Persistent Metabolic Abnormalities in Diabetes in the Absence of Glucagon

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Summary. In order to investigate the contribution of glucagon to the abnormalities of carbohydrate and lipid metabolism in diabetes, hormones and metabolites were measured in response to IV arginine in 5 juvenile onset (control) diabetics and 5 totally pancreatectomised subjects. In the basal state, both control diabetics and pancreatectomised patients showed abnormally elevated levels of plasma glucose, blood 3-hydroxybutyrate, glycerol and plasma free fatty acids (NEFA), although no glucagon was detectable in the plasma of the pancreatectomised subjects. Blood concentrations of the gluconeogenic precursors alanine and glycerol were higher in the pancreatectomised patients than in the diabetics. Following infusion of arginine, the rise in glucagon observed in the diabetics was accompanied by a significant increase in plasma glucose and a fall in blood lactate when compared to the pancreatectomised subjects. In spite of the rise in glucagon in the control diabetics, no significant change was found in the concentrations of ketone bodies, glycerol or NEFA. Thus glucagon does not seem to have a primary role in producing the metabolic abnormalities of diabetes.

Key words: Hyperglucagonaemia, suppression of plasma glucagon, arginine infusion, pancreatectomised subjects, diabetic controls, glucose, 3-hydroxybutyrate, glycerol, alanine, lactate, pyruvate.

suppression of glucagon release in diabetics by somatostatin was observed to retard the development of ketoacidosis following deprivation of insulin [12]. These and other investigations suggested that improvement in diabetic control might be achieved by therapeutic reduction of glucagon secretion. In order to examine further this possibility, we have investigated carbohydrate and lipid metabolism in 5 insulin dependant diabetic patients and 5 totally pancreatectomised subjects who lacked measurable quantities of circulating plasma glucagon.

Materials and Methods

Patients

5 subjects who had undergone total surgical pancreatectomy and 5 age and sex matched insulin-dependent diabetic patients were investigated (Table 1). The 5 pancreatectomised patients were receiving significantly less insulin ($P < 0.05$) than the insulin-dependant diabetics (0.44 and 0.67 units/kg body weight/24 h). Plasma 'C' peptide levels were undetectable in all individuals either in the fasting state or after IV arginine. No patient had detectable levels of glucagon antibodies.

Test Procedures

Patients were investigated after a 14 h fast and before their normal morning dose of insulin. An indwelling cannula was inserted into a vein in the antecubital fossa of each arm and each patient was rested in the sitting position for a minimum of 45 min before baseline blood samples were taken at -15 , -5 min and zero time. Arginine hydrochloride (0.5 g/kg body weight) was then infused into the opposite arm at a

Hyperglucagonaemia has recently been suggested as an essential factor in the pathogenesis of diabetes mellitus [20, 23]. This hypothesis is supported by the fact that administration of pharmacological doses of glucagon to patients with juvenile onset diabetes has been shown to exaggerate many of the biochemical abnormalities of this disorder [17, 21]. In addition,

Table 1. Clinical details of 5 pancreatectomised patients and 5 insulin-dependent diabetic controls

Patients	Sex	Age (yr)	% of ideal weight	Insulin treatment (units)	Total pancreatectomy reason and date
Pancreatectomised					
J D	M	34	63	Sol 12 a m Sol 8 p m	Chronic pancreatitis 1974
M H	F	40	73	Sol 6 a m Sol 4 p m	Chronic pancreatitis 1973
R B	F	49	92	Sol 16 a m Sol 12 p m	Well-differentiated adenocarcinoma of pancreas; May 1975
F W	F	60	99	Sol 10 a m NPH 10 a m	Benign pancreatic cyst; 1969
G B	M	67	54	Sol 10 a m Sol 8 p m	Well-differentiated adenocarcinoma of pancreas; 1974
Diabetic controls					
J M	M	29	99	Sol 24 a m NPH 8 a m Sol 24 p m	
M W	F	37	106	Sol 24 a m PZI 12 a m	
V B	F	48	96	Sol 36 a m PZI 8 a m	
K P	F	56	120	Sol 16 a m NPH 16 a m	
J M	M	60	97	Sol 24 a m PZI 16 a m	

constant rate for 30 min and further blood samples were taken at 5, 10, 20, 25, 30, 40 and 60 min. Blood samples were assayed for glucagon, growth hormone, cortisol, glucose, glycerol, free fatty acids, acetate, 3-hydroxybutyrate, alanine, pyruvate and lactate. Informed consent was obtained from all patients for the procedures carried out in the study.

Assays

Plasma pancreatic glucagon was measured by radioimmunoassay with a pancreatic glucagon-specific (C terminal reacting) antiserum [1, 4]. Plasma growth hormone was measured by radioimmunoassay [15] using WHO 1st IRP (66/217) as standard. Plasma cortisol was measured by competitive protein binding [3]. Plasma glucose was assayed by an automated glucose oxidase method [13]. Blood lactate [16], pyruvate [5], 3-hydroxybutyrate [24], glycerol [10] and alanine [25] were measured by enzymatic methods and plasma non-esterified fatty acids (NEFA) were measured by a radioactive cobalt method [14]. Plasma for glucagon, growth hormone, cortisol and NEFA assays was separated immediately after blood sampling and stored at -20°C so that all samples could be assayed in the same batch.

Statistics

Student's paired and non-paired *t* tests were used to assess the significance of changes in hormones and metabolites.

Results

Plasma and blood concentrations of hormones and metabolites in the fasting state, at the end of a 30 min infusion of arginine and at 60 min are shown in Table 2.

Initial Fasting Values

Hormones. Fasting levels of plasma glucagon were elevated in the diabetic controls but not significantly different from zero in the pancreatectomised subjects. The increase in plasma growth hormone in the diabetics just failed to reach statistical significance when compared with the pancreatectomised subjects. Levels of plasma cortisol were within the normal laboratory range in all subjects.

Metabolites. Plasma glucose, NEFA and blood glycerol and 3-hydroxybutyrate were increased in both

Table 2. Plasma and blood concentrations of hormones and metabolites ($\pm$ SEM) in 5 pancreatectomised subjects and 5 insulin-dependent diabetic controls, in the fasting state and after intravenous infusion of arginine

Hormones and metabolites with Mean $\pm$ SEM for healthy adults	Time mins	Pancreatectomised subjects	Diabetic controls	Significance of difference
Glucagon	0	1.3 $\pm$ 0.6	17.2 $\pm$ 5.3	P < 0.02
pmol/l	30	0.3 $\pm$ 0.3	93.0 $\pm$ 4.5 ^b	P < 0.001
(7.0 $\pm$ 0.6)	60	1.2 $\pm$ 0.6	25.2 $\pm$ 2.6	P < 0.001
Growth hormone	0	3.8 $\pm$ 1.5	8.4 $\pm$ 1.5	NS
mu/l	30	13.6 $\pm$ 5.8	15.9 $\pm$ 5.4	NS
(< 5)	60	12.5 $\pm$ 3.4	17.0 $\pm$ 3.3	NS
Cortisol	0	228 $\pm$ 60	188 $\pm$ 44	NS
nmol/l	30	185 $\pm$ 57	163 $\pm$ 46	NS
(100–500)	60	176 $\pm$ 52	164 $\pm$ 45	NS
Glucose	0	13.9 $\pm$ 3.0	21.6 $\pm$ 1.2	P < 0.05
mmol/l	30	13.1 $\pm$ 3.1	23.3 $\pm$ 1.3	P < 0.02
(4.3 $\pm$ 0.2)	60	13.1 $\pm$ 3.0	24.4 $\pm$ 1.6 ^a	P < 0.02
3 hydroxy butyrate	0	0.56 $\pm$ 0.18	0.84 $\pm$ 0.22	NS
mmol/l	30	0.46 $\pm$ 0.14	0.88 $\pm$ 0.20	NS
(0.03 $\pm$ 0.01)	60	0.45 $\pm$ 0.15	1.02 $\pm$ 0.23	NS
NEFA	0	1.90 $\pm$ 0.28	1.71 $\pm$ 0.51	NS
mmol/l	30	1.32 $\pm$ 0.15	1.58 $\pm$ 0.30	NS
(0.76 $\pm$ 0.08)	60	1.48 $\pm$ 0.18	1.50 $\pm$ 0.16	NS
Glycerol	0	0.25 $\pm$ 0.01	0.15 $\pm$ 0.03	P < 0.02
mmol/l	30	0.22 $\pm$ 0.01	0.15 $\pm$ 0.02	P < 0.02
(0.07 $\pm$ 0.01)	60	0.23 $\pm$ 0.03	0.15 $\pm$ 0.03	NS
Alanine	0	0.56 $\pm$ 0.11	0.24 $\pm$ 0.03	P < 0.05
mmol/l	30	0.56 $\pm$ 0.11	0.23 $\pm$ 0.03	P < 0.05
(0.29 $\pm$ 0.02)	60	0.59 $\pm$ 0.11	0.25 $\pm$ 0.03	P < 0.02
Lactate	0	1.06 $\pm$ 0.35	0.80 $\pm$ 0.07	NS
mmol/l	30	1.06 $\pm$ 0.36	0.71 $\pm$ 0.04	NS
(0.72 $\pm$ 0.04)	60	1.19 $\pm$ 0.52	0.69 $\pm$ 0.04 ^b	NS
Pyruvate	0	0.064 $\pm$ 0.003	0.078 $\pm$ 0.007	NS
mmol/l	30	0.063 $\pm$ 0.004	0.066 $\pm$ 0.004	NS
(0.072 $\pm$ 0.005)	60	0.068 $\pm$ 0.005	0.059 $\pm$ 0.005	NS

Values with asterisks indicate a significant change in comparison with basal values

^a P < 0.05

^b P < 0.02

diabetics and pancreatectomised subjects in comparison with values for healthy adults (Table 2). Fasting blood levels of the gluconeogenic precursors alanine and glycerol were significantly higher in the pancreatectomised subjects than in the diabetics ($p < 0.05$ and $p < 0.02$). Blood levels of lactate and pyruvate were normal in both groups.

There was no indication in either the diabetics or the pancreatectomised subjects that basal levels or changes in metabolites were related to body weight or to differing degrees of hyperglycaemia.

Changes Following Arginine

Hormones. Plasma glucagon rose to maximum at 25 min ($p < 0.02$) during infusion of arginine in the diabetic controls but remained undetectable in the pancreatectomised subjects. Neither group showed a significant change in growth hormone or cortisol following arginine.

Metabolites. In the diabetics, plasma glucose rose significantly after arginine ($p < 0.05$ at 60 min) but did not change in the pancreatectomised subjects. Of the other metabolites, only lactate showed a significant change, falling in the diabetics ($p < 0.02$ at 60 min) but remaining unchanged in the pancreatectomised subjects.

Discussion

The finding of elevated fasting levels of glucose, 3-hydroxybutyrate, NEFA and glycerol in pancreatectomised subjects despite significantly undetectable circulating glucagon indicates that glucagon is not an essential factor in the aetiology of diabetes. In addition, elevation of glucagon by arginine infusion in the diabetics to plasma levels similar in magnitude to those occurring after a protein-rich meal resulted only in a significant rise in plasma glucose and a fall in

blood lactate. Thus fluctuations in plasma glucagon within the physiological range would be unlikely to result in the changes in levels of metabolites that have been observed following administration of large pharmacological doses of this hormone [17, 21]. The failure of physiological changes in glucagon to influence circulating levels of metabolites has previously been described [2, 22].

Fasting blood levels of the gluconeogenic precursors alanine and glycerol were significantly higher in the pancreatectomised patients than in the diabetics. Blood levels of these precursors reflect a balance between muscle release which is inhibited by insulin and hepatic uptake which is enhanced by glucagon [11]. Their increase in the pancreatectomised patients could, therefore, have resulted from a decreased clearance from the circulation. The rise of glucagon in the diabetics after arginine was, however, not followed by a measurable reduction in either alanine or glycerol.

The main objective in improving blood glucose concentrations in diabetic patients by suppressing glucagon would be to prevent the onset of diabetic complications and to improve the clinical manifestations of poor diabetic control. Although reports of total pancreatectomy (usually for malignancy) have appeared in the literature since 1944 [19], few recipients of this operation have survived long enough to evaluate the effect of lack of glucagon on the incidence of diabetic complications. However, diabetic retinopathy was noted in one patient after 3 years [6] and diabetic glomerulosclerosis in another after 8 years [9]. Pancreatectomised subjects continue to suffer from the symptoms of poor diabetic control since they are particularly at risk from hypoglycaemia [18]. In addition, diabetic ketoacidosis has been reported in these patients [7, 8] demonstrating that hyperglycaemia and hyperketonaemia can still occur in situations of inadequate insulin replacement.

The persistence of diabetes in the absence of glucagon in pancreatectomised subjects and indeed the relatively mild diabetes seen in the glucagonoma syndrome where enormously elevated plasma levels of this hormone have been observed, suggest that insulin lack rather than glucagon excess is the primary factor in the aetiology of diabetes. Suppression of glucagon does little to correct many of the metabolic abnormalities seen in this condition and may indeed result in new features such as accumulation of gluconeogenic precursors in the circulation.

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