Molecular Mechanisms of Insulin Resistance That Impact Cardiovascular Biology

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Insulin resistance is concomitant with type 2 diabetes, obesity, hypertension, and other features of the metabolic syndrome. Because insulin resistance is associated with cardiovascular disease, both scientists and physicians have taken great interest in this disorder. Insulin resistance is associated with compensatory hyperinsulinemia, but individual contributions of either of these two conditions remain incompletely understood and a subject of intense investigation. One possibility is that in an attempt to overcome the inhibition within the metabolic insulin-signaling pathway, hyperinsulinemia may continue to stimulate the mitogenic insulin-signaling pathway, thus exerting its detrimental influence. Here we discuss some of the effects of insulin resistance and mechanisms of potentially detrimental influence of hyperinsulinemia in the presence of metabolic insulin resistance. *Diabetes* 53:2735–2740, 2004

Insulin resistance is a prevalent medical condition that accompanies type 2 diabetes, obesity, hypertension, metabolic syndrome, and polycystic ovary disease (1). Furthermore, offspring of insulin-resistant individuals are less sensitive to insulin when compared with control subjects (2), suggesting a hereditary nature of at least some components of insulin resistance.

Insulin resistance has elicited great interest in medical and scientific communities because of its association with cardiovascular disease (3,4). However, the molecular mechanism(s) tying insulin resistance to the development and/or progression of atherosclerosis remains enigmatic.

The term "insulin resistance" as it is used in clinical and experimental settings underscores the inability of insulin to promote normal homeostasis of glucose. In other words, a suboptimal strength of insulin action demands the presence of higher-than-normal concentrations of insulin in order to maintain normoglycemia and normal utilization of glucose by insulin target tissues. Thus, the term "insulin resistance" implies the existence of metabolic insulin resistance, which reflects an inadequate effect of insulin on glucose metabolism, but does not address other aspects of insulin action. However, insulin, the most potent anabolic hormone in the body, exerts a multitude of effects on lipid and protein metabolism, ion and amino acid transport, cell cycle and proliferation, cell differentiation, and nitric oxide (NO) synthesis (5).

Therefore, it is critically important to understand whether insulin resistance affects all aspects of insulin action equally. Physiologically, the fact that the half-maximal effective concentration of insulin action ranges widely, depending on the insulin action studied, has been known for a long time (6). Inhibition of lipolysis appears to be the most sensitive to insulin, while insulin effect on glucose oxidation is among the least sensitive. Conceivably, therefore, insulin resistance could affect certain aspects of insulin action to a greater extent than others.

The second important concept is to distinguish the influence of insulin resistance from that of compensatory hyperinsulinemia that invariably accompanies insulin resistance. If the detrimental influence of insulin resistance is a consequence of reduced insulin action, then compensatory hyperinsulinemia is merely an innocent bystander and has no effect of its own. In contrast, if certain aspects of insulin action are not affected by the diminished strength of insulin, then the presence of compensatory hyperinsulinemia may have its own influence. As a result, compensatory hyperinsulinemia may stimulate or even overstimulate certain aspects of insulin action in various cells and tissues. Clinical and epidemiological studies yielded mixed information and failed to provide definitive evidence either in favor of or against the role of hyperinsulinemia per se.

Therefore, the truly critical point in understanding the role of insulin resistance is to determine whether diminished insulin action (effect of insulin resistance) may coexist with normal or even enhanced insulin action (effect of hyperinsulinemia) within the same tissue and within the same cell. This task became feasible with the unraveling of the intracellular insulin-signaling cascade. Initial studies elucidated the two major postreceptor signaling pathways that convey the insulin signal downstream (7,8). One pathway, involving the phosphorylation of insulin receptor substrate (IRS)-1 and -2 and activation

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ARB, angiotensin receptor blocker; eNOS, endothelial nitric oxide synthase; FTase, farnesyltransferase; GGTase I, geranylgeranyltransferase I; HOPE, Heart Outcomes Prevention Evaluation; IRS, insulin receptor substrate; MAP, mitogen-activated protein; NF-κB, nuclear factor-κB; PDGF, platelet-derived growth factor; PI, phosphatidylinositol; PPAR, peroxisome proliferator–activated receptor; RAAS, renin-angiotensin-aldosterone system; SM-actin, smooth muscle α-actin; VSMC, vascular smooth muscle cell.

of phosphatidylinositol (PI) 3-kinase appears to be absolutely necessary for mediating metabolic effects of insulin (9,10). This pathway also contributes to the mitogenic aspects of insulin action. The second signaling pathway appears to involve the phosphorylation of Shc and activation of Ras, Raf, MEK, and mitogen-activated protein (MAP) kinases (Erk 1 and 2). In contrast to the IRS/PI 3-kinase pathway, activation of the Shc-Ras-MAP kinase intermediates contributes solely to the nuclear and mitogenic effects of insulin and plays no role in conveying the metabolic action of insulin (11,12).

Subsequent experiments have introduced a concept of "selective insulin resistance." This concept has received its first experimental support from the work of Jiang et al. (13) and Cusi et al. (14). Jiang et al. (13) compared insulin signaling via the PI 3-kinase and *Erk* MAP kinase pathways in vascular tissue of lean and obese Zucker rats in both in vivo and ex vivo studies. Both experimental approaches (i.e., in vivo and ex vivo) clearly demonstrated a significant decrease in the ability of insulin to stimulate the phosphorylation of IRS-1, the association of the p85 regulatory subunit of PI 3-kinase with IRS-1, the activity of PI 3-kinase, and the phosphorylation of Akt (a downstream serine kinase of the PI 3-kinase pathway) in the vasculature of obese insulin-resistant rats. In contrast, the stimulatory effect of insulin on *Erk* MAP kinase remained intact in these animals.

Cusi et al. (14) performed somewhat similar experiments in humans and assessed the two pathways of insulin signaling in muscle biopsy samples obtained from patients with type 2 diabetes, obese nondiabetic individuals, and lean control subjects before and after euglycemic-hyperinsulinemic clamp. Insulin stimulation of the PI 3-kinase pathway was dramatically reduced in obese nondiabetic individuals and virtually absent in type 2 diabetic patients. In contrast, insulin stimulation of the *Erk* MAP kinase pathway was normal in obese and diabetic subjects. Subsequent studies have also demonstrated a differential impact of insulin resistance on these two pathways of insulin signaling (15,16).

HYPOTHESIS

Based on these data we hypothesize the following. Compensatory hyperinsulinemia that accompanies insulin resistance generates either a normal or a stronger-than-normal initial signal at the level of the insulin receptor. Progression of this signal downstream is impaired along the IRS-1 and PI 3-kinase pathway (the essence of the metabolic insulin resistance). In contrast, the signal proceeds normally or with greater strength (because of compensatory hyperinsulinemia of various degrees) along the Shc-Ras-MAP kinase pathway, eliciting greater responses of the downstream targets of this pathway.

EXPERIMENTAL CONFIRMATION

To explore this hypothesis we designed an experimental paradigm in vitro that mimics the main defect of the insulin resistance, namely, a blockade of the PI 3-kinase–dependent pathway (17,18). To examine the role of compensatory hyperinsulinemia in vascular biology, we conducted these experiments in endothelial and vascular smooth muscle cells (VSMCs). The cells were treated with PI 3-kinase inhibitors, either wortmannin or LY 294002 or with a *Erk* MAP kinase pathway inhibitor (PD 98059) before the insulin challenge. Effectiveness of these inhibitors in suppressing their corresponding signaling branches was confirmed in control experiments.

In endothelial cells, insulin stimulates the expression and activity of endothelial NO synthase (eNOS), resulting in increased production of NO (19,20). This action of insulin is mediated via the PI 3-kinase pathway (21,22) where Akt, a downstream target of the PI 3-kinase, promotes phosphorylation of eNOS (serine 1179) and its activation (23,24).

Under normal circumstances, NO is not only critically important in the process of vasodilatation, but it also counteracts the stimulatory effect of VEGF on expression of adhesion molecules such as E-selectin, intracellular adhesion molecule, and vascular cellular adhesion molecule, thereby protecting the cells from excessive interactions with circulating monocytes (25). Conceivably, stimulatory effects of insulin on eNOS and NO production may be equally important in preventing endothelial dysfunction and early proatherosclerotic changes in response to oxidized LDL, smoking, and other injurious agents. However, in cells treated with inhibitors of PI 3-kinase, insulin is no longer capable of stimulating eNOS and increasing NO production (21,22,25). Consequently, these cells are no longer protected from the detrimental influence of VEGF, and in fact, compensatory hyperinsulinemia increases the interaction of these cells with circulating monocytes, promoting this very first step in the pathogenesis of atherosclerosis (17,26).

The continuum of existence of VSMCs takes them from the poorly differentiated, highly proliferative state to the well-differentiated, contractile state (27). The latter has been shown to be promoted and maintained by insulin (28). In contrast, proliferation of VSMCs is significantly enhanced by platelet-derived growth factor (PDGF) (29). Because well-differentiated VSMCs express greater amounts of smooth muscle α-actin (SM-actin), a protein responsible for their contractile function, a decline in the amount of SM-actin correlates with their dedifferentiation and progression to a more proliferative state (27).

We used inhibitors of the PI 3-kinase and MAP kinase pathways to determine the role of these signaling pathways in the mechanism of insulin action on VSMCs (18). Well-differentiated VSMCs maintained in a serum-free medium remain quiescent and express certain (control) levels of SM-actin. Insulin further promotes their differentiation and increases SM-actin expression. This effect of insulin is lost when the PI 3-kinase is inhibited but remains unaffected when the *Erk* MAP kinase pathway is blocked. Insulin is also capable of counteracting the effect of PDGF. In both instances, inhibitors of the PI 3-kinase–dependent signaling pathway eliminated the effects of insulin. These experiments indicated that PI 3-kinase–dependent signaling is critically important for insulin to promote and maintain the quiescent, differentiated, and contractile phenotype of VSMCs (18). In contrast, a mild effect of insulin on VSMC migration was blocked by the PD 98059 compound and not by the inhibitors of the PI 3-kinase, strongly suggesting that this function of insulin is preserved in the state of metabolic insulin resistance (18).

Taken together, our experimental paradigm in endothelial cells and VSMCs indicates that under normal conditions insulin is antiatherogenic. In endothelial cells, insulin stimulates NO production and thereby antagonizes the effects of VEGF on expression of adhesion molecules. In the presence of inhibited PI 3-kinase activity, insulin is no longer capable of stimulating NO production or antagonizing VEGF.

In VSMCs, under normal circumstances, insulin promotes their differentiation and antagonizes PDGF. In the presence of inhibited PI 3-kinase activity, insulin is no longer capable of either maintaining a differentiated phenotype of VSMC or antagonizing the proliferative action of PDGF. Furthermore, in the presence of the inhibited PI 3-kinase activity, insulin may continue to exert its action on VSMC migration via unimpaired *Erk* MAP kinase signaling. Thus, in the insulin-resistant state, the sine qua non of which is an impaired PI 3-kinase signaling, insulin is no longer antiatherogenic and its action via unimpaired MAP kinase pathway may contribute to the proatherogenic milieu.

There is one additional aspect of insulin action that has a strong potential proatherogenic influence. This is the ability of insulin to activate the prenyltransferases, farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I) (30). These enzymes promote farnesylation and geranylgeranylation of Ras and Rho proteins, respectively (31). Prenylation of these proteins is a mandatory posttranslational modification step that makes them amenable to activation (31). Attachment of either a farnesyl moiety to Ras or a geranylgeranyl moiety to Rho (both of which are produced in the process of cholesterol synthesis at the steps distal to 3-hydroxy-3-methylglutaril coenzyme A reductase) allows these proteins to anchor at cellular membranes and become targets for activation by GTP loading under the influence of various growth factors. Both enzymes FTase and GGTase I consist of α- and β-subunits. The α-subunit is the same for both enzymes, while the β-subunit confers their specificity in prenylating either Ras or Rho proteins (32).

Insulin promotes the phosphorylation of the α-subunit and thereby increases the activity of both enzymes (30,33). The effect of insulin on the prenyltransferases is mediated via the *Erk* MAP kinase with the PI 3-kinase–dependent signaling pathway being completely unrelated to this aspect of insulin action (33). The upstream elements of the Ras-MAP kinase pathway include Shc, Grb-2-Sos, Ras, and Raf. We determined that in addition to a direct signal from *Erk* MAP kinase, another signal from Shc that involves its SH3 domain and is unrelated to activation of Ras, Raf, and MAP kinase is also necessary for the phosphorylation and activation of the prenyltransferases by insulin (34). The downstream intermediates of this signaling pathway have not yet been identified. In summary, two signals from Shc, one via the MAP kinase and the other via a different mechanism, are necessary for activation of the prenyltransferases by insulin.

The importance of these findings to the role of hyperinsulinemia in the presence of metabolic insulin resistance cannot be underestimated. With the diminished strength of insulin signaling via the PI 3-kinase, a compensatory hyperinsulinemia continues to activate prenyltransferases, thereby increasing the amounts of farnesylated Ras and geranylgeranylated Rho, both of which can be readily activated by a variety of growth-promoting agents (35,36).

We have previously shown the presence of increased amounts of prenylated Ras and Rho in tissues of insulin-resistant hyperinsulinemic animals and in cultured cells under hyperinsulinemic conditions (37,38). Furthermore, hyperinsulinemia-induced increases in the amounts of prenylated Ras and Rho resulted in augmentation of cellular responses to IGF-1, epidermal growth factor, PDGF, and angiotensin II (35,36,39,40). The latter is particularly important in VSMCs where hyperinsulinemia doubled the ability of angiotensin II to transactivate nuclear factor-κB (NF-κB) (39). This effect of hyperinsulinemia was completely blocked by an inhibitor of GGTase I.

To summarize the role of compensatory hyperinsulinemia in vasculature in the presence of insulin resistance, there are three important aspects of its action: *1*) inability to maintain eNOS activity and NO production, *2*) inability to maintain VSMC quiescence and counteract PDGF, and *3*) increased ability to promote prenylation of Ras and Rho proteins and to potentiate action of other growth-promoting agents. All three aspects are a direct consequence of impaired signaling via the PI 3-kinase and stronger signaling via the Shc-Ras-MAP kinase pathway.

The extensive work of Dandona et al. (rev. in 41) has produced a substantial and convincing body of evidence in favor of antiatherogenic and anti-inflammatory influence of insulin. Insulin appears to inhibit expression of adhesion molecules, monocyte chemoattractant protein-1, and activation of NF-κB. However, hyperinsulinemia in the presence of insulin resistance (i.e., impaired PI 3-kinase signaling) may exert a detrimental influence on the arterial wall and could potentiate the development of atherosclerosis. Moreover, these conclusions suggest that amelioration of insulin resistance with exercise, weight loss, or insulin sensitizers would represent a much superior therapeutic approach than simply increasing insulin levels by either administering insulin or insulin secretagogues to patients with insulin resistance. In particular, amelioration of the metabolic insulin resistance (an improvement in insulin signaling via the PI 3-kinase pathway) is expected to remove excessive stimulation of the *Erk* MAP kinase signaling pathway as well as prenylation and thereby improve antiatherogenic properties of insulin. Experimental and clinical studies have provided strong support for these conclusions.

CLINICAL SUPPORT

There are four independent ways of improving insulin sensitivity or reducing insulin resistance, namely lifestyle modification, Metformin, thiazolidinediones, and inhibitors of the renin-angiotensin-aldosterone system (RAAS). Each of these therapeutic approaches has been shown to improve insulin sensitivity. The problem with these studies is that all of these agents exert a profound influence on other aspects of cardiovascular pathophysiology that might explain, at least in part, their beneficial effects on atherosclerosis independently of their effect on insulin resistance.

1) Lifestyle modification

Lifestyle modifications such as weight loss, exercise, and smoking cessation have been shown to improve insulin sensitivity and lipid profile, to lower blood pressure, to decrease the risk of developing type 2 diabetes, and to reduce the risk of coronary heart disease and stroke (42). Lifestyle modification remains the cornerstone of any successful diabetes and cardiovascular prevention program. Invariably, improvement in insulin sensitivity is associated with better insulin signaling along the PI 3-kinase pathway.

2) Metformin

The most dramatic supporting evidence for our hypothesis came from the U.K. Prospective Diabetes Study (43). A decrease in macrovascular disease among all patients in the intensive therapy group was only ~12% and did not reach statistical significance, whereas a decrease in macrovascular complications among diabetic patients on metformin was ~40%. In other words, metformin-induced improvement in insulin sensitivity with a concomitant decline in insulinemia was much more potent in reducing cardiovascular problems than an equally significant reduction in glycemia without improvement in insulin sensitivity. This clearly supports the notion that treatment of hyperglycemia without an attempt to improve insulin sensitivity and reduce insulinemia may not be as cardioprotective as a comprehensive approach that deals with these issues as well.

3) Thiazolidinediones

Presently two drugs, rosiglitazone and pioglitazone, that belong to this class of agents are approved by the Federal Drug Administration for the treatment of diabetes. Thiazolidinediones are synthetic ligands that belong to a family of nuclear receptors known as peroxisome proliferator–activated receptors (PPARs) (44). The thiazolidinediones' binding affinity for PPAR-γ appears to correlate with their glucose-lowering ability. These drugs are also known as insulin sensitizers because they appear to improve insulin sensitivity (45). In addition to lowering blood glucose, both drugs may improve other cardiovascular risk factors, such as lipids, blood pressure, inflammation, and endothelial function (46). These observations suggest that reversal of insulin resistance may be accompanied by an improvement in the cardiovascular risk factors. Because thiazolidinedione receptors are expressed in all major cells of vasculature (47), the direct action of these drugs on arterial wall may be even more important than their effect on glycemia.

4) RAAS

The pathophysiological link between angiotensin II and atherosclerosis has been firmly established (48). The most powerful confirmation was demonstrated in the Heart Outcomes Prevention Evaluation (HOPE) trial. In this study, 9,297 high-risk patients with clinical evidence of vascular disease, diabetes, or other cardiovascular risk factors were randomized to the ACE inhibitor ramipril or placebo. A significant decrease of 21% in the primary end point (a composite of myocardial infarction, stroke or death from cardiovascular disease) was observed in the ACE inhibitor group.

Angiotensin II potently stimulates the expression of adhesion molecules on endothelial cells, an effect that is enhanced in the presence of insulin resistance and reduced NO production (rev. in 49). (Note: NO production is regulated by insulin via the PI 3-kinase signaling pathway.) Activation of the vascular RAAS system may interfere with insulin signaling, promoting and exacerbating preexisting insulin resistance. At the same time, angiotensin II stimulates the generation of reactive oxygen species, resulting in the destruction of NO. The effect of angiotensin II is directed at the inhibition of PI 3-kinase and its downstream kinase, Akt (50). Thus, angiotensin II opposes the action of insulin to enhance glucose uptake in skeletal muscle and may lead to insulin resistance in the vasculature (51).

Angiotensin II also stimulates transactivation of NF-kB, which is known to be important in the pathogenesis of the inflammatory process involved in atherosclerosis. This effect of angiotensin II is potentiated by hyperinsulinemia via its action on GGTase I (39) as described above. Ambient hyperinsulinemia doubles the effect of angiotensin II on activation of NF-kB (39). It is important to note that the HOPE trial demonstrated that despite almost identical levels of blood pressure, patients with diabetes treated with an ACE inhibitor (ramipril) displayed a significant reduction in cardiovascular events (48).

Somewhat similarly, the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial has also shown that inhibition of RAAS, independently of antihypertensive effects, can reduce cardiovascular and renal events (52). The benefits of losartan were even more pronounced in patients with diabetes (24% reduction in the primary end point, 37% reduction in cardiovascular mortality, and 39% reduction in total mortality versus atenolol).

Two additional trials (the Reduction of End Points in Noninsulin Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan [RENAAL] trial and the Irbesartan Diabetic Nephropathy Trial [IDNT]) have provided convincing evidence that angiotensin receptor blockers (ARBs) significantly reduce the rate of death and development of end-stage renal disease (53,54). Finally, the role of RAAS in the development of insulin resistance is suggested by a reduced incidence of type 2 diabetes in animal models and in individuals in clinical trials of ACE inhibitors and ARBs (48,55).

SUMMARY

Insulin under normal circumstances exerts its antiatherogenic action in endothelial cells and VSMCs via the PI 3-kinase signaling pathway. Antiatherogenic aspects of insulin action include stimulation of NO production, counteraction of VEGF and PDGF effects, and maintenance of a differentiated state of VSMCs. In the presence of metabolic insulin resistance (i.e., diminished strength of the PI 3-kinase signaling), the resulting compensatory hyperinsulinemia becomes proatherogenic, stimulating both the MAP-kinase signaling pathway and excessive prenylation of Ras and Rho proteins (Fig. 1). Therefore, treatment of insulin-resistant individuals must include effective measures to reduce insulin resistance (i.e., to improve insulin sensitivity) and to decrease insulinemia.

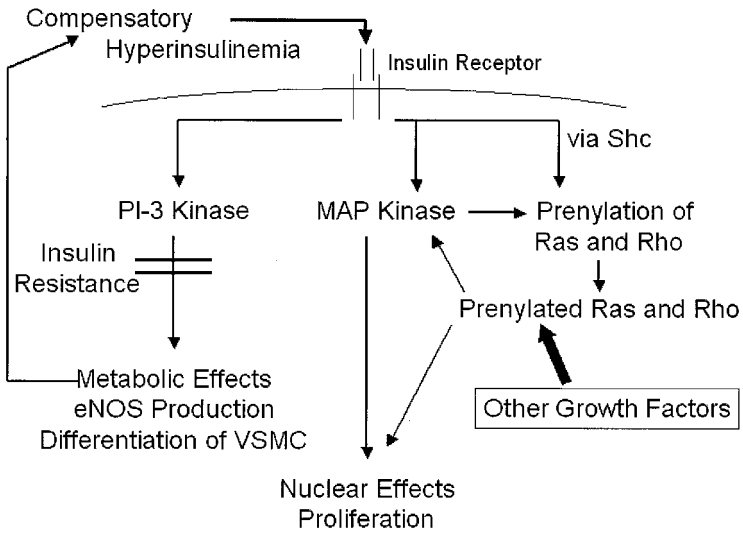

FIG. 1. Proposed influence of insulin resistance and compensatory hyperinsulinemia on proatherosclerotic events in the vascular wall. In the presence of inhibited PI-3 kinase signaling, compensatory hyperinsulinemia continues to stimulate the MAP kinase signaling pathway and increases the amounts of prenylated Ras and Rho proteins that are available for activation by other growth factors.

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