Insulin Receptor Function in Fibroblasts from Patients with Leprechaunism

Differential Alterations in Binding, Autophosphorylation, Kinase Activity, and Receptor-mediated Internalization

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Abstract

Insulin receptor function was examined in cultured skin fibroblasts from three patients with leprechaunism (Ark-1, Minn-1, and Can-1), a rare syndrome of severe insulin resistance and neonatal growth retardation. All three patients cell lines demonstrated insulin binding < 15% of control. This was primarily due to reduced affinity of the receptor in Can-1 and due to reduced number of receptors in the other two cell lines (Ark-1 and Minn-1). When expressed as a fraction of total insulin bound, the percentage of cell-associated insulin internalized and degraded did not differ between the patient cell lines and the controls. However, chloroquine, which inhibited degradation by 50% in the control cells, had no effect in the cells from the patients. When normalized to insulin binding, insulin receptor autophosphorylation was normal in cells from Can-1, but reduced in those of Ark-1 and Minn-1. In contrast, the receptor-associated tyrosine kinase activity toward exogenous substrates was decreased in all three patient cell lines.

These results suggest that leprechaunism is a biochemically heterogenous disease associated with a variety of alterations in receptor function. Cells from Ark-1 and Minn-1 exhibit parallel alterations in receptor autophosphorylation and kinase activity. Cells from Can-1 demonstrate normal receptor autophosphorylation but reduced kinase activity, thus displaying a unique form of a mutant insulin receptor. Despite reduced kinase activity, all three cell lines exhibit normal rates of insulin internalization, but decreased lysosomal-mediated degradation. Our data imply that receptor autophosphorylation and kinase activity may be regulated separately and that kinase activity may be linked to insulin degradation, but not necessarily internalization.

Introduction

Insulin binds to the α-subunit of its receptor and activates a tyrosine kinase activity intrinsic to the β-subunit of the receptor, leading to β-subunit autophosphorylation (1, 2). Autophosphorylation itself further activates the tyrosine kinase (3) and thus both autophosphorylation and kinase activity are regulated in tandem. Site-directed mutagenesis experiments and studies using antiinsulin receptor antibodies have revealed a close link between autophosphorylation, tyrosine kinase activity, and insulin action. In vitro mutagenesis experiments involving the ATP binding site (4, 5) or autophosphorylation sites at tyrosines 1162 and 1163 have resulted in reduction of both autophosphorylation and reduced kinase activity (6). Antibodies directed at intracellular domains of the receptor have also been shown to inhibit receptor autophosphorylation and protein kinase activity[1] (7–9). Likewise, receptors on cells from some patients with type A syndrome of insulin resistance exhibit reduced receptor autophosphorylation and reduced kinase activity secondary to presumed mutations in the receptor (10, 11).

Leprechaunism is a rare genetic syndrome characterized by intrauterine and neonatal growth retardation, reduced subcutaneous tissue, unusual facies, severe insulin resistance with hyperinsulinemia and early death (12, 13). Both receptor and postreceptor defects have been postulated as causes of the insulin resistance (14–16). In the present study, we have characterized insulin binding, receptor autophosphorylation, and tyrosine kinase activity, as well as insulin internalization and degradation in skin fibroblasts from three patients with leprechaunism (17–20). We find that all patient cell lines displayed reduced insulin binding and reduced receptor-mediated degradation but essentially normal insulin internalization. In addition, two of the three cell lines exhibited a decrease in receptor autophosphorylation and insulin stimulated tyrosine kinase activity, whereas cells from one patient have reduced kinase activity with normal autophosphorylation. These data suggest that there are unique and heterogenous defects in the insulin receptor in patients with leprechaunism.

Methods

Materials. $Na^{125}I$-[Tyr^{A14}] monoiodoinsulin (2,000 mCi/μmol) and [γ-^{32}P]ATP (5 mCi/mmol) were purchased from Amersham Corp. (Arlington Heights, IL) and New England Nuclear (Boston, MA), respectively. Agarose bound wheat germ agglutinin was obtained from Vector Laboratories, Inc., Burlingame, CA. Tissue culture plasticware was from Nunc (Copenhagen, Denmark), and Dulbecco's modified Eagle's medium (DME) and FCS were supplied from Gibco Laboratories (Grand Island, NY). All other chemicals used were of analytical grade and obtained from standard suppliers.

Skin fibroblasts of Ark-1 were courtesy of Dr. J. Elders, University of Arkansas, Little Rock, AS; cells of Can-1 were the kind gift of the Department of Genetics, University of Manitoba, Winnipeg, Canada and those of Minn-1 were obtained from the Mutant Cell Repository, Camden, NJ (catalogue No. GM5241). Control skin fibroblasts (n = 9) were obtained from normal volunteers and from American Type Tissue Culture, Bethesda, MD (catalogue No. CRL 1537, CRL 1506,

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CRL 1474, CRL 1477). Fibroblasts were maintained in DME, supplemented with 10% FCS in humidified atmosphere containing 5% CO_2. To standardize the studies, cells were subcultured by splitting 1:3 and then used for experiments 2–3 d after reaching confluence. All cells were used between the 5th and the 16th passages before they become senescent (usually near the 25th passage). Little interpassage variation was present under these conditions.

Insulin binding, internalization, and degradation. Confluent monolayers of fibroblasts were washed with chilled calcium-, magnesium-free PBS, and insulin binding was performed using 0.3 ng/ml of ^{125}I-insulin with 0–1,000 ng/ml of unlabeled insulin in Hepes binding buffer (100 mM Hepes, 1% BSA, 118 mM NaCl, 5 mM KCl, 1.2 $MgSO_4$, and 8.8 mM dextrose, pH 8.0) at 15°C for 3 h.

For internalization and degradation studies, insulin binding was allowed to proceed for 30 min at 37°C. Cell surface bound insulin was removed by a 10-min acid wash (0.3 M acetic acid, 0.15 M NaCl, pH 3.0), and the remaining cell-associated insulin was collected by solubilization of the cells with 0.1% SDS. The acid wash (surface bound tracer) and the SDS-lysates (internalized tracer) were counted in a gamma counter. This method removes 95% of surface bound tracer when insulin binding is performed at 4°C. The amount of ligand degradation was determined by 10% TCA precipitability. In some experiments the effect of a 30-min preincubation with 100 μM chloroquine on internalization and degradation was studied. 2–3% of insulin was degraded in the medium after 30 min at 37°C in all cell lines.

Partial purification of insulin receptor and insulin binding. Confluent monolayers of fibroblasts were solubilized in 50 mM Hepes, 1% Triton X-100, aprotinin (1,000 kallikrein U/ml), 2 mM PMSF at pH 7.4, and ultracentrifuged at 200,000 *g* for 60 min at 4°C. The supernatant was then applied three times to columns containing wheat germ agglutinin coupled to agarose. After extensive washing with 50 mM Hepes, 150 mM NaCl, 0.1% Triton X-100, pH 7.4, the bound material was eluted with the above buffer supplemented with 0.3 M *N*-acetylglucosamine. Protein determination was performed and samples were stored at −70°C. Insulin binding was performed on the partially purified receptor preparations at 4°C for 15 h using the polyethylene glycol precipitation method as previously described (18).

Insulin receptor autophosphorylation. Autophosphorylation of the insulin receptor was studied using partially purified receptor preparations adjusted to give similar insulin receptor concentrations. The insulin binding per milligram protein was improved in the partially purified preparations and thus only one to three times the control concentrations of protein were required for comparable binding capacity. Insulin binding was performed at 4°C for 15 h and then brought to 20°C. Phosphorylation was initiated by adding $MnCl_2$ (4 mM final concentration), 25 μM cold ATP and 5 μCi [^{32}P]ATP for 10 min. The reaction was stopped by addition of Laemmli sample buffer (2% SDS, 100 mM DTT, 0.01% bromphenol blue, 10% glycerol and 10 mM sodium phosphate, pH 7.0) and boiling for 3 min. The proteins were separated on 7.5% PAGE according to Laemmli (21), and the gel was analyzed by autoradiography. ^{32}P-incorporation into the β-subunit of the insulin receptor was quantitated by scanning densitometry of the films or by excising and counting the corresponding bands of the gel in a β-scintillation counter. The amount of phosphorylation was expressed as percentage of control maximum after subtracting the basal value from each stimulated value.

Insulin receptor tyrosine kinase assay. A synthetic peptide, Thr-Arg-Asp-Ile-Tyr-Glu-Thr-Asp-Tyr-Tyr-Arg-Lys, corresponding to 1142-1153 region in the insulin receptor, was purchased from D. Coy, Tulane University, New Orleans, LA. Phosphorylation was conducted using partially purified receptors with equivalent amounts of insulin binding activity. After incubation with insulin (10 μg/ml) at 4°C for 15 h, 4 mM $MnCl_2$, 10 mM $MgCl_2$ and 25 μM ATP were added for 10 min at 20°C to allow prephosphorylation and activation of the insulin receptor. Substrate solution was then added to give a final concentration of 1 mM peptide, 50 mM Hepes, 0.1% Triton X-100, 150 mM NaCl, 4 mM $MnCl_2$, 10 mM $MgCl_2$, 25 μM ATP and 10 μCi of [^{32}P]ATP. The reaction was stopped by addition of 50 μl 5% TCA and 20 μl 1% albumin to a 30-μl aliquot of reaction mixture. Basal and insulin stimulated values were obtained for all time points. A zero time point was also obtained. The samples were then centrifuged to remove precipitated proteins, and two 35-μl aliquots of supernatant were applied to 2 × 2 cm squares of phosphocellulose paper. The papers were extensively washed with 75 mM phosphoric acid and counted in a β-scintillation counter (22, 23). Specific radioactivity of ^{32}P-incorporation into substrate was determined by subtracting the zero time point from all points and then the basal value from the stimulated value.

Insulin stimulated ^{32}P-incorporation into the synthetic peptide, poly-Glu,Tyr (4 Glu:1 Tyr) (Mr. ~ 48,000) was performed as previously described (11). The peptide was used at a concentration of 2.5 mg/ml. Values at 15 min of phosphorylation were used for comparison.

Results

Insulin binding to intact fibroblasts. Insulin binding was performed with control and patient fibroblasts under steady-state conditions at 15°C, and specific insulin binding per milligram protein was plotted against the concentration of insulin (Fig. 1). Control cells (n = 9) displayed maximal insulin binding of 0.7%±0.09/mg protein. Half-maximal inhibition of ^{125}I-insulin binding occurred at 1–2 ng/ml of unlabeled insulin. All three patient cell lines demonstrated reduced specific insulin binding between 9 and 12% of control values. With cells of Can-1, this was accompanied by a significant increase in the concentration of insulin required for half-maximal displacement (20–30 ng/ml) ($P < 0.05$). There were also slight rightward shifts in the competition curves with Ark-1 and Minn-1 (ED_{50} = 2–5 ng/ml).

Scatchard analysis of the data yielded curvilinear Scatchard plots, as expected for insulin binding (Fig. 2). Using a two-site model, cells from Ark-1 and Minn-1 have 80% and 70% reduction of high affinity sites, respectively (Table I). In contrast, cells of Can-1 appeared to have a normal number of high affinity binding sites but with significantly reduced affinity (control $K_D = 2.57 \times 10^{-9}$ M, Can-1 $K_D = 0.69 \times 10^{-9}$ M, P

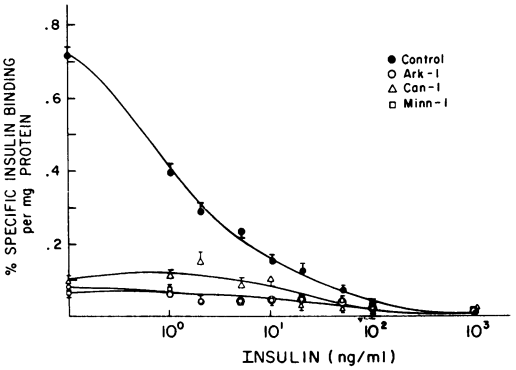

Figure 1. Specific ^{125}I-insulin binding to fibroblasts of patients with leprechaunism. Confluent monolayers of fibroblasts from controls and patients with leprechaunism (Ark-1, Can-1, and Minn-1) were incubated with ^{125}I-insulin (0.3 ng/ml) and various amounts of unlabeled insulin (0–1,000 ng/ml) at 15°C for 3 h. Unbound insulin was removed as described in Methods and the cell associated counts determined. Nonspecific insulin binding was less than 0.3% of total tracer and was not different between patient and control cell lines. The points represent the means±SEM of the results of three to five experiments.

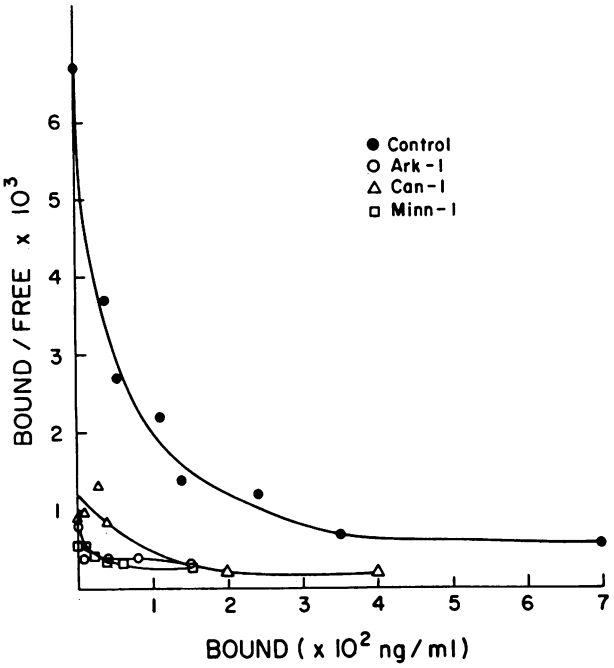

Figure 2. Scatchard analysis of insulin binding. The data from Fig. 1 are replotted as bound/free of insulin as a function of insulin bound.

< 0.05). Due to the low level of binding in fibroblasts, it is difficult to estimate the number of low affinity binding sites.

Insulin internalization and degradation. Insulin internalization and degradation in patient and control cells was determined at 37°C (Fig. 3). In the control cells, 67% of the tracer was internalized by 30 min and of this 31% had been degraded. In the patient cell lines, the percent internalization ranged from 60 to 84% at 30 min and was not distinguishable from normal. Despite apparently normal degrees of internalization, degradation of internalized insulin was slightly reduced in cells of Ark-1 (18%) and Minn-1 (24%), while cells of Can-1 degraded 28% of internalized insulin (normal = 19–35%). It should be noted that Ark-1 and Minn-1 cells had reduced number of high affinity binding sites, whereas Can-1 cells had a normal number of high affinity sites.

Table I. Summary of Data on Insulin Receptor Function in Leprechaunism

	Control	ARK-1	MINN-1	CAN-1
High affinity sites/cell	1,700 (1,300–2,000)	600	300	1,500
ED_{50} (ng/ml)	1–2	2–5	2–5	25
K_d ($\times 10^9$ M)	2.6	1.8	1.5	0.7
Autophosphorylation (% normal)	100	25	27	110
Tyrosine kinase activity (% normal)	100	43	32	33
Insulin processing				
Internalization (%)	66 (60–80)	60	84	75
Degradation (%)	31 (19–35)	18	24	28
Inhibition of degradation with chloroquine (%)	50	<5	<5	<5

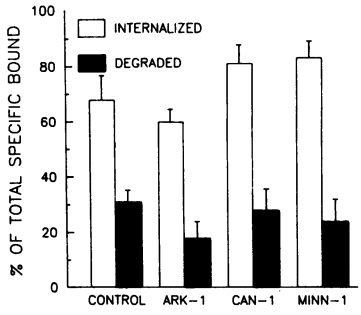

Figure 3. Insulin internalization and degradation. Insulin binding was carried out for 30 min at 37°C on confluent monolayers of fibroblasts from controls and patients and then internalization and degradation of internalized insulin determined as described in Methods. The results represent the mean±SEM (n = 5).

The time course of insulin binding, percent internalization and percent degradation of internalized insulin are depicted in Fig. 4. The effects of chloroquine (100 μM) on these processes is also shown. Specific insulin binding was reduced by 50% in the patient cell lines, but internalization of bound ligand was similar to that observed in controls at 15 and 30 min. Most striking were the differences in chloroquine effects on control and patient cell lines. Chloroquine slightly increased specific binding and initial internalization and reduced degradation in control cells by 50% at 30 min. In contrast, no significant chloroquine effect was seen with the leprechaun cell lines.

Receptor autophosphorylation and kinase activity. To study insulin receptor phosphorylation, partially purified insulin receptor preparations were used (Fig. 5). To avoid effects of variable antigenicity of the patients' cells' insulin receptors, samples were analyzed by direct SDS gel electrophoresis without precipitation by antiinsulin receptor antibodies. When used at equal receptor concentrations, preparations from both Ark-1 and Minn-1 demonstrated only 20% of the insulin stimulated β-subunit phosphorylation observed in control preparations. In contrast, the receptor preparation of Can-1 showed normal insulin stimulated receptor autophosphorylation observed in controls. This was observed in three separate experiments on different lots of receptor preparations. Similar results

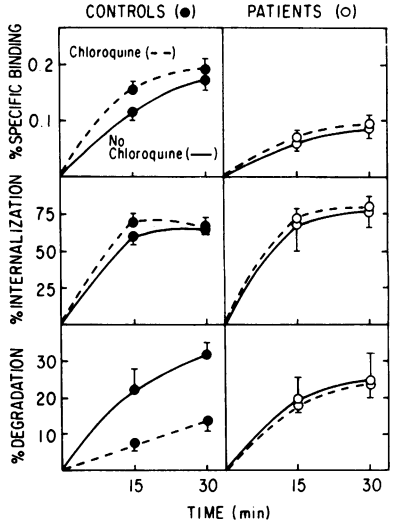

Figure 4. Effects of 100 μm chloroquine on insulin binding, internalization and degradation. ^{125}I-insulin binding, internalization and degradation was determined as described in Methods. The control data are represented on the left, and the patient data on the right. The points represent the means of three to five experiments.

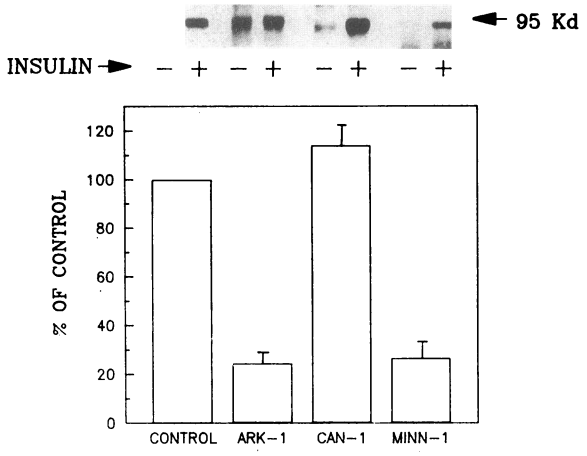

Figure 5. Insulin stimulated insulin receptor autophosphorylation. Partially purified receptor preparations from cells of controls and patients, normalized for binding were used. Autoradiographs of representative experiments are shown at the top, depicting β-subunit phosphorylation with and without insulin (10^3 ng/ml). Below are graphed the amount of insulin stimulated ^{32}P-incorporation into the β-subunit in the various insulin receptor preparations. The values represent the mean±SEM of four experiments. The data represent the value of [insulin-stimulated phosphorylation − basal phosphorylation] expressed as percentage of control stimulation (100%).

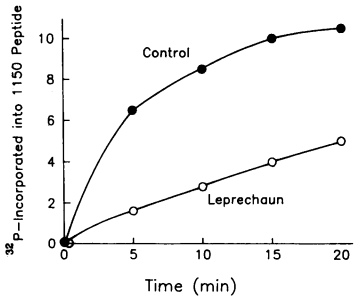

Figure 6. Time course of insulin stimulated ^{32}P-incorporation into synthetic peptide. A dodecapeptide corresponding to the 1142–1153 region of the β-subunit of the insulin receptor (1150 peptide) was used as the substrate for the receptor kinase as described in Methods. ^{32}P-incorporation (arbitrary units) as a function of time (min) is plotted for control and leprechaun receptor (Can-1) preparations.

were observed throughout the dose-response curve of insulin with no alteration in the ED_{50} (data not shown). Thus when corrected for insulin binding, insulin stimulated insulin receptor autophosphorylation appeared normal in Can-1 but reduced in Ark-1 and Minn-1 (Table II).

The ability of the partially purified insulin receptor to phosphorylate an exogenous substrate was also examined using a dodecapeptide corresponding to the 1142-1153 region of the amino acid sequence of the β-subunit. Over a 20-min incubation receptor preparations from all three leprechaun cell lines exhibited reduced tyrosine kinase activity compared to control (Fig. 6). When expressed relative to control, the insulin stimulated ^{32}P-incorporation into the 1142-1153 peptide was 43% for Ark-1, and only 22 and 24% for Can-1 and Minn-1, respectively (Fig. 7, *left*). This reduction in kinase activity in insulin receptors from the patients cells was also observed with another synthetic substrate, poly-Glu,Tyr (4:1) (Fig. 7, *right*). Therefore, although Can-1 receptor has normal β-subunit phosphorylation, it appears to have a defective tyrosine kinase activity. Table II shows the mean moles of ATP incorporation into the receptor or substrate, before and after insulin stimulation. Basal receptor autophosphorylation is higher in the patients with the highest occurring in Ark-1 (Table II).

Table II. Autophosphorylation and Tyrosine Kinase Activity of Insulin Receptors from Patients with Leprechaunism

	Autophosphorylation			Tyrosine kinase activity		
	Basal	+ Insulin	Difference	Basal	+ Insulin	Difference
	mol of ATP/min/mol receptor			*mol of ATP/min/mol receptor*		
Control	0.051	0.147	0.096	1.9	6.3	4.4
ARK-1	0.110	0.129	0.019	1.0	3.0	2.0
CAN-1	0.088	0.188	0.100	0.9	2.4	1.5
MINN-1	0.076	0.111	0.035	1.0	2.3	1.3

Insulin-stimulated insulin receptor autophosphorylation in partially purified receptor preparations from cells of controls and patients. Phosphorylation was carried out for 10 min at 23°C after steady-state insulin binding (10^3 ng/ml) at 4°C. The autophosphorylation data includes experiments for which β-scintillation counting data was available (n = 3). Insulin stimulated ^{32}P-incorporation into synthetic peptides (1150 peptide and poly-Glu,Tyr [4:1]) is tabulated on the right. Conditions are as described in Methods and Fig. 7.

Discussion

Fibroblasts have been useful in investigation of many genetic diseases including some forms of insulin resistance such as type A syndrome and leprechaunism (10–17, 24–28). These syndromes of insulin resistance have been suggested to have mutant forms of the insulin receptor based on family studies and persistence of defects in cultured cells. Characterization of such naturally occurring mutants could lead to many insights

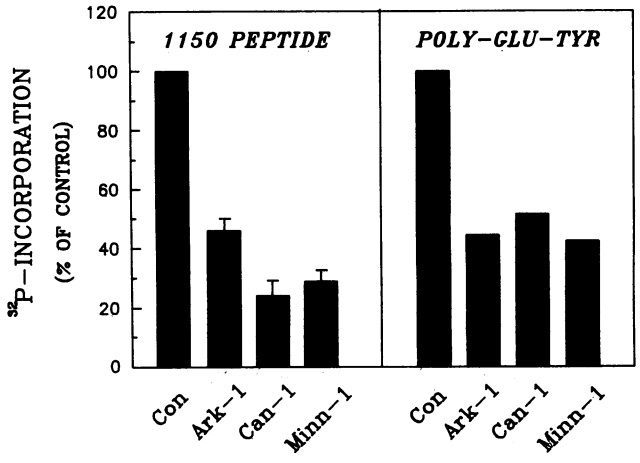

Figure 7. Insulin stimulated ^{32}P-incorporation into synthetic peptides. The tyrosine kinase activity of the partially purified receptors from the patients' cells is compared to that of the control insulin receptors. On the left are shown the 10-min points for the phosphorylation of the 1150 peptide, and on the right are shown the 15-min points for poly-Glu,Tyr (4:1) phosphorylation. The data represent the values of [total insulin-stimulated phosphorylation minus basal kinase activity] expressed as percentage of control stimulation.

into insulin action. We therefore have characterized insulin receptor function in three fibroblast cell lines of leprechaunism in an attempt to correlate alterations in insulin action with possible biochemical genetic defects in the insulin receptor.

Of the three leprechaun cell lines studied, Ark-1 and Minn-1 have a significant reduction of high affinity binding sites with minimal change in receptor affinity, while cells from Can-1 demonstrate only a slight reduction in number of high affinity binding sites but a significant decrease in affinity. Our findings with the cells of Ark-1 are in agreement with those of Elsas et al. who studied binding and cross-linking of insulin to fibroblasts (28, 29). With the exception of two studies (16, 26), all have shown a significant decrease in amount of insulin binding in fibroblasts of patients with leprechaunism. These findings have also been corroborated by other reports examining erythrocytes and EB virus transformed lymphocytes from Ark-1 and Minn-1 (19, 30, 31).

Affected cells have also been shown to be resistant to many but not all actions of insulin. In one cell line, thymidine incorporation only was reduced while in the cells of Can-1, glucose uptake and glycogen synthase activity were affected but not amino acid transport. Also, in the fibroblasts of Can-1, hydrogen peroxide but not insulin, was able to stimulate glucose uptake normally (14). In another cell line, the sensitivity of amino acid transport was reduced, while both sensitivity and responsivity of glucose incorporation were reduced (27). Thus the insulin resistance of leprechaunism can not be explained solely by a decrease in receptor number only. This has led some investigators to postulate postbinding defects in insulin action.

Over the past six years, the insulin receptor tyrosine kinase activity has been well characterized biochemically and increasing evidence has supported its role in mediating insulin action (4–9, 32, 33). With the cloning of the insulin receptor cDNA (32, 33) specific site-directed mutagenesis experiments (4–6, 33), as well as inhibition studies with specific antibodies (7–9), have determined that loss of the kinase activity can induce an insulin-resistant state. It has also been shown that insulin receptor autophosphorylation is critical in activating the receptor tyrosine kinase activity.

In this study we find that insulin stimulated autophosphorylation of the β-subunit is normal in Can-1 but reduced by nearly 80% in Ark-1 and Minn-1. On the other hand, when the tyrosine kinase activity was examined using two different synthetic substrates, all three patients' receptors displayed greater than a 60% decrease in insulin-stimulated kinase activity. From the results of the autophosphorylation data, one expected reduced kinase activity in the receptors of Ark-1 and Minn-1, but not in the insulin receptor of Can-1. Thus Can-1 demonstrates a new type of insulin receptor defect with intact autophosphorylation and reduced tyrosine kinase activity. Interestingly, some of the in vitro mutant cell lines with a defective insulin receptor kinase also had higher basal levels of autophosphorylation (6).

Insulin-stimulated autophosphorylation in leprechaunism has been previously studied only in EB virus transformed lymphocytes (34) and erythrocytes of Ark-1 (30). Although the assays were not corrected for insulin binding activity, autophosphorylation of the insulin receptor was within the normal range in the transformed cells but significantly reduced in erythrocytes. Different antiinsulin receptor antibodies (B-10 and B-8) used in the two studies may be the cause of these discordant results, since the antigenicity of the insulin receptor of leprechaunism may be different (30, 34). In this study we avoided this variable by directly analyzing the receptor preparations without immunoprecipitation. Despite the quantitative differences in phosphorylation, no difference in mobility of the β-subunit of the patient cell lines was noted.

Schilling et al. found IGF-I binding to be reduced in the fibroblasts of Can-1 (14), and Craig et al. found reduced MSA binding in the fibroblasts of Ark-1 (27) suggesting that the role of IGF-I receptors in confounding the data is likely to be minimal. Scatchard analyses gave curvilinear plots typical of the insulin receptor while IGF-I binding to IGF-I receptors normally produces linear Scatchards (14). Also, our dose-response curves of receptor autophosphorylation, did not reveal any rightward shift that might be expected if the IGF-I receptor was significantly contributing to the observed activity.

Insulin internalization and degradation has not been previously studied in leprechaunism; however, one might predict that these processes would be hampered by the reduced specific insulin binding and/or might be altered in parallel with the kinase activity. Interestingly, the percent internalization was not significantly different between the cells of the patients and those of controls, while degradation of internalized insulin in the patients' cell lines correlated with the number of high affinity receptors/cell and amount of insulin stimulated receptor autophosphorylation. Thus the cells of Can-1 had normal amounts of degradation when normalized for insulin binding, while cells of Ark-1 and Minn-1 had slightly reduced levels of degradation. Also, it was found that the degradation of internalized insulin was reduced by 50% in control cells treated with 100 μM chloroquine, whereas the degradation was unaffected in the patients' cell lines. Whether this indicates different contributions of lysosomal and nonlysosomal degradative pathways in the different cell lines (perhaps secondary to reduced receptor-mediated endocytosis) or a reduced sensitivity to chloroquine is not known.

In contrast to our findings, recent evidence from in vitro mutagenesis experiments (35, 36) and isolated rat adipocyte experiments (37), showed that mutants with no tyrosine kinase activity have reduced rates of internalization and that the adipocyte insulin receptors with activated kinase are preferentially internalized. Also, a hybrid receptor in which the β-subunit is replaced by a heterologous tyrosine kinase, v-ros, displayed essentially normal internalization and processing (38). These data suggest that some form of tyrosine kinase activity is important in internalization or that there may be a critical level of tyrosine phosphorylation needed for internalization. In contrast, in the patient cell lines, despite a 70% reduction in tyrosine kinase activity and in autophosphorylation, internalization proceeded normally. Thus, it is clearly possible to dissociate the tyrosine kinase activity and the internalization process. Perhaps, the exact nature of the mutation is important. Receptor phosphorylation does not appear to be necessary for receptor-mediated ligand internalization of the growth hormone (39) or EGF (40).

Regarding the inheritance of leprechaunism, the cells of the parents of Ark-1 have been studied by several laboratories (29–31). Both parents are phenotypically normal, but the father has a moderate degree of insulin resistance and the transformed cells of the mother have been shown to have qualitative defects in insulin binding. It is thought that Ark-1 may have inherited an abnormal recessive insulin receptor allele

from each parent. Restriction fragment length analysis of the insulin receptor gene from the parents and Ark-1 has not revealed any abnormalities.[2]

In conclusion, we have demonstrated biochemical heterogeneity of the insulin receptor in three cultured cell lines of leprechaunism. Two different types of defects of insulin receptor autophosphorylation and tyrosine kinase activity exist in these cell lines. Two cell lines (Ark-1 and Minn-1) exhibit reduction in both autophosphorylation and kinase activity while the cells of Can-1 exhibit a decrease in kinase activity with no reduction in autophosphorylation. This is a new type of insulin receptor defect with discordance between receptor autophosphorylation and tyrosine defect and implies that insulin receptor autophosphorylation and tyrosine kinase activity, though very closely associated, can be controlled selectively. Thus decreased receptor autophosphorylation will lead to decreased tyrosine kinase activity, but the converse is not necessarily true. There appears to be some correlation with the receptor defects and insulin processing by the fibroblasts. Possible structural differences in their insulin receptors are currently being investigated.

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Note added in proof. Since submission of this manuscript, the insulin receptor cDNA from patient Ark-1 has been cloned and sequenced (41). Mutations have been found in both alleles in the region of the α-subunit. One mutation leads to a substitution of lysine at position 460 by glutamic acid; the other leads to a premature stop codon.

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