The HIV Protease Inhibitor Indinavir Decreases Insulin- and Contraction-Stimulated Glucose Transport in Skeletal Muscle

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In many patients with human immunodeficiency virus (HIV) treated with HIV protease inhibitors, a complication develops that resembles abdominal obesity syndrome, with insulin resistance and glucose intolerance that, in some cases, progresses to diabetes. In this study, we tested the hypothesis that indinavir, an HIV-protease inhibitor, directly induces insulin resistance of glucose transport in skeletal muscle. Rat epitrochlearis muscles were incubated with a maximally effective insulin concentration (12 nmol/l) and 0, 1, 5, 20, or 40 μmol/l indinavir for 4 h. In control muscles, insulin increased 3-O-[^3H]methyl-[2-^3H]-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-[2-^3H] (D-mannose-4-yloxy)-2-propylamine extracellular photolabeling technique, was reduced by ~70% in the presence of 20 μmol/l indinavir. Insulin stimulation of phosphatidylinositol 3-kinase activity and phosphorylation of protein kinase B were not decreased by indinavir. These results provide evidence that indinavir inhibits the translocation or intrinsic activity of GLUT4 rather than insulin signaling. Diabetes 50:1397–1401, 2001

RESEARCH DESIGN AND METHODS

Materials. Indinavir was a gift from Merck Pharmaceuticals (Nutley, NJ). Pork insulin was purchased from Eli Lilly (Indianapolis, IN). 3-O-[^3H]methyl-[2-^3H]-4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis-[2-^3H] (D-mannose-4-yloxy)-2-propylamine extracellular photolabeling technique, was reduced by ~70% in the presence of 20 μmol/l indinavir. Insulin stimulation of phosphatidylinositol 3-kinase activity and phosphorylation of protein kinase B were not decreased by indinavir. These results provide evidence that indinavir inhibits the translocation or intrinsic activity of GLUT4 rather than insulin signaling. Diabetes 50:1397–1401, 2001

In many individuals with human immunodeficiency virus (HIV) treated with HIV protease inhibitors, a condition develops that is similar to the central/visceral obesity–insulin resistance syndrome (1–6), which is characterized by a shift in body fat distribution to the central abdominal region, insulin resistance, hyperinsulinemia, dyslipidemia, and in some, type 2 diabetes (1, 4–13). It has been reported that this metabolic syndrome develops in 60–80% of patients treated with HIV protease inhibitors (1,6,9,12,13). The mechanism responsible for these metabolic abnormalities is unknown. One proposed mechanism is that HIV protease inhibitors induce development of central/visceral obesity, which in turn causes insulin resistance (2). In the present study, we tested the alternative possibility that HIV protease inhibitors directly induce insulin resistance of skeletal muscle, which is the major site of insulin-stimulated glucose disposal (14,15).
INSULIN RESISTANCE AND INDINAVIR

RESULTS

Insulin-stimulated glucose transport. As shown in Fig. 1, incubation of epitrochlearis muscles for 4 h with a maximally effective concentration of insulin (12 mmol/l) resulted in a sixfold increase in glucose transport above basal. When indinavir was included in the incubation medium, insulin-stimulated glucose transport was inhibited in a dose-dependent manner. The increase in glucose transport induced by insulin was reduced by 40% in the presence of 5 mmol/l indinavir, 58% with 20 mmol/l indinavir, and 72% in the presence of 40 mmol/l indinavir (Fig. 1). Indinavir (40 mmol/l) had no effect on basal glucose transport (0.12 ± 0.05 and 0.10 ± 0.04 mmol · min⁻¹ · 10⁻⁶ g⁻¹ for basal and basal + indinavir, respectively). The inhibitory effect of indinavir on the stimulation of glucose transport by insulin is not muscle fiber type–specific, as a similar ~65% reduction was observed in soleus muscles incubated in the presence of 20 mmol/l indinavir (Fig. 2).

FIG. 1. Effect of various concentrations of indinavir on insulin-stimulated 3MG transport in rat epitrochlearis muscle. Glucose transport was measured after 4 h incubation in the absence or presence of a maximally effective concentration of insulin (12 mmol/l) and 0, 1, 5, 20, or 40 mmol/l of indinavir (IDV) as described in RESEARCH DESIGN AND METHODS. Results are expressed as mean ± SE for 7–16 muscles per treatment. *P < 0.001 compared with insulin.

FIG. 2. Effect of indinavir on insulin-stimulated 3MG transport in rat soleus muscle. Glucose transport was assessed in muscles after a 4-h incubation in the absence or presence of a maximally effective concentration of insulin (12 mmol/l) with or without 20 mmol/l of indinavir (IDV) as described in RESEARCH DESIGN AND METHODS. Results are expressed as mean ± SE for 8–11 muscles per treatment. *P < 0.001 compared with insulin.
Effect of indinavir on insulin-stimulated cell surface GLUT4. The exofacial label ATB-[2-3H]BMPA was used to quantify GLUT4 transporters at the cell surface after insulin stimulation in the absence or presence of 20 \( \mu \)mol/l indinavir. Insulin induced an approximate sevenfold increase in GLUT4 labeling (Fig. 3). When 20 \( \mu \)mol/l indinavir was included in the incubation medium, the increase in cell surface GLUT4 labeling in response to insulin was reduced by \(~70\%\) (Fig. 3).

Effect of indinavir on contraction-stimulated glucose transport. 3MG transport was increased approximately sevenfold in epitrochlearis muscle in response to stimulation of contractile activity (Fig. 4). The contraction-stimulated increase in glucose transport was reduced by \(~70\%\) when muscles were incubated with 20 \( \mu \)mol/l indinavir for 4 h before stimulation (Fig. 4).

PI 3-kinase activity. Activation of PI 3-kinase is an essential step in the signal-transduction pathway by which insulin stimulates glucose transport (26–29). Therefore, we determined whether indinavir inhibits PI 3-kinase activity. As shown in Fig. 5, incubation of muscles with 12 nmol/l insulin for 4 h resulted in an approximate fourfold increase in PI 3-kinase activity, and 20 \( \mu \)mol/l indinavir did not inhibit the stimulation of PI 3-kinase activity by insulin (Fig. 5).

Phosphorylated PKB. The next step in the insulin-signaling pathway is mediated by PKB (30,31). PKB is activated by dual phosphorylation of threonine 308 and serine 473. Phosphorylation of the threonine 308 residue on PKB, by 3-phosphoinositide-dependent protein kinase (PDK)-1, with subsequent phosphorylation of serine 473, results in activation of the kinase (32,33). As shown in Fig. 6, insulin-stimulated phosphorylation of PKB, as measured using an antibody specific for the phosphorylated serine 473 residue of PKB, was not decreased in muscles incubated with 20 \( \mu \)mol/l indinavir (Fig. 6).

DISCUSSION
Our results show that the HIV protease inhibitor indinavir induces severe insulin resistance of muscle glucose transport within 4 h in rat skeletal muscle incubated in vitro. Skeletal muscle is responsible for \(~90\%\) of insulin-stimu-
One potential mechanism by which indinavir could be decreasing insulin-stimulated glucose transport is inhibition of one or more steps in the signaling pathway by which insulin stimulates glucose transport. The first step in the insulin-signaling cascade is the binding of insulin to the insulin-receptor, which results in activation of the kinase. Involvement of PI 3-kinase activation in the stimulation of muscle glucose transport by insulin is evidenced by the finding that inhibition of PI 3-kinase with wortmannin blocks stimulation of glucose transport by insulin (27–29). In the present study, we found that activation of PI 3-kinase by insulin is not inhibited by a concentration of indinavir that markedly reduces insulin-stimulated glucose transport.

This finding also provides evidence that the earlier, tyrosine phosphorylation insulin-signaling steps are not inhibited in the presence of indinavir.

The next step in the insulin-signaling pathway involves threonine/serine phosphorylation of PKB, resulting in its activation (37). PI-3,4-biphosphate and PI-3,4,5-triphosphate, produced by the action of PI 3-kinase, result in phosphorylation of threonine 308 of PKB by PDK-1, with subsequent phosphorylation of serine 473 and activation of the kinase (32,33,37). Our finding that insulin-stimulated phosphorylation of PKB is not inhibited by indinavir suggested that indinavir acts at a step beyond the insulin-signaling pathway. This possibility was supported by the finding that contraction-stimulated glucose transport was also inhibited in the presence of indinavir. Therefore, it seemed likely that indinavir is acting on a step common to both pathways, i.e., GLUT4 translocation to the cell surface.

Our finding that cell-surface labeling of GLUT4 in maximally insulin-stimulated muscle was decreased ~70% in muscles treated with indinavir is compatible with the hypothesis that indinavir inhibits GLUT4 translocation. However, while this paper was in revision, Murata et al. (38) reported that indinavir inhibits glucose transport in adipocytes without affecting GLUT4 translocation to the plasma membrane. They used both a cell-fractionation procedure and the plasma membrane sheet method to show that GLUT4 translocation was not inhibited. On the basis of these results and their finding that indinavir reduced the glucose transport activity of GLUT4 expressed in oocytes, Murata et al. (38) concluded that indinavir acts by inhibiting GLUT4 intrinsic activity. In this context, our finding that cell-surface GLUT4 labeling by ATB-[2-3H]BMPA is markedly reduced by indinavir in insulin-stimulated muscle could be explained by a direct effect of indinavir on GLUT4 rather than on GLUT4 translocation. Such an effect could be mediated either by 1) binding of indinavir to the glucose- and ATB-[2-3H]BMPA-binding site on GLUT4, thus interfering with the ability of GLUT4 to interact with glucose and ATB-[2-3H]BMPA, or 2) by a conformational change in GLUT4 induced by indinavir that interferes with its ability to transport glucose and bind ATB-[2-3H]BMPA.

In conclusion, the results of this initial study show that exposure of skeletal muscle to the HIV protease inhibitor indinavir causes a large decrease in insulin responsiveness of glucose transport within 4 h. This finding raises the possibility that the first event in the development of the insulin-resistance syndrome that occurs in patients with HIV treated with the HIV protease inhibitor indinavir is insulin resistance of skeletal muscle glucose transport.

FIG. 6. Effect of 4-h exposure to 20 µmol/l indinavir on insulin-stimulated Ser473 phosphorylation of PKB in rat epitrochlearis muscle. A: Western blot analysis was performed on muscles treated for 4 h with insulin (12 nmol/l) in the absence or presence of 20 µmol/l indinavir (IDV) as described in RESEARCH DESIGN AND METHODS. Data are arbitrary optical density (O.D.) units, with insulin set at 1.0, and are presented as mean ± SE with n = 8 in each treatment. B: Representative Western blot showing phosphorylation of PKB in the absence or presence of 20 µmol/l indinavir.
Reckamp for expert assistance with preparation of the manuscript.

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