Imidapril, an Angiotensin-Converting Enzyme Inhibitor, Improves Insulin Sensitivity by Enhancing Signal Transduction via Insulin Receptor Substrate Proteins and Improving Vascular Resistance in the Zucker Fatty Rat

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Angiotensin-converting enzyme (ACE) inhibitors are antihypertensive agents, that inhibit the conversion of angiotensin I to angiotensin II, resulting in smooth-muscle relaxation and a reduction of vascular resistance. Recently, it has been suggested that ACE inhibitors improve insulin resistance in diabetic patients. To investigate the effect of an ACE inhibitor on insulin sensitivity, insulin signaling, and circulation, imidapril was administered orally or intraduodenally to Zucker fatty rats. Oral administration of imidapril improved insulin sensitivity based on the results of an oral glucose tolerance test (OGTT) and a decrease in urinary glucose secretion. Phosphatidylinositol 3-kinase (PI 3-kinase) activity associated with hepatic insulin receptor substrate-1 (IRS-1) in the insulin-stimulated condition was significantly enhanced 110% without a significant alteration in tyrosine phosphorylation of IRS-1 in the imidapril-treated group. In muscle, IRS-1 tyrosine phosphorylation and PI 3-kinase activity associated with IRS-1 in the insulin-stimulated condition were enhanced 70% and 20%, respectively, in the imidapril-treated group. In contrast, an alteration of the IRS-2 pathway was observed only in liver; a significant insulin-induced increase in the IRS-2–associated PI 3-kinase over the basal level was observed in the imidapril-treated group but not in the control. In addition, treatment with imidapril was shown to significantly reduce blood pressure and increase blood flow in the liver and muscle. These results suggest that the ACE inhibitor imidapril may improve insulin sensitivity not only by acting directly on the insulin signaling pathway but also by increasing blood flow in tissues via normalization of vascular resistance, a major cause of hypertension.

IN TYPE II DIABETES MELLITUS, hypertension is present at the time of diagnosis of diabetes in about one third of the patients.[1-3] The common coexistence of glucose intolerance, hypertension, hyperlipidemia, obesity, and susceptibility to cardiovascular disease suggests that they may relate to a common underlying mechanism, such as insulin resistance.[1-7] In addition, it is well known that hypertension markedly accelerates the progression of diabetic nephropathy.[8,9] Thus, the treatment of hypertension is considered particularly important in diabetic patients. In diabetic patients with underlying nephropathy, an angiotensin-converting enzyme (ACE) inhibitor is considered the first-choice antihypertensive agent. ACE inhibitors inhibit the conversion of angiotensin I to angiotensin II, resulting in smooth-muscle relaxation and a reduction of vascular resistance and mean arterial blood pressure (MAP).[10-12] Recently, it has been suggested that ACE inhibitors improve insulin resistance in diabetic patients and animals.[1,2,8-19]

In this study, to investigate the effect of an ACE inhibitor on insulin sensitivity, insulin signaling, and blood circulation, imidapril was administered orally or intraduodenally to Zucker fatty rats, a model of insulin resistance characterized by hyperinsulinemia, obesity, hypertension, and hyperlipidemia in association with glucose intolerance.[14-17] Impaired insulin-induced phosphatidylinositol 3-kinase (PI 3-kinase) activation has been suggested as the molecular mechanism causing insulin resistance in Zucker fatty rats in previous reports.[20] In addition, it was reported that Zucker fatty rats exhibit significantly higher blood pressure than lean (fa/-) rats.[14,17] If the hypertension is due to vascular resistance and a resultant decrease in blood flow, this circulatory impairment could be one of the mechanisms of insulin resistance in Zucker fatty rats, since the supply of insulin to peripheral tissues would be decreased.

This is the first report on the effect of an ACE inhibitor administered orally on insulin-induced PI 3-kinase activation in insulin-resistant diabetic obese rats. We will discuss the possible mechanisms by which ACE inhibitors induce an improvement of insulin resistance.

MATERIALS AND METHODS

Animals

Female Zucker fatty rats aged 6 weeks were purchased from Charles River Japan (Tokyo, Japan). To investigate the effect of imidapril on MAP and vascular resistance, 12 rats were divided into two groups, one treated intraduodenally with imidapril with 10 mg/kg and the other with vehicle. Furthermore, six additional rats were orally administered 2.5% carboxymethyl cellulose (CMC) aqueous solution with or without imidapril 5 mg/kg once daily for 10 weeks to investigate the long-term effects of imidapril on insulin sensitivity and insulin signaling.

Effects of Imidapril on Blood Flow Rate and MAP

Rats were anesthetized with thiobutabarbital 100 mg/kg intraperitoneally, and then treated intraduodenally with vehicle with or without imidapril 10 mg/kg immediately. To investigate the effect of imidapril on the blood flow rate, laser Doppler probes were placed directly on the liver and skeletal muscle and the measurement was performed as

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described previously.[21] MAP was continuously monitored with a transducer connected to a vital-signs monitor. The mean of five MAP values was used as the representative MAP. The heart rate was monitored with a heart rate counter triggered by arterial pressure pulses. The representative heart rate was the mean of five values.

Both hepatic and skeletal muscle vascular resistance were calculated by dividing the MAP (millimeters of mercury) by the hepatic blood flow (liters per minute) or skeletal muscle blood flow (liters per minute), expressed as the percentage of stable values obtained at time 0.

Oral Glucose Tolerance Test

An oral glucose tolerance test (OGTT) was performed on the rats after an approximately 18-hour overnight fast 10 weeks after initiation of imidapril treatment. Glucose 4.5 g/kg was administered by oral gavage, and blood from the tail vein was collected at 0, 30, 60, and 120 minutes. Blood glucose was assayed by the glucose oxidase method, and plasma insulin was determined by radioimmunoassay.[22]

Immunoblotting and PI 3-Kinase Assay

Food was withdrawn 12 to 14 hours before the terminal experiment, and the rats were anesthetized with pentobarbital sodium 60 mg/kg intraperitoneally before the abdominal cavity was opened. The portal vein was exposed, and 4 mL saline (0.9% NaCl) with or without 10^{-5} mol/L insulin was injected after blood sampling. Thirty and 90 seconds later, the liver and skeletal muscle were respectively removed and immediately homogenized in ice-cold solubilization buffer (1/10 wt/vol) with a Polytron (Kinematica, Switzerland) as described previously.[20,23] The supernatants were obtained by centrifugation at 10,000 rpm for 30 minutes. Equal protein amounts of the supernatants were immunoprecipitated with anti–insulin receptor substrate-1 ([IRS-1] 10 μg/mL), anti-IRS-2 (10 μg/mL), or anti-phosphotyrosine (10 μg/mL) antibodies.[20,23] Aliquots of the samples were processed for sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and immunoblotting was performed with anti-IRS-1, anti-IRS-2, or anti-phosphotyrosine antibodies.

Aliquots of the same samples were used for PI 3-kinase assay. The immunoprecipitates with anti-IRS-1 and anti-IRS-2 were washed three times in solution 1 (1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride [PMSF], and 1 mmol/L sodium vanadate in phosphate-buffered saline, pH 7.4), three times in solution 2 (10 mmol/L $LiCl_2$, 1 mmol/L PMSF, and 1 mmol/L sodium vanadate, pH 7.4), and twice in solution 3 (10 mmol/L Tris hydrochloride, and 1 mmol/L $MgCl_2$, pH 7.4) and suspended in kinase buffer (10 mmol/L Tris hydrochloride, 1 mmol/L $MgCl_2$, 0.2 mmol/L adenosine triphosphate [ATP], and 0.2 μmol/L [γ^{-32}P]ATP). PI 3-kinase activity in the immunoprecipitates was measured as previously described.[20,23]

Analytical Methods

Body weight was measured in the rats every 7 days. Four days before the end of the experiment, blood pressure was measured by the tail-cuff method.[8] Three days before dissection, the animals were placed in metabolic cages for 24 hours to collect urine. The urine was centrifuged at 3,000 rpm for 10 minutes, and the supernatant was removed and frozen until measurement. Plasma and urinary parameters were measured using an automatic analyzer (model 7150; Hitachi, Tokyo, Japan).

Statistical Analysis

All experimental data are expressed as the mean ±SE. Student's unpaired *t* test was used to compare control and imidapril treatment data using the statistical software package SuperANOVA (Abacus Concepts, Berkeley, CA). A P value less than .05 was considered significant.

RESULTS

Effect of Imidapril Injection on Blood Flow Rate in Liver and Muscle of Zucker Fatty Rats

First, an experiment was performed to confirm that imidapril has a direct effect on the circulatory system in Zucker fatty rats. The circulation was confirmed to be stable, and then every 5 minutes after injection of imidapril, the MAP, heart rate, and vascular resistance were measured and plotted as a percentage of their corresponding values obtained at time 0. In both the liver and skeletal muscle, vascular resistance decreased by a maximum of approximately 20% in a time-dependent manner. MAP also significantly decreased by 25% and the heart rate increased by 9% after injection of imidapril. These results indicate that imidapril has an acute relaxation effect on vessels in both the liver and skeletal muscle of Zucker fatty rats (Fig 1).

Effect of Oral Imidapril Treatment for 10 Weeks on Body Weight, Biochemical Parameters, and Blood Pressure

The body weight, blood glucose, plasma insulin, and urinary glucose excretion in rats with and without imidapril treatment are listed in Table 1. The body weight was slightly higher for rats treated with imidapril versus control rats, but this difference was not significant. Blood pressure was significantly lower in rats treated with imidapril versus control rats ($P < .05$). In addition, the nonfasting plasma glucose concentration and urinary glucose excretion were significantly decreased by imidapril treatment ($P < .05$), while the urinary volume was not different for imidapril and vehicle treatment. Urinary protein excretion was also significantly decreased by 65% in rats treated with imidapril compared with controls ($P < .05$). The change in nonesterified fatty acids was not statistically significant.

Effect of Imidapril on OGTT Results in Zucker Fatty Rats

Since the results (Table 1) suggested an improvement of hyperglycemia with imidapril in Zucker fatty rats, we performed an OGTT in rats with and without imidapril treatment to examine the effect of imidapril on insulin sensitivity (Fig 2). Fasting glucose and insulin concentrations did not differ between the two groups. However, the glucose concentration was lower after glucose intake in the imidapril group versus the controls (difference at 60 and 90 minutes, $P < .05$; Fig 2A). In addition, the insulin concentration was also significantly lower at 60 and 90 minutes in the imidapril group versus the controls ($P < .05$; Fig 2B). These results indicate that oral administration of imidapril for 10 weeks improved the insulin resistance in Zucker fatty rats.

Effect of Imidapril on Phosphorylation of the Insulin Receptor, IRS-1, and IRS-2 in Liver and Skeletal Muscle

Expression levels of IRS-1 and IRS-2 were determined by immunoprecipitation and immunoblotting with specific antibodies against IRS-1 and IRS-2, respectively. As a result, the expression levels of IRS-1 and IRS-2 in both the liver and skeletal muscle were not different between vehicle- and imidapril-treated rats (data not shown).

Subsequently, we investigated the tyrosine phosphorylation levels of the insulin receptor, IRS-1, and IRS-2 in the liver and

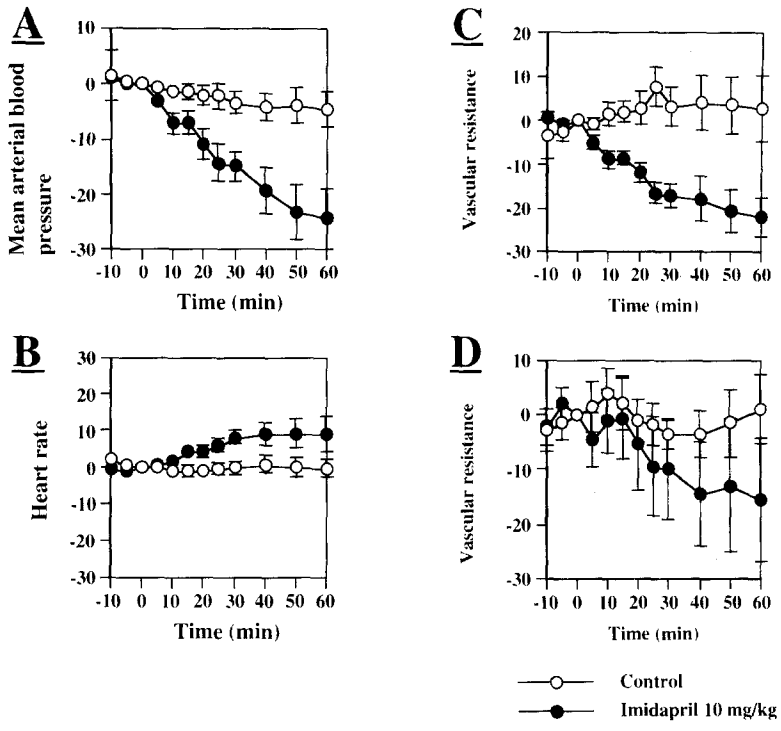

Fig 1. Effect of imidapril on MAP, heart rate, and vascular resistance in Zucker fatty rats. Imidapril (●) or vehicle (○) was administered intraduodenally at the time of operation after these parameters stabilized. Blood flow rate was measured using a laser Doppler probe. Data points indicate MAP (A), heart rate (B), vascular resistance in liver (C), and vascular resistance in skeletal muscle (D). Values are the percentage of values obtained at time 0, and are shown as the mean ± SE (n = 6).

muscle under basal and insulin-stimulated conditions. Insulin-induced phosphorylation of the insulin receptor was slightly increased by 42% and 26% in the liver (Fig 3A) and muscle (Fig 4A), respectively, although these differences were not significant. The insulin-induced tyrosine phosphorylation of IRS-1 was increased by 40% but nonsignificantly in the liver (Fig 3B) and increased significantly by 72% in muscle (Fig 4B). In contrast, the insulin-induced tyrosine phosphorylation level of IRS-2 was not significantly different between the imidapril and control groups (Figs 3C and 4C).

Table 1. Posttreatment Body Weight, Blood Pressure, and Biochemical Parameters in Zucker Fatty Rats With and Without Imidapril Treatment for 10 Weeks

Variable	Control	Imidapril
No. of animals	6	6
Body weight (g)	425 ± 15	453 ± 19
Plasma parameters		
Glucose (mg/dL)	192 ± 5	169 ± 4*
Insulin (ng/mL)	10.6 ± 1.1	10.0 ± 1.1
Triglyceride (mg/dL)	413 ± 127	415 ± 105
Phospholipid (mg/dL)	236 ± 20	204 ± 14
NEFA (μEq/L)	341 ± 58	200 ± 31
Total cholesterol (mg/dL)	73 ± 3	65 ± 8
Urine parameters (24-h excretion)		
Water intake (mg)	27.3 ± 4.1	24.0 ± 3.1
Urinary volume (mL)	8.7 ± 0.9	9.3 ± 1.5
Total protein (mg)	14.7 ± 5.9	5.1 ± 2.2*
Glucose (mg)	8.6 ± 2.3	4.9 ± 1.4*
Blood pressure (mm Hg, tail-cuff)	136.1 ± 4.4	110.1 ± 4.8*
Heart rate (bpm)	386.3 ± 6.2	380.8 ± 21.9

NOTE. Values are the mean ± SE.
Abbreviation: NEFA, nonesterified fatty acid.
*$P < .05$ *v* control.

Effect of Imidapril on PI 3-Kinase Activity Associated With IRS-1 and IRS-2 in Liver and Skeletal Muscle

Hepatic and muscle PI 3-kinase activation in response to insulin stimulation was investigated in immunoprecipitates with antibodies against IRS-1 and IRS-2, respectively (Figs 5 and 6). In the liver and skeletal muscle of rats treated with imidapril, basal PI 3-kinase activity associated with IRS-1 was comparable to the value in controls. In the insulin-stimulated condition, IRS-1–associated PI 3-kinase activity was increased by 110% in the liver (Fig 5A), and by 20% in the muscle of rats treated with imidapril (Fig 6A) compared with controls. Thus, it is possible that imidapril treatment enhanced certain insulin-induced tyrosine phosphorylation sites on IRS-1 that increased the associated PI 3-kinase activity.

In addition, a significant insulin-induced increase in muscle IRS-2–associated PI 3-kinase above the basal level was observed in the imidapril-treated group ($P < .05$) but not in the controls, although no significant difference in either basal or insulin-stimulated PI 3-kinase activity associated with IRS-2 was observed between the imidapril group and the control group (Fig 5B). In the muscle, no significant difference in either basal or insulin-stimulated PI 3-kinase activity associated with IRS-2 was observed between the imidapril group and the control group (Fig 6B).

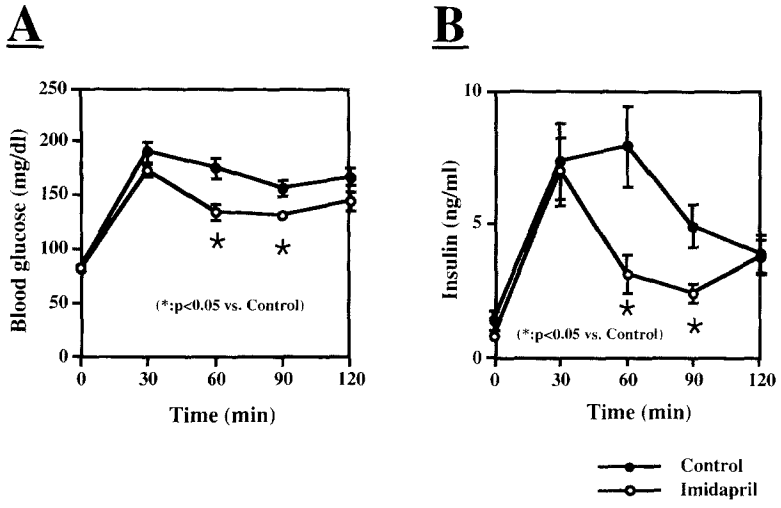

Fig 2. Effect of imidapril on OGTT results in Zucker fatty rats. An OGTT was performed after oral administration of vehicle (●) or imidapril (○) for 10 weeks. After fasting, glucose 4.5 g/kg was administered by oral gavage and tail-vein blood was collected. Blood glucose (A) and plasma insulin (B) were determined. Values are the mean ± SE (n = 6). *$P < .05$ *v* control.

DISCUSSION

Insulin is a hormone secreted from pancreatic β cells that acts on insulin-sensitive tissues such as muscle and liver. In muscle and fat cells, insulin induces translocation of the glucose transporter from the intracellular pool to the cell surface, resulting in an increase of glucose uptake by the cells.[24-26] For this step, insulin-induced activation of PI 3-kinase has been shown to be essential.[27-32] On the other hand, in hepatocytes, insulin plays an important role in glycogen synthesis via the action of PI 3-kinase on c-*Akt* and also suppresses the gene expression of phosphoenolpyruvate carboxykinase, the rate-limiting enzyme of gluconeogenesis via PI 3-kinase activation.[33-35] Therefore, the process of PI 3-kinase activation is considered particularly important for insulin-induced glucose metabolism.

Diabetes is defined as a disorder characterized by an elevated blood glucose level due to insufficient insulin action, and is classified into two major groups, insulin-dependent and non–insulin-dependent (NIDDM). NIDDM is largely due to insulin resistance, which can be caused by numerous factors including unknown genetic factors, obesity, a high-fat diet, and insufficient physical exercise.[36-39] To date, it has been reported that insulin-induced PI 3-kinase activation is impaired in most insulin-resistant diabetic animal models,[20,22,23,40,41] as well as obese humans.[42,43] On the other hand, insulin-sensitizing agents, such as troglitazone, pioglitazone, and JTT-501 improve insulin-

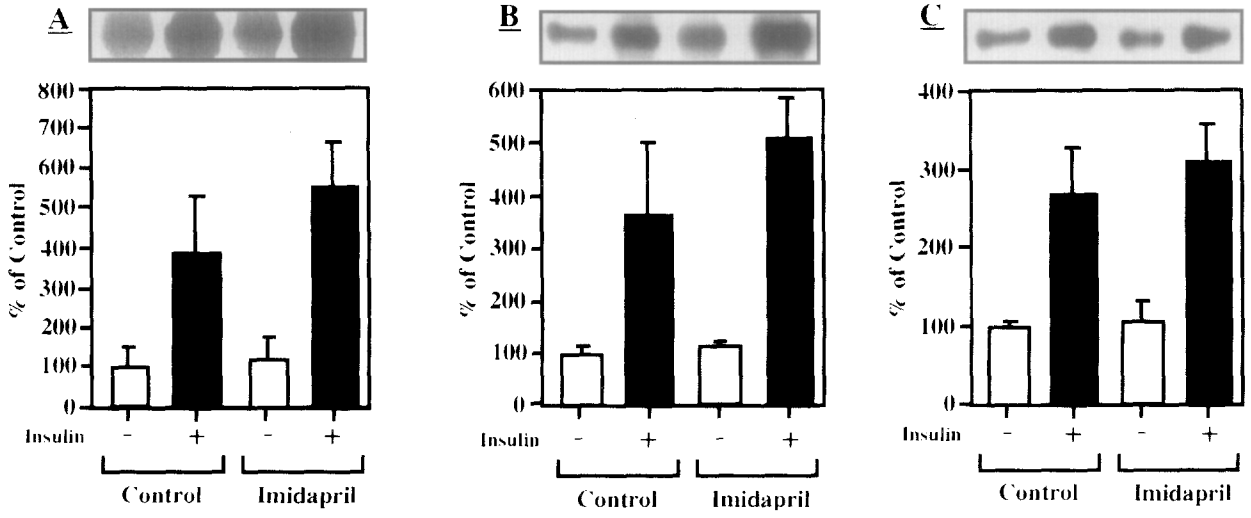

Fig 3. Effect of imidapril on phosphorylation of the insulin receptor, IRS-1, and IRS-2 in Zucker fatty rat liver. Imidapril or vehicle were administered orally for 10 weeks and saline (0.9% NaCl) with or without 10^{-5} mmol/L insulin was injected via the portal vein of Zucker fatty rats. The liver was homogenized in extraction buffer. After centrifugation, aliquots with the same amount of protein were immunoprecipitated with anti-phosphotyrosine (A), anti-IRS-1 (B), or anti-IRS-2 (C) antibodies. The immunoprecipitated proteins were used for immunoblotting with anti-phosphotyrosine antibody using an ECL kit (Amersham Pharmacia Biotech Japan, Tokyo, Japan). Quantitation of the bands was performed with a Bio-Rad Molecular Imager (Bio-Rad Laboratories, CA). Top panels are the representative immunoblotting data, and bottom panels are bar graphs representing the results of 3 independent experiments. Values are the mean ± SE (n = 3).

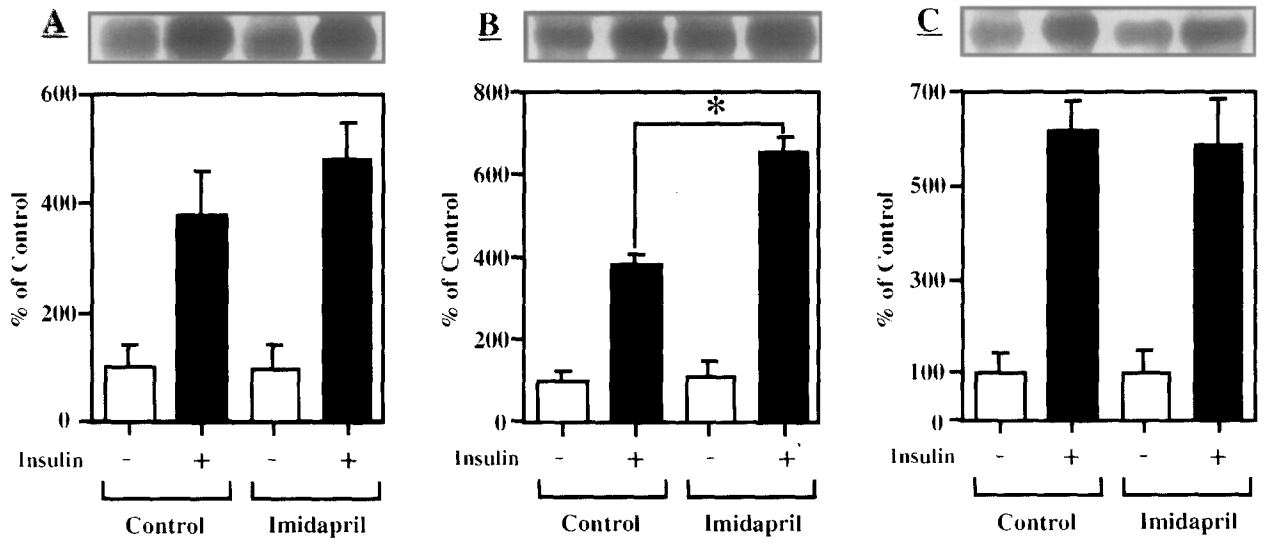

Fig 4. Effect of imidapril on tyrosine phosphorylation of the insulin receptor, IRS-1, and IRS-2 in Zucker fatty rat muscle. Imidapril and vehicle were administered orally for 10 weeks and saline (0.9% NaCl) with or without 10^{-5} mol/L insulin was injected via the portal vein of Zucker fatty rats. Muscle was homogenized in extraction buffer. After centrifugation, aliquots with the same amount of protein were immunoprecipitated with anti-phosphotyrosine (A), anti–IRS-1 (B), or anti–IRS-2 (C) antibodies. The immunoprecipitated proteins were used for immunoblotting with anti-phosphotyrosine antibody using an ECL kit. Quantitation of the bands was performed with a Bio-Rad Molecular Imager. Top panels are represent immunoblotting data, and bottom bar graphs represent results of 3 independent experiments. Values are mean ± SE (n = 3). *$P < .05$ *v* control (stimulated with insulin).

induced tyrosine phosphorylation of the insulin receptor and IRS-1, leading to PI 3-kinase activation in various cell lines and in diabetic animal tissues.[23,44-47] These findings suggest that the degree of insulin-induced tyrosine phosphorylation of IRS proteins and PI 3-kinase activation may be an important factor determining insulin sensitivity.

Imidapril, a non–sulfhydryl-containing ACE inhibitor, is an antihypertensive agent that leads to smooth-muscle relaxation and a reduction of vascular resistance and MAP.[8-12] Recently, it has been reported that ACE inhibitors improve insulin resistance in diabetic patients, although the mechanism of this effect is not fully understood.[8-19] The Zucker fatty rat (fa/fa) used in this study is a model of insulin-resistant mild NIDDM characterized by hyperinsulinemia, obesity, and hypertension.[14-17] The results of an OGTT and the amount of urinary glucose excretion indicate that oral administration of imidapril improved the insulin sensitivity, consistent with the findings of previous reports using other ACE inhibitors.[15-17]

In Zucker fatty rats, insulin-induced PI 3-kinase activation has been shown to be impaired in muscle and in liver.[20] In this study, after injection of insulin via the portal vein, the tyrosine phosphorylation level of the insulin receptor was slightly higher

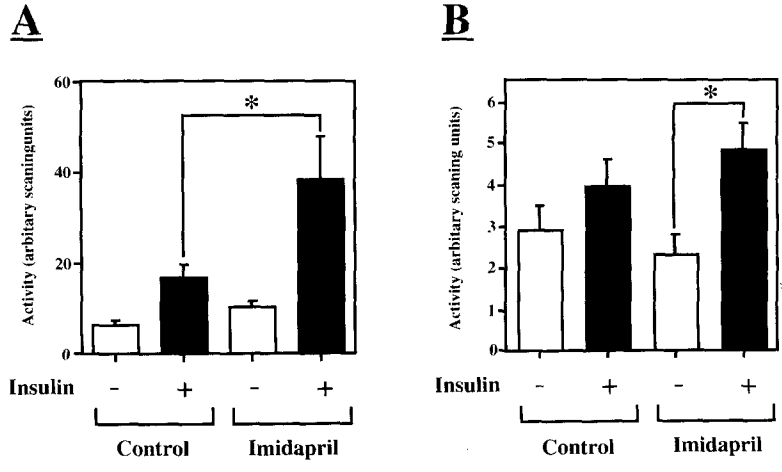

Fig 5. Effect of imidapril on PI 3-kinase activity associated with IRS-1 and IRS-2 in Zucker fatty rat liver. Imidapril or vehicle was administered orally for 10 weeks and saline (0.9% NaCl) with or without 10^{-5} mol/L insulin was injected via the portal vein of Zucker fatty rats. Liver homogenates were immunoprecipitated with anti–IRS-1 or anti–IRS-2 antibody. Measurement of PI 3-kinase activity associated with IRS-1 (A) and IRS-2 (B) immunoprecipitates from the liver was performed. The resulting labeled lipids were extracted, separated by thin-layer chromatography, then quantified and visualized with a Bio-Rad Molecular Imager. Values are mean ± SE (n = 3). *$P < .05$ *v* control (stimulated with insulin).

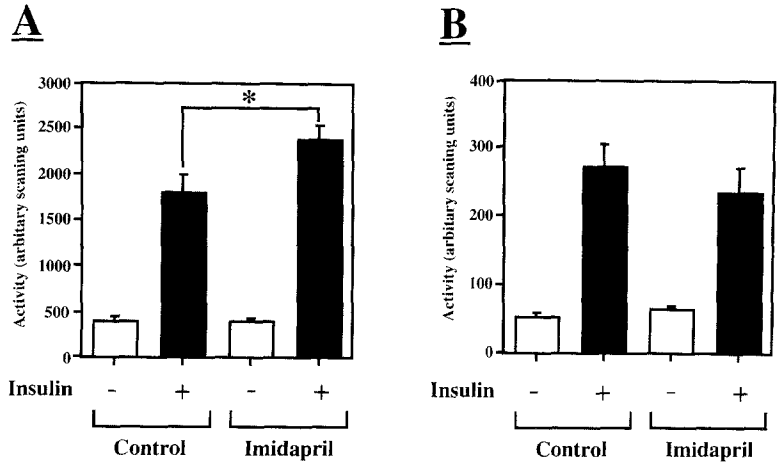

Fig 6. Effect of imidapril on PI 3-kinase activity associated with IRS-1 and IRS-2 in Zucker fatty rat muscle. Imidapril or vehicle was administered orally for 10 weeks and saline (0.9% NaCl) with or without 10^{-5} mol/L insulin was injected via the portal vein of Zucker fatty rats. Muscle homogenates were immunoprecipitated with anti-IRS-1 or anti-IRS-2 antibody. Measurement of PI 3-kinase activity associated with IRS-1 (A) and IRS-2 (B) immunoprecipitates from the liver was performed. The resulting labeled lipids were extracted, separated by thin-layer chromatography, then quantified and visualized with a Bio-Rad Molecular Imager. Values are mean ± SE (n = 3). *$P < .05$ *v* control (stimulated with insulin).

in both the liver and muscle of the imidapril-treated group; however, these differences were not significant. Hepatic IRS-1–associated PI 3-kinase activity was significantly enhanced 110% without a significant alteration in the tyrosine phosphorylation level of IRS-1 in the imidapril-treated group. In muscle, the IRS-1 tyrosine phosphorylation level and PI 3-kinase activity associated with IRS-1 were also significantly enhanced 70% and 20%, respectively, in the imidapril-treated group. In contrast, an alteration of the IRS-2 pathway was observed only in liver; a significant insulin-induced increase in IRS-2–associated PI 3-kinase over the basal level was observed in the imidapril-treated group but not in the control. Thus, although imidapril treatment seems to enhance IRS-1 and IRS-2, which mediate insulin signaling, alterations in the tyrosine phosphorylation level and associated PI 3-kinase activation of IRS proteins did not appear linear. This suggests that insulin-induced phosphorylation levels of the individual tyrosine residues in IRS-1 and IRS-2 may be regulated differently. To date, although such a change in the insulin-induced phosphorylation tyrosine residues in IRS-1 and IRS-2 was suggested in previous reports,[20,23] the causative molecular mechanism has not been identified. We speculate that the conformational change induced by phosphorylation in some serine or threonine residues in IRS proteins is one of the most probable mechanisms. Similarly, we speculate that the interaction of the pleckstrin homology (PH) domain of IRS protein with phospholipid also may induce a conformational change of IRS protein, and this is another possible mechanism. We anticipate that the clarification of this mechanism may be a breakthrough in understanding the regulatory system of insulin sensitivity in the living body.

As regards the mechanism of enhancement of insulin signaling by an ACE inhibitor, two major hypotheses have been suggested. Carvalho et al[48] reported that injection of captopril or bradykinin, but not losartan, enhanced the insulin-induced increase in insulin receptor phosphorylation, IRS-1 phosphorylation, and PI 3-kinase association with IRS-1 in normal rats. Thus, one possible mechanism was suggested to be that bradykinin produced by treatment with an ACE inhibitor inhibits dephosphorylation of the insulin receptor, which results in enhancement of insulin receptor phosphorylation and subsequently PI 3-kinase activation.[17,48] In this respect, the increases in insulin-induced phosphorylation of the insulin receptor and IRS-1 in this study agree well with the previous results by Carvalho et al[48] and support this hypothesis. In this study, although the alteration in the phosphorylation level of IRS-2 and its associated PI 3-kinase activation were less dramatic than the values for IRS-1, a significant insulin-induced increase in IRS-2–associated PI 3-kinase above the basal level was observed in the imidapril-treated group, but not in the control.

Despite their considerable structural similarity, several different properties have been reported for IRS-1 and IRS-2. First, although both IRS-1 and IRS-2 contain the NPXY motif, which is involved in the association with the insulin receptor, IRS-2 possesses an additional domain bound to the insulin receptor.[49,50] Second, IRS-2 is dephosphorylated more rapidly and activates PI 3-kinase more transiently than IRS-1,[51] which suggests that IRS-1 and IRS-2 may be dephosphorylated by different tyrosine phosphatases. In addition, the intracellular distribution in adipocytes is reportedly different between IRS-1 and IRS-2.[52] Taking these previous reports into consideration, we propose two possibilities. One is that IRS-1 and IRS-2 associate with different parts of the insulin receptor, and thus, the insulin receptor activated by imidapril treatment may phosphorylate IRS-1 more efficiently than IRS-2. The other possibility is that bradykinin produced by an ACE inhibitor leads to inhibition of the tyrosine phosphatase for IRS-1 but less marked inhibition of that for IRS-2. Although we speculate that the latter possibility is more likely, further study is required to clarify this issue.

As a result, imidapril treatment induced partial normalization mainly of IRS-1– and IRS-2–mediated PI 3-kinase activation. Although it remains controversial as to whether IRS-1 or IRS-2 is more important for various insulin actions in each tissue, Rother et al[53] have clearly demonstrated that, at least in

hepatocytes, IRS-2 functions as the main effector of insulin actions through PI 3-kinase activation. Based on this finding, we speculate that only a partial improvement of insulin resistance with imidapril may be explained by insufficient normalization of the IRS-2 pathway.

The other possible mechanism for the insulin-sensitizing effect of ACE inhibitors is that the increased blood flow due to the normalization of vascular resistance by an ACE inhibitor may contribute to improved insulin sensitivity.[10-12] Our results demonstrate that imidapril acutely increased blood flow in the liver and skeletal muscle of Zucker fatty rats by reducing vascular resistance. Since Zucker fatty rats exhibit elevated blood pressure compared with lean controls as reported previously[14-16] and confirmed in our study, it seems likely that ACE inhibitors such as imidapril increase tissue blood flow, which results in an increase of insulin and glucose contact with insulin-sensitive cells. In fact, captopril treatment reportedly improved endothelium-dependent vasodilation and glycemic control in NIDDM subjects, accompanied by an increase in forearm blood flow.[54-56] Thus, we speculate that the beneficial effect of imidapril on vascular resistance (as well as blood flow) in Zucker fatty rats may also contribute to the reduction of insulin resistance.

In conclusion, imidapril treatment improved the impaired insulin sensitivity in Zucker fatty rats. The enhancement of insulin signaling, in which IRS-1 and IRS-2 are involved, is suggested as one of the mechanisms leading to the improvement of insulin sensitivity. In addition, a study of the circulation suggested that not only a direct effect on insulin signaling but also an increase in blood flow in insulin-sensitive tissues may contribute to the improvement of insulin sensitivity. Although further investigation is needed to fully clarify the mechanism, our data support the recommended use of ACE inhibitors for the treatment of diabetic patients with insulin resistance and hypertension.

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