Protein tyrosine phosphatases: the quest for negative regulators of insulin action

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Asante-Appiah, Ernest, and Brian P. Kennedy. Protein tyrosine phosphatases: the quest for negative regulators of insulin action. Am J Physiol Endocrinol Metab 284: E663–E670, 2003; 10.1152/ajpendo.00462.2002.—Type 2 diabetes is increasing at an alarming rate worldwide, and there has been a considerable effort in several laboratories to identify suitable targets for the design of drugs against the disease. To this end, the protein tyrosine phosphatases that attenuate insulin signaling by dephosphorylating the insulin receptor (IR) have been actively pursued. This is because inhibiting the 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 protein tyrosine phosphatases (PTPs) have been implicated in the dephosphorylation of the IR. These phosphatases include PTP\(1B\), LAR, CD45, PTP\(2\), SHP2, and PTP1B. In most cases, there is evidence for and against the involvement of the phosphatases in insulin signaling. The most convincing data, however, support a critical role for PTP1B in insulin action. PTP1B knockout mice are not only insulin sensitive but also 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 PTP1B knockout mouse is reproduced when the phosphatase is also knocked down with an antisense oligonucleotide in obese mice. Thus PTP1B appears to be a very attractive candidate for the design of drugs for type 2 diabetes and obesity.

insulin receptor; protein tyrosine phosphatase; type 2 diabetes

IF CURRENT TRENDS CONTINUE, it is predicted that 300 million people worldwide will suffer from type 2 diabetes by the year 2025 (79). This sobering statistic makes the search for agents to intervene in type 2 diabetes ever more pressing. Because type 2 diabetes is characterized by an impaired insulin action, research efforts have focused on understanding the insulin-signaling pathway in an attempt to identify suitable therapeutic target(s) for drug intervention. Although significant gaps exist in our current understanding of the insulin signal transduction pathway, much has been accomplished since the ground-breaking discovery of the hormone by Banting and Best in 1921 (6), in particular, the signal transduction pathway leading up to the translocation of GLUT4 to the plasma membrane and subsequent uptake of glucose into cells. The mechanism(s) by which the activated insulin receptor (IR) is returned to the basal state has, however, lagged behind. In this review, we discuss protein tyrosine phosphatases (PTPs) that have been implicated in insulin receptor dephosphorylation. We especially focus on PTP-1B, a PTP that is receiving tremendous attention as an attractive target for the design of drugs to intervene in type 2 diabetes and obesity.

INSULIN SIGNALING

Insulin is secreted from pancreatic \(\beta\)-cells in response to increasing glucose concentrations in the blood. The hormone binds to its receptor, a tetrameric complex composed of two \(\alpha\)- and two \(\beta\)-subunits (for a review on insulin signaling see Ref. 64). Binding of the hormone to the extracellular \(\alpha\)-subunits triggers a conformational change that activates the intrinsic tyrosine kinase activity of the intracellular \(\beta\)-subunit via autophosphorylation of specific tyrosine residues in the activation loop. Some phosphorylated residues (outside the activation loop) act as docking sites for IR substrates (IRs), which in turn become phosphorylated by the receptor tyrosine kinase (RTK). The phosphor-
glycogen by phosphorylating glycogen synthase kinase 3 (GSK3) to promote glycogen synthesis via glycogen synthase (GS) (18, 29, 76). GSK3 is constitutively active and phosphorylates GS to inactivate this enzyme, which is required for the incorporation of glucose (in the form of UDP-glucose) into glycogen. Phosphorylation of GSK3 by Akt inactivates the kinase and relieves its block on GS. In addition to the above pathway, a PI3K-independent pathway appears to be required for insulin-dependent glucose uptake into cells. This c-Cbl-associated protein (CAP)/Cbl-dependent pathway apparently provides a second signal that influences GLUT4 vesicle translocation via lipid rafts to effect glucose uptake (for a recent review see Ref. 9).

As we have indicated, a substantial amount of knowledge has accrued regarding processes that initiate and propagate insulin signaling to influence glucose uptake; events that lead to signal termination, however, are not that well understood. An emerging hypothesis that is gaining acceptance involves the dephosphorylation of key tyrosine residues in the activation loop of the receptor. It has been postulated that the level of receptor activation is determined by the opposing actions of receptor phosphorylation vis-à-vis dephosphorylation. The role of PTPs in the deactivation of IR is therefore taking on much significance in insulin signaling. Thus inhibition of the IR phosphatase should provide an attractive approach for intervention in type 2 diabetes. The search for the enzyme that dephosphorylates the IR has implicated a number of PTPs.

**PTPs IN INSULIN SIGNALING**

Early studies with the nonspecific inhibitor vanadate, which acts as an insulin-mimetic agent, implicated PTPs in insulin signaling (11, 13, 24). Because many of the signaling proteins downstream of the IR tend to be phosphorylated on serine and threonine residues, research efforts focused on the identification of the PTP(s) that dephosphorylated the IR. This is because PTPs are enzymes that remove phosphate groups from key tyrosine residues on signaling proteins in vivo. PTPs will, however, nonspecifically dephosphorylate a number of synthetic organophosphates in vitro. As such, they show only moderate selectivity when studied in vitro. The enzymes are classified as PTPs on the basis of an invariant and catalytically essential cysteine residue that is part of a unique signature motif: (I/V)HCX₅R(S/T) (7). Enzymes in this family fall into two main groups, receptor or nonreceptor, depending on whether they possess or do not possess a transmembrane domain. Although very little substrate specificity is observed among PTPs in vitro, accumulating evidence suggests that this is not the case in vivo. For example, whereas CD45-null mice show a deficit in thymocyte development and B cell maturation (12, 30), SHP1 knockout (KO) mice show a striking phenotype in autoimmunity (68, 75). Using gene knockout approaches, transgenic mice in which specific PTP genes have been overexpressed, and other biochemical approaches, several PTPs, including PTPΔ, PTPε, CD45, SHP2, LAR, and PTP1B, have

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**Fig. 1. Insulin-signaling pathway.** The metabolic arm of the insulin transduction pathway is shown schematically. Larger round circles represent insulin binding to its receptor; smaller circles depict glucose being taken up by the membrane-embedded GLUT4 transporter. Other key protein molecules have been identified: PTP1B, protein tyrosine phosphatase 1B; PI3K, phosphatidylinositol 3-kinase; PDK1, phosphoinositide-dependent kinase-1; GSK3, glycogen synthase kinase 3; PKCζ/α, protein kinase Cζ/α; GS, glycogen synthase; IRS, insulin receptor substrate.
been implicated as negative regulators of insulin signaling.

**PTPα**

The involvement of PTPα in the dephosphorylation of the IR is controversial. Initial experiments in which the phosphatase was overexpressed in cells showed that it negatively affected cell surface expression of GLUT4 and attenuated insulin signaling (17, 38). Subsequent experiments, however, have not substantiated those initial observations. For example, the use of antisense oligonucleotide (ASO) to specifically knock down PTPα in adipocytes has not shown an effect on insulin signaling (4). The gene encoding for the phosphatase has also been disrupted in mice; no effects on insulin signaling or glucose homeostasis have been reported for these mice. The primary target of PTPα involved in insulin signaling or glucose homeostasis have been reported. The effects observed in these knockout mice are different from those observed in the transgenic null mice. The phenotypic defects were primarily observed in mammary gland development and function (67). Hence, it is still not clear what contribution LAR plays in insulin signaling.

**LAR**

The negative regulation of the IR by LAR is similarly controversial. Several studies have implicated the receptor PTP in insulin signaling (1, 2, 36, 47). As in the case of PTPα, overexpression of LAR showed that it negatively regulates insulin signaling (40, 83). Subsequently, a role for LAR in insulin signaling has been muddied by conflicting results from LAR-deficient mice. In one study, in which LAR was disrupted in transgenic mice, “secondary” defects in glucose homeostasis were observed (58). For a phosphatase implicated in the negative regulation of insulin signaling, sensitivity to insulin would be expected in its absence rather than the resistance to insulin-stimulated glucose uptake that was observed. From that study, the role of LAR would not be directly on IR per se but downstream of the receptor, if indeed it influences the signaling pathway. A recent study seems to support this notion. When LAR was overexpressed in the skeletal muscle of mice, whole body insulin resistance was observed (82). The effect of the PTP was proposed to be most likely on downstream targets such as IRS-1. An alternate LAR disruption study in mice has been reported. The effects observed in these knockout mice were different from those observed in the transgenic null mice. The phenotypic defects were primarily observed in mammary gland development and function (67). Hence, it is still not clear what contribution LAR plays in insulin signaling.

**CD45**

The list of PTPs that have been implicated in the dephosphorylation of the IR also includes CD45. In vitro, CD45 has been demonstrated to be capable of dephosphorylating the IR (74). The phosphatase also dephosphorylates the IR when overexpressed in cells (35). However, the tissue distribution of CD45 is relegated to B and T cells, and knockout studies in mice clearly show that a primary role of CD45 is in thymocyte development and B cell maturation (12, 30). Furthermore, the specificity of IR dephosphorylation by CD45 in vitro appears to be quite different from that of PTP1B (56), a phosphatase whose gene disruption affects insulin action (see PTP1B IN INSULIN SIGNALING). It is highly likely, then, that CD45 does not have any role in IR dephosphorylation, and the results that show activity on the IR are artifactual because the CD45 in vivo specificity is lost when overexpressed in cells.

**PTPe**

As in the case of the PTPs just described, overexpression of PTPε also inhibits insulin signaling (3). In baby hamster kidney cells overexpressing the IR, PTPε can block insulin-dependent cell rounding and detachment. Mice lacking the gene that codes for PTPε show a defect in the regulation of the respiratory burst (72). Bone marrow-derived macrophages show a reduced ability to produce TNFα and IL-10 when challenged with LPS. The absence of the phosphatase in mice also apparently influences the activation of Kv channels and affects Schwann cell function in early postnatal development (51). No defects in insulin signaling have been reported for PTPε KO mice.

**SHP2**

Another phosphatase that has been implicated in insulin signaling is SHP2. The presence of two NH2-terminal Src homology 2 (SH2) domains suggested that this phosphatase may be involved in signaling mediated through receptor tyrosine kinases. Expression of SHP2 in cells leads to a negative regulation of insulin signaling and downstream functional responses, such as GS (50). The insulin-mimetic effects of vanadate have also been partly attributable to inhibition of SHP2 (53). Other reports have also suggested a direct interaction between SHP2 and IR (59, 71). In addition to IR, the IRSs have also been suggested as targets through which SHP2 may modulate insulin signaling (34, 62). Furthermore, when SHP2 is expressed in a transgenic mouse model, an insulin-resistant phenotype is observed that implicates the PTP as a negative regulator of insulin signaling (42). Unfortunately, other reports do not confirm a role for SHP2 in insulin signaling. The most significant piece of evidence comes from the observed phenotype of the knockout mouse. Disruption of the SHP2 gene in mice results in embryonic lethality (5, 66). However, hemizygotes are viable and show no defects in insulin signaling. Plasma insulin levels and glucose uptake are not affected (5). It is not clear whether a >50% decrease in protein levels is perhaps required to observe an effect on insulin signaling. Knockdown studies with ASO may provide insights into effects on insulin signaling by SHP2. Nevertheless, genetic studies have rather implicated SHP2 in limb, lymphoid, and hematopoietic cell development among others (54, 55, 65). The evidence therefore does not support SHP2...
as an important negative regulator of insulin signaling at the present time.

One PTP that accumulating biochemical, structural, and genetic evidence has implicated in insulin action is PTP1B.

**PTP1B IN INSULIN SIGNALING**

The most convincing evidence that PTP1B is involved in the insulin-signaling pathway originates from the phenotype of the PTP1B KO mouse (23, 32) and, more recently, from results of PTP1B ASO treatments in diabetic rodents (60, 84). The PTP1B KO mouse has generated a number of surprising results and has provided insights into a number of presumptive roles for the phosphatase in vivo. It was assumed that the disruption of the PTP1B gene in mice would result in either lethality or a significant susceptibility to tumor formation, since this phosphatase has been shown, at least in cell culture, to be involved in the attenuation of many growth factor receptor kinase-signaling pathways, including IGF-IR, PDGFR, EDGFR, and IR, to name a few (25, 39, 41, 44, 61). Neither of these possibilities was observed; the mice were viable and long-lived without a significant increase in tumor formation. The reason for this may be that results derived from cell culture studies may not accurately reflect the function of PTP1B in vivo. It also seems possible that there may be a compensation for the PTP1B deficiency by other PTPs. Although we cannot completely rule out compensatory effects, the phenotype we have observed in the PTP1B KO mice, to date, appears to be associated with metabolic functions with no overt mitogenic effects. For example, when insulin was injected into the portal vein of PTP1B−/− mice, a significant increase in IR tyrosine phosphorylation was observed in muscle and liver compared with their wild-type littermates. In contrast, when IGF-I was injected into the mice, although a significant increase in IGF-I receptor tyrosine phosphorylation was measured in the lungs of these animals, there was no difference in the phosphorylation levels between PTP1B KO and wild-type mice (unpublished observations). From these results, it appears that PTP1B deficiency primarily affects the metabolic actions of insulin signaling and perhaps leptin signaling (see PTP1B AND OBESITY RESISTANCE), with very few (if any) effects on mitogenic signaling responses.

**PTP1B AS A NEGATIVE REGULATOR OF INSULIN SIGNALING**

PTP1B deficiency in mice results in enhanced insulin sensitivity, as demonstrated by a significant reduction in fed glucose levels that is maintained with one-half the circulating insulin levels (23). Additionally, there is increased insulin-stimulated phosphorylation of the IR in muscle and liver and an improved glucose clearance in glucose and insulin tolerance tests. The loss of PTP1B potentiates insulin’s activity, which would suggest that PTP1B is a negative regulator of insulin signaling. This would place PTP1B downstream of the IR, and presumably it functions to dephosphorylate and inactivate the IR. Alternatively, or in addition to its activity on the IR, PTP1B potentially may attenuate insulin signaling by dephosphorylating IRSs or possibly other phosphotyrosyl insulin-dependent signaling molecules yet to be identified. Although proof that PTP1B directly interacts with the IR in a cellular or in vivo context is not unequivocal, there is a significant amount of evidence to suggest that this is probably the case.

Catalytically inactive mutants of PTP1B can “trap” the activated IR in immunoprecipitation protocols (25, 77). It has been suggested that an NH2-terminal domain of PTP1B that includes tyrosines 152 and 153 is required for IR binding (20). Additional evidence supporting a direct interaction between PTP1B and IR comes from kinetic and structural studies with the IR activation segment (56, 63). A very interesting observation was on the order of dephosphorylation of a triphosphorylated peptide derived from the activation segment of the receptor by various PTPs. Ramachandran et al. (56) found that the receptor-type PTPs CD45 and LAR preferentially dephosphorylated the single phosphotyrosine residue (for example Tyr 1158 in the IR sequence), whereas TC-PTP, the most closely related PTP to PTP1B, displayed no phosphotyrosyl preference. In contrast, PTP1B showed a very strong preference for the tandem pTyr motif, suggesting that this feature was a strong determinant for PTP1B substrate binding and specificity. The recent crystallization of the IR activation segment in complex with PTP1B has established a structural basis for this selectivity (63). It was observed that there are extensive interactions between the tandem pTyr residues and PTP1B such that pTyr 1162 is located within the catalytic site and pTyr-1163 is positioned in the adjacent secondary pTyr-binding site. From these data, it seems likely that PTP1B may directly dephosphorylate the activated IR.

An outstanding issue that remains to be resolved involves how the endoplasmic reticulum (ER)-localized PTP1B can actually interact with the IR that becomes activated on the plasma membrane. As a possible explanation, it has been reported that the ER can come into close proximity with the plasma membrane and that, under some conditions (i.e., phagocytosis), both membranes may even fuse together (26). Thus it seems possible that PTP1B and the IR can come within close proximity for dephosphorylation to take place. It is also possible that, once the activated IR is internalized into endosomes, it may be directed to specific locations on the ER, where it becomes dephosphorylated, as has been suggested for the dephosphorylation of the platelet-derived and epidermal growth factor receptors by PTP1B (27).

**FUNCTIONAL RELATIONSHIP BETWEEN PTP1B AND IR**

Both the PTP1B KO mouse and the PTP1B ASO-treated diabetic animals display increased insulin sensitivity (23, 32, 84). Treatment of ob/ob and db/db mice
with PTP1B-specific ASO reduced protein levels of the phosphatase in liver and fat by 50% and resulted in normalization of glucose levels in these preclinical insulin-resistant mouse models. Hence, a ≥50% reduction in PTP1B protein level by genetics or ASO is sufficient to cause insulin sensitization and alleviate insulin resistance. Currently, it is not clear whether the improvement in the insulin resistance observed in these models is a consequence of a correction in the dysregulation in the insulin receptor/PTP1B equilibrium or is due to an overall enhancement in insulin action that results from a reduction in the levels of a negative regulator. More research is warranted to clarify what controls the nature of the interaction between PTP1B and the IR. It will also be interesting to determine whether or not there are alterations in this relationship during the development of insulin resistance. For example, how much PTP1B is active within the cell? And are there specific pools or specific intracellular locations for PTP1B to interact with the IR?

Recently, it was reported that reversible oxidation of PTP-1B may control the amount of functionally active PTP1B available in the cell. Goldstein and colleagues (Mahadev et al., 43) have shown that activation of IR results in the production of H₂O₂ and a concomitant transient oxidation and inactivation of PTP-1B. They have also demonstrated that, depending on the type of fat depot, there was a significant difference in the level of oxidized-inactive PTP1B, and they suggested that increased levels of active PTP1B could contribute to insulin resistance (80). Several groups have also reported that phosphorylation of PTP1B by both the IR and other protein kinases also affects PTP1B enzyme activity (19, 45, 57, 73). Unfortunately, many of the data are conflicting, and it is presently not clear whether phosphorylation results in activation or deactivation of PTP1B's enzyme activity. Understanding what regulates the in vivo activity of PTP-1B and how the phosphatase interacts with the IR would enhance efforts to develop potent inhibitors for the enzyme.

**PTP1B AND OBESITY RESISTANCE**

An unexpected phenotype of the PTP1B KO mouse was its resistance to diet-induced obesity (DIO). Because insulin promotes the storage of glucose and fat, it was expected that PTP1B KO mice would be rather more susceptible to obesity. Not only are the homozygous mice resistant to DIO, but the heterozygotes also display this phenotype, suggesting that an ~50% reduction in PTP1B levels would be sufficient for insulin sensitization and obesity resistance. In fact, this was recently validated by studies with the PTP1B ASO (60). Several factors appear to contribute to the obesity resistance phenotype. For instance, PTP1B KO mice have been reported to exhibit enhanced leptin sensitivity (15, 81). It has been suggested that this may be due to PTP-1B acting as a negative regulator of leptin signaling by dephosphorylating the leptin receptor-associated kinase Jak2 (15, 49, 81). Although a role for PTP1B in leptin signaling seems possible, the studies reported with the PTP1B KO mouse do not conclusively implicate a role for the phosphatase in leptin signaling. This is because in both the ob/ob PTP1B double knockout and the PTP1B−/− mice treated with gold thioglucose to ablate leptin-responsive hypothalamic neurons, leptin signaling is absent. The resulting mice, however, were more resistant to obesity than their respective controls (15, 81). Because leptin signaling is absent and therefore cannot be influenced by increased signaling (via an absence of PTP1B), the results would indicate that other mechanisms besides enhanced leptin signaling contribute to the obesity resistance. Indeed, in both models of the PTP1B KO mice that lacked leptin signaling, insulin sensitivity was maintained at the level of the control lean mice. Because insulin and leptin sensitivity are very tightly coupled (8, 14), the enhanced leptin sensitivity observed in the PTP1B−/− mice could be an indirect effect of the insulin-sensitive phenotype and not necessarily a direct effect of PTP1B on leptin signaling. More work is required to show definitively that PTP1B has a direct role in leptin signaling.

An additional factor of the PTP1B KO mice that may be influencing their obesity resistance is that these animals display tissue-specific insulin sensitivity. Liver and muscle are sensitive to insulin-stimulated phosphorylation of the IR, whereas adipose tissue sensitivity is not any different from that of wild-type littermates (23, 32). If adipose tissue were as hypersensitive to insulin as liver and muscle, then an increased ability to store fat would be expected. The fact that the PTP1B KO mice fail or have a decreased ability to store fat suggests a different role for the phosphatase in fat tissue. It is also possible that other PTPs play a compensatory role in this tissue, unlike in liver or muscle. However, recent results in ob/ob mice treated with the PTP1B ASO suggest that it is more likely that PTP1B has a different role in adipose tissue (60). Adipose tissue of ob/ob mice that were treated with the PTP1B ASO had a significant decrease in adiposity that was associated with a downregulation of genes involved in lipogenesis; insulin sensitivity in this tissue was not changed relative to ob/ob control mice. Therefore, a reduction in PTP1B levels in adipose tissue by genetic or ASO methods affects fat storage but does not enhance insulin sensitivity. Is it possible that, in adipose tissue, PTP1B functions in insulin signaling downstream of the IR in pathways that control fat metabolism? Recently, Bluher et al. (10) reported on the adipose tissue-specific knockout of the IR and found that these mice (FIRKO mice) were resistant to obesity and obesity-induced insulin resistance. They concluded that insulin signaling in fat is critical for the development of obesity. Perhaps reduction of PTP1B levels in adipose blunts insulin signaling in this tissue and, as in the case of the FIRKO mouse, this leads to obesity resistance. Further efforts to understand the role of PTP-1B in adipose tissue and fat metabolism should clarify these outstanding questions. Presently, it seems likely that the loss of PTP1B activity specifi-
cally in adipose tissue is a contributing factor to the obesity resistance observed in the PTP1B KO mouse.

PERPECTIVE

Protein tyrosine phosphatases have a very important role in insulin signaling and metabolism. Although other phosphatases such as SHP2 and LAR have been implicated in both the positive and negative regulation of insulin signaling, there is substantial evidence supporting PTP1B as the critical PTP controlling insulin action. Furthermore, recent genetic evidence has shown that human PTP1B gene variants are associated with changes in insulin sensitivity (21, 22, 46). Because of this, a significant amount of effort has gone into generating PTP1B-specific inhibitors for the treatment of type 2 diabetes. A number of recent publications have described the design of various PTP1B inhibitors, but there have been no reports of in vivo efficacy (for a review see Ref. 31). The development of potent, bioavailable PTP1B inhibitors will be a challenge, but the possible benefits for an overweight, insulin-resistant North American population cannot be overemphasized.

REFERENCES


