circulation.[18] In a previous study,[11] PI comprised 1.5 per cent of insulin peak in nondiabetic pancreases. In the present study the proportion of proinsulin ranged from 0.2 to 5 per cent and was not different in diabetics. There is thus no defect in conversion of proinsulin to insulin in diabetics.

ACKNOWLEDGMENT

This work was supported by a grant in aid from the Indian Council of Medical Research, New Delhi. The human insulin standard was kindly provided by the Division of Biological Standards, National Institute of Medical Research, London. Bovine proinsulin was a gift from Dr. D. F. Steiner, Chicago. We thank Mr. B. R. Sharma, Mr. S. C. Nakra and Miss S. Bedi for technical assistance.

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