Recent studies have identified a β-cell insulin receptor that functions in the regulation of protein translation and mitogenic signaling similar to that described for insulin-sensitive cells. These findings have raised the novel possibility that β-cells may exhibit insulin resistance similar to skeletal muscle, liver, and fat. To test this hypothesis, the effects of tumor necrosis factor-α (TNFα), a cytokine proposed to mediate insulin resistance by interfering with insulin signaling at the level of the insulin receptor and its substrates, was evaluated. TNFα inhibited p70s6k activation by glucose-stimulated β-cells of the islets of Langerhans in a dose- and time-dependent manner, with maximal inhibition observed at ~20–50 ng/ml, detected after 24 and 48 h of exposure. Exogenous insulin failed to prevent TNFα-induced inhibition of p70s6k, suggesting a defect in the insulin signaling pathway. To further define mechanisms responsible for this inhibition and also to exclude cytokine-induced nitric oxide (NO) as a mediator, the ability of exogenous or endogenous insulin ± inhibitors of nitric-oxide synthase (NOS) activity, aminoguanidine or N-nitro-L-arginine, was evaluated. Unexpectedly, TNFα and also interleukin 1 (IL-1)-induced inhibition of p70s6k was completely prevented by inhibitors that block NO production. Western blot analysis verified inducible NOS (iNOS) expression after TNFα exposure. Furthermore, the ability of IL-1 receptor antagonist protein, IRAp, to block TNFα-induced inhibition of p70s6k indicated that activation of intra-islet macrophages and the release of IL-1 that induces iNOS expression in β-cells was responsible for the inhibitory effects of TNFα. This mechanism was confirmed by the ability of the peroxisome proliferator-activated receptor-γ agonist 15-deoxy-Δ12,14-prostaglandin J2 to attenuate TNFα-induced insulin resistance by down-regulating iNOS expression and/or blocking IL-1 release from activated macrophages. Overall, TNFα-mediated insulin resistance in β-cells is characterized by a global inhibition of metabolism mediated by NO differing from that proposed for this proinflammatory cytokine in insulin-sensitive cells.

A hallmark of human type 2 diabetes is an initial attenuated responsiveness of cells to insulin (a term designated as insulin resistance) that is countered or compensated by the ability of pancreatic β-cells to secrete more insulin than normally required to achieve euglycemia (1–3). Sustained hyperglycemia, however, occurs when the ability of β-cells to over-secrete insulin fails, resulting in overt diabetes. This sequence of events has generated the concept that a specific defect in the insulin-signaling pathway of insulin-sensitive cells such as skeletal muscle, fat, and liver is a primary cause for insulin resistance. As a secondary effect of insulin resistance, β-cell failure or the loss of the ability of β-cells to compensate has been proposed to be mediated by glucose toxicity, cellular exhaustion, and/or other undefined cellular mechanisms (4–6). More recent findings using gene knockout approaches have shown that disruption of insulin receptor substrate protein-2 (IRS-2) in mice results in defects in both insulin action and insulin secretion by β-cells, which closely mimics the development of human type 2 diabetes (7). Of particular significance, IRS-2 knockout mice exhibit a reduced mass of β-cells compared with wild-type mice, suggesting that this defect in the insulin signaling pathway also produces a functional defect in mitogenic signaling, resulting in decreased β-cell replication and growth. These novel results have altered this previous concept for the development of type 2 diabetes by demonstrating that disruption of a single gene product, i.e. IRS-2, involved in insulin signaling may be responsible for insulin resistance in insulin-sensitive tissues and also in pancreatic β-cells. Thus, β-cell failure may be because of insulin resistance rather than β-cell toxicity or exhaustion that occurs in parallel with the development of peripheral insulin resistance in type 2 diabetes.

Evidence in support of a functional β-cell insulin receptor has been reported recently by several groups. Studies by Rothenberg et al. (8) characterize an insulin-activated cell surface receptor tyrosine kinase and IRS-1 that associates with the 85-kDa α subunit of phosphoinositide 3-kinase in the β-cell line βTC3. Overexpression of the human insulin receptor in the β-cell line βTC6-F7 also regulates insulin gene expression and insulin content (9). Harbeck et al. (10) also describe expression of insulin receptor mRNA and IRS-1 in single primary rat β-cells. Furthermore, Leibiger et al. (11) show that endogenous insulin secreted from the β-cell line HIT promotes insulin bio-

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synthesis by enhancing insulin gene transcription in an autocrine manner. In addition, our laboratory presented findings suggesting that glucose-stimulated phosphorylation of the translational factors PHAS-I (also known as 4EBP-1) and p70S6K by β-cells of the islet is mediated via insulin interacting in an autocrine manner with its own insulin receptor (12). Our more recent studies show that amino acids, in particular branched-chain amino acids, stimulate phosphorylation of PHAS-I and p70S6K and are also essential for insulin and other growth factors to activate these same translational regulators (13).

An understanding of causes responsible for the development of insulin resistance in insulin-sensitive cells has focused on the close relationship between obesity and defects in insulin signaling. A large number of studies have identified the proinflammatory cytokine tumor necrosis factor (TNFα) as a potential mediator of insulin resistance associated with obesity. Overexpression of TNFα by fat tissue in both obese animals and humans has been correlated with the development of peripheral insulin resistance (14–19). A key observation indicating that TNFα may exert a role in the development of insulin resistance is that neutralization of TNFα in obese rats increases insulin sensitivity (20). In addition, TNFα also interferes with insulin signaling in vitro by inhibiting insulin receptor tyrosine kinase activity and tyrosine phosphorylation of insulin receptor substrates (17). In this latter case, TNFα induces serine phosphorylation of IRS-1, which then is believed to function as an inhibitor of insulin receptor tyrosine kinase activity.

Another important feature of insulin resistance is the ability of the PPARγ agonists troglitazone and a derivative of prostaglandin D3, 15-deoxy-Δ2,14-prostaglandin J2 (15d-PGJ2), to correct this defect (20, 21). The anti-diabetic agent troglitazone also ameliorates both insulin resistance and hyperinsulinemia in diabetic animals and type 2 diabetics (22, 23). Furthermore, troglitazone normalizes TNFα-induced inhibition of insulin-stimulated glucose disposal in rodents (24). Troglitazone and 15d-PGJ2 also prevent TNFα-induced inhibition of the most proximal steps in insulin signaling i.e. tyrosine phosphorylation of the insulin receptor and its substrate IRS-1 and activation of phosphoinositide 3-kinase in vitro (25). Of particular importance, troglitazone also improves the reduced response of β-cells to glucose observed in subjects with impaired glucose tolerance (26) and decreases the ratio of plasma proinsulin to immunoreactive insulin in type 2 diabetics (27). Overall, these findings suggest that troglitazone may improve β-cell function in addition to insulin sensitivity.

To further assess if defects in insulin signaling may negatively impact β-cells in a manner analogous to that described for insulin-sensitive cells, we have examined whether TNFα, an important mediator of insulin resistance in insulin-sensitive cells, also mediates insulin resistance in pancreatic β-cells. As a measure of β-cell insulin resistance, we have examined the phosphorylation level of p70S6K in response to stimulation by glucose or amino acids. This parameter has been shown to be a reliable measure of insulin action (28).

**EXPERIMENTAL PROCEDURES**

**Materials**—Male Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). CMRL-1066 and RPMI 1640 tissue culture media, penicillin, streptomycin, Hanks’ balanced salt solution, L-glutamine, minimum essential medium amino acid solution, and nonessential minimum essential medium solution were obtained from Life Technologies, Inc., Fetal bovine serum was from Hyclone (Logan, UT). Human recombinant interleukin-1β (IL-1β) was from Cistron Biotechnology (Pine Brook, NJ). Collagenase type P and human recombinant TNFα were from Roche Molecular Biochemicals. Purine insulin was from ICN (Aurora, OH). Ficoll and aminoguanidine were from Sigma.

NMMA was from Calbiochem. IRAP was from R&D Systems (Minneapolis, MN). Mouse macrophage nitric oxide synthase (iNOS) antibody and 15d-PGJ2 were from Cayman Chemical (Ann Arbor, MI). The antibody for p70S6K was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The secondary antibody was peroxidase-conjugated donkey anti-rabbit IgG, Jackson Immunoresearch Laboratories (West Grove, PA). Trolgitazone was a kind gift from John Johnson, Parke-Davis (Ann Arbor, MI). All other chemicals were from commercially available sources.

**Islet Isolation and Culture**—Islets were isolated from male Sprague-Dawley rats (200–250 g) by collagenase digestion as described previously (12, 13). Briefly, pancreases were inflated with Hank’s balanced salt solution, and the tissue was isolated, minced, and digested with 7 mg of collagenase/pancreas for 7 min at 38 °C. Islets were separated on a Ficoll step density gradient and then selected with a stereomicroscope to exclude any contaminating tissues. Islets were cultured overnight in an atmosphere of 95% air, 5% CO2 in “complete” CMRL-1066 tissue culture medium containing 5.5 mM glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Where CMRL is stated, the culture medium does not contain fetal bovine serum.

**Pancreatic β-Cell Line—RINm5F cells, an insulin secreting β-cell line (29), were cultured by the Washington University Tissue Culture Support Center in RPMI 1640 containing 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin, 0.1 mM nonessential amino acids, 16.8 mM glucose, 1 mM sodium pyruvate, and 10 mM HEPES. Cells were subcultured in complete CMRL in Petri dishes (60 × 15 mm) at a concentration of 3 × 105 cells/ml and incubated at 37 °C under an atmosphere of 95% air, 5% CO2 for 24 h before initiating experiments.

**p70S6K and iNOS Assays**—Islets (200/1 ml) and RINm5F cells (3 × 106 cells/ml) were washed free of fetal bovine serum and incubated in CMRL, 3 mM glucose, 0.1% BSA at 37 °C, 95% air, 5% CO2 for 48 h ± TNFα (50 ng/ml), IL-1β (5 units/ml), L-arginine (1 mM), L-NAME, NMMA, 15d-PGJ2, NMMA, IRAP, troglitazone, and inhibitors. The islets were added 30 min before TNFα or IL-1β. Culture media was collected for accumulated nitrite determinations. Islets were then incubated for 30 min with either 3 or 20 mM glucose ± cytokines, insulin, and inhibitors. RINm5F cells were incubated for 2 h in Krebs-Ringer bicarbonate buffer (KRBB) in the absence of glucose and amino acids, followed by a 30-min incubation ± 1 × 10−6 M amino acid mixture (13) and TNFα. Following experimental treatments, cells or islets were washed with phosphate-buffered saline and solubilized in 300 μl or 30 μl, respectively, of Laemmli sample buffer, heated at 100 °C for 5 min, and centrifuged at 10,000 × g for 15 min to remove insoluble materials. The supernatants were processed for SDS-PAGE and Western blotting of iNOS or p70S6K as described previously (13, 30). Detection was performed using ECL reagents from Amersham Pharmacia Biotech. Quantitation of Western blots was performed by densitometry using a Molecular Dynamics personal densitometer scanning instrument (Sunnyside, CA). Data are expressed as percent of phosphorylated p70S6K over total.

**Glucose-stimulated Insulin Secretion**—Isolated islets (150/1 ml) were cultured for 48 h at 37 °C in CMRL medium, 9 mM glucose, 0.1% BSA ± TNFα (50 ng/ml), ± aminoguanidine (0.5 mM). In the absence of fetal calf serum, the glucose concentration was raised to 8 mM to maintain the glucose-stimulated insulin secretory response of β-cells. Culture medium was collected for accumulated nitrite measurements, and islets were washed and incubated for 2 h in CMRL, 0.1% BSA, minus glucose. Following this period, islets were washed in KRBB, 3 mM glucose, 0.1% BSA, and groups of 20 islets were placed into 10 × 75-mm siliconized, borosilicate tubes. KRBB was replaced with 200 μl of fresh KRBB, 3 mM glucose, 0.1% BSA, and the islets were preincubated for 30 min at 37 °C under 95% air, 5% CO2, in a shaking water bath. Buffer was replaced with 200 μl of KRBB containing 3 or 20 mM glucose, and islets were incubated for 30 min. TNFα and aminoguanidine were present during the entire experiment. Insulin secretion was determined in the incubation buffer by insulin radioimmunoassay at the Radioimmunoassay Core Facility, Diabetes Research Training Center, Washington University Medical Center.

**Nitrite Determination**—Culture media was removed, and 50-μl aliquots were mixed with 50 μl of Griess reagent (31). Nitrite production was determined at an absorbance of 540 nm using a Molecular Devices Thermomax plattereader (Sunnyside, CA).
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RESULTS

Effects of TNFα on p70<sup>65k</sup> Phosphorylation—Our initial focus was to determine whether preincubation of pancreatic islets in the presence of TNFα would inhibit insulin signaling at concentrations and periods of exposure similar to that used previously with insulin-sensitive cells. As shown in Fig. 1A (lanes 1 and 2), islets incubated under control conditions for 48 h and subsequently stimulated with an insulin stimulatory concentration of glucose (20 mM) showed enhanced phosphorylation of p70<sup>65k</sup> compared with basal glucose (3 mM), as determined by gel shift analysis. Pretreatment of islets for 48 h with TNFα concentrations of 20 ng/ml or greater reduced phosphorylation of p70<sup>65k</sup> to basal or lower levels. As shown in Fig. 1B, the inhibitory effect of TNFα was detected at 24 h (lanes 5 and 6) and 48 h (lanes 7 and 8). Thus, both the dose and time dependence of TNFα-induced inhibition of p70<sup>65k</sup> by pancreatic islets is quite similar to that described previously for TNFα on other aspects of insulin signaling in insulin-sensitive cells. To determine whether TNFα-induced inhibition of p70<sup>65k</sup> represents a specific defect in insulin signaling by islets, the ability of exogenous insulin to reverse TNFα-mediated inhibition of p70<sup>65k</sup> was assessed in Fig. 2. As shown in Fig. 2, 48 h pretreatment of islets with TNFα (50 ng/ml) for 48 h completely prevented the inhibition of glucose-stimulated activation of p70<sup>65k</sup>. Furthermore, exogenous insulin (200 nM) at basal glucose levels (3 mM) in the presence of an inhibitor of iNOS activity, aminoguanidine, completely reversed this inhibition compared with the absence of aminoguanidine (lanes 4 and 5). Also shown in Fig. 4 (lane 6), exogenous insulin (200 nM) at basal glucose (3 mM) enhanced phosphorylation of p70<sup>65k</sup> similar to elevated glucose (20 mM). These results indicate that although TNFα-induced NO formation inhibits endogenous insulin secretion (Fig. 3B), β-cell insulin resistance is reversed by exogenous insulin providing aminoguanidine is present to prevent inhibition of cellular metabolism (Fig. 4, lanes 4 and 5). Thus, β-cell insulin resistance is not because of a decrease in secreted insulin but a defect in insulin signaling by β-cells.

Previous studies by Spiegelman et al. (15) demonstrate that other proinflammatory cytokines, in particular IL-1, interfered with insulin signaling in cultured 3T3-L1 adipocytes, similar to the effects produced by TNFα (15). Similar comparisons with pancreatic islets (Fig. 5) indicated that IL-1 also markedly inhibited glucose-stimulated activation of p70<sup>65k</sup> (lanes 2 and 6). Furthermore, the inhibitory effects produced by both TNFα and IL-1 were completely prevented by inhibitors of iNOS activity, aminoguanidine and NMMA.

iNOS Expression and the Cellular Target of TNFα—To further establish a role for NO as a mediator of TNFα-induced β-cell insulin resistance, Western blot analysis of iNOS expression was performed. As shown in Fig. 6, TNFα (1–50 ng/ml) dose-dependently induced iNOS expression by islets following a 48-h pretreatment. TNFα-induced iNOS expression was...
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3. A. aminoguanidine prevents TNFα-mediated inhibition of p70<sub>6k</sub> phosphorylation by rat islets. Islets were incubated for 48 h in the presence or absence of 50 ng/ml TNFα and/or various concentrations of aminoguanidine as indicated. Islets were then stimulated with either 3 or 20 mM glucose for 30 min. Islets were then processed for SDS-PAGE, followed by Western blot for p70<sub>6k</sub> as described under "Experimental Procedures." Results are representative of three individual experiments.

4. TNFα-induced insulin resistance is reversed by aminoguanidine and exogenous insulin. Islets were incubated for 48 h in the presence or absence of 50 ng/ml TNFα and/or various concentrations of aminoguanidine and/or various concentrations of insulin as indicated. Islets were then stimulated with either 3 or 20 mM glucose for 30 min. Islets were then processed for SDS-PAGE, followed by Western blot for p70<sub>6k</sub> as described under "Experimental Procedures." Results are representative of three individual experiments.

5. TNFα and IL-1-induced inhibition of p70<sub>6k</sub> is reversed by aminoguanidine and NMMA. Islets were treated with ±50 ng/ml TNFα, ±5 unit/ml IL-1, ±5 mM aminoguanidine (AG), ±5 mM NMMA for 48 h as indicated. After incubation, islets were stimulated with either 3 or 20 mM glucose for 30 min. Islets were then processed for SDS-PAGE, followed by Western blot for p70<sub>6k</sub> as described under "Experimental Procedures." Results are representative of three individual experiments.

6. TNFα induces iNOS expression by rat islets. Islets were treated with various concentrations of TNFα for 48 h as indicated. For lanes 7 and 8, 1 µg/ml IRAP and 1 µM actinomycin D (Act D), respectively, were added in addition to 50 ng/ml TNFα. After incubation, islets were processed for SDS-PAGE, followed by Western blot for iNOS. Results are representative of three individual experiments.

Findings shown in Fig. 7A, indicating that IRAP reversed in a dose-dependent manner TNFα-induced inhibition of p70<sub>6k</sub> (lanes 3 and 4–6). As expected, IRAP also prevented IL-1-induced inhibition of p70<sub>6k</sub> (lanes 7 and 8).

Further support for this paracrine mechanism was obtained by assessing the direct effects of TNFα on β-cell function. Exposure of the β-cell line, RINm5F, to TNFα (1–100 ng/ml) for 48 h did not result in any detectable nitrate production as an indicator of NO formation (data not shown). Our previous studies examining signaling pathways in β-cells show that amino acids are capable of activating p70<sub>6k</sub> in RINm5F cells via the mammalian target of rapamycin (mTOR) pathway (13). As shown in Fig. 7B, TNFα (1–100 ng/ml) had no effect on the ability of a complete supplement of amino acids normally present in tissue culture media to stimulate p70<sub>6k</sub> phosphorylation by RINm5F cells. Thus, TNFα mediates activation of intra-islet macrophages, but TNFα does not exert any direct effects on pancreatic β-cells.

Protection by PPARγ Agonists—Recent studies show that PPARγ agonists including troglitazone and a derivative of prostaglandin D<sub>2</sub>, 15d-PGJ<sub>2</sub>, suppress monocyte production of inflammatory cytokines including IL-1 (33). Furthermore, these agents, by their ability to up-regulate PPARγ, also inhibit expression of iNOS by activated monocytes (34). These findings suggest that if NO mediates the effects of TNFα because of activation of resident intra-islet macrophages, both 15d-PGJ<sub>2</sub> and troglitazone should protect β-cells of the islet from TNFα-induced insulin resistance. As expected, 15d-PGJ<sub>2</sub> at 5 and 10 µM inhibited TNFα-induced iNOS expression by rat islets (Fig. 8A, lanes 3 and 4). These same concentrations of 15d-PGJ<sub>2</sub> also inhibited nitrate levels in the incubation media (data not shown). As shown in Fig. 8B, troglitazone at 50 and 100 µM also appeared to significantly inhibit TNFα-induced iNOS expression. However, troglitazone at these concentrations resulted in the loss of islet integrity, resulting in cells escaping from the islet capsule. Also, 50 µM troglitazone inhibited glucose-stimulated insulin secretion following a 48-h pretreatment (data not shown).
Fig. 7. IRAP reverses TNFα-induced inhibition of p70S6K. Islets were incubated for 48 h in the presence and absence of 50 ng/ml TNFα ± increasing concentrations of IRAP as indicated. For lanes 7 and 8, 5 units/ml IL-1 ± 1 μg/ml IRAP was added as a control. After incubation, islets were stimulated with either 3 or 20 mM glucose for 30 min. Results are representative of three individual experiments.

Fig. 8. Effects of 15d-PGJ2 and troglitazone on iNOS expression and p70S6K phosphorylation. A, islets were treated with 50 ng/ml TNFα ± 5 or 10 μM 15d-PGJ2 for 48 h as indicated. Islets were processed for SDS-PAGE, followed by Western blot for iNOS. B, islets were treated with 50 ng/ml TNFα ± 1–100 μM troglitazone for 24 h as indicated. C, islets were incubated in the presence and absence of 50 ng/ml TNFα ± 5 or 10 μM 15d-PGJ2 for 48 h. After incubation, islets were stimulated with either 3 or 20 mM glucose for 30 min. Results are representative of three individual experiments.

These in vitro effects produced by troglitazone at these higher concentrations preclude an interpretation of its actions in islets. The ability of 15d-PGJ2 to prevent the inhibitory effects of TNFα on p70S6K phosphorylation was also evaluated. Fig. 8C demonstrates that 15d-PGJ2 (10 μM) prevents the inhibitory effects of TNFα on p70S6K phosphorylation, although not completely. This partial protection is expected because 15d-PGJ2 (10 μM) was not able to prevent completely TNFα-induced iNOS expression as shown in Fig. 8A. Troglitazone was ineffective in reversing the inhibitory effects of TNFα on p70S6K phosphorylation (data not shown). These findings suggest that combinations of NOS inhibitors and PPARγ agonists may be more efficient in reversing insulin resistance compared with using PPARγ agonists alone.

**DISCUSSION**

Our approach in this study was to determine whether TNFα-induced insulin resistance could be demonstrated in β-cells of the islet as described previously for insulin-sensitive cells. In general, many of the characteristics of the inhibitory effects of TNFα on insulin signaling by islets mimic closely that of insulin-sensitive cells. For example, exposure of islets to TNFα for periods up to 6 h was ineffective, whereas longer exposure periods of 24 and 48 h produced significant inhibition of p70S6K activation. In addition, the concentration dependence of TNFα (5–50 ng/ml) to inhibit p70S6K activation was quite similar to that reported with insulin-sensitive cells (15, 17, 25). Other proinflammatory cytokines, in particular IL-1, also inhibited insulin signaling in islets as effectively as TNFα. Spiegelman and co-workers (15) also report that IL-1 inhibits insulin signaling in murine 3T3-F442A adipocytes as potently as TNFα. An observation in this comparison was the ability of the PPARγ agonist, 15d-PGJ2, to reverse TNFα-induced inhibition of insulin signaling by islets. The ability of PPARγ agonists to normalize defects in insulin signaling associated with insulin resistance is well documented (14, 24, 25).

In contrast, the cellular mechanism whereby TNFα induces insulin resistance in islets differed markedly from that currently proposed for insulin-sensitive cells. As opposed to a specific defect in the upstream signaling pathway of insulin-sensitive cells, TNFα appears to cause a global inhibition of metabolism by the overproduction of the free radical nitric oxide (NO). NO potently inhibits cellular metabolism by inactivating iron-sulfur-containing enzymes primarily localized to the mitochondria as well as attenuating glycolytic flux (35–38). Thus, both TNFα- and IL-1-induced inhibition of p70S6K activation was prevented completely by inhibitors of NOS activity, aminoguanidine, and NMMA. In addition, TNFα stimulated the expression of iNOS protein by islets in a manner consistent with its inhibitory effects on insulin signaling. Because the pancreatic islet represents a heterogeneous population of endocrine and nonendocrine cells, studies were performed to determine the identity of cell(s) targeted by TNFα. The ability of IRAP to prevent both TNFα-induced inhibition of p70S6K and also block iNOS protein expression identified intra-islet resident macrophages as the probable target of TNFα. Thus, activation of resident macrophages by TNFα alone or with other endogenous islet cytokines results in the release of IL-1 from macrophages that then interact directly with β-cells to induce iNOS expression and the overproduction of NO. This paracrine interaction between intra-islet resident macrophages and β-cells has been documented in both rodent and human pancreatic islets (32, 39). Furthermore, TNFα appears to exert no direct effect on the β-cell population of the islet. Consistent with this mechanism, 15d-PGJ2 attenuated TNFα-induced inhibition of insulin signaling by islets. Recent studies by Ricote et al. (34) indicate that ligand activation of PPARγ by troglitazone or 15d-PGJ2 prevents iNOS expression and NO production by monocytes and macrophages (34). Furthermore, Jiang et al. (33) also demonstrate that these same PPARγ agonists...
strongly inhibit cytokine production, i.e., IL-1, TNF-α, and IL-6, by human monocytes (33). It is also of interest in this context that the ability of troglitazone to lower triglyceride content in islets of Zucker diabetic fatty rats also improves β-cell function and is suggested to be possibly a consequence of decreased NO production by β-cells in this animal model of type 2 diabetes (40). Thus, the ability of troglitazone or 15d-PGJ2 to block production of either iNOS expression or IL-1 release would be consistent with the ability of these agents to prevent TNFα-induced inhibition of insulin signaling by islets. Alternatively, the ability of 15d-PGJ2 to down-regulate iNOS expression may be mediated by blocking NFκB activation (30). In our studies, we were unable to evaluate the ability of troglitazone to alter iNOS expression through PPARγ activation because of its lower potency and a requirement for increased concentrations in vitro that affected islet integrity. Ongoing studies using other PPARγ agonists are in progress to more definitively determine whether these agents down-regulate iNOS expression in islets.

Evidence obtained from both cultured cells and whole animal studies have clearly demonstrated that TNFα produces insulin resistance similar to that observed in obesity and type 2 diabetes (19, 24, 41). However, the mechanism whereby TNFα mediates this effect is less clear. A general mechanism put forth to explain the action of TNFα proposes that this proinflammatory cytokine produces defects in autophosphorylation of the insulin receptor and insulin receptor-mediated phosphorylation of its substrates i.e. IRS-1/IRS-2 at tyrosine residues (17). Thus, the ability of TNFα to stimulate serine phosphorylation of these substrates is believed to interfere with normal insulin signaling. However, a recent re-examination of this mechanism by Stephens et al. (42) finds no evidence of defects in insulin receptor autophosphorylation or in insulin-stimulated substrate phosphorylation after prolonged exposure of 3T3-L1 adipocytes to TNFα. These investigators suggested a more global effect, possibly mediated at the level of the regulation of gene expression to explain the action of TNFα (42). Interestingly, another model of insulin resistance involving the hexosamine biosynthetic pathway in insulin-sensitive cells is also characterized by an impairment in insulin receptor autophosphorylation and IRS-1 phosphorylation (4, 43). In this model, glucosamine, which enters the hexosamine pathway downstream of the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase mimics closely insulin resistance caused by chronic hyperglycemia (43). Although the mechanism responsible for glucosamine-induced insulin resistance is not yet defined, it is presumed to reflect in some manner the deleterious effects caused by chronic hyperglycemia. However, recent studies have suggested that glucosamine-induced insulin resistance in 3T3-L1 adipocytes may result from the rapid lowering of cellular ATP levels that closely correlates with decreases in insulin responsiveness (44). As a whole, these proposed mechanisms are consistent with our results indicating that TNFα-induced insulin resistance in islets is mediated by overproduction of NO, which produces a global inhibition of metabolism including decreases in cellular ATP levels. Furthermore, our findings also suggest that defects in the upstream insulin signaling cascade may be secondary to a decrease in cellular metabolism.

In summary, a proposed model of TNFα-induced insulin resistance in insulin-sensitive tissues and β-cells is illustrated in Fig. 9. Currently, it is generally accepted that TNFα released by adipocytes interferes with insulin signaling in fat, muscle, and liver, resulting in insulin resistance. Our findings suggest that TNFα alone or in the presence of other endogenous cytokines may also activate intra-islet resident macrophages, resulting in the release of IL-1, which induces iNOS expression and the overproduction of NO in β-cells. The free radical, NO, then down-regulates cellular metabolism primarily by interfering with mitochondrial function that leads to inhibition of insulin and other growth factor signaling associated with β-cell growth and proliferation. Furthermore, these defects in the signaling pathway are normalized by the PPARγ agonist, 15d-PGJ2, and also the iNOS inhibitor, aminoguanidine. It is proposed that β-cell insulin resistance may be a major determinant in β-cell failure and the inability of β-cells to overcompensate in the development of type 2 diabetes. This study also suggests that a global inhibition of metabolism may be a major factor in the pathogenesis of insulin resistance in β-cells and possibly other insulin-sensitive cells.

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