Insulin signaling and glucose homeostasis in mice lacking protein tyrosine phosphatase α

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Received 30 October 2003

Abstract

Studies in cultured cells have implicated protein tyrosine phosphatase α (PTPα) as a potential regulator of insulin signaling. The physiological role of PTPα in insulin action was investigated using gene-targeted mice deficient in PTPα. PTPα-null animals had normal body weights and circulating levels of glucose and insulin in random fed and fasted states. In glucose and insulin tolerance tests, their efficiency of blood glucose clearance was comparable to wild-type mice. Kinetics and extents of insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation were similar in wild-type and PTPα null liver, muscle, and adipose tissue. However, the association of IRS-1 and PI 3-K was altered in PTPα null liver, with increased insulin-independent and reduced insulin-stimulated association compared to wild-type samples. This did not affect activation of the downstream signaling effector Akt. Our data indicate that PTPα is not a negative regulator of insulin signaling and does not perform an essential role in mediating the physiological action of insulin.

Keywords: Insulin action; Insulin receptor; IRS; PI 3-K; Tyrosine phosphorylation; PTP

Non-insulin-dependent (Type 2) diabetes mellitus is characterized by insulin resistance that can arise as a result of several factors, including post-receptor defects in the insulin signaling pathway [1,2]. Negative regulators of insulin signaling are thus potential targets for therapeutic intervention, as the inhibition of such targets could ameliorate insulin resistance. Since protein tyrosine phosphorylation of the insulin receptor (IR) and the insulin receptor substrate (IRS) proteins is key to insulin signal propagation and maintenance [3–5], the identification of protein tyrosine phosphatases (PTPs) that can reverse these events in a physiological setting is clearly a key step in this therapeutic strategy.

Several PTPs act as IR and/or IRS phosphatases in vitro or cell culture systems, including the receptors LAR, PTPα, PTPε, and CD45, and the intracellular PTP1B, TC PTP, and SHP-2 [6–9]. Furthermore, wild-type or catalytically inactive ‘substrate-trapping’ mutants of LAR [10], PTP 1B [11–15], TC PTP [9,15], and SHP-2 [16] associate with IR/IRS. However, heterologous PTP and/or substrate expression, possibly inappropriate cell type, and the culture setting, are all factors that limit the ability of these studies to evaluate the physiological role of a particular PTP in insulin action. Studies in mice with modified PTP expression have provided additional insight into the roles of various PTPs in insulin signaling and metabolic effects. PTP 1B-deficient mice have increased insulin sensitivity, with enhanced insulin-induced tyrosine phosphorylation of the IR in liver and muscle but not in adipose tissue [17,18]. These mice also exhibited a resistance to diet-induced obesity that was associated with higher metabolic rate and energy expenditure. In another animal-based approach, diabetic mice treated with PTP 1B antisense oligonucleotide had reduced PTP 1B
expression and decreased hyperglycemia and hyperinsulinemia, as well as increased insulin sensitivity [19]. Insulin signaling pathways were activated in the livers of the antisense-treated ob/ob mice, including increased IR, IRS-1, and IRS-2 tyrosine phosphorylation [19,20]. These studies strongly support a physiological role for PTP1B as an IR phosphatase in liver and muscle, and as a negative regulator of insulin signaling. On the other hand, differing phenotypes of LAR-deficient mice have not clearly defined a role for this PTP in normal insulin signaling [21–23]. However, a causal link between increased LAR and insulin resistance noted in obese subjects is suggested by the finding that transgenic mice with muscle-specific overexpression of LAR have whole body insulin resistance, probably due to IRS dephosphorylation [24]. SHP-2-null mice die during embryonic development, but SHP-2 hemizygous mice did not exhibit any alterations in insulin signaling and metabolism [25].

There are no reported studies of insulin action in PTPα-null mice, despite the many studies that have been conducted in cultured cells, with mixed results, to investigate PTPα as a potential regulator of insulin signaling. Expression of PTPα in BHK cells expressing a high level of the IR (BHK-IR) counteracted the detachment and growth inhibition of these cells by insulin [26]. In contrast, the expression of PTP1B, known to negatively regulate insulin signaling, did not abrogate the insulin-induced effects on BHK-IR cells. It may be that in this system PTPα counterbalanced insulin-induced cell detachment through actions independent of insulin signaling that promoted cell attachment, akin to the increased substrate adhesion induced by PTPα expression in A431 cells [27]. Tyrosine phosphorylation of the highly expressed IR β-subunit was reduced by PTPα expression in BHK-IR cells, although this was similarly reduced by expression of the receptor PTP CD45 that had little ability to rescue insulin-stimulated cell detachment and growth inhibition. Cell type and heterologous protein expression levels may influence the ability of PTPα to dephosphorylate the IR, since in 293 cells co-expression of PTPα and the IR resulted in lower insulin-stimulated IR and Shc phosphorylation [28,29], but in IR-expressing GH4 pituitary cells transient or stable expression of PTPα had no effect on insulin-induced IR, IRS-1 or Shc tyrosine phosphorylation or IR kinase activity [30]. However, in the latter cells, PTPα inhibited insulin-stimulated expression of a prolactin reporter plasmid. Other studies have employed naturally insulin-responsive cells. Rat adipose cells transiently expressing PTPα had lower cell surface amounts of the glucose transporter GLUT4 compared to parent cells and correspondingly reduced insulin-stimulated translocation of GLUT4 to the cell surface [31]. Antisense oligonucleotide-mediated reduction of PTPα to undetectable levels in 3T3-L1 adipocytes had no effect on IR and IRS tyrosine phosphorylation upon insulin stimulation or on dephosphorylation following insulin withdrawal. Insulin-stimulated Erk 2 activation and DNA synthesis were also unaffected by PTPα depletion [32]. In contrast, and in accordance with the action of PTPα as an upstream activator of src [33], PTPα depletion greatly reduced the activity of this tyrosine kinase. In L6 myoblasts, insulin-stimulated DNA synthesis was enhanced in cells overexpressing PTPα and reduced in cells in which PTPα expression was decreased using antisense cDNA [34]. Elevated PTPα expression did not influence insulin-induced IR (or IGF-1R) tyrosine phosphorylation. In summary, enhanced or ablated expression of PTPα in cultured cells exerts negative, positive, or no effects on insulin-stimulated cellular responses, and is sometimes accompanied by alterations in insulin-responsive IR tyrosine phosphorylation. PTPα has continuously been discussed as a candidate regulator of IR phosphorylation and insulin action [6–8]. To address the question of whether PTPα is a physiological regulator of insulin action, we have investigated insulin signaling and parameters of glucose homeostasis in PTPα-deficient mice.

Materials and methods

Reagents and antibodies. Human insulin was purchased from Eli Lilly. Anti-IRβ and anti-IRS-1 antibodies were obtained from Santa Cruz Biotechnology. Anti-phosphotyrosine clone 4G10 and antibodies to PI-3 kinase p85, IRS-2, and SHP-2 were from Upstate Biotechnology. Anti-phospho-Akt (Ser473 or Thr308), anti-Akt, anti-phospho-p44/42 MAPK (Thr202/Tyr204), and anti-p44/42 MAPK were from Cell Signaling. Antibodies to LAR, PTP 1B, Grb2, and Shc were purchased from BD Transduction Laboratories. All chemicals were from Sigma.

Mice. Mice deficient for PTPα were generated as described [35] and maintained as an advanced intercross line (129SvE/J × Black Swiss, 50:50 mixed background). Animal care and use followed the guidelines of the Canadian Council on Animal Care and the University of British Columbia. All experiments were carried out with male mice unless indicated otherwise.

Experimental protocol. Mice (10–14 weeks old) were fasted overnight and anesthetized with tribromoethanol (250 mg/kg, i.p.). The abdominal cavity was opened, the portal vein was exposed, and insulin (5 U/mouse) or saline was injected into the vein. At selected times post-injection, pieces of liver, skeletal muscle, and abdominal fat pad were collected, frozen in liquid nitrogen, and stored at −80°C until use.

Preparation of tissue lysates. Liver, muscle, and adipose tissues were homogenized using a Polytron PT 3100 in lysis buffer containing 20 mM Tris–HCl (pH 7.4), 2 mM EDTA, 150 mM NaCl, 1% Triton X-100, 50 mM sodium fluoride, 1 mM sodium vanadate, 10 μg/ml aprotinin, 0.1 μM leupeptin, 5 μM pepstatin, and 0.2 μM phenylmethylsulfonyl fluoride. The tissue homogenate was incubated on ice for 1 h and then centrifuged at 55,000 rpm for 30 min. The resulting supernatant was collected and assayed for protein concentration using Biorad reagent.

Immunoprecipitation and immunoblotting. Equal amounts of lysate protein were subjected to immunoprecipitation with anti-IRβ, anti-IRS-1, or anti-IRS-2 antibodies (2–3 μg) overnight at 4°C. Protein G Plus Protein A Agarose (Oncogene) was added for 2 h at 4°C and the immunocomplexes were washed three times with lysis buffer and eluted with SDS sample buffer at 100°C for 5 min. Immunoprecipitated
proteins were resolved by 7.5% SDS–PAGE and transferred to PVDF membranes. The membrane was blocked with 2% BSA or 5% skim milk, subjected to anti-phosphotyrosine immunoblotting if appropriate, and then stripped and probed for other proteins of interest. To examine the activation of Akt and MAPK, total lysates (50 μg) were analyzed with anti-phospho Akt and anti-phospho-MAPK or anti-Akt and anti-MAPK antibodies.

Determination of blood glucose and plasma insulin concentrations. Blood samples were collected from the tail vein. Blood glucose levels were measured with a One Touch Ultra Glucose meter (Lifescan). Blood was also collected into heparin-coated capillary tubes (Sarstedt, Germany), and serum was separated by centrifugation and used to determine plasma insulin concentrations using a Mercodia Mouse Insulin ELISA kit (Alpco Diagnostics, USA).

Fig. 1. PTP expression in insulin-responsive tissues of wild-type and PTPα−/− mice. Lysates of tissues from wild-type (+/+) and PTPα−/− (−/−) mice were probed for (A) PTPα (upper panel) and actin (lower panel), (B) LAR, PTP 1B, SHP-2, and actin as indicated.

Fig. 2. Metabolic parameters in PTPα−/− mice. Wild-type (+/+) and PTPα−/− (−/−) mice as indicated were used for measurements. (A) Body weights of male mice (+/+, solid circles, n = 14; −/−, open circles, n = 14) that were weighed weekly from 4 to 10 weeks of age. (B) Body weights of female mice (+/+, solid circles, n = 16; −/−, open circles, n = 9) that were weighed weekly from 4 to 10 weeks of age. (C) Blood glucose of 8- to 12-week-old male mice fed ad libitum (+/+, n = 11; −/−, n = 12) or fasted overnight (+/+, n = 12; −/−, n = 15). (D) Serum insulin of 12- to 15-week-old male mice fed ad libitum (+/+, n = 5; −/−, n = 5) or fasted overnight (+/+, n = 6; −/−, n = 6). (E) Glucose tolerance tests were conducted on 8- to 10-week-old male mice (+/+, solid circles, n = 12; −/−, open circles, n = 12) that were fasted overnight and then injected i.p. with 1.5 mg/g glucose. (F) Insulin tolerance tests were conducted on 10- to 12-week-old male mice (+/+, solid circles, n = 11; −/−, open circles, n = 9) that were fasted overnight and then injected i.p. with 0.75 U/kg insulin.
Glucose and insulin tolerance tests. Animals were fasted overnight (15–16 h) prior to each experiment but allowed free access to water. Animals were injected intraperitoneally with glucose (1.5 mg/g, Sigma) or human insulin (0.75 U/kg, Eli Lilly), and blood glucose was measured prior to and at 15, 30, 60, and 120 min after injection.

Results and discussion

PTPα expression in the major insulin-responsive tissues of liver, muscle, and adipose was evaluated in comparison to that in other mouse tissues (Fig. 1A). Mouse brain, followed by kidney and stomach, expressed the highest levels of PTPα. PTPα expression in liver and adipose tissues was comparable to that in lung and spleen. Low levels of PTPα were detected in muscle and heart, and it was almost undetectable in intestine. PTPα was confirmed to be absent in all tissues of PTPα−/− mice. The lack of PTPα did not result in altered expression of certain other PTPs implicated in insulin signaling such as PTP 1B, LAR, and SHP-2 (Fig. 1B).

Wild-type and PTPα−/− mice were weighed weekly, starting at 4 weeks of age, and found to have similar initial body weights and rates of weight gain (Figs. 2A and B). No differences in fed and fasted blood glucose levels (Fig. 2C) or circulating insulin (Fig. 2D) were apparent between wild-type and PTPα-null animals. Blood glucose clearance following intraperitoneal injection of glucose occurred with normal efficiency in mice lacking PTPα (Fig. 2E). Likewise, wild-type and PTPα−/− mice demonstrated similar alterations in blood glucose in response to intraperitoneal insulin injection (Fig. 2F). In conjunction, the results indicate that the lack of PTPα does not render the mice more sensitive to insulin nor does it alter insulin-regulated glucose homeostasis.

Insulin-stimulated tyrosine phosphorylation of the IR was examined in liver, muscle, and adipose tissues of WT and PTPα−/− mice. In WT liver, enhanced phosphorylation of the IR was detected by 1 min post-insulin injection and sustained for at least 10 min. Liver IR phosphorylation was unaffected by the absence of PTPα (Fig. 3A). In WT muscle, IR phosphorylation increased up until at least 6 min following insulin stimulation and remained readily detectable to 11 min, with no alterations observed in PTPα−/− muscle (Fig. 3B). In adipose tissues from WT and PTPα−/− mice we observed comparable insulin-induced tyrosine phosphorylation of the IR up to at least 7 min post-injection that decreased by 12 min post-injection (Fig. 3C). Probing muscle and liver lysates with an antibody that specifically recognizes phosphoTyr-972 within the NPEY sequence, a recognition site for IRS-1, demonstrated efficient insulin-stimulated phosphorylation of this site in PTPα−/− muscle and liver that was comparable to that in WT tissues (data not shown). These findings suggest that PTPα is not a critical regulator of IR phosphorylation in insulin-responsive tissues.

Although IR phosphorylation appeared normal in PTPα-deficient settings, any undetected changes could be reflected by altered downstream signaling. Insulin signaling phosphotyrosyl intermediates could also be direct substrates for PTPα, with consequent effects on protein–protein interactions. We therefore examined the insulin-induced phosphorylation of IRS-1, an early and central effector of insulin signaling, and its insulin-stimulated association with PI 3-K. IRS-1 was immunoprecipitated from liver (A) and muscle (B), probed with anti-phosphotyrosine antibody (upper panels), and then reprobed with anti-IR antibody (lower panels). (C) Lysates of adipose tissue were probed with anti-phosphotyrosine antibody (upper panel) and then reprobed with anti-IR antibody (lower panel).
phosphorylation levels between WT and PTPα−/− control samples (saline-treated), and between WT and PTPα−/− insulin-treated samples, revealed no statistically significant differences (p > 0.05) (Fig. 5C). However, the non-significant increase in IRS-1 tyrosine phosphorylation in the PTPα−/− saline-treated control liver (relative to saline-treated WT liver) resulted in a lower-fold stimulation of IRS-1 phosphorylation by insulin in the PTPα−/− liver compared to the insulin-induced fold-stimulation in WT liver. Indeed by 10 min after insulin treatment, IRS-1 phosphorylation had returned to control level in PTPα−/− samples, but in WT samples it was still ~2-fold higher than the WT control. Analysis of the association of IRS-1 with PI 3-K revealed a reduced insulin-stimulated interaction in PTPα−/− liver (Fig. 5D). This was in part due to a significant 2-fold increase in insulin-independent IRS-1 and PI 3-K association in control non-insulin-treated PTPα−/− liver compared to the insulin-induced fold-stimulation in WT liver. Thus, insulin induced a more than 3-fold enhanced association over saline-treated control liver at 5 min, and only a ~1.5-fold enhanced association over that in saline-treated PTPα−/− liver. The difference was more pronounced after 10 min insulin treatment, with a 4-fold increase in IRS-1 and PI 3-K association in WT liver over the saline-treated WT samples, but a much lesser 1.3-fold increase in PTPα−/− liver over saline-treated control PTPα−/− samples. PI 3-K expression was not altered in the absence of PTPα (data not shown). These results indicate that the insulin-stimulated interaction of IRS-1 and PI 3-K in liver is either reduced in extent and/or occurs over a shorter time in the absence of PTPα. IRS-2 plays an important role in regulating hepatic insulin action [36], however, there were no significant differences in insulin-induced IRS-2 tyrosine phosphorylation or interaction with PI 3-K between WT and PTPα−/− liver (Fig. 6).

The enhanced association of IRS-1 and PI 3-K in control non-insulin-treated PTPα−/− liver, that partially contributed to the reduced effect of insulin in stimulating association of these two proteins (Fig. 5D), was not observed in non-insulin-treated muscle nor with IRS-2/PI 3-K association in control PTPα−/− liver. Similarly, in liver of LAR−/− mice, an increased basal level of anti-phosphotyrosine immunoprecipitable PI 3-K activity was observed [21]. The converse insulin-independent effect was reported in adipose cells upon overexpression of PTPα, whereupon the cell surface level of the glucose transporter GLUT4 was reduced compared to control liver.
transfected cells [31]. This was attributed to the possible attenuation of minor signaling by unoccupied insulin receptors. It is likewise possible that PTPα performs such a role in liver, with the phosphatase maintaining basal phosphorylation of IRS-1. This would be consistent with the small but not significant increase in insulin-independent IRS-1 tyrosine phosphorylation that we observed in PTPα/C0 liver compared to WT liver (Fig. 5C). Nevertheless, independent of the increased control IRS-1/PI 3-K interaction in the PTPα-deficient liver, there is still a small but significant reduction in the level of the IRS-1/PI 3-K complex in PTPα/C0 liver at the later time of 10 min when directly compared to that in WT liver (Fig. 5D).

Insulin, acting via the IR and the IRS proteins, stimulates two major protein kinase-dependent signaling pathways, the PI 3-K/Akt pathway and the Ras-MAPK (p42/44) pathway. Alterations in insulin signaling that occur upstream of or at Akt or MAPK may be manifested by the altered activation of these kinases, as with the enhanced insulin-stimulated Akt activation observed in PTP1B-depleted mouse liver [19]. However, insulin-independent activation of Akt, as measured by monitoring Akt S473 phosphorylation (data not shown), was not apparently different in WT and PTPα−/− liver, muscle, or adipose tissue at earlier (5, 6, and 7 min, respectively) (data not shown) or later (10, 11, and 12 min, respectively) (Figs. 7A–C) times. Insulin-dependent MAPK activation was almost undetectable in liver and appeared similar in WT and PTPα−/− muscle and fat at earlier (5, 6, and 7 min, respectively) (data not shown) or later (10, 11, and 12 min, respectively) (Figs. 7D–F) times.

In conclusion, mice lacking PTPα exhibit no differences from wild-type mice in terms of glucose homeostasis or insulin signal transduction events, with the exception of a reduced, or more rapidly attenuated, insulin-stimulated interaction between IRS-1 and PI 3-K in PTPα−/− liver. This contrasts with the predicted consequences of loss of a negative regulatory PTP, namely an increased or prolonged protein tyrosine phosphorylation and ensuing signaling protein interactions. Normal insulin-stimulated IRS-1 phosphorylation but reduced IRS-1/PI 3-K association or PI 3-K activity was previously observed in LAR-deficient liver, and proposed to account for a complex phenotype of secondary insulin resistance arising in compensation for initial insulin sensitivity [21]. Subsequently, muscle-spe-
Specific overexpression of LAR was also reported to reduce insulin-dependent association of PI 3-K with IRS-1 without altering IRS-1 tyrosine phosphorylation [24] and linked to insulin resistance. However, in PTPα−/− animals, any metabolic or signaling effects of the reduced IRS-1/PI 3-K interaction, including downstream Akt activation, were not apparent. While it cannot be ruled out that other PTPs compensate for the absence of PTPα to maintain normal insulin action, our study indicates that PTPα does not play an essential role, if indeed any, in the physiological mediation or regulation of insulin action.
Acknowledgments

We thank J. Wang for mouse maintenance and genotyping, G. Soukhateheva and L. Marzban for advice and help with glucose-insulin tolerance tests and insulin measurements. H.T.L. is the recipient of a BC Research Institute for Children’s & Women’s Health Graduate Studentship. This work was supported by funding from the Jhohal Program in Pediatric Oncology Basic and Translational Research (C.J.P.).

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