Vasopeptidase Inhibitor Omapatrilat Induces Profound Insulin Sensitization and Increases Myocardial Glucose Uptake in Zucker Fatty Rats

Studies Comparing a Vasopeptidase Inhibitor, Angiotensin-Converting Enzyme Inhibitor, and Angiotensin II Type I Receptor Blocker

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Background—ACE inhibitors (ACEIs) improve insulin resistance and prevent type 2 diabetes, possibly mediated by inhibition of bradykinin (BK) degradation. The vasopeptidase inhibitor omapatrilat (OMA) raises BK to a greater extent than ACEIs by dual enzyme inhibition, whereas its insulin-sensitizing effects and mechanisms have not been investigated.

Methods and Results—We compared the insulin-sensitizing effects of OMA, ramipril (an ACEI), losartan (an angiotensin II type 1 receptor blocker), and placebo by 2-step euglycemic hyperinsulinemic clamp in insulin-resistant Zucker fatty rats (n = 6 to 7 in each group). OMA resulted in a lower rate of endogenous glucose production than placebo at baseline (35±5 versus 54±4 mmol · kg⁻¹ · min⁻¹, P<0.01), greater suppression of endogenous glucose production by low-dose insulin (73±11% versus 27±18%, P<0.05), and greater glucose disposal at high-dose insulin (135±5 versus 92±4 mmol · kg⁻¹ · min⁻¹, P<0.01). Ramipril tended to improve insulin sensitivity, but losartan did not. OMA significantly increased 2-deoxyglucose uptake by myocardium, fat, and skeletal muscle. Ramipril increased 2-deoxyglucose uptake only by some skeletal muscles, but losartan did not. The insulin-sensitizing effects of OMA were blocked significantly by HOE-140 (a BK, B₂ receptor antagonist) and N⁵-nitro-L-arginine methyl ester (a nitric oxide synthase inhibitor) in all tissues except myocardium.

Conclusions—OMA induces profound insulin sensitization and increases myocardial glucose uptake in Zucker fatty rats. This effect is greater than that of ramipril and probably occurs at least in part via stimulation of the B₂ receptor. OMA has the potential for greater type 2 diabetes prevention than ACEI. (Circulation. 2003;107:1923-1929.)

Key Words: insulin | bradykinin | glucose | hypertension | diabetes mellitus

Insulin resistance is an important risk factor for the development of diabetes, hypertension, atherosclerotic heart disease, and heart failure.¹ ACE inhibitors (ACEIs), frequently the first line of treatment of hypertension in people with type 2 diabetes, have been shown to improve insulin sensitivity, prevent cardiovascular events, and reduce the number of new cases of type 2 diabetes.²,³ The effect has been postulated to be a result of the bradykinin (BK)-raising effects of ACEIs.²,³ The vasopeptidase inhibitor omapatrilat (OMA) inhibits both neutral endopeptidase and ACE with similar potency.⁴ In addition to decreasing angiotensin II production, OMA also potently inhibits the metabolism of BK to a greater extent than ACEIs.⁵ In heart failure patients, OMA has been shown to improve both symptoms and prognosis compared with ACEIs.⁶ The major side effect of OMA appears to be angioedema, which is currently being addressed before the widespread use of this promising agent.⁷,⁸ Despite the concern about angioedema, clinical studies of OMA and a number of other vasopeptidase inhibitors currently in development are ongoing in an attempt to define a safe-dose range and to better predict which individuals are at the greatest risk of developing side effects.

Most of the beneficial and the adverse effects of vasopeptidase inhibitors are believed to be caused by the decreased metabolism of BK. There is increasing evidence to suggest that BK enhances skeletal and cardiac muscle glycolytic flux and glucose uptake.⁹,¹⁰ In addition, plasma BK levels have

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been found to be reduced in patients with diabetes.\textsuperscript{12,13} It is clinically important to determine whether the dual inhibitory effects of OMA on BK degradation can improve whole-body and myocardial glucose metabolism, perhaps to a greater extent than ACEIs, and to study the mechanism of this putative beneficial effect.

The purpose of the present study was first, to assess the effects of OMA on insulin sensitivity in an animal model of insulin resistance, the Zucker fatty rat. We compared the insulin-sensitizing effects of OMA, ramipril (an ACEI), and losartan (an angiotensin II type 1 receptor blocker [ARB] that does not raise BK). Potentially, if this agent is shown to be a more effective insulin sensitizer than ramipril, it may prove to have greater cardioprotective and diabetes-prevention properties than ACEIs in clinical trials. Second, a BK B\textsubscript{2} receptor antagonist and a nitric oxide (NO) synthase inhibitor were used to investigate the mechanism of the putative insulin-sensitizing effects of OMA.

**Methods**

**Experimental Animals**

Male Zucker fatty rats (fa/fa) and their lean (fa/− and −/−) littermates (Charles River, Quebec, Canada) were obtained at the age of 9 weeks and were housed individually in the Animal Care Facility of the Toronto General Hospital with a 12-hour light/dark cycle. The rats received chow (Purina 5001, 4.5% fat, Ralston Purina Co) and water ad libitum. After 3 to 5 days of adaptation to the facility, the animals were studied at \(\sim\)10 weeks of age. All procedures were approved by the Animal Care Committee of the University Health Network, University of Toronto.

**Effects of OMA, Ramipril, and Losartan on Insulin Sensitization**

The rats were anesthetized by intraperitoneal injection of ketamine/xylazine/acepromazine (20:2:1 mg/mL, 1 \(\mu\)L/g body weight), and indwelling catheters were inserted into the right internal jugular vein for later infusion and the left carotid artery for later blood sampling. Both catheters were tunneled subcutaneously and exteriorized to the back of the neck. The catheters were filled with a mixture of 60% polyvinylpyrrolidone and heparin (1000 USP/mL) to maintain patency. After 3 to 5 days of adaptation to the facility, the animals were studied at \(\sim\)10 weeks of age. All procedures were approved by the Animal Care Committee of the University Health Network, University of Toronto.

**Experimental Procedures**

**Two-Step Euglycemic Hyperinsulinemic Clamp**

Experiments were started at 6 AM after an overnight fast (food was removed at 6 PM after the final dose of medications on the day before the clamp). A 2-step euglycemic hyperinsulinemic clamp was performed after a 2-hour tracer equilibration period in unrestrained conscious rats. The first step (low-dose insulin) was conducted with a continuous infusion of human insulin (Humulin R, Eli Lilly) at a rate of 100 pmol \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) for 100 minutes, followed by a second step (high dose) at a rate of 300 pmol \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\) for 100 minutes. The lower-insulin-dose first step of the euglycemic hyperinsulinemic clamp allowed us to examine the suppressive effects of insulin on endogenous (largely hepatic) glucose production, whereas the higher-dose insulin infusion (second step) allowed us to examine insulin-mediated glucose disposal. The infusion rate of dextrose was adjusted according to the blood glucose measurements (assessed at 5- to 10-minute intervals) to maintain blood glucose at basal concentrations. A primed-continuous infusion of high-performance liquid chromatography–purified [\(3\)-H\]glucose (10 \(\mu\)Ci bolus, 0.15 \(\mu\)Ci/min; DuPont-NEN) was started 120 minutes before the beginning of the low-dose clamp (ie, during the basal period) to allow equilibration of the tracer and continued throughout both low- and high-dose clamps. To maintain specific activity within 30% of the baseline values, 12 \(\mu\)Ci [\(3\)-H\]glucose was added to the dextrose infusate. Blood samples were taken at 10-minute intervals for the final 30 minutes of the basal period and low-dose and high-dose clamps for the determination of plasma [\(3\)-H\]glucose and plasma insulin concentrations.

**Tissue Glucose Flux**

Insulin-stimulated glucose uptake by individual tissues was estimated by use of 2-deoxy-\([\text{1-}\text{\textsuperscript{14}C}]\)glucose (2-[\(\text{14}\text{C}\)]DG; NEN Life Science), a nonmetabolizable analogue, and by determining the tissue content of 2-deoxyglucose-6-phosphate. A bolus of 2-[\(\text{14}\text{C}\)]DG (10 \(\mu\)Ci) was administered 45 minutes before the end of the high-dose clamp. Blood samples were taken at 57, 60, 65, 70, 80, 90, and 100 minutes after the start of the high-dose clamp for the determination of plasma 2-[\(\text{14}\text{C}\)]DG concentrations. At the end of the study, animals were anesthetized with sodium pentobarbital. Within 5 minutes, 3 muscles (soleus, gastrocnemius, and tibialis anterior), epididymal white adipose tissue, and heart were taken, frozen immediately with liquid N\(_2\)-cooled aluminum blocks, and stored at \(-70\text{°C}\) for later analysis.

**Calculations**

Rates of basal glucose turnover and whole-body glucose uptake at the end of the basal period and during the final 30 minutes of the low- and high-dose euglycemic hyperinsulinemic clamp were calculated by use of a modified form of Steele’s equation, which takes into account the rates of basal glucose turnover and whole-body glucose uptake at the end of the basal period and during the final 30 minutes of the low- and high-dose euglycemic hyperinsulinemic clamp.
account the extra tracer infused with the glucose infusate. Endogenous glucose production rate (EGP) during clamps was determined by subtracting the glucose infusion rate from the whole-body glucose uptake. Glucose uptake in individual tissue was calculated from the plasma 2-[14C]DG profile, which was fitted with a double-exponential curve using MLAB (Civilized Software), and tissue 2-DG-6-P content.

Statistical Analyses
Data are presented as mean±SEM. The group of lean Zucker rats was compared separately with Fatty Control by unpaired t test. All other groups of Zucker fatty rats were compared by ANOVA with Fisher’s least-significant-difference test. A probability value of P<0.05 was considered statistically significant.

Results

Effects of OMA, Ramipril, and Losartan on Fasting Plasma Glucose, FFA, and Insulin Concentration and Glucose Infusion Rates During the Euglycemic Hyperinsulinemic Clamp Study
Compared with the lean rats, the Fatty Contr had significantly higher glucose, insulin, and FFA concentrations at baseline and lower glucose infusion rates at low-dose and high-dose insulin infusion (Table; Figure 2, A and B). During insulin infusion, either low-dose or high-dose, there was no significant difference in the plasma insulin and glucose concentrations between groups at each step of the clamp (Figure 1, A and B). Plasma glucose levels were clamped at baseline levels (≈6 mmol/L). The rate of glucose infusion necessary to maintain euglycemia was significantly higher in the OMA group versus Fatty Contr both at low-dose and at high-dose insulin infusion (Figure 2, A and B). The rate of glucose infusion in the ramipril and losartan treatment groups was not significantly different from Fatty Contr.

In a separate group of Zucker fatty rats at age 12 weeks, OMA significantly decreased plasma insulin (2226±396 versus 3693±507 pmol/L, P<0.05) and FFA (639±42 versus 778±45 μmol/L, P<0.05) concentrations compared with the Fatty Contr, although there was no significant difference in food intake between the 2 groups (88±3 versus 79±2 g·kg⁻¹·d⁻¹, P=0.05).

EGP and Glucose Disappearance
Compared with the lean rats, the Fatty Contr had significantly higher EGP at baseline (Table). Only OMA significantly improved the baseline EGP rate (by 35%, almost to the rate seen in the lean Zucker rats) compared with Fatty Contr (Table). During the euglycemic hyperinsulinemic clamp, the glucose specific activities were maintained within 30% of the baseline levels in all groups (not shown). During low-dose insulin infusion, EGP was 100% suppressed in the lean rats, and glucose disappearance rate (Rd) was greater than that seen in the Fatty Contr (P<0.01) (Figure 2C). Compared with Fatty Contr, OMA resulted in greater suppression of EGP from baseline (by 47%) during the low-dose insulin clamp but did not result in a significant increase in Rd above that observed in Fatty Contr (Figure 2E). During high-dose clamp, EGP was completely (100%) suppressed with OMA, which was not significantly different from Fatty Contr (Figure 2D). Rd was significantly greater, by 46% and 30%, in the OMA and ramipril treatment groups versus Fatty Contr (Figure 2F). Losartan had only an insignificant trend toward an increase in Rd (15%). OMA partially normalized the Rd by up to 62% of the Rd in Zucker lean rats.

Tissue Glucose Uptake
Insulin-stimulated tissue glucose uptake was much higher in myocardium than skeletal muscles and adipose tissues (Figure 3; note the different scales). Insulin-stimulated glucose uptake in myocardium was 42% higher with OMA treatment versus Fatty Contr (Figure 3A). The increases in the ramipril and losartan groups did not reach statistical significance (31% and 25%, respectively). In epididymal white adipose tissue, only OMA resulted in greater insulin-stimulated glucose uptake versus Fatty Contr (Figure 3B). In gastrocnemius muscle, insulin-stimulated glucose uptake was significantly greater, by 66% and 64%, in the OMA and ramipril treatment groups versus Fatty Contr but not with losartan treatment (Figure 3C). In soleus muscle, only OMA improved glucose uptake significantly, by 54% (Figure 3D). Both OMA and ramipril increased glucose uptake by tibialis anterior muscle, by 47% and 45%, respectively (Figure 3E). With Zucker lean rats used as normal controls, OMA partially normalized glucose uptake, by 82% in heart, 68% in fat, and 41% to 80% in skeletal muscles.
Antagonistic Effects of HOE-140 and L-NAME on OMA

HOE-140 alone did not have significant effects on glucose kinetics at baseline or during either low- or high-dose insulin clamps (Figure 2). During the low-dose euglycemic hyperinsulinemic clamp, L-NAME treatment alone (ie, in the absence of OMA) induced hepatic and generalized insulin resistance, as evidenced by a significantly diminished percentage suppression of EGP (14 ± 7% suppression from baseline with L-NAME versus 27 ± 18% suppression in Fatty Contri, \( P = 0.51 \)) and a lower glucose infusion rate (\( P < 0.01 \)), and lower Rd (\( P < 0.05 \)) (Figure 2, A, C, and E), but no significant effect on glucose kinetics was evident during the high-dose insulin clamp (Figure 2, B, D, and F). Both HOE-140 and L-NAME significantly abolished the insulin-sensitizing effect of OMA in the Zucker fatty rats, reducing insulin sensitivity to levels seen in Fatty Contri animals. This was evidenced by similar glucose infusion rates required to maintain euglycemia in OMA+HOE-140, OMA+L-NAME, and Fatty Contri at low-dose (Figure 2A) and high-dose (Figure 2B) insulin (differences between OMA and OMA+HOE-140 and between OMA and OMA+L-NAME were significant, as indicated in Figure 2, A and B). The reduction of EGP at baseline with OMA was abolished only by HOE-140 (Table). Both HOE-140 and L-NAME significantly blocked the effects of OMA on EGP at low-dose insulin (Figure 2C) and Rd at both low- and high-dose insulin (Figure 2, E and F). However, compared with L-NAME alone, OMA+L-NAME still had a significantly higher glucose infusion rate and percentage suppression of EGP at low-dose insulin. HOE-140 or L-NAME alone did not have significant independent effects on tissue glucose uptake compared with Fatty Contri. In myocardium, there was no significant blockade of the OMA-enhanced glucose uptake by either HOE-140 or L-NAME (Figure 3A). Conversely, both inhibitors significantly blocked the stimulatory effect of OMA on glucose uptake in epididymal white adipose tissue and skeletal muscles except for the tibialis anterior (Figure 3, B–E).

Discussion

In the present study, we have shown that OMA, a member of the vasopeptidase inhibitor class of vasoactive agents, has profound insulin-sensitizing properties in Zucker fatty rats. OMA increased insulin-stimulated glucose uptake both at the whole-body level and specifically in insulin-responsive tissues (myocardium, fat, and skeletal muscle), reduced basal EGP, and potentiated the acute suppressive effect of insulin on EGP, but not quite to the level of the Zucker lean. Furthermore, these effects were greater than those of ramipril, whereas losartan had no demonstrable insulin-sensitizing effect. The insulin-sensitizing effects of OMA were greatly diminished in the presence of a BK \( B_2 \) receptor antagonist (HOE-140) and to a slightly lesser extent by the NO synthase inhibitor (L-NAME), implicating the BK pathway in the insulin-sensitizing effects of OMA. In addition, OMA normalized the insulin-desensitizing effect of L-NAME. Generally, short-term administration of OMA partially normalized whole-body insulin sensitivity of Zucker fatty rats up to 62% of lean Zucker rats and even up to 80% in some muscles, such as myocardium and soleus muscle.

In accordance with previous reports on ACEIs,2–4,6 our data showed that ramipril had a strong tendency to improve whole-body insulin sensitivity with respect to glucose uptake, and in some skeletal muscles, ramipril showed significant enhancement of glucose uptake. Compared with ramipril, however, OMA had very consistent and potent insulin-sensitizing effects, whereas losartan had no such effect. Our findings are in keeping with those of Aguilar-Salinas et al14 and extend those observations by providing a more extensive examination of the mechanisms of the effect of OMA. Although some investigators have shown previously that ARBs increase muscle glucose uptake in vitro,15 a recently published report by Ishizawa et al16 showing no insulin-sensitizing effect of ARBs, is consistent with our findings. Our data suggest that dual enzyme inhibition with OMA, which results in a more profound effect than ACEI in prolonging BK half-life systemically or locally in myocardium,17 is the likely explanation for the greater insulin-sensitizing effect of OMA versus ramipril and for the lack of effect of losartan, which does not raise BK levels. We also showed that L-NAME reduced the insulin-sensitizing properties of OMA, but its effect was slightly less than that of
HOE-140. This could be explained by the fact that NO is one of a number of stimulatory products of BK. Other products, such as prostacyclin, have also been shown to improve tissue glucose uptake.

Previous reports of the beneficial effects of ACEIs on insulin sensitivity and glucose metabolism have generated much speculation regarding potential mechanisms, including inhibition of angiotensin II formation, inhibition of BK degradation, vasodilatation of insulin-sensitive tissues, or reduction in circulating catecholamines. There is, however, increasing experimental evidence to suggest that the BK-NO pathway plays a major role, partly independent of changes in muscle blood flow. In experimental models of diabetes, exogenous BK and ACEI were found to enhance insulin sensitivity and increase glucose uptake by both skeletal and cardiac muscle. Furthermore, infusion of a BK antagonist abolishes this insulin-sensitizing effect. At the molecular level, BK and NO were found to stimulate glucose uptake through both insulin-dependent and insulin-independent pathways. In contrast, the effect of ARBs on insulin sensitization is more controversial, with some but not all studies showing a beneficial effect. Our studies showed that NO synthase inhibition alone significantly reduced insulin sensitivity, a finding that is compatible with a previous report by Arbin et al. An interesting observation is that OMA still maintained some of its beneficial effects even in the presence of L-NAME treatment. These findings demonstrate that OMA has NO-independent in addition to NO-dependent effects in improving either hepatic or systemic insulin sensitivity.

Under basal conditions, glucose uptake by myocardium was only 2-fold greater than that of skeletal muscle, as has been shown previously by others. With supraphysiological insulin infusion, however, glucose uptake by myocardial tissue was 5 and 40 times higher than that of skeletal muscle and adipose tissue, respectively. Because HOE-140 and L-NAME did not completely reverse the positive effects of OMA on glucose uptake in myocardium, as they did in skeletal muscles, the myocardial insulin-sensitizing effects of OMA are unlikely to be mediated entirely by the BK-NO pathway. The role of BK in mediating the insulin-sensitizing effect of OMA on myocardium might not be as great as its role in skeletal muscles and adipose tissues. Reduction of angiotensin II levels by OMA could have directly affected the myocardium (OMA and HOE-140 inhibitors induced the same insulin-stimulated glucose uptake as losartan), because angiotensin II has both glycogenolytic and gluconeogenic properties and may play an important role in myocardial glycogen-rich tissue. In addition, the NO effect in myocardium is still controversial, with some reports showing that NO actually decreases myocardial glucose uptake.
Plasma insulin concentrations in the OMA group were lower at baseline. Because peripheral insulin concentrations are determined not only by insulin secretion but also to a large extent by insulin clearance and because C-peptide (insulin secretion) was not lower with OMA, the most likely explanation for the lower insulin levels with OMA is that OMA treatment resulted in enhanced hepatic and whole-body clearance of insulin. Insulin sensitization is commonly accompanied by improved insulin clearance, and this finding supports the beneficial effects of OMA on hepatic insulin sensitization. This finding is further supported by the effect of OMA in decreasing plasma FFA concentrations, a finding that may also be closely related to improvement in insulin sensitivity. The reduction in plasma FFA flux from adipose tissue to muscle, liver, and other tissues could in turn enhance the insulin-sensitizing effects of OMA. Another potential mechanism whereby OMA could enhance insulin sensitivity is by lowering intracellular long-chain fatty acyl-CoA accumulation in insulin-sensitive tissues through BK and NO stimulation of fatty acid oxidation. These putative mechanisms, however, remain highly speculative and require further testing in future studies.

What are the clinical implications of the finding that OMA improves insulin sensitivity to a greater extent than the ACEI ramipril? Considering the importance of metabolic control at the time of an acute myocardial infarction, as shown in the DIGAMI (Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction) and ECLA (Estudios Cardiológicos Latinoamérica) glucose-insulin-potassium trials, there is hope that the insulin-sensitizing effects of OMA will result in improved myocardial protection against ischemia, particularly in patients with diabetes and insulin resistance. Finally, given the impressive ~30% reduction in new cases of type 2 diabetes demonstrated with ramipril treatment in the HOPE study, we speculate that this novel vasoactive agent may also have the clinically important benefit of preventing the occurrence of type 2 diabetes in those who are at risk, such as those with insulin resistance and impaired fasting blood glucose, perhaps to an even greater extent than ramipril. The putative cardioprotective and diabetes-protective effects of OMA require further testing in large prospective randomized clinical trials in humans.

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