Small Molecule Insulin Receptor Activators Potentiate Insulin Action in Insulin-Resistant Cells

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In type 2 diabetes, impaired insulin signaling leads to hyperglycemia and other metabolic abnormalities. To study a new class of antidiabetic agents, we compared two small, nonpeptide molecules that activate insulin receptor (IR) β-subunit tyrosine kinase activity: Merck L7, a direct IR agonist, and Telik's TLK16998, an IR sensitizer. In rat hepatoma cells (HTCs) that overexpress the IR (HTC-IR), IR autophosphorylation was directly activated by L7 in the absence of insulin. TLK16998 did not directly activate IR autophosphorylation, but it enhanced IR autophosphorylation in the presence of insulin. Tyrosine phosphorylation of an endogenous 185-kDa IR substrate was also significantly enhanced by both Merck L7 alone and TLK16998 plus insulin. Adding TLK16998 to L7 produced synergistic effects, further indicating that these two compounds act on the IR through separate mechanisms. We next studied HTC-IR$_{\Delta 485–599}$ cells, which overexpress a mutant IR with a deletion in the α-subunit connecting domain that does not undergo autophosphorylation in response to insulin binding. L7 was able to directly activate autophosphorylation of the deletion mutant IR in these cells, whereas TLK16998 had no effect. Compounds were then tested in three other cell models of impaired IR function. Both TLK16998 and Merck L7 improved IR autophosphorylation in cells with diminished IR signaling due to either treatment with tumor necrosis factor-α or overexpression of membrane glycoprotein PC-1. However, in TPA (tetradecanoylphorbol acetate)-treated cells, TLK16998 but not Merck L7 was able to significantly reverse the impaired insulin-stimulated IR autophosphorylation. In summary, these two classes of IR activators selectively increased IR function in a variety of insulin-resistant cell lines. *Diabetes* 50:2323–2328, 2001

The cellular response to insulin is mediated through the insulin receptor (IR), which is a tetrameric protein consisting of two identical extracellular α-subunits that bind insulin as well as two identical transmembrane β-subunits that have intracellular tyrosine kinase activity (1,2). When insulin binds to the α-subunit of the receptor, the β-subunit tyrosine kinase is activated, resulting in autophosphorylation of β-subunit tyrosine residues (3). After autophosphorylation, the activated receptor phosphorylates endogenous substrates, such as insulin receptor substrate (IRS)-1 and -2 (4,5). Phosphorylated tyrosine residues on these substrates then bind to a variety of other substrates and insulin action ensues (1–6). It is well established that insulin signaling, including activation of IR tyrosine kinase activity, is impaired in most patients with type 2 diabetes (7,8). This resistance to insulin then leads to hyperglycemia and other metabolic abnormalities of the disease (9,10).

Therefore, pharmacological agents that enhance IR β-subunit tyrosine kinase activity would be useful in the treatment of type 2 diabetes. Recently, two small nonpeptide molecules that enhance insulin action in cultured cells have been reported: Merck L-7, a direct IR agonist (11), and Telik 16998, an IR sensitizer (12). Both molecules have been reported to interact with the IR β-subunit. In conditions where IR signaling is impaired, however, it is unclear whether these compounds could overcome defects in IR signaling. In the present study, therefore, we studied and compared these agents in cultured cells with induced defects in IR activation.

RESEARCH DESIGN AND METHODS

Compounds. Compounds Merck L7 and TLK16998 were kindly provided by Merck (Rahway, NJ) and Telik (South San Francisco, CA), respectively. Monoclonal anti-IR α-subunit antibody, MA 20, was produced as previously described (13). Biotin-conjugated anti-phosphotyrosine antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Phorbol 12-myristate 13-acetate (TPA) was from Sigma Chemical (St Louis, MO), and recombinant tumor necrosis factor (TNF)-α was from Biosource International (Camarillo, CA). All other reagents were of the highest quality commercially available.

Cell culture. Rat hepatoma cells (HTCs) that overexpress human IR, or HTC-IR cells, as well as HTC-IR PC1 cells (HTC-IR cells transfected with and overexpressing membrane glycoprotein PC-1) and HTC-IR$_{\Delta 485–599}$ cells (HTC-IR cells transfected with and overexpressing IR, with a deletion of amino acids 485–599) were produced as previously described (14,15). All cell lines were cultured in Dulbecco's modified Eagle's medium containing 1.0 g/l glucose supplemented with 10% fetal calf serum. Penicillin (10 units/ml), fungizone (0.25 μg/ml), and streptomycin (10 μg/ml) were routinely added to cultures, and all cell lines were cultivated at 37°C in a 5% CO_2–enriched humidified atmosphere.

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B.B.Z. is an employee of and holds stock in Merck.

ANOVA, analysis of variance; CD, connecting domain; ELISA, enzyme-linked immunosorbent assay; HTC, hepatoma cell; IR, insulin receptor; IRS, insulin receptor substrate; TNF, tumor necrosis factor; TPA, phorbol 12-myristate 13-acetate.

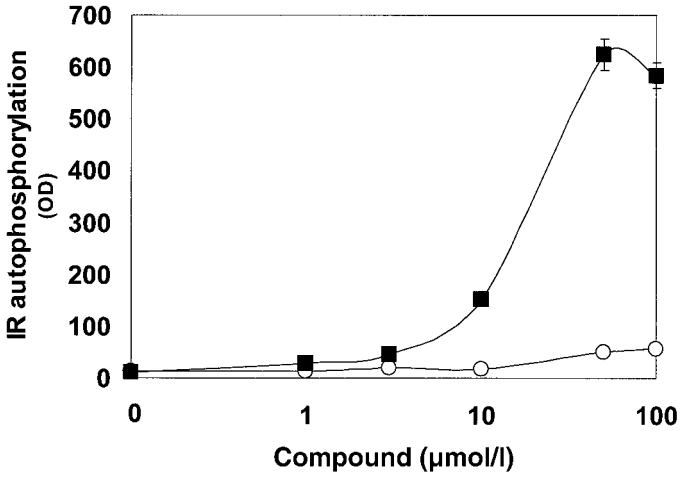

FIG. 1. Effects of Merck L7 and TLK16998 on IR β-subunit autophosphorylation of HTC-IR cells. Cells were incubated for 10 min at 37°C in the presence of Merck L7 (■) or TLK16998 (○) at concentrations indicated before solubilization. IR β-subunit autophosphorylation was measured by ELISA. Shown is one representative experiment of four total. Values are means ± SE of the OD (×1,000) from triplicate determinations.

Insulin stimulation and treatment of cells with IR activators. Cells were grown in 12-well plates until confluent and were then serum starved for 2 h before preincubation with the compounds. For studies involving Merck L7, a stock solution of 20 mmol/l L7 in DMSO was prepared before each experiment. Merck L7 was then diluted to desired concentrations in cell medium with ascorbic acid (final concentration 200 μg/ml) to improve the stability of the compound. Cells were then exposed to various concentrations of Merck L7 for 10 min at 37°C. Untreated control cells were incubated in 0.1% DMSO and 200 μg/ml ascorbic acid. TLK 16998 stock solution was prepared by dilution in DMSO alone. The final concentration of DMSO in treated and control cells was 0.1%. Studies on the maximal effects of Merck L7 produced final DMSO concentrations >0.1%. The treatment of control cells in these studies with DMSO concentrations ≤0.5% had no effect on IR autophosphorylation (data not shown). Incubation with TLK16998 was for 10 min at 37°C. After treatment with the compounds, insulin (0–100 nmol/l final concentration) was added to the cell media for 10 min at 37°C. After treatment, the cells were washed three times with ice-cold phosphate-buffered saline and solubilized in lysis buffer (50 mmol/l HEPES, pH 7.6, 150 mmol/l NaCl, 1% Triton X-100, 1 mmol/l phenylmethylsulfonyl fluoride, and 2 mmol/l sodium orthovandate) for 60 min at 4°C. After removal of cellular debris (15,000*g* for 30 min at 4°C), the protein content in each sample was measured using Bio-Rad (Richmond, CA) protein assay reagent according to the manufacturer's instructions.

Enzyme-linked immunosorbent assay for whole-cell IR autophosphorylation. The enzyme-linked immunosorbent assay (ELISA) for tyrosine phosphorylation of the IR was performed as previously described (16). Equal amounts of cell lysates (50 μg of protein) were applied to 96-well ELISA plates coated with monoclonal anti–human IR antibody, MA-20. IRs from the samples were allowed to bind during an 18 h incubation at 4°C. Next, the 96-well ELISA plates were washed with Tris-buffered saline with Tween (NaCl 150 mmol/l, Tween 20 0.05%, Tris 20 mmol/l, pH 7.4). Biotin-conjugated anti-phosphotyrosine antibody was added to the wells for a 2-h incubation at 22°C. Wells were washed again and then incubated with streptavadin-horseradish peroxidase. After the addition of a peroxidase substrate, 3,3′,5,5′-tetramethylbenzidine (TMB), the degree of tyrosine phosphorylation was quantified by reading the optical density at 451 nm by a plate reader.

Western blotting. After treatment with insulin in the presence or absence of various compounds, cells were solubilized and cell lysates were normalized for protein. Equal amounts of the lysates (30 μg of protein) were diluted in 2× Laemmli reducing buffer and were subjected to SDS-PAGE, followed by transfer to nitrocellulose membranes (Amersham). The membranes were blocked with 5% nonfat milk and then incubated with phosphotyrosine-specific antibody (anti-phosphotyrosine). Immunoreactive proteins were visualized with horseradish peroxidase–coupled anti-mouse IgG and developed with enhanced chemiluminescence reagents as instructed by the manufacturer (NEN Life Science Products). The signals on the blot were quantified by scanning densitometry.

Statistics. The effects of the compounds, in combination with insulin or across multiple cell lines, were examined by a two-factor analysis of variance (ANOVA). Comparison of multiple treatment conditions in a single cell line was accomplished by single-factor ANOVA. Post hoc analysis was by paired Student's *t* test when a significant interaction was obtained. All analyses were performed with significance at $P < 0.05$.

RESULTS

Studies in normal cells.

Direct effects of compounds on IR autophosphorylation of HTC-IR cells. HTC-IR cells, which are differentiated rat HTCs that express the human IR, were incubated with various concentrations of Merck L7 and TLK16998 in the absence of insulin. Merck L7 increased IR β-subunit autophosphorylation with a detectable effect at 10 μmol/l and a maximal effect at 50 μmol/l (Fig. 1). In contrast, IR β-subunit autophosphorylation was not increased by incubation with TLK16998 (Fig. 1).

Effects of compounds on insulin-stimulated IR tyrosine autophosphorylation of HTC-IR cells. To examine the ability of the compounds to potentiate the effects of insulin, HTC-IR cells were pretreated with either Merck L7 or TLK16998, followed by incubation with various concentrations of insulin. Exposure of HTC-IR cells to insulin alone activated IR β-subunit autophosphorylation with a detectable effect at 300 pmol/l and maximal effects at 100 nmol/l (Fig. 2). At 20 μmol/l, TLK16998 increased the autophosphorylation of the IR induced by each concentration of insulin (Fig. 2). This insulin-potentiating effect of TLK16998 was dose-dependent (Fig. 2) and reached a maximal effect at 50 μmol/l (data not shown).

At 50 μmol/l, Merck L7 increased IR β-subunit autophosphorylation to a value similar to that of a maximal concentration of insulin (100 nmol/l) (Fig. 3). When cells were incubated with both 50 μmol/l Merck L7 and 100 nmol/l insulin, IR autophosphorylation was significantly greater than with L7 alone. However, the combined stimulatory effect of insulin and Merck L7 just missed statistical significance compared with the effect of insulin alone ($P =$

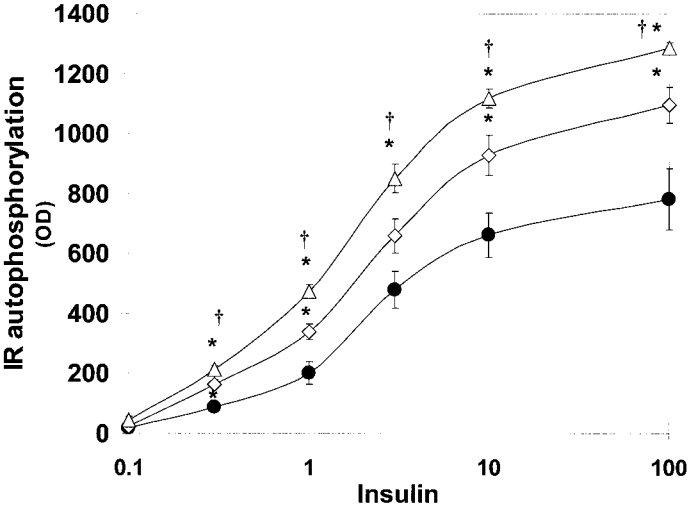

FIG. 2. Effects of TLK16998 on the insulin dose-response of IR β-subunit autophosphorylation in HTC-IR cells. Cells were incubated in the presence or absence of TLK16998 at the concentrations indicated for 10 min before insulin stimulation (additional 10-min incubation with insulin at doses from 0 to 100 nmol/l at 37°C). IR β-subunit autophosphorylation was measured by ELISA. Values are means ± SE of the OD (×1,000) from four experiments. *Significantly greater than insulin alone; †significantly greater than insulin plus 20 μmol/l TLK16998 ($P < 0.05$). ●, control; ◇, 20 μmol/l; △ 50 μmol/l.

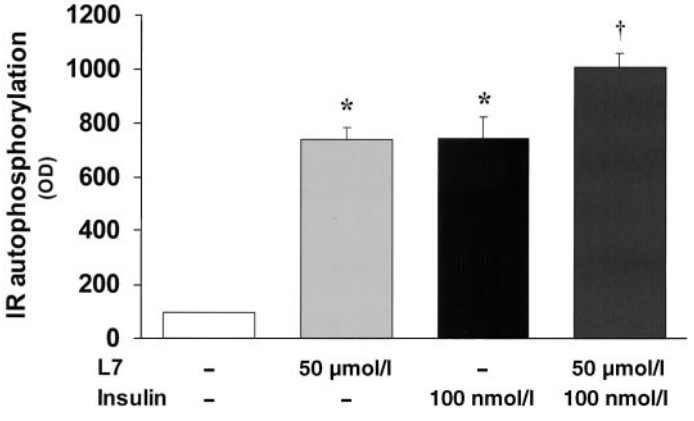

FIG. 3. Effects of Merck L7 on insulin-stimulated IR β-subunit autophosphorylation in HTC-IR cells. HTC-IR cells were stimulated with 100 nmol/l insulin for 10 min at 37°C with or without pretreatment for 10 min with Merck L7 (50 μmol/l). Cells were lysed, and IR β-subunit autophosphorylation was measured by ELISA. Values are means ± SE of the OD (×1,000). *Significantly greater than basal; †significantly >50 μmol/l Merck L7 ($P < 0.05$).

0.052) and did not represent a fully additive effect of the IR agonists.

Merck L7 and TLK16998 were then added in combination (Fig. 4). At 10 μmol/l, TLK16998 had no effect on IR β-subunit autophosphorylation. However, this concentration of the compound potentiated the effects of submaximal (10 μmol/l) and maximal (50 μmol/l) concentrations of Merck L7. Thus, TLK16998, though unable to induce IR autophosphorylation alone, was able to potentiate the effects of submaximal and maximal doses of two IR agonists, insulin and Merck L7.

Western blots. The effects of the two compounds on IR signaling in HTC-IR cells was further analyzed by Western blot experiments. Lysates of cells stimulated with insulin in the absence or presence of Merck L7 or TLK16998 were subject to SDS-PAGE and probed with anti-phosphotyrosine antibodies. A band of ~185 kDa was detected, with a signal intensity that was minimal in unstimulated cells and significantly enhanced by insulin (Fig. 5). This band was not positively identified but likely represents the tyrosine-phosphorylated form of IRS-1 and/or IRS-2. The magnitude of pp185 tyrosine phosphorylation was dose-dependent on insulin. Incubation of cells with 50 μmol/l Merck L7 increased tyrosine phosphorylation of pp185. Tyrosine phosphorylation of pp185 was unaffected by incubation with 20 μmol/l Telik 16998. In cells exposed to both insulin and TLK16998, the tyrosine phosphorylation of the pp185 band was greatly increased over cells treated with insulin alone. Thus, the pattern of IR autophosphorylation induced by Merck L7 and TLK16998, as well as insulin, is representative of the ability of these compounds to stimulate downstream signaling by the IR. Together, these studies in insulin-responsive HTC-IR cells indicate that Merck L7 is an IR agonist, whereas TLK16998 is an IR potentiator. Both compounds were able to increase IR signaling in HTC-IR cells concurrently with exposure to submaximal and maximal insulin concentrations.

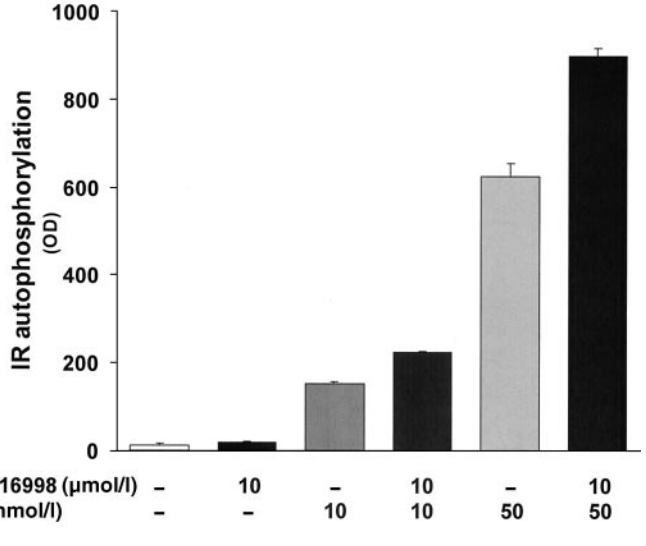

FIG. 4. TLK16998 potentiates the effects of Merck L7 on IR β-subunit autophosphorylation in HTC-IR cells. HTC-IR cells were pretreated for 10 min with Merck L7 at submaximal (10 μmol/l) or maximal (50 μmol/l) concentrations. Cells were then incubated in the presence or absence of 10 μmol/l TLK16998. Cells were lysed, and the ELISA for IR β-subunit autophosphorylation was performed as described. Shown is one representative experiment of four total. Values are means ± SE of the OD (×1,000) from triplicate determinations.

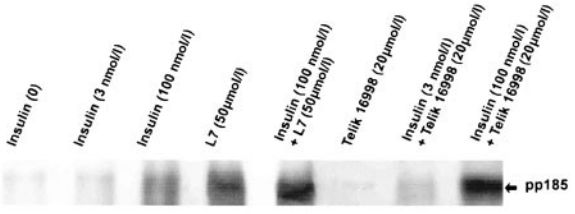

FIG. 5. Immunoblotting of p185 of HTCs. Lysates were prepared from cells incubated with insulin (0, 3, and 100 nmol/l) for 10 min or incubated with Merck L7 (50 μmol/l) or TLK16998 (20 μmol/l) for 10 min and then insulin (0, 3, and 100 nmol/l) for an additional 10 min. Equal amounts of soluble protein from each preparation were loaded onto polyacrylamide gels, resolved by electrophoresis, and transferred to nitrocellulose membranes that were subsequently incubated with phosphotyrosine-specific antibody (anti-phosphotyrosine). Tyrosine phosphorylation of p185 was visualized with horse-radish peroxidase-coupled anti-mouse IgG and ECL reagents as described in RESEARCH DESIGN AND METHODS.

Effect of compounds on an IR deletion mutant. HTC-$IR_{\Delta 485–599}$ cells have a deletion at the tyrosine kinase regulatory site in the α-subunit connecting domain (CD), located in amino acids 485–599 (15). In these cells, binding of insulin to the α-subunit does not lead to activation of the β-subunit tyrosine kinase (15). The small effect of insulin in these cells (Fig. 6) in the present study is likely the result of endogenous rat IR. Whether alone or in addition to insulin, TLK16998 had no effect on IR autophosphorylation in HTC-$IR_{\Delta 485–599}$ cells (Fig. 6). Incubation of these cells with 50 μmol/l Merck L7, however, resulted in a significant increase in IR autophosphorylation.

Effects of compounds on IR tyrosine autophosphorylation in insulin-resistant cells.

To investigate the effects of Merck L7 and TLK19668 on insulin-resistant cells, we exposed HTC-IR cells to either TNF-α or TPA. In addition, we used transfected HTC-IR cells that overexpress membrane glycoprotein PC-1. Each of these treatments resulted in a significant impairment in the ability of insulin to stimulate IR autophosphorylation (Fig. 7). We then tested the effects of pretreating insulin-resistant cells with Merck L7 or TLK16998 before exposure to 100 nmol/l insulin. Treatment of cells overexpressing

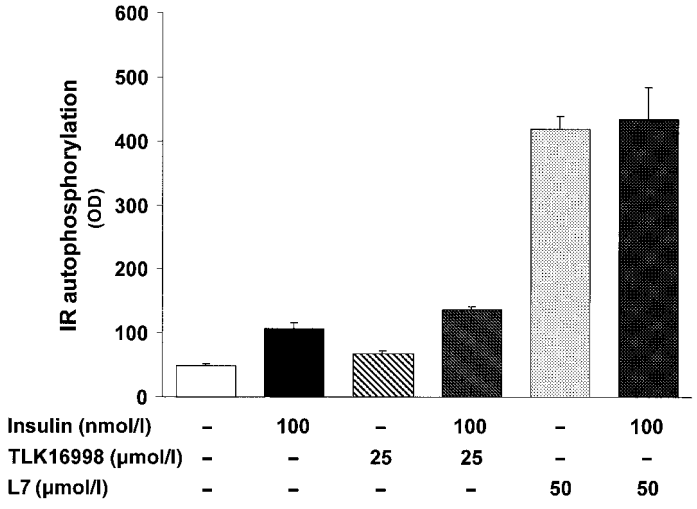

FIG. 6. Effects of Merck L7 and TLK16998 on insulin dose-response of HTC-IR$_{\Delta 485-599}$ cells autophosphorylation. HTCs transfected with and overexpressing CD deletion mutant IR were preincubated for 10 min with either Merck L7 (50 μmol/l) or TLK16998 (25 μmol/l). Cells were then stimulated with 100 nmol/l insulin for 10 min at 37°C. Cells were solubilized, and the ELISA for IR β-subunit autophosphorylation were performed as described in RESEARCH DESIGN AND METHODS. Shown is one representative experiment of three total. Values are means ± SE of the OD (× 1,000) from triplicate determinations.

PC-1 and cells exposed to TNF-α with either Merck L7 or TLK16998 resulted in IR autophosphorylation levels significantly greater than those induced by insulin alone (Table 1). In contrast, although Merck L7 was not able to significantly increase IR signaling above the effect of insulin alone, TLK16998 was able to enhance insulin-stimulated IR autophosphorylation in TPA-treated cells.

DISCUSSION

In this study, we observed that micromolar concentrations of both Merck L7 and TLK16998 activated the IR in insulin-sensitive HTC-IR cells and reversed impaired IR function in insulin-resistant HTC-IR cells. This drug effectiveness was evidenced by the increased autophosphorylation of IR β-subunit tyrosine kinase as well as endogenous substrate phosphorylation. These findings are in agreement with the recent report by Zhang et al. (11), which described a direct agonist effect of Merck L7 on insulin-induced IR tyrosine kinase activity and other functions in CHO-IR cells. Merck L7 also lowered blood glucose levels in diabetic mice (11). These findings are also in agreement with the study of Manchem et al. (12), in which TLK16998 potentiated insulin-stimulated IR autophosphorylation with subsequent enhancement of other functions in 3T3-L1 adipocytes as well as lowered blood glucose values in diabetic mice.

Based on structure, it is expected that the two compounds would have different mechanisms of action on the IR. Merck L7 has a quinone-like structure, whereas TLK16998 is a polysulfonic acid. Both compounds do not act on the IR binding site but rather act on the IR β-subunit, where they specifically enhance β-subunit autophosphorylation and subsequent downstream signaling (11,12). Phosphopeptide mapping studies demonstrate that TLK16998 produces a peptide phosphorylation pattern distinct from that of insulin (11), which may help to explain how this compound is able to produce a greater degree of tyrosine phosphorylation than insulin alone and why it can function independently of insulin in insulin-resistant cells.

TABLE 1
Insulin (100 nmol/l)-induced IR autophosphorylation in the presence of either TLK16998 (50 μmol/l) or Merck L7 (50 μmol/l) in insulin-resistant cells.

	Insulin (100 nmol/l)	Insulin + L7 (50 μmol/l)	Insulin + TLK16998 (50 μmol/l)
Control	100 ± 13	135 ± 7*†	165 ± 2*†
TNF-α–treated	50 ± 3‡	164 ± 16*†	102 ± 1*
TPA-treated	39 ± 5‡	52 ± 9‡	93 ± 24*
HTC-IR PCI	43 ± 4‡	67 ± 3*‡	71 ± 8*

Data are means ± SE of the percent of insulin-stimulated IR tyrosine phosphorylation of control (untreated cells). *Significant effect of the compound plus insulin over insulin alone in each cell line; †values significantly greater than those of insulin-stimulated control cells; ‡values significantly reduced compared with insulin-stimulated control cells ($P < 0.05$).

Although it is established that both compounds function to enhance IR autophosphorylation in normal cell lines, it was not clear whether these compounds could act to overcome insulin resistance in cells where IR signaling is impaired. To address this question, we first studied HTC-IR cells that overexpress a mutant IR from which the amino acids 485–599 are deleted. This mutant receptor binds insulin, but the binding does not activate tyrosine kinase activity (15). Luo et al. (17) described a model of the tertiary structure of the human IR based on scanning transmission electron microscope. They elucidated a CD in the α-subunit that transmits the activation of the receptor by ligand binding to the β-subunit. The amino acids 485–599, which are deleted in the mutant IR, are in the CD. Thus, autophosphorylation in the mutant IR is blocked due to an inability of this receptor to produce a conformational change in response to insulin binding. Merck L7 was able to activate the IR$_{\Delta 485-599}$ mutant, demonstrating the inde-

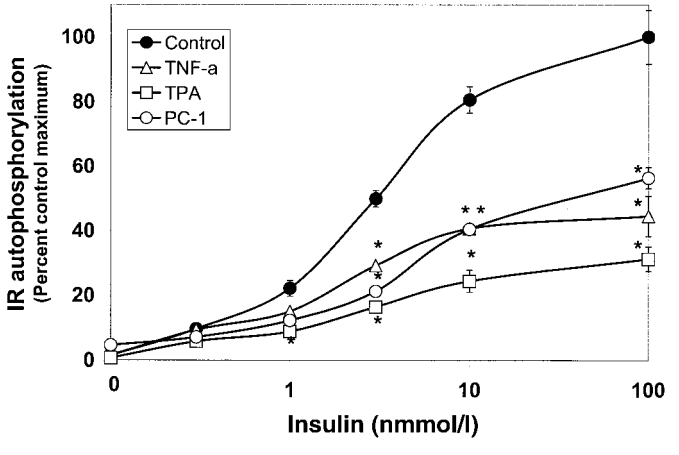

FIG. 7. Induction of impaired IR function in HTC-IR cells. Insulin resistance in HTC-IR cells was induced by incubating cells with either TNF-α (1.2 nmol/l for 18 h), TPA (1 μg/ml for 30 min), or regular medium (control). HTC-IR PC1 cells were transfected with and overexpressed human PC-1. Cell lines thus treated were then incubated with various concentrations of insulin for 10 min at 37°C. Cells were lysed, and IR β-subunit autophosphorylation was measured by ELISA. Values are means ± SD of the fold increase of IR autophosphorylation over basal from three to five experiments per condition. *Significantly diminished compared with control cells ($P < 0.05$).

pendence of this compound on the conformational change of the IR. This is consistent with the earlier finding that L7 is capable of activating the tyrosine kinase of a chimeric receptor comprised of the extracellular domain of the IR-related receptor and the intracellular domain of the IR (11). These distinct methods of activating the IR β-subunit provide potential explanations for the partial additivity of insulin and Merck L7. In contrast, the fact that TLK16998 is unable to induce autophosphorylation of $IR_{\Delta 485–599}$ suggests that the interaction of the Telik compound with the IR may be dependent on the conformational change in the IR induced by insulin.

We next studied HTC-IR cells made insulin resistant by three different mechanisms: PC-1 overexpression, incubation with TNF-α, and incubation with phorbol esters. These studies were carried out to evaluate the potential of the compounds to overcome separate models of impaired insulin-stimulated IR signaling. The membrane glycoprotein PC-1 is overexpressed in cells of insulin-resistant patients and inhibits insulin activation of the IR by binding to the CD of the α-subunit (14,18,19). When HTC-IR cells were made to overexpress PC-1, IR autophosphorylation was decreased by 57%. Both Merck L7 and TLK16998 increased insulin-stimulated IR autophosphorylation in HTC-IR PC-1 cells. In the case of cells treated with TLK16998, insulin-stimulated IR autophosphorylation was not different from that in control cells not transfected by PC-1.

TNF-α inhibits IR function in cells most likely via several mechanisms. One is via ceramide production and serine phosphorylation and inactivation of the IR and IRS proteins (20–22). Treatment of HTC-IR cells with TNF-α decreased the maximal insulin stimulation of IR autophosphorylation by 50%. In TNF-α cells that were exposed to either TLK 16698 or Merck L7, insulin-stimulated IR autophosphorylation was significantly increased by exposure to either TLK16698 or Merck L7. Insulin-stimulated IR autophosphorylation in TNF-α–treated cells exposed to Merck L7 significantly exceeded insulin-stimulated values from insulin-sensitive control cells. IR autophosphorylation in insulin-stimulated TNF-α–treated cells exposed to TLK16998 was not different from that in insulin-sensitive control cells.

Phorbol esters cause insulin resistance via activation of protein kinase C isoforms (23–25). The exact isoforms involved, however, are not well defined. TPA treatment decreased IR autophosphorylation of HTC-IR cells. In contrast to other insulin-resistant conditions, TLK16698, but not Merck L7, effectively improves insulin-stimulated IR autophosphorylation.

It is not clear why the compounds have varying degrees of effectiveness at overcoming different models of impaired IR function. PC-1 has been demonstrated to interact with the 485–599 region of the IR, apparently blocking the conformational change in the IR normally induced by insulin binding (17). In this model, PC-1 overexpression and subsequent interaction with the IR might be considered to function similarly to the deletion and inactivation of the CD region of the IR. Thus, it is not surprising that the Merck L7 compound is able to activate IR in both $HTC\text{-}IR_{\Delta 485–599}$ and PC-1–overexpressing cells. TLK16998, however, was able to potentiate the effect of insulin in PC-1–overexpressing cells but not $HTC\text{-}IR_{\Delta 485–599}$ cells. This is likely due to the fact that, unlike $HTC\text{-}IR_{\Delta 485–599}$ cells, insulin was effective in significantly increasing IR autophosphorylation in HTC-IR PC-1 cells, albeit to a much lower extent than in control HTC-IR cells. The explanation is likely that in these cells that overexpress both the IR and PC-1, only some fraction of the total cellular IR are interacting with and being inhibited by PC-1. It is unknown whether TLK16998 exerts its effects in these cells by overcoming the inhibition of PC-1 on inhibited IR, or whether the potentiating effects of the compound result from interaction with the normally functioning IR. It is clear, however, that TLK16998 was able to potentiate the effects of insulin on these insulin-resistant cells to the extent that there was no significant difference in insulin-stimulated IR autophosphorylation between control and HTC-IR PC-1 cells treated with TLK16998.

In summary, we found that two new, nonpeptide, small molecules that act directly on the IR β-subunit are active in various cultured cells that are insulin resistant at the level of IR activation. These two compounds are of different structure and have different mechanisms of action. Because insulin resistance is a major feature of type 2 diabetes, it is likely that similar agents may eventually prove useful in the treatment of this disease.

ACKNOWLEDGMENTS

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