Angiotensin II-Induced Insulin Resistance and Protein Tyrosine Phosphatases

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Abstract—Although the importance of protein tyrosine phosphorylation by tyrosine kinases in mitogenic signaling is well-accepted, recent studies also suggest that tyrosine dephosphorylation by protein tyrosine phosphatases (PTPases) play an equally important role. For example, both angiotensin II (Ang II) and insulin are known to mediate protein tyrosine phosphorylation and dephosphorylation events. These apparently paradoxical effects of Ang II and insulin suggest that both convergent and divergent intracellular signaling cascades are stimulated downstream of their respective receptors, producing diverse cellular responses. In this review, we discuss the hypothesis that the protein tyrosine phosphatase (PTPase), PTP-1B, plays a central role in Ang II-induced insulin resistance by inhibiting activation of the insulin receptor. We hypothesize that Ang II-induced PTP-1B activation leads to dephosphorylation of the insulin receptor and that this signaling pathway underlies the maladaptive responses observed in diabetic vascular and renal tissue during type II diabetes. (Arterioscler Thromb Vasc Biol. 2004;24:1-5.)

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Cardiovascular and renal complications are the leading causes of morbidity and mortality in patients with diabetes mellitus, and Ang II has been implicated in the pathogenesis of maladaptive growth in both of these end organs.1-3 For example, inhibition of Ang II-induced growth of vascular smooth muscle cells (VSMC) and glomerular mesangial cells (GMC) with angiotensin-converting enzyme (ACE) inhibitors and Ang II AT1 receptor blockers (ARB) reduces cardiovascular and renal disease in diabetic patients and animal models.4,5 High glucose has also been shown to augment Ang II-induced responses, ie, growth, contraction, Ca2+ signaling, and AT1 receptor density. Furthermore, hyperglycemia is associated with an increased incidence of neointimal hyperplasia (VSMC proliferation) and progression of nephropathy (glomerular mesangial cell [GMC] growth) in patients with diabetes.6 The AT1 receptor has been linked to mitogenic signaling cascades (ie, JAK/STAT, p21ras/Raf-1/MAP kinase, and PLC-γ1), and investigators and clinicians have been able to prevent VSMC and GMC growth and proliferation by inhibiting many of these Ang II-mediated events using molecular, biochemical, and pharmacological approaches.8,9

Both ACE inhibitors and ARB are first-line therapeutic agents for the treatment of hypertension in patients with the cardiometabolic syndrome and those with diabetes. Therapy with ACE inhibitors and ARBs reduces microvascular and macrovascular complications in diabetes and appears to improve insulin sensitivity and glucose metabolism. Several recent studies indicate that ACE inhibitor and ARB therapy also reduce the development of type II diabetes in persons with essential hypertension, a population with a high prevalence of insulin resistance.10 ACE inhibition and ARBs have been shown to improve potential surrogates of chronic cardiovascular disease (eg, vascular compliance, endothelial-derived nitric oxide production, vascular relaxation and plasma markers of inflammation, oxidative stress, and thrombosis) and reduce progression of cardiovascular and renal disease, and occurrence of stroke.10

Ang II Signaling Pathways

The actions of Ang II are mediated through 2 types of cell surface receptors (AT1 and AT2). Most physiological responses triggered by Ang II in VSMC occur via the AT1 subtype.7 For AT1 receptors, activation by Ang II results in G protein-mediated signaling, including phospholipase C-dependent activation of protein kinase C and release of calcium from intracellular stores.7 AT1 receptors also activate signaling pathways traditionally associated with growth factor and cytokine receptors, which induce the production of early growth response genes, and the Ang II-induced expression of these early growth response genes is under control of intracellular signal transduction pathways.7 Three intracellular signaling pathways have been implicated in the activation of proto-oncogenes, the JAK/STAT, p21ras/Raf-1/MAP kinase, and the PLC-γ1 signaling cascades.7 From multiple studies focusing on AT1 receptor signal transduction pathways, it has become apparent that the temporal arrangement of agonist stimulated signaling varies from seconds (ie, the...
activation of PLC-γ1 and generation of inositol phosphates) to minutes (e.g., MAP kinase activation) to hours (e.g., JAK/STAT pathway). The exact mechanism(s) by which the AT1 receptor differentially couples to disparate signal transduction pathways is not clear, but presumably involves a complex series of steps that selectively recruits, activates, and then inactivates each signaling system in a time-dependent manner.

**Insulin Signaling Pathways**

Similar to many cytokine receptors, the insulin receptor (IR) is a heterotetramer of IRα and IRβ subunits. Whereas IRβ has an active cytoplasmic tyrosine kinase domain, these are inactive in IRα. Only one receptor heterodimer needs to engage insulin for intracellular signaling to be initiated. This results in mutual phosphorylation of the tyrosine kinase domains, allowing binding and phosphorylation of a variety of intracellular proteins, including insulin receptor substrate-1 (IRS-1), IRS-1 and other proteins bind transiently, via PTB-domains, to the insulin receptor. Once bound, they are phosphorylated on an YXXM/YMXM motif. Each phosphorylated IRS-1 molecule can then act as a docking site for SH2-domains of a variety of signaling proteins, including JAK2, STAT1, STAT3, PLC-γ, Grb2, and phosphatidyl inositol 3 kinase (PI3K). As these signaling molecules assemble and become activated (or display increased susceptibility for phosphorylation), a distinct signaling cascade is triggered. Tyrosine phosphorylation activates JAK2, which phosphorylates STAT proteins resulting in the formation of homodimers. Phosphorylated PLC-γ degrades phosphorylated inositol bisphosphate to produce 1,4,5-IP3 and DAG, which in turn activate protein kinase C, respectively. PI3K converts phosphatidyl inositol 3-phosphate to PI3K, which in turn activates phosphatidylinositol 3 kinase (PI3K) phosphatase. PI3K is a key enzyme in insulin-induced signaling cascades. For example, inhibition of PI3K activity using dominant-negative mutants or pharmacological agents, such as wortmannin or LY294002, abolishes insulin-induced glucose uptake. In addition, many other cellular effects of insulin, such as antipolysis, activation of fatty acid synthesis, Akt phosphorylation, and stimulation of protein and DNA synthesis, are also dependent on PI3K. Finally, insulin stimulates an IRS-1/Grb2/SOS complex that activates ras, a small G-protein in the chain of signaling events leading to activation of MAP kinase.

**Insulin Resistance**

Increased insulin action, associated with hyperinsulinemia, is thought to contribute to atherogenesis through smooth muscle cell hypertrophy and hyperplasia, and increased synthesis of extracellular matrix proteins. Evidence in support of this concept derives from studies of insulin in cell culture demonstrating increased smooth muscle cell mitogenesis, protein synthesis, and production of matrix proteins, such as type I collagen. However, insulin is a weak agonist for these effects and supraphysiologic concentrations of insulin were required in most studies. By contrast, the vasodilatory actions of insulin are well-recognized, and there is evidence that this effect may be, at least in part, mediated by nitric oxide (NO). The ability of insulin to increase NO generation has been shown in cultured endothelial cells, and the proposed mechanism involves PI3K-dependent activation of endothelial NO synthase. NO has antiatherogenic functions, including diminished expression of cell adhesion molecules and inhibition of smooth muscle cell migration and growth.

Because insulin may have both pro-atherogenic and anti-atherogenic effects in the vasculature, information about the balance of insulin action in insulin-resistant states is important. Insulin action is assumed to be increased in vascular tissues under these conditions, but there is little or no in vivo evidence to support this assumption. Conversely, reports show that the ability of insulin to induce vasodilation is reduced in insulin-resistant states and diabetes, potentially because of diminished endothelial synthesis and/or enhanced inactivation of NO. Alternatively, this and possibly other actions of insulin may be diminished by specific mechanisms involved in insulin resistance.

In summary, insulin resistance and hyperinsulinemia are closely associated with several disease processes such as hypertension, noninsulin-dependent diabetes, neointimal hyperplasia, and dyslipidemia (syndrome X). However, the pathogenic role of insulin resistance and/or hyperinsulinemia in the development of hypertension and other cardiovascular diseases is still not clear.

**Angiotensin II Signaling, Insulin Resistance, and Diabetes**

Studies have shown that Ang II infusion induces insulin resistance, and that ACE inhibitors and ARB enhance insulin sensitivity. Therefore, overactivity of the renin-angiotensin system as observed in cardiovascular diseases is likely to impair insulin signaling and contribute to insulin resistance. However, the molecular mechanisms by which Ang II inhibits insulin signaling remain unclear. Nevertheless, there are data suggesting that Ang II acting through AT1 receptor inhibits the actions of insulin in vascular tissue, in part, by interfering with insulin signaling through PI3K and downstream Akt signaling pathways via generation of reactive oxygen species (ROS). In addition, other factors in the diabetic microenvironment may also impact Ang II signaling. For example, we have recently found that high glucose and advanced glycoxidation end products (AGEs), known to accumulate in diabetic tissues (including the vasculature), potentiate Ang II intracellular signaling. The latter occurs via AGE interaction with the receptor for AGE (Figure 1). Rector for AGE is a multiligand member of the immunoglobulin-like receptor superfamily expressed at increased levels in cells of the vessel wall in diabetes.

**Protein Tyrosine Phosphatases**

Protein tyrosine phosphorylation-dependent mitogenic signals are, of necessity, transient in nature. To reset or “switch off” mitogenic signaling pathways that are activated via phosphorylase and phosphotyrosine-binding domain interactions (e.g., src homology [SH2] domains), a PTPase is required to extinguish the signal initiated by protein tyrosine kinases. PTPases are divided into 2 types: low-molecular-
weight (<80 kDa) cytosolic PTPases and high-molecular-weight (>80 kDa) membrane-associated (transmembrane) PTPases. Cytosolic PTPases, like SHP-1 and SHP-2, have a single PTPase catalytic domain, whereas those present in the cell membrane have 2 PTPase domains. PTP-1B belongs to the low-molecular-weight cytosolic PTPases family. PTPases have also been shown to play a role in Ang II receptor-mediated signaling. For example, recent studies from our laboratory show that Ang II regulates activation and tyrosine phosphorylation of SHP-1 and SHP-2 in VSMC and GMC.

Protein Tyrosine Phosphatases and Insulin Resistance

Although initially viewed as housekeeping enzymes, research over the past 15 years has revealed that the PTPases are critical regulators of tyrosine phosphorylation-dependent signaling events and may represent novel targets for therapeutic intervention in a variety of human diseases. The significance of protein phosphorylation on tyrosine residues in mitogenic signaling is well-accepted. However, tyrosine dephosphorylation by PTPases may also have a crucial role. For example, our laboratory and other investigators have shown that PTPase inhibition with vanadium promotes mitogenesis and cell proliferation, and potentiates the mitogenic signaling cascades of Ang II, insulin, IGF-1, EGF, and PDGF in several cell types, including VSMC. Probably because of their insulin-like effects, vanadium compounds have recently been shown to ameliorate glucose homeostasis in type II diabetic patients and animals. In view of the alarming increase in occurrence of type II diabetes, there has been considerable effort in many laboratories to identify a spectrum of suitable targets as a basis for drug design. In this context, PTPases that attenuate insulin signaling by dephosphorylating the insulin receptor have been actively pursued. The rationale underlying this approach is that inhibition of phosphatases would be expected to prolong insulin signaling and thereby facilitate glucose uptake and, presumably, result in a lowering of blood glucose. Targeting the IR protein tyrosine phosphatase, therefore, has the potential to be a significant disease-modifying strategy. Several PTPs have been implicated in the dephosphorylation of the IR. These phosphatases include PTPα, LAR, CD45, PTPε, SHP-2, and PTP-1B. In most cases, there is evidence for and against the involvement of these phosphatases in insulin signaling. The most convincing data, however, support a critical role for PTP-1B in insulin action. PTP-1B knockout mice display increased insulin sensitivity and maintain euglycemia (in the fed state) with one-half the level of insulin observed in wild-type littermates. Interestingly, these mice are also resistant to diet-induced obesity when fed a high-fat diet. The insulin-sensitive phenotype of the PTP-1B knockout mouse is reproduced when expression of this phosphatase is suppressed using an antisense oligonucleotide approach in obese mice. Thus, PTP-1B appears to be an attractive candidate for the design of drugs for type II diabetes and obesity.

Ang II Activation of PTP-1B and Insulin Resistance

Ang II signaling, via AT1 receptors, induces the activation of the vascular NADPH oxidase, leading to increased generation of ROS. ROS are important intracellular second messengers that activate many downstream signaling molecules, such as PTPases and protein tyrosine kinases. Activation of these signaling cascades leads to VSMC growth and migration, modulation of endothelial function, expression of pro-inflammatory mediators, and modification of extracellular matrix. We have previously reported that Ang II stimulates tyrosine phosphorylation and activation of the PTPase SHP-2 in VSMC. In addition, we have found that SHP-2 has a positive role in Ang II-induced JAK/STAT signaling, whereas the other cytosolic PTPase, SHP-1, has a negative role in Ang II induction of the JAK/STAT pathway. However, it is not currently known whether Ang II might also
have an impact on the activation of PTP-1B in VSMC. Nevertheless, we have recently shown that Ang II induces the activation of PTP-1B in VSMC.33 We observed that PTP-1B was activated in response to Ang II. Furthermore, we also found that by depleting the VSMC of PTP-1B, during pre-incubation of the cells with PTP-1B antisense, insulin-induced phosphorylation of IRS-1 in VSMC was not significantly inhibited by Ang II. These results suggest that PTP-1B plays a key role in the Ang II-induced blockade of insulin-induced signaling events.33

Effects of H-89 (PKA Inhibitor) on Insulin-Induced Tyrosine Phosphorylation of Its Receptor

Previous studies have shown that PTP-1B is activated via PKA-dependent serine phosphorylation and deactivated via tyrosine phosphorylation.34 Therefore, to determine whether Ang II blocks insulin-induced autotyrosine phosphorylation of the insulin receptor, via a PKA-dependent mechanism, we have generated new data, in which we used the PKA-specific inhibitor H-89.11 VSMC were incubated for 48 hours in serum-free medium followed by pretreatment for 60 minutes with Ang II (100 nM) with or without H-89 (10 μmol/L). As shown in Figure 2, insulin (10 nM) induces autotyrosine phosphorylation of its receptor. In contrast, when VSMCs were pre-incubated with Ang II, tyrosine phosphorylation of the IR did not occur, suggesting that Ang II blocks insulin-induced auto-activation of its own receptor (Figure 2). However, in the presence of the PKA inhibitor H-89, there was significant (P<0.01) inhibition of Ang II-mediated blockade of the insulin-induced IR auto-tyrosine phosphorylation (Figure 2).

Ang II Activates PKA Via a cAMP-Independent Mechanism

Most recently, a novel mechanism for PKA activation by Ang II and other vasoactive peptides has been described that is related to the NF-kB pathway.35,36 Under basal conditions, NF-κB exists in an inactive state bound to its natural inhibitor IκB in the cytoplasm. Activation of IκB occurs as a result of agonist-induced phosphorylation, followed by release of free NF-κB, which migrates to the nucleus. Apparently, a certain pool of the catalytic subunit of PKA (PKAc) also exists in a complex with IκB. In the quiescent state, IκB retains PKAc in an inactive state, presumably by masking its ATP binding site. Therefore, in VSMC, Ang II might induce the tyrosine phosphorylation of IκB, potentially resulting in release and activation of PKAc. In support of this contention, evidence has recently emerged that NF-κB can be activated via JAK2-dependent tyrosine phosphorylation of IκB, resulting in the dissociation of the IκB/NF-κB complex and activation of NF-κB.37 Because Ang II stimulates the tyrosine phosphorylation and activation of JAK2 in VSMC,21,22 these data might suggest a key role for JAK2 in IκB tyrosine phosphorylation leading to cAMP-independent Ang II-induced activation of PKA in VSMC.

Conclusion

The need for research into the pathogenesis, prevention, and treatment of diabetes is highlighted by the profound financial, societal, and personal impact of this devastating disease. Because Ang II has been implicated in the pathogenesis of diabetic vascular disease, it is crucial to examine how Ang II affects insulin-induced signaling events. Based on the results of recent studies from our33 and other28,34 laboratories, we advance the hypothesis that Ang II activation of the PTPase PTP-1B plays a central role in the Ang II-induced insulin resistance in vascular tissues (Figure 3).

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References


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