Immunoreactive Insulin in the Portal and the Peripheral Venous Blood after Intravenous Tolbutamide Administration

Rolf Erwald, M. L., Ragnar Hed, M.D., Arne Nygren, M. L., Sven Röjdmark, M. L., Lars Sundblad, M.D., and Karl-Ludvig Wiechel, M.D., Stockholm, Sweden

SUMMARY

Immunoreactive insulin has been assayed in portal and peripheral venous blood in fourteen patients with hepatic, pancreatic and gastric disease before and after tolbutamide administration. The difference between portal and peripheral insulin varied considerably in different patients. In some of the patients the portal insulin seemed to increase at a time when peripheral venous insulin had started to decrease. The implication of this finding is discussed. DIABETES *20*:686-90, October, 1971.

Deductions regarding insulin release from the pancreas have been made from peripheral vein insulin concentrations by assuming that the fractional removal rate of insulin from the blood is independent of insulin concentration, does not vary from time to time in any one subject and is not grossly affected by disease states.[1-3] These assumptions may introduce a serious error, since insulin is secreted into the portal system and must traverse the hepatic bed before reaching the periphery.

A few studies concerning the relationship between portal and peripheral vein insulin concentrations have been published.[1-3] According to these studies there is rough agreement between portal and peripheral vein insulin responses to glucose and secretin.[1,2] Blackard and Nelson found, however, evidence indicating that the peripheral insulin response may not accurately reflect secretory patterns after glucose infusions.[3]

In this investigation immunoreactive insulin (IRI) has been assayed in blood samples drawn simultaneously from portal and peripheral venous blood after intravenous administration of tolbutamide. The insulin curves thus obtained in the portal and peripheral veins have been compared in order to estimate the difference between the portal and the peripheral venous IRI after tolbutamide stimulation.

MATERIAL

The material comprised six women (aged 46-86 years) and eight men (aged 23-60 years) admitted to the Surgical Department II, Södersjukhuset, because of jaundice and/or various kinds of abdominal complaints (see table 1). None of the patients showed glucosuria or elevated fasting glucose in peripheral venous blood. Three patients (cases 3, 6 and 9) had body weights slightly exceeding the normal range given in Documenta Geigy 1960.[4] Two patients (cases 3 and 4) had an elevated axillary temperature (37.8° C. and 38.8° C. respectively) at examination.

METHODS

Blood glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi Reagents, Sweden).

Immunoreactive insulin (IRI) was assayed by a double-antibody procedure essentially as described by Soeldner and Slone.[5] Pork insulin (10 x crystallized) was used for immunization. All insulin concentrations were

From the Department of Clinical Chemistry, Medical Clinic II and Surgical Clinic II, Södersjukhuset, Stockholm, Sweden.

TABLE 1

Laboratory values and diagnosis. GOT = glutamic-oxaloacetic transaminase GPT = glutamic-pyruvic transaminase. Normal values: GOT = 0-40, GPT = 0-35, alkaline phosphatase 2-10 units.

Case	Sex	GOT	GPT	Alkaline phosphatase	Bilirubin	Liver biopsy	Diagnosis
1	male	43	19	10.0	0.5	—	Acute pancreatitis with stenosis of the common bile duct
2	male	13	15	3.5	0.6	Slight infiltration by lymphocytes	Chronic cholecystitis and stenosis of duodenum
3	male	21	20	3.8	0.7	—	Acute pancreatitis
4	male	16	16	3.4	0.3	—	Pyloric ulcer with hematemesis
5	male	10	12	3.8	0.3	—	Relapsing pancreatitis with pancreatic abscess
6	female	53	26	16.8	13.5	Slight biliary cirrhosis	Carcinoma of the papilla of Vater
7	female	23	13	7.6	0.7	—	Chronic alcoholism and gastric ulcer
8	female	33	19	7.2	0.9	Hepatic cirrhosis	Cholecystolithiasis and hepatic cirrhosis
9	female	63	37	20.4	9.0	Cholangitis and hepatic cirrhosis	Carcinoma of the common bile duct
10	male	25	25	3.4	1.0	Normal	Chronic alcoholism and hematemesis
11	male	20	17	12.4	0.4	—	Primary carcinoma of the liver
12	female	27	25	14.5	3.4	Slight fatty infiltration in the portal zones	Carcinoma of the pancreas
13	female	15	12	5.6	0.6	—	Simple cyst of the liver and benign pulmonary tumor
14	male	30	24	3.6	0.7	—	Acute pancreatitis

determined by reference to a standard of 2 x crystallized human insulin (obtained by the courtesy of Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen).

Liver biopsy specimens were obtained at abdominal operation in cases 2, 6, 8, 9 and 12, and with a Vim-Silverman needle in case 10.

Umbilical vein catheterization was performed for diagnostic purposes (portal venography and manometry). Under local anesthesia the umbilical vein was uncovered extraperitoneally and reopened and probed into the left portal branch, whereupon a catheter could be placed in the main portal trunk with the aid of fluoroscopic control.[6] The catheter was left for some days without any essential discomfort for the patients, who were able to move freely in the ward and eat regular hospital meals.

Tolbutamide was administered intravenously after an overnight fast. The examinations were performed at least one day after the umbilical vein catheterization. One gram of tolbutamide (20 ml. 5 per cent Rastinon, Hoechst) was injected into the antecubital vein over a period of two minutes. The midpoint of the injection was taken as zero time. Blood samples from the portal and the antecubital veins were drawn simultaneously over a period of one minute for determinations of IRI and glucose concentrations (the beginning of each sampling period has been indicated in table 2 and figures 1, 2). IRI was assayed in triplicate and glucose in duplicate, and mean values were calculated. A few milliliters of blood were drawn and discarded before each blood sample was collected.

RESULTS

Fasting IRI varied in the fourteen cases between 6 and 58 μU./ml. (mean $\pm$ S.E.M. = 23.8 $\pm$ 4.3 μU./ml.) in the portal blood, and between 1 and 32 μU./ml. (mean $\pm$ S.E.M. = 10.1 $\pm$ 2.3 μU./ml.) in the peripheral venous blood.

After the tolbutamide injection IRI increased both in portal and in peripheral venous blood in all patients (table 2). There was, however, a great variation in the relationship between portal and peripheral IRI of different patients.

Case 14, with pancreatitis, displayed a distinct IRI peak in the portal blood in response to tolbutamide. The corresponding insulin curve in the periphery was, however, low (table 2). Case 5, also with pancreatitis,

TABLE 2

IRI and glucose in portal and peripheral venous blood after intravenous tolbutamide administration

Case		Insulin (μU./ml.)					Glucose (mg./100 ml.)				
		—2′	2′	5′	10′	30′	—2′	2′	5′	10′	30′
1	Po.v:	48	240	262	186	112	99	92	88	78	73
	Pf.v:	4	112	100	74	32	95	92	87	82	73
2	Po.v:	8	215	235	140	32	50	48	44	40	31
	Pf.v:	4	96	90	70	22	63	51	48	35	20
3	Po.v:	38	141	160	146	83	96	95	92	88	69
	Pf.v:	17	111	75	79	37	97	93	88	79	70
4	Po.v:	58	180	216	244	78	122	117	115	100	72
	Pf.v:	32	80	76	78	60	93	92	74	69	45
5	Po.v:	10	26	44	52	20	82	79	75	73	60
	Pf.v:	4	32	26	30	16	84	79	75	68	57
6	Po.v:	20	104	120	114	—	75	71	67	55	—
	Pf.v:	1	76	60	52	—	75	71	67	61	—
7	Po.v:	18	136	140	144	60	87	84	83	78	61
	Pf.v:	20	50	68	52	24	78	78	78	65	52
8	Po.v:	10	48	50	40	—	79	72	68	68	—
	Pf.v:	4	14	8	12	—	89	81	68	68	—
9	Po.v:	46	230	290	302	274	95	95	90	88	78
	Pf.v:	12	144	240	122	78	100	90	90	86	67
10	Po.v:	20	142	136	108	32	81	79	75	66	55
	Pf.v:	22	76	46	32	4	79	92	77	73	59
11	Po.v:	10	148	180	172	—	67	64	79	60	—
	Pf.v:	6	38	42	40	—	65	64	79	70	—
12	Po.v:	6	94	84	90	40	112	102	97	94	94
	Pf.v:	10	42	42	32	40	95	95	95	81	74
13	Po.v:	32	124	118	92	48	85	80	76	76	68
	Pf.v:	4	58	50	48	28	89	89	89	80	70
14	Po.v:	19	129	75	45	48	87	84	84	82	68
	Pf.v:	8	28	26	26	9	89	89	83	78	68

TOLBUTAMIDE

showed a low insulin response to tolbutamide in portal as well as in peripheral venous blood (table 2).

Blood glucose concentrations in portal and peripheral venous blood in response to tolbutamide are given in table 2.

DISCUSSION

In the present series the fasting insulin content of peripheral venous blood was found to vary between 1 and 32 μU./ml., i.e. close to the range found for normal persons in this laboratory.[7] The insulin level in portal blood showed a variation of 6 to 58 μU./ml., similar to the range of values reported by White and Dupré.[2] The difference between fasting IRI in portal and peripheral blood (mean values 23.8 ± 4.3 and 10.1 ± 2.3 μU./ml. respectively) indicates that an appreciable amount of insulin is removed from the blood during its passage from the portal vein, through the liver, to the antecubital vein. In a study designed to determine whether or not a significant amount of insulin is retained by the liver during the initial transhepatic passage, it was found that about 50 per cent of the total insulin was removed by the human liver in a single transhepatic circulation.[8] Probably this capacity of the liver varies in the presence of liver disease.[9] In the present series a great variation in the relationship between portal and peripheral IRI was observed in the different patients under fasting conditions, as well as in response to tolbutamide. This might well be due to the fact that some of the patients had significant liver impairment. The values in seven cases (cases 1-4, 6, 7 and 9) suggest, however, that the portal vein IRI continued to increase at a time when peripheral vein IRI had started to decrease (a representative study is shown in figure 1 or 2). This could be due to an altered dilution of IRI in the portal vein as a result of sudden changes in splanchnic blood flow. A sudden increase in peripheral insulin utilization under the influence of tolbutamide might also explain results as shown in figures 1 and 2. There are evidences indicating that

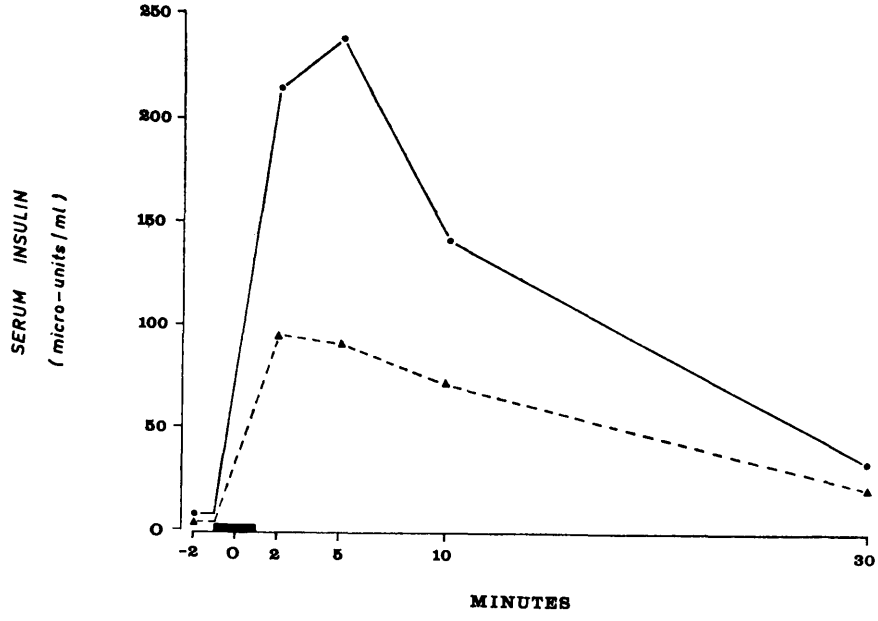

FIG. 1.

IRI in the peripheral venous blood (▲ - - - - - - ▲) and in the portal blood (●————●) in case 2 after intravenous tolbutamide administration (■).

tolbutamide may have an extrapancreatic effect upon skeletal muscle tissue. However, tolbutamide had to be administered for at least twenty-four hours before this effect could be demonstrated.[10] Therefore it is unlikely that results as shown in figures 1 and 2 reflect increased peripheral insulin utilization due to an extrapancreatic

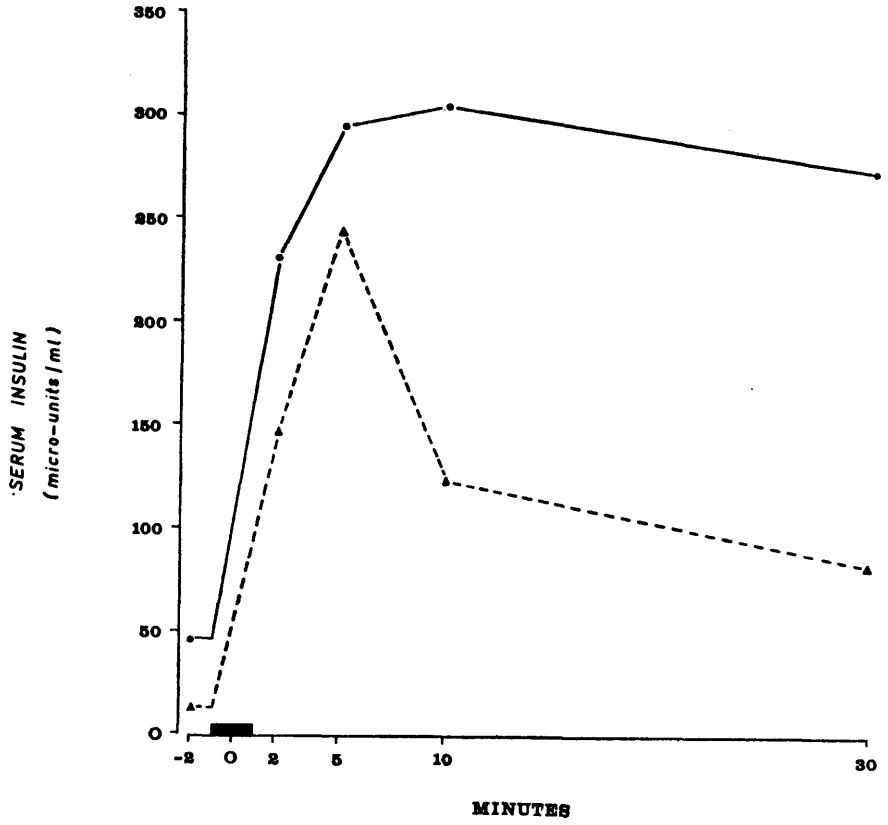

FIG. 2.

IRI in the peripheral venous blood (▲ - - - - - - ▲) and in the portal blood (●————●) in case 9 after intravenous tolbutamide administration (■).

action of tolbutamide. Finally a change in the hepatic uptake of insulin might have occurred, but to solve this question measurements of portal IRI, hepatic venous IRI and blood flow across the liver must be performed.

In an investigation of patients with pancreatic diabetes, Bank et al. were impressed by the great variation in the peripheral insulin response to glucose stimulation.[11] McKiddie et al. found that some patients with pancreatic diabetes had low insulin levels while others were capable of producing normal or greater-than-normal insulin levels in response to glucose.[12] The patients with pancreatic diseases in the present series showed a wide variation with regard to peripheral IRI concentrations after tolbutamide administration (table 2). Case 14 is of special interest displaying a low peripheral insulin level although the pancreatic release of insulin was fairly good judging from the peak IRI value in the portal blood. These findings may well imply that a high degree of removal of insulin between portal and peripheral venous blood sometimes contributes to a poor peripheral insulin response to tolbutamide in pancreatic disease.

ACKNOWLEDGMENT

This investigation was supported by research grants from Nordisk Insulinfond and the Swedish Medical Research Council. The authors are grateful to Mrs. Birgit Grunditz for technical assistance.

REFERENCES

[1] Dupré, J., Rojas, L., White, J. J., Unger, R. H., and Beck, J. C.: Lancet *2*:26, 1966.

[2] White, J. J., and Dupré, J:: Surgery *64*:204, 1968.

[3] Blackard, W. G., and Nelson, N. C.: Portal and peripheral vein immunoreactive insulin concentrations before and after glucose infusion. Diabetes *19*:302, 1970.

[4] Documenta Geigy, Wissenschaftliche Tabellen, p. 588, 1960.

[5] Soeldner, J. S., and Slone, D.: Critical variables in the radioimmunoassay of serum insulin using the double antibody technic. Diabetes *14*:771, 1965.

[6] Wiechel, K-L., Blanck, C., Erwald, R., Lindberg, K., and Marions, O. Nord. Med. *84*:956, 1970.

[7] Hed, R., Kallner, G., Nygren, A., and Sundblad, L.: To be published.

[8] Madison, L. L., Combes, B., Unger, R. H., and Kaplan, N.: Ann. N.Y. Acad. Sci. *74*:548, 1959.

[9] Samols, E., and Ryder, J. A.: J. Clin. Invest. *40*:2092, 1961.

[10] Feldman, J. M., and Lebovitz, H. E.: An insulin dependent effect of chronic tolbutamide administration on the skeletal muscle carbohydrate transport system. Diabetes *18*: 84, 1969.

[11] Bank, S., Jackson, W. P., Keller, P., and Marks, I. N.: Postgrad. Med. J. *44*:214, 1968.

[12] McKiddie, M. T., Buchanan, K. D., McBain, G. C., and Bell, G.: Postgrad. Med. J. *45*:726, 1969.

[13] Porte, D., and Bagdade, J. D.: Ann. Rev. Med. *21*:219, 1970.