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SHORT COMMUNICATIONS

Determination of Pancreatic and Gut Glucagon-Like Immunoreactivity (GLI) in Normal and Diabetic Subjects

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Summary. Oral glucose tolerance tests (1.75 g glucose/kg ideal body weight) were performed in 29 normal subjects, 17 maturity-onset diabetics (D I) and 8 juvenile type diabetics (D II). Glucose, IRI, pancreatic and gut glucagon-like immunoreactivity (GLI) were determined at various intervals. The basal levels of pancreatic GLI in the three groups were as follows: normals: 0.33 ± 0.03; D I: 0.43 ± 0.06; and D II: 0.50 ± 0.05 ng equiv./ml (mean ± s.e.m.). After glucose, a significant decrease in pancreatic GLI was observed in the normals, whereas the diabetics showed no significant change. An increase in gut GLI was observed in all persons. The importance of the GLI levels and variations is discussed.

Key words: glucagon, insulin, glucose, pancreatic GLI, gut GLI, oral glucose tolerance test, normals, diabetics.

Introduction

The interest in determining glucagon-like immunoreactivity (GLI) in animal and human plasma has been greatly stimulated since the possibility of determining specifically pancreatic GLI was described (Heding, 1968; Aguilar-Parada *et al.*, 1969a). Several papers have been published during the past few years, chiefly by Unger's group in Dallas, Texas, describing the variations in pancreatic GLI during a series of tests. It has been shown that infusions of amino acids and pancreozymin increased the level of pancreatic GLI in normal subjects (Unger and Eisentraut, 1969), that glucose decreased the level of pancreatic GLI in normals (Heding, 1971) but not in diabetics (Müller *et al.*, 1970), that 3 days' starvation increased the level of pancreatic GLI (Aguilar-Parada *et al.*, 1969b) and so did insulin-induced hypoglycaemia (Persson *et al.*, 1971). In diabetic subjects, the same level of pancreatic glucagon was found as in normals despite the fact that the diabetics had hyperglycaemia (Unger *et al.*, 1970). A possible explanation for the lack of suppression of the diabetic α-cell was given by Müller *et al.* (1971), who showed that the normal suppression of glucagon release did not occur when severe insulin deficiency was produced.

This paper is a follow-up of the previous publication on glucose tolerance tests performed in 7 normal subjects (Heding, 1971). The IRI (immunoreactive insulin), glucose, pancreatic and gut GLI were determined in 29 normal subjects and 25 diabetics during an oral glucose load.

Table 1. *Age and sex of normals and diabetics*

Group	Sex	Number	Age		Excretion of glucose in urine (during OGTT, g)	
			range	mean	range	mean
Normals	f	17	22–69	46	0	
	m	12	15–67	17	0	
Diabetics I	f	8	22–75	45	0–14	5.5
	m	9	22–72	50	0–19	4.1
Diabetics II	f	1	51		13	
	m	7	19–70	45	12–44	27.9

Materials and Methods

The GLI estimates were done by radioimmunoassay according to Heding (1971). The two anti-glucagon sera were raised in rabbits (Heding, 1969). One of these anti-

sera (K 47) showed hardly any reaction with a crude gut GLI extract whereas the other antiserum (K 36) reacted with gut GLI giving parallel dilution curves for gut and pancreatic GLI. Twice crystallized pork glucagon (NOVO) was used as a standard. Pork glucagon was iodinated using a modification of the Hunter and Greenwood procedure (1962). The modification and purification was developed by Jørgensen and Larsen (1972). IRI was determined according to Heding (1972) and glucose was determined using a glucose oxidase method. The oral glucose loads were performed at the Hvidøre Diabetes Hospital in the following manner: after an overnight fast, a catheter was placed in the antecubital veins of the trial subjects and they ingested a cold solution of glucose containing 1.75 g of glucose per kg of ideal body weight. The

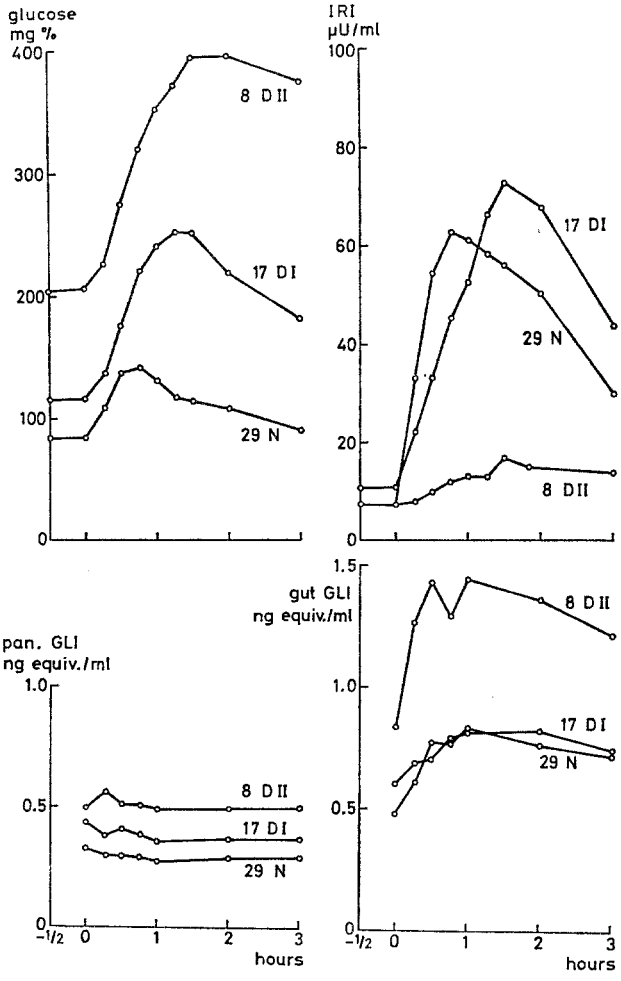

Fig. 1. Oral glucose tolerance test (1.75 g glucose per kg of ideal body weight) in 29 normal subjects, 8 juvenile-type diabetics (D II) and 17 maturity-onset-type diabetics (D I)

blood was collected into heparinized glass tubes containing 1 ml of trasylol (5000 KIE/ml, Bayer) per 10 ml of blood. The plasma was pipetted into plastic tubes and stored at −18°C until used. The diabetic patients were all newly diagnosed and untreated. The 25 diabetics were divided into two groups as described by Rasmussen *et al.* (1969): group II, juvenile-type diabetics, had IRI values below 30 μU/ml before and during the test, and group I, maturity-onset-type diabetics, comprised all the remaining diabetic subjects. Four of the eight juvenile-type diabetics had ketosis while none of the 17 maturity-onset diabetics showed ketosis during the two weeks of observation. Table 1 shows the age and sex of normals and diabetics as well as the excretion of urine glucose during the OGTT.

Results

Fig. 1 and Table 2 show the results. The increase in IRI was of the same order in the normals and in Diabetics I, whereas Diabetics II showed a small increase. There was a significant drop in the pancreatic GLI in the normal subjects. There was also a decrease in pancreatic GLI in Diabetics I, but this decrease was not significant. Five of the eight Diabetics II showed an increase in pancreatic GLI. The gut GLI increased both in normals and diabetics.

The basal levels of pancreatic GLI were 0.33 ± 0.03 ng equiv./ml in normal subjects, 0.43 ± 0.06 ng equiv./ml in Diabetics I, and 0.50 ± 0.05 ng equiv./ml in Diabetics II. The difference in the three groups is hardly significant but it should be noted that the highest basal pancreatic GLI values were found in the group with the highest glucose levels, and vice versa.

Discussion

The levels of pancreatic GLI reported here are higher than those obtained by Unger's group. The reason for this difference is unknown, but it might be methodological, or due to the differences in the antibodies used. For instance, the antibody K 47 used in this study does react with fragments of pancreatic glucagon. On the other hand, the results agree reasonably well with the findings of Müller *et al.* (1970), who showed that a dietary carbohydrate load of approximately 200 g resulted in an approximately 0.04 ng/ml decrease in the pancreatic GLI level in the normals whereas no decrease was observed in the juvenile and maturity-onset diabetics.

In the diabetic group — all newly diagnosed, yet untreated cases — there was a clear tendency to higher plasma pancreatic GLI levels than in the normal subjects, despite their hyperglycaemia. This lends further support to the theory put forward by Unger to the effect that the function of the α-cell is augmented in Diabetes mellitus. But one should bear in mind that the specificity as claimed for the various anti-glucagon sera stands only for their not reacting with a crude gut extract. The term specificity may lead to the false assumption that the antibodies react only with the intact glucagon molecule. This is very unlikely considering the fact that different antibodies react in a practically identical manner both with glucagon and with deshistidine glucagon (no biological activity) (Sundby, 1970) and mono-desamidoglucagon (about 60% biological activity) (Heding, 1972).

Elevated gut GLI levels were found after an oral glucose load in all the trial subjects, both diabetic and normal. Three juvenile-type diabetics showed an exceptionally marked increase.

The significance of gut GLI in blood remains obscure. Crude gut extracts containing less than 1% of GLI have been shown to have insulin-releasing effect in the isolated rat islet system, but no correlation was found between the GLI and the insulin-releasing activity (Moody *et al.*, 1970). Marco *et al.* (1971) showed that an enhanced secretion of endogenous gut GLI caused by, e.g., galactose failed to induce a rise in insulin. Glycogenolytic activity was found in peak II of a crude canine jejunal extract containing gut GLI as well as many other unknown substances (Valverde *et al.*, 1970). The possible insulin-releasing or glycogenolytic activity of this substance cannot be studied properly until a pure gut GLI preparation has been made.

Table 2. *Variations in glucose, IRI, pancreatic and gut GLI in normals and diabetics during OGTT, 1.75 g glucose per kg ideal body weight*

Test group	glucose mg%		IRI μU/ml		pancreatic GLI ng equiv./ml		gut GLI ng equiv./ml	
	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.	mean	s.e.m.
29 normal subjects								
basal level (−5 min)	84.6	1.0	7.4	0.9	0.33	0.03	0.48	0.06
increase after glucose	63.3	4.9	69.4	6.4	−0.07	0.02	0.45	0.06
sign. of increase (pairwise t-test)	$p < 0.1\%$		$p < 0.1\%$		$p < 0.2\%$		$p \ll 0.1\%$	
17 Diabetics I (IRI values > 30 μU/ml)								
basal level (−5 min)	116	5.0	11.5	2.1	0.43	0.06	0.61	0.09
increase after glucose	150	9.6	69.5	18.2	−0.07	0.03	0.43	0.06
sign. of increase (pairwise t-test)	$p < 0.1\%$		$p < 0.2\%$		n.s.		$p \ll 0.1\%$	
8 Diabetics II (all IRI values < 30 μU/ml)								
basal level (−5 min)	205	25	6.8	1.6	0.50	0.05	0.84	0.13
increase after glucose	210	13	12.4	2.9	0.03	0.05	0.86	0.35
sign. of increase (pairwise t-test)	$p < 0.1\%$		$p < 0.2\%$		n.s.		$p < 5\%$	

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