Decreased Insulin Binding and Antilipolytic Response in Adipocytes from Patients with Cushing’s Syndrome

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Human adipocytes from patients with chronic endogenous hypercortisolism (Cushing’s syndrome) showed a statistically significant decrease in insulin binding at low unlabelled-insulin concentrations but no change in receptor numbers (Cushing’s 180,000 ± 48,000 (3) receptors/cell and controls 189,000 ± 30,000 (7)) together with a fourfold decrease in apparent receptor affinity (ED50: Cushing’s 2.25 × 10⁻⁹ M and controls 0.57 × 10⁻⁹ M) and a decreased sensitivity to the antilipolytic effect of insulin. These events could represent the final situation of a chronic and endogenous regulation by high levels of cortisol of insulin receptors in human adipose tissue.

INTRODUCTION

In humans, insulin binding studies after in vivo glucocorticoid-treatment have provided controversial results in circulating cells (1–11) and no changes in isolated adipocytes (12). Direct in vitro effects of glucocorticoids on the human insulin receptor have been studied in a monocyte-like cell line (U-937) (13), cultured lymphocytes (IM-9 line) (4,14,15), cultured human urinary bladder carcinoma cells (JTC-32) (16), human fibroblast cultures (17), and in human adipose tissue in culture (18), also rendering ambiguous results.

The present study investigates the binding of insulin to isolated human adipocytes.

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from patients with chronic endogenous hypercortisolism. Data from other authors (6,19,20) have, to our knowledge, been obtained from circulating human cells. In addition, we studied the antilipolytic action of insulin in these human adipocytes as a post-binding event probably involved in the process.

MATERIALS AND METHODS

Three female patients with chronic endogenous hypercortisolism (Cushing's syndrome) were studied. They were diagnosed on the basis of clinical signs and symptoms and evaluated by a routine endocrine-function test. Their ages ranged from 39 to 59 yr. All of them were within 15% of their ideal body weight. Seven other patients (2 male and 5 female) were also included as controls. This group comprised patients undergoing cholecystectomy for gallstones. Their ages ranged from 31 to 59 yr. None of the patients had known endocrine or malignant disorders. All the patients received a normal hospital diet. Studies were performed in the morning following an overnight fast.

The adipose tissue was obtained from the subcutaneous tissue of the right upper quadrant of the abdomen. Isolated adipocytes were prepared by collagenase digestion according to the method reported by Rodbell (21). There was no size difference in adipocytes from Cushing's syndrome and control patients.

Mono-[125I]insulin was prepared (22) with specific activities of 200–300 μCi/μg using Na125I (Radiochemical Centre, Amersham).

Human adipocytes (0.1–0.3 × 10^6 cells/ml) were incubated at a final volume of 350 μl with mono-[125I]insulin (0.1–0.2 × 10^-9 M) at 30°C for 30 min in Krebs-Hepes buffer pH 7.4 containing glucose (3.3 mM), HSA (1%) (Behring Co., Marburg) and bacitracin (0.9 mM) (Sigma Co., St Louis) either in the absence or the presence of unlabelled insulin (Novo Ind., Bagsvaerd) at increasing concentrations from 0.25 × 10^-9 M to 0.5 × 10^-7 M. Adipocytes were separated from the medium by centrifugation through dinonyl phthalate according to the method of Gliemann et al. (23). The amount of 125I-insulin bound in the presence of 0.25 × 10^-6 M unlabelled insulin was considered as non-specific binding; this was not different between groups. Binding data are expressed as 125I-insulin specifically bound per 10^6 cells/ml. Insulin degradation at the time of binding was determined by the amount of TCA-precipitable labelled insulin remaining after incubation using appropriate controls where fat cells were omitted.

For the antilipolysis studies, 100 μl of isolated adipocytes (0.1–0.3 × 10^6 cells/ml) were incubated with Krebs-Hepes buffer pH 7.4 without glucose and with 1% FFA-free BSA (Sigma Co., St Louis) and bacitracin (0.9 mM). The cells were incubated at 37°C either in the absence or in the presence of 10^-6 M isoproterenol or 10^-6 M isoproterenol plus different concentrations of insulin (1.25 × 10^-11 M–1.25 × 10^-10 M) for 120 min. The glycerol content was determined by the method of Lambert and Neish (24) with some of our own modifications (25). Glycerol data are expressed as nmol/10^5 cells/2 h.

The results were expressed as mean ± SEM. For statistical comparison student’s "t"-test was used.
RESULTS

Circulating insulin levels were similar in patients with hypercortisolism and controls (11 ± 3 (3) μU/ml vs. 10 ± 0.7 (7) μU/ml; p = NS). However, the patients with Cushing’s syndrome were hyperglycemic as compared to controls (161 ± 20 (3) mg/dl vs. 77 ± 2 (7) mg/dl; p < 0.02).

In the chronic endogenous hypercortisolemic patients, insulin binding to adipocytes was significantly decreased compared with controls. Figure 1(a) shows the displacement curves for $^{125}$I-insulin binding to adipocytes in the two groups. The effect is statistically significant at low concentrations of unlabelled insulin (below $2.5 \times 10^{-10}$ M) and disappears at higher concentrations. The amount of unlabelled insulin necessary to decrease the initial binding by 50% was fourfold greater in cells from patients with Cushing’s syndrome as compared to controls ($2.25 \times 10^{-9}$ M vs. $0.57 \times 10^{-9}$ M), suggesting a decrease in the apparent affinity of the insulin receptor. Figure 1(b) shows a Scatchard analysis (26) of the binding data. A significant decrease in binding at low insulin concentrations was observed. However, the total number of insulin receptors was practically the same in the patients with Cushing’s syndrome and the controls: $180,000 \pm 48,000 (3)$ receptors/cell and $189,000 \pm 30,000 (7)$ receptors/cell respectively.

Adipocytes from the patients with adrenal hyperfunction (3) were found to inactivate insulin to the same extent as the controls (7): 0.5–1%.

Basal values of glycerol release (Fig. 2) were $60 \pm 16$ nmol/10$^5$ cells/2 h in adipocytes from the patients with Cushing’s syndrome (3) and $40 \pm 22$ nmol/10$^5$ cells/2 h in controls (3); the differences were not statistically significant. Isoproterenol ($10^{-6}$ M) significantly stimulated the rate of glycerol release: 60% in adipocytes from patients with hypercortisolism and 81% in controls. Fat cells prepared from the patients with Cushing’s syndrome were significantly less sensitive to the antilipolytic effect of insulin. The percentages of inhibition in the presence of insulin concentrations of $1.25 \times 10^{-11}$ M; $2.5 \times 10^{-11}$ M and $1.25 \times 10^{-10}$ M were: 23%, 35% and 27% respectively in adipocytes from patients with Cushing’s syndrome and 53%, 36% and 41% in the controls. The differences between the actual values were statistically significant except in the presence of $2.5 \times 10^{-11}$ M insulin.

DISCUSSION

We have previously studied the regulation by glucocorticoids of insulin (27,28) and glucagon (29,30) binding and action in isolated rat adipocytes. In the present study we investigated the binding and action of insulin in human adipocytes from patients with chronic endogenous hypercortisolism.

Our data concerning decreased insulin binding and the decreased affinity of the insulin receptor in isolated adipocytes from patients with Cushing’s syndrome are not in agreement with previous data from other authors who also studied patients with chronic endogenous hypercortisolism but obtained their data in circulating cells (6,19,20). In this sense, Muggeo et al. (6) reported that patients with adrenocortical hyperfunction had increased insulin binding due to an increase in receptor affinity in
Fig. 1. (a) Displacement curves of Mono-[125I]insulin binding in human adipocytes from patients with Cushing's syndrome as compared to controls. (b) Scatchard plot of the binding data. The asterisks (*) represent statistically significant differences between values of the control and Cushing's syndrome patients.
monocytes and decreased insulin receptor concentrations accompanied by an increase in receptor affinity in erythrocytes. Wajchemberg et al. (19) reported that in patients with Cushing’s disease insulin binding to erythrocytes and insulin receptor affinity was unaffected. Likewise, Pallardo et al. (20) did not detect insulin receptor defects in erythrocytes from patients with Cushing’s syndrome. This diversity of results may be due to the known differential tissue effects of glucocorticoids and also to the influence of obesity and hyperinsulinemia detected in the patients studied by some of the aforementioned authors (19,20). The insulin levels and body weight in our Cushing’s syndrome patients were found to be within normal ranges. Thus, these parameters do not seem to influence our results on insulin binding. Nor were any differences detected in insulin degradation during the binding experiments, suggesting that this parameter does not affect our results concerning insulin binding either.

Our binding results in adipocytes from patients with Cushing’s syndrome can also be compared with the data of Pagano et al. (12) in adipocytes from patients after an in vivo prednisone treatment, or with the data of Cigolini and Smith (18) using human adipocytes cultured in vitro for one week with cortisol. In their work, Pagano et al. (12) did not detect any change either in receptor concentrations or in receptor affinity, and Cigolini and Smith (18) detected a decreased insulin binding that was mainly related to a decrease in receptor number. The discrepancies between our results and those of these authors could be explained on the basis of different factors. These would involve the dose and type of glucocorticoid used, the duration of the in vivo treatment and the time-course of the in vitro exposure to the glucocorticoid.
Additionally, in our experiments the decrease in insulin binding was accompanied by a decreased sensitivity to the antilipolytic action of insulin in adipocytes from patients with Cushing’s syndrome and related to the insulin-resistant state present in patients with hypercortisolism. Concerning this, it has been reported recently that insulin resistance in Cushing’s syndrome is characterized by both decreased insulin responsiveness and decreased insulin clearance (31,32); similar results were obtained in patients with Cushing’s disease (19). Moreover, a decrease in glucose transport in human adipocytes after in vivo prednisone administration (12), together with an unimpaired insulin action on lipolysis in humans after in vivo cortisol administration (33) and a decrease in the sensitivity to the antilipolytic effect of insulin in explants of human adipose tissue in culture (18) have also been described previously.

In summary, the results of the present study, although based on a small number of patients, appear to be homogeneous and support the concept that decreased insulin binding with decreased receptor affinity and antilipolytic activity in adipocytes from patients with Cushing’s syndrome could represent a final situation of a chronic and endogenous regulation by high levels of cortisol of insulin receptors in human adipose tissue.

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