Hyperproinsulinaemia in patients with myotonic dystrophy

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Summary. Hyperinsulinaemia is a reported feature of the inherited multisystem disorder myotonic dystrophy. This phenomenon has been attributed to a compensatory beta cell response to tissue insulin resistance. In this study, circulating concentrations of insulin, proinsulin, and split proinsulin molecules were determined after an overnight fast in ten patients with myotonic dystrophy using two-site monoclonal antibody-based immunoradiometric assays. Results were compared with ten healthy control subjects matched for age, gender, and body mass index. Oral glucose tolerance (75 g), as defined by World Health Organization criteria, was normal in all subjects. Fasting plasma immunoreactive insulin concentration, as determined using a conventional radioimmunoassay, was almost three times higher ($p < 0.005$) in the myotonic dystrophy patients than the healthy control subjects. By contrast, fasting concentrations (mean $\pm$ SEM) of C-peptide (0.75 ± 0.09 vs 0.52 ± 0.03 nmol/l, $p = 0.07$) and immunoradiometrically-determined insulin (60 ± 12 vs

38 ± 4 pmol/l, $p = 0.09$) were not significantly different between the groups. Fasting concentrations of proinsulin (10.3 ± 2.9 vs 1.6 ± 0.3 pmol/l, $p < 0.01$), and 32–33 split proinsulin (7.8 ± 2.5 vs 2.9 ± 0.4 pmol/l, $p < 0.05$) were significantly elevated in the patients with myotonic dystrophy. Accordingly, the mean fasting proinsulin:insulin ratio, expressed as a percentage, was significantly increased in the myotonic patients (20 ± 5 vs $4 \pm 1\%$, $p < 0.01$). The overall C-peptide response to the oral glucose challenge was significantly greater in the myotonic patients compared with the healthy control subjects ($p < 0.001$). These results provide corroborative evidence of increased beta-cell secretion in myotonic dystrophy. In addition, myotonic dystrophy is characterised by elevated plasma concentrations of proinsulin-like molecules which may cross-react in insulin radioimmunoassays.

Key words: Myotonic dystrophy, proinsulin, insulin resistance.

Insulin resistance is a characteristic metabolic feature of the multisystem disorder myotonic dystrophy [1, 2]. While oral glucose tolerance is usually normal or only mildly impaired, markedly elevated plasma immunoreactive insulin concentrations, as determined by radioimmunoassay, are a well-documented feature of the disorder [1]. As in other insulin-resistant states, hyperinsulinaemia in myotonic dystrophy has been attributed to a compensatory beta-cell response to insulin resistance in target tissues [2, 3]. No intrinsic abnormalities of the insulin molecule have been described and the ratio of circulating proinsulin to insulin, as determined by chromatographic separation followed by radioimmunoassay, is reported to be normal [4].

Recent studies in other insulin-resistant disorders including impaired glucose tolerance [5] and Type 2 (non-insulin-dependent) diabetes mellitus [6] have indicated elevated plasma concentrations of beta-cell conversion intermediates with lower biological potencies than insulin. In

this study, fasting concentrations of insulin and proinsulin-like were determined in a group of patients with myotonic dystrophy using two-site immunoradiometric assays.

Patients and methods

Patients

Ten fully ambulant patients with unequivocal features of myotonic dystrophy were studied. Eight kindreds were studied; two of the patients were brothers while two others were uncle and nephew, respectively. One patient had a family history of diabetes in a first degree relative. Ten healthy volunteers served as control subjects. The two groups (myotonic dystrophy vs control subjects) were well matched for age (36 ± 9 vs 36 ± 13 years, $p > 0.1$), gender (male:female, 7:3 for each group), and body mass index (25 ± 3 vs 24 ± 3 kg/m², $p > 0.1$). No subjects had evidence of active hepatic or renal disease as judged by clinical and biochemical criteria, although serum aspartate transaminase was elevated in several of the patients with myotonic dystrophy. Serum creatine kinase was also elevated in these individuals suggest-

Fig. 1. Fasting concentrations (mean and SEM) of insulin (top), proinsulin (middle), and 32–33 split proinsulin (bottom) in patients with myotonic dystrophy ($n=10$) and healthy control subjects ($n=10$) as determined by two-site immunoradiometric assays. There was no significant difference between the groups in plasma insulin concentrations ($p=0.09$). Fasting plasma proinsulin ($p<0.01$), and 32–33 split proinsulin ($p<0.05$) concentrations were significantly elevated in the patients with myotonic dystrophy

ingskeletal muscle was the site of origin of the transaminase. No subject was receiving medication known to interfere with endogenous insulin release. Informed written consent for the study was obtained from all subjects, which was approved by the Ethical Committee of the Central Birmingham Health Authority.

Protocol

Following a 10-h overnight fast, an antecubital vein was cannulated in each subject and kept patent by flushing with 1–2 ml saline (150 mmol/l). After discarding the first 2 ml, free-flowing blood was

drawn and placed in tubes containing lithium heparin (LIP; Shipley, West Yorkshire, UK). The blood was centrifuged at 4°C and plasma separated and stored at –20°C pending assay for beta-cell products. Glucose tolerance was assessed using a 75-g oral glucose load under standardised conditions with measurement of immunoreactive insulin and C-peptide at 0, 15, 30, 60, 90, 120, 180, 240, and 300 min.

Assays

Plasma immunoreactive insulin concentration was determined using a double-antibody radioimmunoassay with a sensitivity of 12 pmol/l (Guildhay, University of Surrey, UK). Cross-reactivity was 100% for porcine insulin, human insulin and proinsulin, 29% for 32–33 split proinsulin, and 50% for 65–66 split proinsulin. Plasma C-peptide concentrations were measured by a double antibody radioimmunoassay (Novo Industries, Bagsvaerd, Denmark) with a cross-reactivity of 80% with proinsulin.

Fasting plasma concentrations of insulin, proinsulin, and split proinsulin products were measured using two-site monoclonal antibody-based immunoradiometric assays with between assay coefficients of variation of 15% or less and detection limits ranging between 0.8 to 2.5 pmol/l [7]. Cross-reactivity of the proinsulin assay was 66% with 65–66 split proinsulin, but zero with insulin and 32–33 split proinsulin. Cross-reactivity of the 32–33 split proinsulin assay was 84% with proinsulin, and 60% with 65–66 split proinsulin. Cross-reactivity of the insulin assay was 5.0%, 5.3%, and 62% with 32–33 split proinsulin, intact proinsulin, and 65–66 split proinsulin, respectively [7]. Whole blood glucose concentrations at 0 and 120 min were determined using a hexokinase/glucose 6-phosphate dehydrogenase method [1].

Statistical analysis

Clinical characteristics and fasting metabolite and hormone concentrations were compared using unpaired *t*-tests after logarithmic transformation of data where appropriate. Correlations were examined using the least squares method. Differences in overall immunoreactive insulin and C-peptide concentrations between the groups during the glucose tolerance tests were analysed by repeated measures analysis of variance. Results are presented in the text as the mean and standard error of the mean (SEM).

Results

Fasting glucose concentration was normal [8] in all subjects with mean values of 4.8 ± 0.2 vs 4.7 ± 0.1 mmol/l ($p>0.1$) for the myotonic dystrophy patients and healthy control subjects, respectively. Oral glucose tolerance, as defined by World Health Organization criteria [8] was normal in all of the myotonic patients and healthy control subjects.

Fasting plasma immunoreactive insulin concentration, as determined by double antibody radioimmunoassay, was significantly elevated in the myotonic patients, being nearly three times higher than that for the healthy control subjects (45.0 ± 9.6 vs 16.2 ± 2.4 pmol/l, $p<0.005$). By contrast, fasting plasma insulin concentration, as determined by the immunoradiometric assay (Fig. 1), was approximately 50% higher in the myotonic patients, a difference which did not reach statistical significance ($p=0.09$). Consistent with the latter observation, the difference in fasting C-peptide levels between the groups did not reach conventional statistical significance (0.75 ± 0.09 vs 0.52 ± 0.03 nmol/l, $p=0.07$). A significant correlation between plasma C-peptide and immunoradiometrically-

determined insulin concentrations was observed for both the myotonic patients ($r = 0.88$, $p < 0.01$) and the healthy control subjects ($r = 0.71$, $p < 0.05$).

The fasting concentration of proinsulin (Fig. 1) was significantly elevated in the patients with myotonic dystrophy (10.3 ± 2.9 vs 1.6 ± 0.3 pmol/l, $p < 0.01$). Accordingly, the circulating proinsulin:insulin ratio, calculated from the individual data and expressed as a percentage, was significantly increased in these patients (20 ± 5 vs $4 \pm 1\%$, $p < 0.01$). Plasma 32–33 split proinsulin concentration (Fig. 1) was also significantly higher in the patients with myotonic dystrophy (7.8 ± 2.5 vs 2.9 ± 0.4 pmol/l, $p < 0.05$). Plasma concentrations of 65–66 split proinsulin were below the detection limit of the assay in the majority of subjects in each group (data not shown).

The overall response of immunoreactive insulin ($p < 0.001$) and C-peptide ($p < 0.001$) to the oral glucose challenge was significantly greater in the myotonic patients than in the healthy control subjects. Peak C-peptide concentrations at 60 min were 3.99 ± 0.38 vs 2.02 ± 0.26 nmol/l ($p < 0.001$) for the myotonic patients and the healthy control subjects, respectively.

Discussion

Fasting plasma immunoreactive insulin concentration, as determined using a conventional radioimmunoassay, was significantly elevated in the patients with myotonic dystrophy, being nearly three times higher than that for the healthy control subjects. By contrast, fasting concentrations of C-peptide and immunoradiometrically-determined insulin were only ~50% higher in the myotonic dystrophy patients. Fasting plasma proinsulin and 32–33 split proinsulin concentrations were significantly elevated in the myotonic dystrophy patients suggesting that cross-reactivity of proinsulin-like molecules in the insulin radioimmunoassay led to an overestimation of the degree of fasting hyperinsulinaemia in these patients. Consistent with this view, significant correlations were observed between fasting concentrations of plasma C-peptide and immunoradiometrically-determined insulin.

Overall plasma immunoreactive insulin and C-peptide responses to the 75-g oral glucose challenge were significantly greater in the myotonic patients. Since C-peptide does not undergo hepatic extraction, the concentration of this molecule in the systemic circulation is held to be a reliable marker of endogenous insulin secretion. The C-peptide response therefore provides corroborative evidence of increased beta-cell secretion in myotonic dystrophy [1].

The elevated plasma concentrations of proinsulin-like molecules suggest that beta-cell processing of proinsulin may be defective in myotonic dystrophy. Alternatively, differences in clearance rates of these molecules from the circulation could explain the elevated plasma concentrations of beta-cell products [9]. However, data from glucose clamp studies and our own incremental insulin infusion studies [2] suggest that the metabolic clearance rate of insulin is normal in myotonic dystrophy. Few data are available concerning clearance rates of proinsulin-like molecules in insulin-resistant states.

Elevated plasma concentrations of beta-cell conversion intermediates have been described in some other insulin-resistant disorders including impaired glucose tolerance and Type 2 diabetes [5, 6]. In view of putative toxic effects of hyperglycaemia on beta-cell function [10] the elevated concentrations of proinsulin-like molecules in the present study is noteworthy since oral glucose tolerance, as defined by World Health Organization criteria, was normal in all the myotonic dystrophy patients [8].

In summary, in addition to increased insulin secretion, myotonic dystrophy is characterised by elevated fasting plasma concentrations of the beta-cell conversion intermediates proinsulin and 32–33 split proinsulin. While the pathological significance of this phenomenon is unclear, the elevated concentrations of proinsulin-like molecules in myotonic dystrophy is consistent with recent observations in other insulin-resistant disorders in man.

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