Qualitative abnormalities in insulin binding in a patient with extreme insulin resistance: Decreased sensitivity to alterations in temperature and pH

(cultured human lymphocytes/insulin receptors/leprechaunism)

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ABSTRACT Cultured lymphocytes transformed by Epstein–Barr virus were employed to study insulin receptors from a patient with extreme insulin resistance associated with the syndrome of leprechaunism. With cultured lymphocytes from normal subjects, insulin binding to its receptor is exquisitely sensitive to changes in temperature and pH. In cells from normal subjects, insulin binding was increased by approximately 250% as the temperature was decreased from 37°C to 12°C. In contrast, with cells from the leprechaun, insulin binding was only approximately 30% higher at 12°C than at 37°C. Similarly, insulin binding to cells from the leprechaun was markedly less sensitive to changes in pH, as compared to cells from normal subjects. Binding studies suggested that the number of insulin receptors per cell was within the normal range in this patient. Despite the unusual characteristics of insulin binding in cells from this insulin-resistant patient, the receptors were typical in at least two respects: (i) binding was inhibited normally by antibodies to the receptor; and (ii) the specificity for insulin analogs was normal (chicken insulin > porcine insulin >> guinea pig insulin > porcine proinsulin). This patient has an inborn error affecting insulin receptor function. The receptor’s binding function was abnormal in having decreased sensitivity to alterations in temperature and pH. However, the level of insulin binding to cells from the leprechaun was within normal limits. Consequently, the hormonal resistance probably results from a decreased ability of the receptor to couple insulin binding to insulin action.

Surface receptors in the plasma membrane play an important role in many physiological processes such as hormone action, transport across the plasma membrane, and the immune response. Primary genetic defects in surface receptors have been recognized to cause such diverse diseases as familial hypercholesterolemia (1) and extreme insulin resistance (2).

Moderate insulin resistance contributes importantly to glucose intolerance in common diseases such as obesity and type II diabetes (3, 4). The special interest in the rare syndromes of extreme insulin resistance resides in their potential to give insight into the molecular mechanisms of insulin resistance and insulin action. A variety of pathophysiological mechanisms may eventuate in insulin resistance (5–13). This paper is concerned primarily with the forms of extreme insulin resistance (e.g., leprechaunism) caused by primary inborn defects in hormone responsiveness at the level of the target tissue (2, 7–13). Some patients with leprechaunism have a decreased number of insulin receptors as the cause of insulin resistance (10, 11), whereas others have normal levels of insulin binding and are said to have “postreceptor” defects (12, 13).

We have studied insulin binding in a leprechaunism patient with extreme insulin resistance previously attributed to a postreceptor defect (12). However, our studies with cultured lymphocytes from this patient provide evidence for an abnormality in insulin binding manifested as a markedly decreased sensitivity to changes in pH and temperature (cf. refs. 14–16). Thus, this patient appears to have not a postreceptor defect but rather an inborn error affecting insulin receptor function and thereby causing insulin resistance.

METHODS

Patients. All studies were approved by the human studies committee of the National Institute of Arthritis, Metabolism, and Digestive Diseases, and informed consent was obtained. The patient referred to as "leprechaun/Ark-1" has been described in more detail by Elders and her collaborators (12, 17).

Growth of B-Lymphocyte Cell Lines. Cell lines were established by infecting peripheral blood lymphocytes with Epstein–Barr virus (18). Mononuclear cells were obtained by centrifugation through Ficoll/Hypaque. After being washed three times with phosphate-buffered saline, cells were resuspended at a density of 1–3 × 10³ cells per ml in RPMI 1640 medium with 20% fetal calf serum. The cells were infected with Epstein–Barr virus obtained from the conditioned medium of marmoset B-95-8 cells (1 ml per 5 ml of cell suspension). Cells began to proliferate after 2–16 weeks of incubation at 37°C in an atmosphere of 5% CO₂ in air with a relative humidity of 85%. Cells were grown in RPMI 1640 medium containing 10% fetal calf serum (Flow Laboratories).

₁²⁵I-Labeled Insulin Binding Studies. Binding of ₁²⁵I-labeled insulin (₁²⁵I-insulin) to cultured lymphocytes was studied according to described methods (19). For investigation of the effects of temperature (Figs. 1–3), the following buffer was employed: 120 mM NaCl/1.2 mM MgSO₄/2.5 mM KCl/15 mM sodium acetate/10 mM glucose/1 mM EDTA/100 mM HEPES/bovine serum albumin at 10 mg/ml (pH 7.8). In the experiments in which pH was varied (Figs. 4–6), various ethanesulfonylic acid buffers (50 mM) were added to the following medium: 120 mM NaCl/4.5 mM KCl/1.2 mM MgSO₄/1 mM EDTA/10 mM glucose/bovine serum albumin at 10 mg/ml.

RESULTS

Abnormal Temperature Sensitivity of ₁²⁵I-Insulin Binding in Leprechaun/Ark-1 Cells. In cultured lymphocytes from leprechaun/Ark-1, insulin binding showed remarkably reduced sensitivity to changes in temperature (Figs. 1–3) and pH (Figs. 4–6). At 37°C, insulin binding to cultured lymphocytes from leprechaun/Ark-1 was as well as from normal subjects was rapid.

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achieving a maximum within 45 min (Fig. 1B). At 12°C, 125I-insulin bound more slowly to both cell types, requiring several hours to achieve steady state (Fig. 1A). With the normal cells at 12°C, 125I-insulin binding was 250% of that observed at 37°C. This was highly reproducible in cell lines from a wide variety of patients (Fig. 1C). Cells from leprechaun/Ark-1 were unique: 125I-insulin binding increased by less than 30% as the temperature was decreased from 37°C to 12°C.

The reduction of 125I-insulin binding at 37°C results from a reduction of the receptor's affinity without any major alteration.

Fig. 2. Steady-state insulin binding at 12°C and 37°C. Cultured lymphocytes (10⁶ cells per ml) were incubated at either 12°C for 3 hr or 37°C for 30 min in the presence of 125I-insulin (0.1 ng/ml) and various concentrations of unlabeled insulin. 125I-Insulin binding as a function of the total concentration of added insulin is shown in A and C. Scatchard plots (20) for specific binding of insulin are presented in B and D.
in receptor number (ref. 15; Fig. 2 B and D). Detailed steady-state binding studies to cells from leprechaun/Ark-1 showed two major abnormalities: temperature had an abnormally small effect on the shape of the binding-competition curve; even at 12°C, the binding competition curve in cells from leprechaun/Ark-1 was shifted to the left. The concentration of unlabeled insulin required to displace 50% of the 125I-insulin (i.e., IC50) was lower by a factor of 1/(2.9 ± 0.3) (n = 6, P < 0.001) in cells from leprechaun/Ark-1 as compared to cells from normal subjects (Fig. 2 A and C). Moreover, the number of receptors per cell as well as the binding of tracer concentrations of 125I-insulin (0.1 ng/ml) in leprechaun/Ark-1 are within the normal range for cultured lymphocytes (Figs. 1C and 2 B and D).

**Kinetics of Dissociation of 125I-Insulin.** The abnormality of steady-state binding of insulin to cells from leprechaun/Ark-1 results from abnormal kinetics of dissociation. Increasing the temperature from 12°C to 37°C increased the initial rate of spontaneous dissociation of 125I-insulin by approximately 20-fold in cells from normal subjects (Fig. 3). In contrast, in cells from leprechaun/Ark-1 the initial rate of dissociation was increased by only 3-fold at the higher temperature. In all cells studied (including leprechaun/Ark-1), excess unlabeled insulin (1 µg/ml) increased the rate of dissociation of 125I-insulin both at 12°C and at 37°C (Fig. 3). Because of the abnormally slow spontaneous rate of dissociation of 125I-insulin in cells from leprechaun/Ark-1 at 37°C, the effect of insulin to accelerate dissociation was particularly marked at this temperature. In cells from leprechaun/Ark-1, increasing the temperature from 12°C to 37°C caused an increase in the rate of dissociation of 125I-insulin similar to that for the addition of unlabeled insulin at 12°C. In all other cells we have studied, increasing the temperature had a larger effect on the dissociation rate than did the addition of unlabeled insulin (Fig. 3).

**Abnormal pH Profile.** 125I-Insulin binding is exclusively sensitive to the pH of the incubation medium. At 12°C the optimal pH was approximately 7.4; cells from all the subjects we have studied, including leprechaun/Ark-1 (Fig. 4). However, the insulin binding to cells from leprechaun/Ark-1 was markedly less sensitive to alterations in pH. For example, a decrease in the pH from 8.0 to 6.8, which reduced insulin binding by 80–90% in all other cells we have studied, reduced binding by only 60% in cells from leprechaun/Ark-1.

More detailed binding studies were carried out at two values of pH: 6.8 and 8.0. The time courses of association of 125I-insulin (0.1 ng/ml) to several cell lines, including leprechaun/Ark-1, were studied and found to be similar at 12°C at both values of pH (data not shown). Binding achieved steady-state levels within 3 hr in all cases. In steady-state binding studies, lowering the pH from 8.0 to 6.8 decreased 125I-insulin binding by 85–90% in cell lines from normal subjects (Fig. 5). Moreover, the Scatchard plot approached linearity at pH 6.8. In contrast, with cells from leprechaun/Ark-1, 125I-insulin binding fell by only 60% and the Scatchard plot remained curvilinear even at pH 6.8.

Similar to what we observed with temperature, the abnormality in the effect of pH on insulin binding to the cells from leprechaun/Ark-1 was the result of an abnormality in the kinetics of dissociation of 125I-insulin. In cells from normal subjects, decreasing the pH from 8.0 to 6.8 increased the rate of dissociation by approximately 7-fold. In cells from leprechaun/Ark-1, the effect was quantitatively much smaller, only about

![Fig. 3. Dissociation of 125I-insulin from cultured lymphocytes at 12°C and 37°C. Cultured lymphocytes (9 × 10⁶ cells per ml) were incubated with 125I-insulin (0.1 ng/ml) at 12°C in a total volume of 10 ml. After 3 hr, 35 ml of ice-cold incubation medium was added. The cells were immediately separated from the medium by centrifugation at 250 × g for 8 min. After the supernatant had been discarded, the cells were resuspended in 10 ml of ice-cold incubation medium. Aliquote (0.2 ml) of cell suspension were added to 12 × 75 mm plastic test tubes (Falcon) containing 4.8 ml of incubation buffer with (C, D) or without (A, B) unlabeled insulin (1 µg/ml) preequilibrated at 12°C or 37°C. "Dil" indicates dilution; "Dil + Ins," dilution with insulin. Duplicate tubes were removed for assay of cell-associated 125I-insulin as indicated. After centrifugation of the tubes at 900 × g for 5 min at room temperature and aspiration of the supernatant, cell-associated 125I-insulin was determined by placing the tubes directly into a Scintillation model 1285 gamma counter. In similar experiments with three cell lines obtained from control subjects, initial rates of dissociation at 37°C were faster by a factor of 2.9 ± 0.4 (mean = SEM) as compared to leprechaun/Ark-1.](image)

![Fig. 4. Effect of pH on 125I-insulin binding to cultured lymphocytes. Cultured lymphocytes (9 × 10⁶ cells per ml) were suspended in incubation media to which one of the following buffers had been added: 50 mM 2-(2-amino-2-oxoethyl)amino)ethanesulfonic acid (Acetate) (pH 6.8), 50 mM 2-(bis(2-hydroxyethyl)amino)ethane sulfonic acid (Bis) (pH 7.2), 50 mM Hepes (pH 7.6), 50 mM 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (Hepes) (pH 8.0), or 50 mM 3-[(triethanol amino) methyl]propanesulfonic acid (Taps) (pH 8.5). After cells were incubated at 12°C for 4 hr, specific binding of 125I-insulin was determined as usual. Results are presented as B/F ratios for specific 125I-insulin binding relative to the B/F values observed at pH 8.0. The normal range, defined as the mean ± 2 SD for observations with cells from five control subjects (two normal volunteers, one patient with type B extreme insulin resistance, one patient with lipotropic diabetes, and one patient with familial extreme insulin resistance), is presented (○), along with data from leprechaun/Ark-1 cells (●). Because the SEMs of observations with leprechaun/Ark-1 cells are smaller than the size of symbols, they are not depicted on the graph.](image)
Its ability to increase the rate of dissociation of $^{125}$I-insulin at the more acid pH. This, too, fits well with a negative cooperativity model. With the exception of leprechaun/Ark-1, in all cell lines we have studied, dissociation of $^{125}$I-insulin is more markedly stimulated by decreasing the pH from 8.0 to 6.8 than by the addition of unlabeled insulin (1 µg/ml) at pH 8.0. In contrast, in cells from leprechaun/Ark-1, addition of unlabeled insulin has a more marked effect on the dissociation of $^{125}$I-insulin than does reduction of the pH.

**Normal Specificity of Insulin Receptors in Leprechaun/Ark-1.** In light of all of the abnormal features of $^{125}$I-insulin binding to cells from leprechaun/Ark-1, we wished to see whether certain other characteristic features of insulin receptors were preserved. Antibodies to the insulin receptor (6) had a normal affinity for the receptors from leprechaun/Ark-1 as shown by their ability to inhibit $^{125}$I-insulin binding at the usual concentration (data not shown). In addition, insulin receptors from leprechaun/Ark-1 showed normal specificity for insulin analogs: chicken insulin > porcine insulin >> guinea pig insulin > porcine proinsulin (data not shown).

**DISCUSSION**

Because the number of insulin receptors appeared normal, Kobayashi et al. (12) concluded that the insulin resistance in leprechaun/Ark-1 resulted from a postreceptor defect. In contrast, our studies with cultured cells from this patient show that insulin binding is qualitatively normal in the response to alterations in temperature and pH. However, the abnormal properties of the receptor in this patient do not result in decreased insulin binding under physiological conditions (37°C, pH 7.4).

**Nature of the Structural Abnormality in Leprechaun/Ark-1.** The extensive binding data for leprechaun/Ark-1 are compatible with many possible molecular mechanisms. The simplest hypothesis is that the qualitative abnormalities in insulin binding result from a point mutation in a single gene coding for a protein involved in the pathway of insulin responsiveness—either the receptor itself, or, alternatively, in a protein that interacts with the insulin receptor. The latter possibility is based on the analogy to the C/F subunit, which not only couples hormone binding to hormone action (24) but regulates receptor affinity as well (25). In the case of the insulin receptor, there is indirect evidence for the existence of an "affinity regulator" that alters the affinity of the receptor for insulin (26). It is possible that an abnormality in such an affinity regulator might desensitize the receptor to changes in pH and temperature. Other more complicated mechanisms are possible as well. For example, there might be an abnormality in an enzyme involved in the posttranslational modification (e.g., glycosylation) of the receptor. Alternatively, there might be an abnormality in the structure of the membrane in which the receptor is embedded.

**Analysis of Binding Data.** In some systems (particularly at 37°C), a portion of the cell-associated radioactivity represents hormone that has been internalized rather than hormone that is bound to surface receptors (27). Some of our results might be explained if the cells of leprechaun/Ark-1 internalized more hormone at 37°C than was internalized by cells from normal subjects under similar conditions. This possibility cannot yet be ruled out with certainty, but we do not believe this was the case because cultured lymphocytes have been shown previously not to internalize significant quantities of $^{125}$I-insulin, even at 37°C (28). It seems even less likely that the decreased sensitivity of binding to alterations in pH could be explained on this basis.

**Conclusions.** This patient with extreme insulin resistance has...
a novel abnormality in insulin binding. Previously, extreme insulin resistance in some patients has been attributed to a decreased number of qualitatively normal receptors (11, 29). In other patients with extreme insulin resistance, insulin receptors have been normal, both quantitatively and qualitatively (30, 31). The novelty in this patient lies in the finding of a normal number of receptors but with a clear-cut qualitative abnormality in insulin binding.

Abnormalities in temperature sensitivity have been described previously in other receptor systems. For example, in the testicular feminization syndrome, a temperature-sensitive mutation in the androgen receptor has been described (32). Androgen binding was normal at the low temperature but depressed at higher temperature. Therefore, if the in vitro experiments reflect the situation in vivo, androgen resistance in these patients may result from decreased hormone binding at physiological temperature. Unlike the situation in androgen resistance, temperature insensitivity of insulin binding per se does not explain insulin resistance in the case of leprechaun/Ark-1. Rather, it merely provided a marker that allowed identification of a defect in receptor function. Insulin resistance almost certainly results from a defect in coupling of hormone binding to hormone action. Cultured cells from leprechaun/Ark-1 may provide an important tool in elucidation of the biochemical details of that coupling process.

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