Pancreatic A and B Cell Hyperfunction in the Mendenhall Syndrome

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Summary. A 16-year-old boy with persistent hyperglycaemia (approximately 16 mmol/l in the fasting state) and acanthosis nigricans had insulin resistance and received daily up to 2800 U of short-acting, soluble, highly purified porcine insulin. The number and affinity of insulin receptors were markedly decreased. No significant insulin binding to IgG could be detected. Immunoreactive insulin varied between 1344 and 2400 mU/l. Endogenous insulin secretion and proinsulin levels were grossly elevated in the fasting state (C-peptide 2.2–3.5 pmol/ml; proinsulin approximately 1 pmol/ml). After an oral glucose tolerance test and intravenous arginine infusion, B cell hypersecretion was confirmed. The molar ratio of C-peptide to immunoreactive insulin, normally approximately 7, was about 0.3, clearly indicating that most of the immunoreactive insulin was exogenous. The molar ratio of proinsulin to C-peptide, which is about 0.05 in fasting control subjects, was 0.23–0.45, clearly showing that too high a proportion of proinsulin was being secreted. This may indicate that the constant hyperstimulation of the B cell leads to reduced conversion of proinsulin to insulin. Immunoreactive glucagon levels were within normal limits fasting but were above normal after intravenous arginine infusion. Thus, in this case of diabetes with acanthosis nigricans, the severe insulin resistance, probably caused by a receptor defect, was associated with markedly increased B cell function.

Key words: Acanthosis nigricans, insulin resistance, C-peptide, proinsulin, diabetes mellitus, glucagon.

Recent research using receptor assays has shown that insulin-resistant states associated with the so-called acanthosis nigricans syndromes are mostly due to defects at the receptor level or are post-receptor binding defects [14, 15]. In a patient suffering from a peculiar variant of acanthosis nigricans syndrome and severe insulin-resistant diabetes (Mendenhall syndrome) [4, 6, 16, 24, 25], we described the virtual disappearance of insulin receptor in erythrocytes [18]. The reaction of the endocrine pancreas to prolonged and extreme endogenous insulin resistance is unknown and therefore the present study was undertaken to investigate A and B cell responses to oral glucose and intravenous arginine infusion in this patient.

Material and Methods

Patient

The patient was a 16-year-old Caucasoid boy, born to a non-consanguineous marriage after a normal pregnancy. Delivery was complicated by respiratory distress requiring prompt resuscitation. Examination at birth showed striking phenotypic features: abdominal disten-

sion, acromegaloid facial features, macroglossia, acanthosis nigricans around the neck and in flexure areas, as well as anal and lingual papillomata. Psychomotor development from the perinatal period up to the age of 16 years was estimated to be only moderately retarded. At the age of 3 years, peptic ulcer-like symptoms lead to a radiological and endoscopic diagnosis of diffuse gastric papillomata; the symptoms subsided with antacid therapy. At the age of 5 years macrogenitosomy was prominent and a testicular biopsy revealed an advanced degree of maturity for his age, but no hormonal studies at that time are available. Growth was estimated to be in the 25th percentile (Stuart chart) at the age of 7 years, reaching the third percentile when the patient was aged 16 years. Occasional bouts of sudden unconsciousness without seizures were recorded at the age of 3 years, and an increased focal electric activity of the right hemisphere was established in the electroencephalogram. However, an air-encephalogram performed when the patient was 4 years old, and computerized axial tomography of the skull carried out at age 16 years, failed to detect any significant intracranial abnormality other than pineal calcification. Retarded dentition with tooth dysplasia was a prominent additional feature observed at the age of 5 years.

Marked polydipsia, polyphagia and polyuria lead to the diagnosis of diabetes mellitus at the age of 11 years. Insulin therapy was started using four to five injections a day of short-acting monocomponent porcine preparations, but within a few months increased doses of up to 2500–2800 U daily were required. Despite these high doses, hyperglycaemia (> 11 mmol/l) without ketonuria persists up to the present. Careful assessment of endocrine function was carried out when the patient was 12 years old. FSH, LH, testosterone, TSH and prolactin
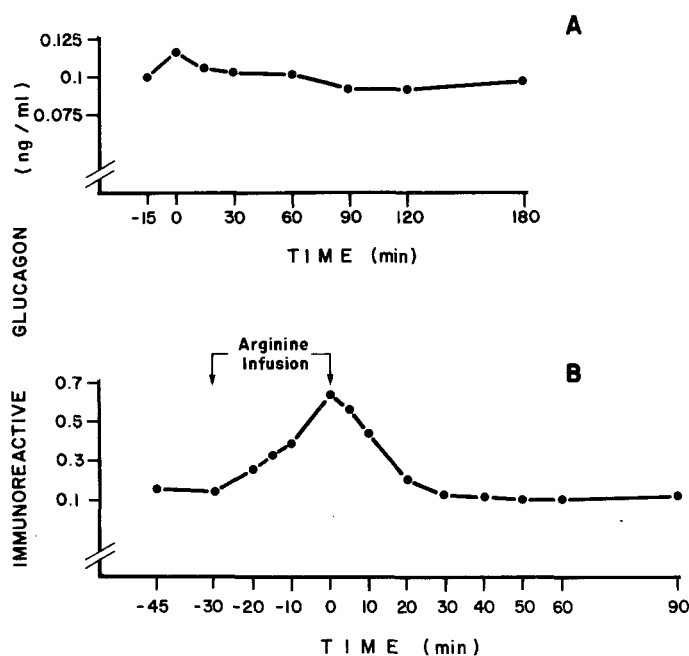
Fig. 1. Immunoreactive glucagon during (A) an oral glucose tolerance test (1.75 g/kg body weight), and (B) arginine infusion (500 mg/kg body weight) in the patient

Table 1. Serum glucose, immunoreactive insulin, C-peptide and proinsulin levels in the patient

Time (min)	Glucose (mmol/l)	IRI (mU/l)	C-peptide (pmol/ml)	Proinsulin (pmol/ml)
After oral glucose tolerance test (1.75 g/kg body weight)				
− 15	16.6	1344	2.25	1.00
0	16.1	1344	1.75	0.80
15	24.0	2240	5.00	1.20
30	28.2	2304	4.50	1.30
60	34.4	2272	5.00	1.50
90	29.8	2176	3.75	1.30
120	25.6	2016	3.25	0.90
180	19.2	1728	1.75	0.90
After arginine infusion (500 mg/kg body weight, −30–0 min)				
− 45	17.6	1696	3.50	0.80
− 30	17.8	1600	3.00	0.60
− 20	17.8	2112	5.25	1.00
− 15	18.0	2112	4.50	1.30
− 10	17.9	1856	3.75	1.00
0	19.3	2400	5.00	1.10
5	19.6	2272	4.25	1.00
10	19.8	2048	3.50	1.50
20	19.5	1728	2.75	0.70
30	18.7	1632	2.75	0.70
40	17.9	1536	2.50	0.80
50	16.9	1444	2.25	0.70
60	16.0	1344	2.25	0.40
90	15.6	1408	2.50	0.60

responses to TRH and LHRH were normal. Evaluation of thyroid and adrenal function was reported as normal, as well as basal and arginine-stimulated growth hormone release.

At the time of our study (September 1981), acanthosis nigricans, lingual papillomatosis and insulin-resistant diabetes mellitus were prominent features. There was no evidence of retinopathy by conventional funduscopy or fluorescein angiography, nor could peripheral

neuropathy be detected. Renal function was within normal limits. Conventional haematological and blood chemistry studies showed no significant abnormalities apart from hyperglycaemia and mild hypertriglyceridaemia. Family history revealed that the patient's parents and a younger brother were healthy and of normal appearance. However, an elder brother suffering from insulin-resistant diabetes and acanthosis nigricans had died aged 2 years. A first degree cousin has also been diagnosed as insulin-resistant with defective insulin receptors and acanthosis nigricans [17].

Special Studies

An oral glucose tolerance test (1.75 g glucose/kg body weight) and arginine hydrochloride infusion (500 mg/kg body weight) were carried out 7 days apart, after 12 h of fasting and withdrawal of insulin therapy for 24 h. For each test, blood samples were taken at predetermined times into precooled tubes (with/without heparin-aprotinin) for either serum or plasma as appropriate. Aliquots of serum were used for immediate glucose assay (in triplicate) by the glucose oxidase method using a calibrated Beckman autoanalyzer. The appropriate aliquots (serum or plasma) were immediately frozen at − 20 °C for radioimmunoassay of: (1) total insulin (antibody-bound immunoreactive insulin + 'free' immunoreactive insulin) (IRI) — interassay coefficient of variation 10.4%, lower detection limit < 1 mU/l [9]; (2) C-peptide, determined after removal of proinsulin by Sepharose-bound insulin antibodies (S-AIS) [10] – inter-assay coefficient of variation 9.2%, lower detection limit of 0.03 pmol/ml; (3) Proinsulin, bound to S-AIS [11], determined using antiserum K 1219 in an improved assay in which human proinsulin shows about 66% cross-reactivity with insulin in the range 0–0.50 pmol/ml — inter-assay coefficient of variation 10%, lower detection limit 0.002 pmol/l [12]; (4) glucagon (8) – inter-assay coefficient of variation 8.6%, lower detection limit < 0.026 ng/ml in plasma samples. Intra-assay coefficients of variation were approximately 2.5% in all assays. Assays were carried out within 3 months after sampling at Dr. L. Heding's laboratory, Novo Research Institute, Copenhagen. Insulin binding IgG was determined by radioimmunoelectrophoresis according to Christiansen, non-specific binding being 40 mU/l [3].

Insulin binding to erythrocytes was carried out according to Gambhir et al. [7]. Insulin binding to cultured fibroblasts obtained from skin biopsy was studied by the method of Rechler and Podskalny [21]. Mono-[125]I-(tyr A14)-insulin was the tracer in the binding studies [13], and monocomponent cristalline porcine insulin (26.9 U/mg) was used as standard (0–10^5 ng/ml) in the competition experiments. Both preparations were a gift from Novo Reserach Institute. Results of the competition studies were interpreted using the Scatchard plot method. Results are expressed as mean ± SD for erythrocyte assays and as mean ± SEM for fibroblast assays.

The precision of the insulin binding assays was determined on the basis of the ID_{50}, that is the amount of unlabelled insulin required to inhibit 50% of the ^{125}I-insulin binding to the receptors. For insulin binding to erythrocytes the ID_{50} was 4.33 ± 1.3 ng/ml (mean ± SD; n = 17); in the case of insulin binding to fibroblasts, the ID_{50} was 2 ± 1.37 ng/ml (mean ± SD; n = 3). The coefficients of variation (intra-assay) for our insulin binding studies were < 10%.

Control Subjects

For radioimmunological determinations of total insulin, C-peptide and proinsulin, control samples consisted of serum obtained from 13 fasting normal subjects. For immunoreactive glucagon assays control samples from 26 fasting healthy volunteers were tested; with regard to control samples for insulin binding to erythrocytes, 17 normal volunteers (age 24–43 years) were studied. The control fibroblast lines were derived from three normal subjects. In none was there a family or personal history of diabetes mellitus.
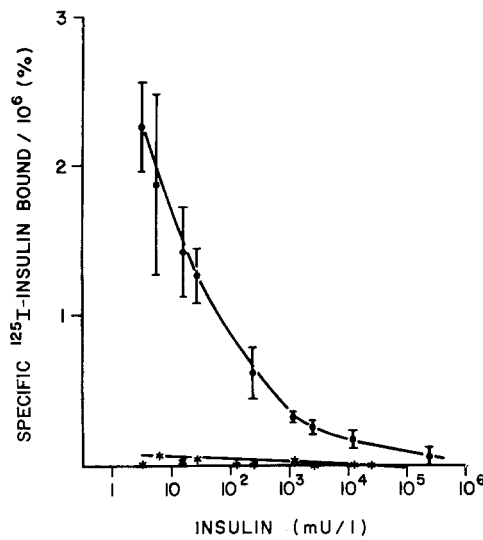
Fig. 2. ^{125}I-insulin binding to fibroblasts. Competition-inhibition curve. Binding was determined using $1.5-3.5 \times 10^6$ cells/ml, incubated during 3 h at 15 °C with 125–150 pg/ml of [125]I-(tyr A14)-insulin and the indicated concentration of porcine insulin, in 0.5 ml of buffer assay (pH 8) containing 1% of bovine serum albumin. Values expressed as mean ± SEM in the control group ●——●, ($n=3$); Mendenhall syndrome ✳——✳

Table 2. Molar ratios in response to an oral glucose tolerance test

Time (min)	C-peptide: IRI (pmol/pmol)	Proinsulin: C-Peptide (pmol/pmol)
− 15	0.27	0.44
0	0.21	0.45
15	0.45	0.24
30	0.31	0.29
60	0.36	0.30
90	0.27	0.35
120	0.26	0.27
180	0.16	0.51

Results

Insulin antibodies were detectable at low concentrations, actual values 1 year apart being 96 and 159 mU/l. The B cell response to oral glucose is shown in Table 1. Serum glucose rose from 16.6 to 34.4 mmol/l. IRI varied from 1344 to 2304 mU/l and C-peptide from approximately 2 to 5 pmol/ml. Fasting proinsulin was very high (approximately 1 pmol/ml) and increased slightly during the test. Before and during the intravenous arginine test (Table 1) glucose, IRI, C-peptide and proinsulin levels fluctuated, but all four parameters showed a decrease after the infusion was discontinued.

The molar ratios between C-peptide and IRI, and between C-peptide and proinsulin after the oral glucose tolerance test are shown in Table 2. The C-peptide: IRI ratio was far higher than that observed in the normal subjects. The same was the case for the proinsulin: C-peptide ratio. After the arginine infusion, similar ratios

were found for C-peptide:IRI (0.26–0.39) and for proinsulin: C-peptide (0.18–0.42).

Immunoreactive glucagon levels, fasting and after each test, are shown in Figure 1 (normal fasting values varied between 0.070 and 0.120 ng/ml; mean ± SD 0.091 ± 0.013 ng/ml, $n=26$). Basal levels of immunoreactive glucagon were normal, but after the oral glucose tolerance test no significant suppression of immunoreactive glucagon was observed (Fig. 1A). On the other hand, arginine infusion elicited a normal time-response pattern with markedly elevated absolute values at all times (Fig. 1B), specially when taking the glucose values into account.

Data concerning insulin binding to erythrocytes have been partially presented [22]. Insulin binding to erythrocytes and fibroblasts is shown in Table 3 and Figure 2 respectively. ^{125}I-insulin binding is dramatically diminished in erythrocytes (specific maximal bound = 0.99 ± 0.41%; $n=4$ different assays) when compared with the control group (specific maximal bound = 12.01 ± 1.51%; mean ± SD; $n=17$ males). In cultured fibroblasts the specific maximal bound/ 10^6 cells is 0.065% in comparison with 2.27 ± 0.21% (mean ± SEM; $n=3$) in the control subjects. The calculated receptor number in erythrocytes was markedly reduced (32 ± 7 sites/cell) when compared with controls (79 ± 11 sites/cell), whereas receptors where virtually absent in the cultured fibroblasts. In both systems, the extremely low maximal binding, particularly in fibroblasts, made the interpretation of results using the Scatchard plot method even more complicated, it being almost impossible to derive the average affinity profile according to De Meyts and Roth [5].

Discussion

Insulin-resistant diabetic patients with acanthosis nigricans have been classified as types A, B and C according to the level of the receptor defect [1, 2, 14]. Our patient, having an acanthosis nigricans insulin-resistant state with a decreased number and affinity of the insulin receptors in two systems (erythrocytes and cultured fibroblasts) and no anti-receptor factors [22], should be considered a type A genetic variant. Furthermore, the low levels of IgG insulin antibodies make it unlikely that the resistance was caused by insulin antibodies. Further studies recently carried out by Taylor et al. [23] in this patient have also established abnormally low levels of insulin binding to cultured lymphocytes and to cultured skin fibroblasts, with a concomitant decrease in the affinity of ^{125}I-insulin binding to the latter cell system. All these findings are in agreement with the observations of Podskalny and Kahn [19, 20] in cultured fibroblasts from patients suffering from other syndromes of extreme insulin resistance.

On the other hand, our findings established a striking over-activity of both A and B cells. We found very

Table 3. ^{125}I-insulin binding to erythrocytes in vitro

	Specific ^{125}I-insulin bound (%)	Receptor concentration			Receptor affinity	
		In 3.52×10^9 red blood cells (ng/ml)	Binding (sites/cell)	ID_{50} (ng/ml)	$(\overline{K}_e/10^8 mol^{-1} \cdot l)$	$(\overline{K}_f/10^8 mol^{-1} \cdot l)$
Mendenhall syndrome	0.99 ± 0.41	1.3 ± 0.25	32 ± 7	$40 - 100$	0.54 ± 0.29	0.19 ± 0.05
Control group ($n = 17$, males)	12.01 ± 1.51	2.71 ± 0.37	79 ± 11	4.33 ± 1.30	2.99 ± 0.47	0.47 ± 0.13

Results expressed as mean $\pm$ SD

high levels of circulating IRI activity, consisting of a mixture of exogenous insulin (2800 U of short action monocomponent porcine insulin/day), endogenous insulin (C-peptide in the fasting but hyperglycaemic condition being 1.75–3.50 pmol/ml) and endogenous proinsulin (0.60–1.00 pmol/ml). In fact, the molar ratio between C-peptide and IRI, which in normal fasting subjects is approximately 7:1, was 0.21–0.39, clearly showing that most of the IRI was exogenous. This indicated that despite the high dose of exogenous insulin and the resulting high plasma concentration of IRI, endogenous B cell secretion was excessive and not suppressed, as shown by a fasting C-peptide concentration of 1.75–3.50 pmol/ml when plasma glucose was approximately 17 mmol/l, which markedly increased after oral glucose. Fasting C-peptide levels in the 13 normal subjects were 0.49 ± 0.22 pmol/ml (mean $\pm$ SD). Basal proinsulin was increased (0.60–1.00 pmol/ml) compared with normal subjects (0.024 ± 0.020 pmol/ml) [12]. In addition, the ratio between proinsulin and C-peptide (normally 0.05 ± 0.03 [12]) was increased tenfold. This again clearly indicates that a much higher proportion of unconverted proinsulin is being secreted than in normal subjects. The molar plasma proinsulin concentration was at least fivefold higher than that of the endogenously secreted insulin. Such hyperproinsulinaemia has not been reported previously under these circumstances. In one study by Bar et al. [2] using a filtration method for the evaluation of the proinsulin, it was found that proinsulin was less than 10% of the IRI, and the authors concluded that the resistance could not be caused by an excessive concentration of proinsulin.

Interestingly enough, A cell hyperfunction was also present, as indicated by the arginine-induced hyperglucagonaemia although the normal glucose-induced fall in glucagon release was barely present in the oral glucose tolerance test. These findings lead us to conclude that the insulin resistant state of our Mendenhall syndrome patient is characterized by a certain A cell hyperfunction as well as by marked B cell responses occurring concomitantly with a lack of peripheral insulin receptors. Of paramount interest is the hyperproinsulinaemic state present, but whether this is a primary defect or a consequence of the long-standing, extremely hyperglycaemic, insulin resistant state cannot be established by the present study. Neither is it possible to draw any

conclusions about the apparently autonomous hyperfunction of A and B cells. Further studies on this patient and his family are currently being conducted to try to elucidate whether we are dealing with a hitherto undescribed combination of two genetic defects: abnormal proinsulin to insulin conversion, and a concomitant genetically determined defect in insulin receptors in several cell systems.

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