

Insulin Stimulates Tyrosine Phosphorylation and Inactivation of Protein-tyrosine Phosphatase 1B *in Vivo**

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Protein-tyrosine phosphatase (PTP) 1B has been implicated in negative regulation of insulin action, although little is known of the ability of insulin to regulate PTP1B itself. The ability of insulin to regulate phosphorylation and activation of PTP1B was probed *in vivo*. Challenge with insulin *in vivo* provoked a transient, sharp increase in the phosphotyrosine content of PTP1B in fat and skeletal muscle that peaked within 15 min. Insulin stimulated a decline of 60–70% in PTP1B activity. In mouse adipocytes, the inhibition of PTP1B activity and increased tyrosine phosphorylation of the enzyme were blocked by the insulin receptor tyrosine kinase inhibitor AG1024. Phosphoserine content of PTP1B declined in response to insulin stimulation. Elevation of intracellular cyclic AMP provokes a sharp increase in PTP1B activity and leads to increased phosphorylation of serine residues and decreased tyrosine phosphorylation. Suppression of cyclic AMP levels or inhibition of protein kinase A leads to a sharp decline in PTP1B activity, a decrease in phosphoserine content, and an increase in PTP1B phosphotyrosine content. PTP1B appears to be a critical point for insulin and catecholamine counter-regulation.

Activation of the intrinsic tyrosine kinase of the insulin receptor is the paradigm of insulin action (1). Insulin receptor substrates (*e.g.* IRS-1 and IRS-2) are downstream targets of the insulin receptor tyrosine kinase that, upon specific phosphorylation of tyrosine residues, create motifs and modules for the docking and regulation of other effectors, further downstream, via protein-protein interactions (2, 3). The molecular basis for insulin signaling involves tyrosine kinase activation (4) but fails to provide an explanation for the full range of signaling that modulates insulin action, either directly or indirectly (5).

Protein-tyrosine phosphatases (PTP)¹ constitute a family of phosphatases, including PTP1B, PTP1C, PTP1D, and LAR, that acts to reverse tyrosine kinase action (6). Expression of PTP1B can block the actions of the *neu* oncogene (7), regulate development in zebrafish (8), and attenuate insulin signaling (9–11). Levels of PTP1B have been reported to be decreased (12) or increased (13) in diabetes associated with insulin resistance. The *fa/fa* genetic model of insulin resistance and obesity

and the ZDF/*fa/fa* model of insulin-resistant diabetes display increased PTP1B expression (14). These and other data provide a compelling linkage between PTP1B and insulin signaling defects associated with diabetes (15, 16).

PTP1B has been extensively studied since it was first identified (6), purified (17), and subjected to molecular cloning (18). The crystal structure of PTP1B has been solved (19), and its substrate specificity has been characterized (20). This protein-tyrosine phosphatase itself is a phosphoprotein. PTP1B is phosphorylated on serine residues during mitosis, although this phosphorylation does not alter enzymatic activity (21). Activation of the stress pathway leads to phosphorylation of PTP1B on Ser-352 and Ser-386 (22). Activation of protein kinase A leads to phosphorylation of serine residues in region 283–364, most likely Ser-352 within the motif Lys-Gly-Ser-Pro-Leu (23). Activation of protein kinase A in HeLa cells increases PTP1B activity severalfold. The epidermal growth factor receptor also phosphorylates PTP1B, on a tyrosine kinase substrate motif flanking Tyr-66 (24), although the consequences of this phosphorylation of tyrosine residues on enzymatic activity is poorly understood. Studies of the regulation of PTP1B have been confined largely to cells in culture; little is known of its regulation *in vivo*.

Herein we report on the regulation of PTP1B activity by insulin *in vivo* using a mouse model. The data clearly demonstrate tyrosine phosphorylation and inhibition of PTP1B in fat and skeletal muscle in response to challenge with insulin. This insulin-sensitive response is rapid and accompanied by an increase in phosphotyrosine content and a modest decline in phosphoserine content. Elevation of intracellular cyclic AMP levels increases PTP1B activity. Suppression of cyclic AMP levels or inhibition of protein kinase A reduces PTP1B activity.

MATERIALS AND METHODS

Mice—FVB mice were used in these studies. All animals were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee at SUNY/Stony Brook. Mice were maintained on a normal light/day cycle. The mice used in these experiments were 8–16 weeks of age, with no age-related differences observed in the parameters measured.

Administration *In Vivo* of Insulin and Other Agents—Four-month-old FVB mice were fed *ad libitum* and maintained on normal light/dark cycles. During the evening prior to experiments, mice were allowed access to drinking water only. Experiments were routinely conducted between 8:00 a.m. and 10:00 a.m. Mice were anesthetized and insulin (160 units/kg), isoproterenol (2.0 μ g/kg), or forskolin (10 mg/kg) was administered via intravenous injection as described previously (25). Control mice received injections with vehicle alone.

Immunoblotting Analysis—Mice were administered with insulin as described, and epididymal white fat and hind limb skeletal muscle were removed at the times indicated. The fat and skeletal muscle samples were immediately homogenized in ice-cold lysis buffer (130 mM NaCl, 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 2 mM Na₃VO₄, 20 mM β -glycerophosphate, 10 mM sodium molybdate, 10 mM NaF, 2 mM sodium

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¹ The abbreviations used are: PTP, protein-tyrosine phosphatase; BSA, bovine serum albumin; CPT-cyclic AMP, cyclophenylthio-cyclic AMP.

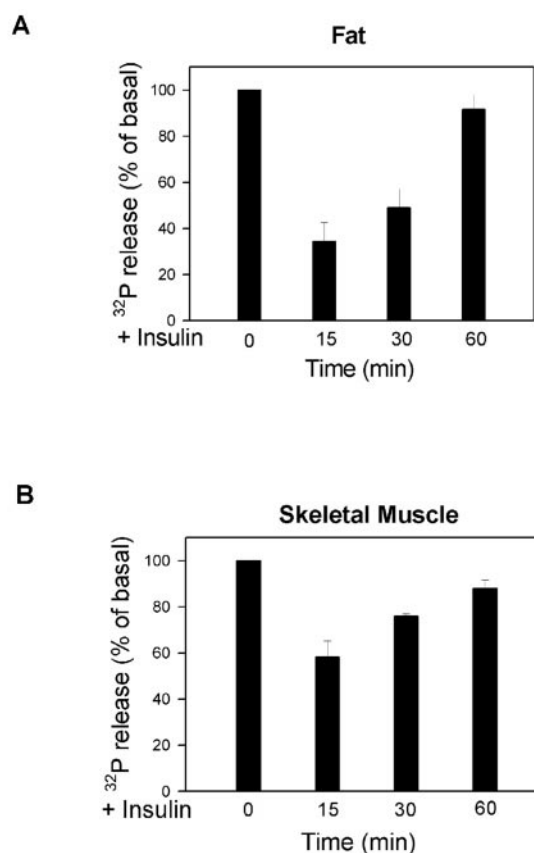


FIG. 1. Insulin administration *in vivo* leads to activation of fat and skeletal muscle protein-tyrosine phosphatase 1B. Mice were challenged with a bolus of insulin via injection into the vena cava, and fat (A) and skeletal hind muscle (B) tissues were sampled at the time intervals indicated. Whole-tissue extracts were prepared, and immunoprecipitates of PTP1B were subjected to direct assay of activity using the Raytide-labeled substrate. The assays were performed in tissue samples derived from four to eight separate mice in each group. The data in the bar graphs are mean values \pm S.E.

pyrophosphate, 0.5% Nonidet P-40, 1% Triton X-100, supplemented with 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, and 0.2 mM phenylmethylsulfonyl fluoride). After 60 min on ice, the lysate was centrifuged at 14,000 \times g for 10 min. Aliquots of protein were subjected to 10% SDS-polyacrylamide gel electrophoresis, and the separated proteins were transferred electrophoretically from the gel to the nitrocellulose membrane. Antibodies to the following antigens employed in these studies were obtained from the indicated sources: PTP1B (product P18020 from Transduction Laboratories, San Diego, CA); anti-P-Tyr antibodies (product SC-7020, Santa Cruz Biotechnology, Santa Cruz, CA); anti-phosphoserine antibodies (product 61-8100, Zymed Laboratories Inc., San Francisco, CA). The immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography of Kodak X-Omat film.

PTP1B Activity Assay—Mice were administered with insulin as described above, and epididymal white fat and hind limb skeletal muscle were removed at the times indicated. The fat and skeletal muscle biopsies were immediately homogenized in ice-cold lysis buffer (130 mM NaCl, 20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 20 mM β -glycerophosphate, 10 mM NaF, 2 mM sodium pyrophosphate, 0.5% Nonidet P-40, 1% Triton X-100, supplemented with 5 μ g/ml aprotinin, 5 μ g/ml leupeptin, and 0.2 mM phenylmethylsulfonyl fluoride) (25). After 60 min on ice, the lysate was centrifuged at 14,000 \times g for 10 min. An aliquot (1 mg of protein) of the clarified lysate was resuspended to 0.5 ml in the same tissue lysis buffer and incubated with 1 μ g of anti-PTP1B antibody at 4 $^{\circ}$ C for 2 h with constant rotation. Then, 10 μ l of Protein A/G Plus-agarose (Santa Cruz Biotechnology, Santa Cruz, CA) was added, and the incubation was continued for an additional 2 h. The immunoprecipitates were collected by centrifugation at 14,000 \times g for 5 min. The pellets were washed twice in radioimmune precipitation buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100) and then washed

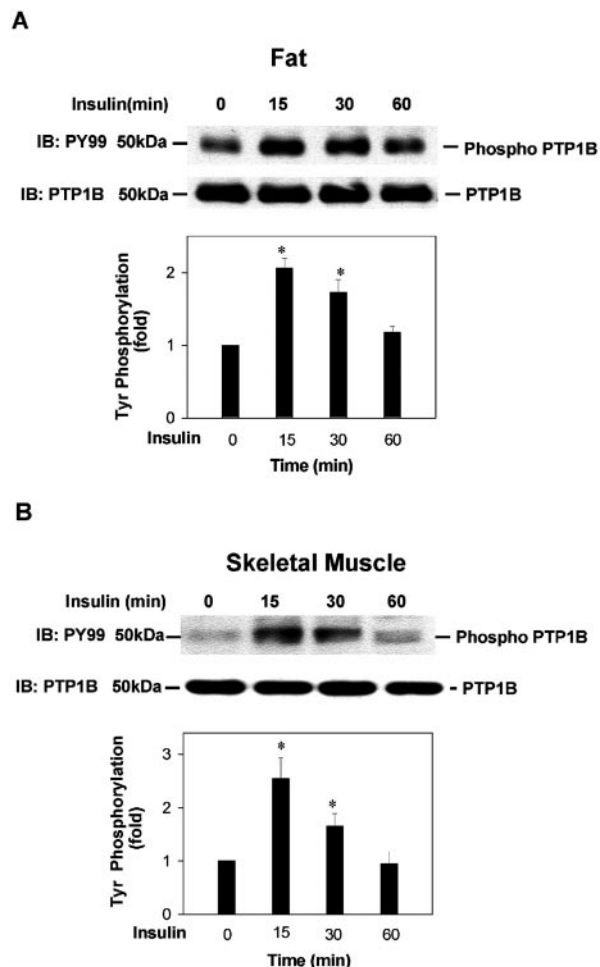


FIG. 2. Phosphotyrosine content of protein-tyrosine phosphatase 1B is increased transiently in fat and skeletal muscle of mice administered insulin *in vivo*. Mice were challenged with a bolus of insulin via injection into the vena cava, and tissues were sampled at the time intervals indicated. Whole-tissue extracts of fat (A) and skeletal hind muscle (B) were prepared, and immunoprecipitates of PTP1B were subjected to immunoblotting. The blots were stained with antibodies against PTP1B as well as with anti-phosphotyrosine antibodies (PY99). The immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography of Kodak X-Omat film. Shown are the results of a typical immunoblot as well as a compilation of the relative changes in phosphotyrosine content (-fold over control) obtained from four to eight blots prepared from as many separate animals (bar graphs). The data in the bar graphs are mean values \pm S.E.

twice with PTP1B assay buffer (25 mM imidazole, pH 7.5, 0.1 mg/ml bovine serum albumin (BSA)). The phosphatase activity of the immobilized PTP1B was assayed as follows: either PTP1B or mouse IgG immobilized on protein-A beads was resuspended in 50 μ l to assay buffer. The reaction mixture (25 mM imidazole, pH 7.5, 0.1 mg/ml BSA, 10 mM dithiothreitol, 10 nM ³²P-labeled substrate Raytide) was then added to a final volume of 80 μ l and incubated at 30 $^{\circ}$ C for 30 min. The reaction was terminated, and the ³²P released was quantified by a charcoal binding assay as described previously (26). The synthetic peptide Raytide (Oncogene Science) was phosphorylated at its unique tyrosine residue, by following the manufacturer's instructions.

Preparation of Adipocytes—Adipocytes were released from epididymal fat pads of male FVB mice by digestion for 30 min at 37 $^{\circ}$ C in KRB buffer (120 mM NaCl, 4.8 mM KCl, 2.6 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 1 mM sodium phosphate buffer, pH 7.4, 5 mM HEPES, 2.5 mM D-glucose, 3% BSA) with 1 mg/ml collagenase (Calbiochem). Adipocytes were washed twice in KRB buffer without collagenase and twice in HBM buffer containing 0.1 mM cyclic AMP-PDE inhibitor (Ro-20-1724) and 10 mM NaF. Adipocytes were then incubated in either the absence or the presence of 50 μ M forskolin or 20 μ M 8-CPT-cAMP at 37 $^{\circ}$ C for 15 min, then stopped with a 300- μ l aliquot of lysis buffer.

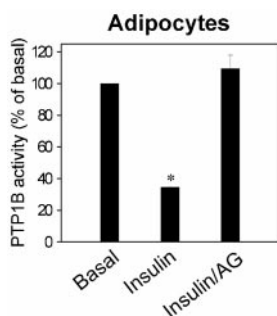


FIG. 3. The activity of protein-tyrosine phosphatase 1B is decreased in adipocytes treated with insulin: reversal by the insulin receptor kinase inhibitor tryphostin AG1024. Mice adipocytes were challenged with either insulin (100 nM) alone or insulin in combination with the tryphostin AG1024 (10 μ M) for 15 min *in vitro*. Whole-cell extracts of adipocytes were prepared, and immunoprecipitates of PTP1B were subjected to direct assay of activity using the Raytide-labeled substrate. The data in the bar graph are mean values \pm S.E.

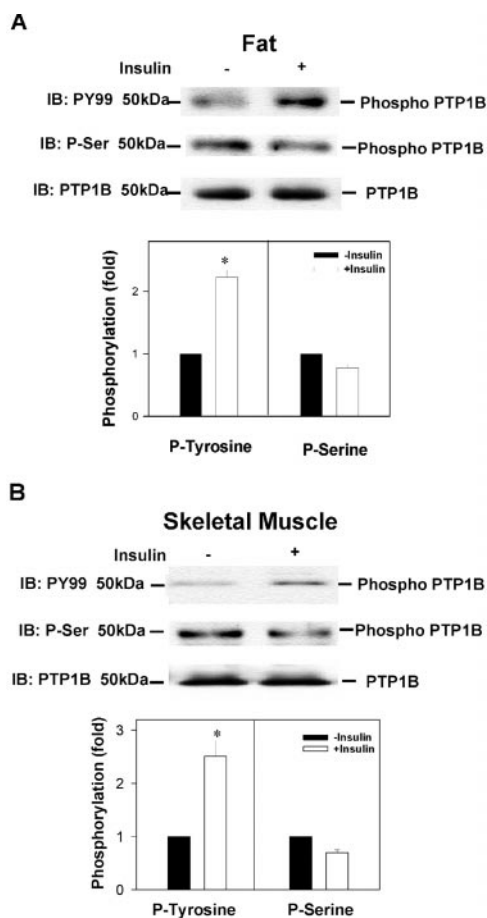


FIG. 4. Content of phosphotyrosine increases and phosphoserine decreases for protein-tyrosine phosphatase 1B in fat and skeletal muscle of mice administered insulin *in vivo*. Mice were challenged with a bolus of insulin via injection into the vena cava, and tissues were sampled at 15 min post-injection. Whole-tissue extracts of fat (A) and skeletal hind muscle (B) were prepared, and immunoprecipitates of PTP1B were subjected to immunoblotting. The blots were stained with antibodies against PTP1B as well as with anti-phosphotyrosine antibodies (PY99) and anti-phosphoserine antibodies (P-Ser). The immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography of Kodak X-Omat film. Shown are the results of a typical immunoblot as well as a compilation of the relative changes in phosphotyrosine content (-fold over control) obtained from four to eight blots prepared from as many separate animals (bar graphs). The data in the bar graphs are mean values \pm S.E.

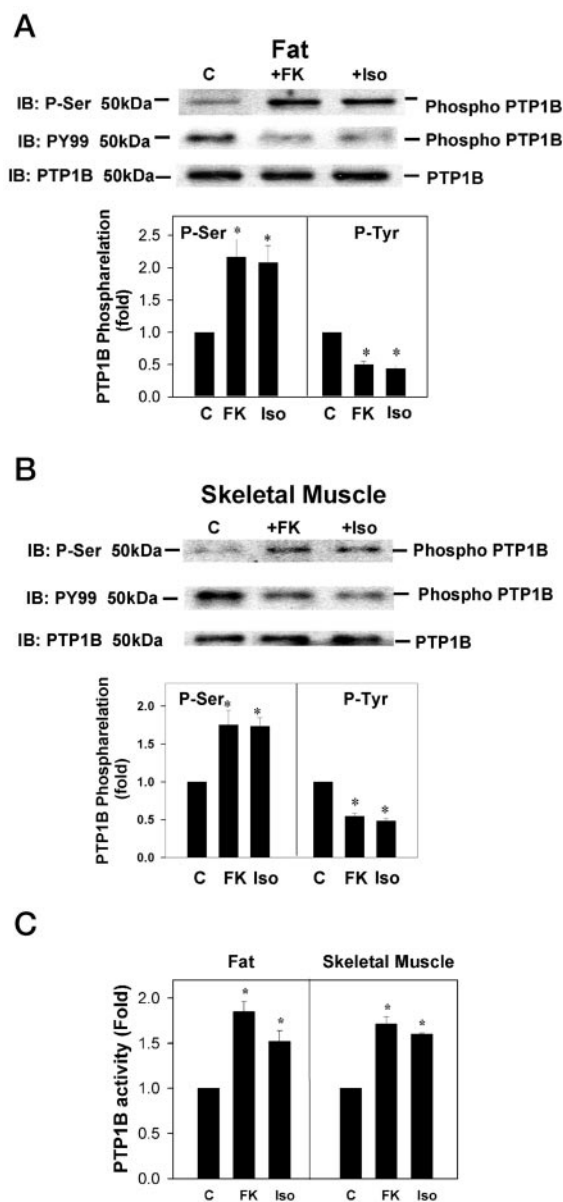


FIG. 5. Content of phosphoserine of protein-tyrosine phosphatase 1B is increased transiently while phosphotyrosine content declines in fat and skeletal muscle of mice administered either forskolin or isoproterenol *in vivo*. Mice were challenged with a bolus of either forskolin or the beta-adrenergic agonist isoproterenol via injection into the vena cava, and tissues were sampled at 15 min post-injection. Whole-tissue extracts of fat (A) and skeletal hind muscle (B) were prepared, and immunoprecipitates of PTP1B were subjected to immunoblotting or to direct assay of PTP1B activity using the labeled Raytide substrate (C). The blots shown in A and B were stained with antibodies against PTP1B as well as with anti-phosphotyrosine antibodies (PY99) and anti-phosphoserine antibodies (P-Ser). The immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography of Kodak X-Omat film. Shown are the results of a typical immunoblot as well as a compilation of the relative changes in phosphotyrosine content (-fold over control) obtained from four to eight blots prepared from as many separate animals (bar graphs). Measurements of PTP1B activity in the fat and skeletal muscle samples were obtained from four separate animals each. The data in the bar graphs are mean values \pm S.E.

RESULTS

FVB mice were anesthetized deeply and administered insulin as a bolus, directly to the vena cava. The protein-tyrosine phosphatase 1B was immunoprecipitated from extracts prepared from fat and skeletal muscle sampled in the mice follow-

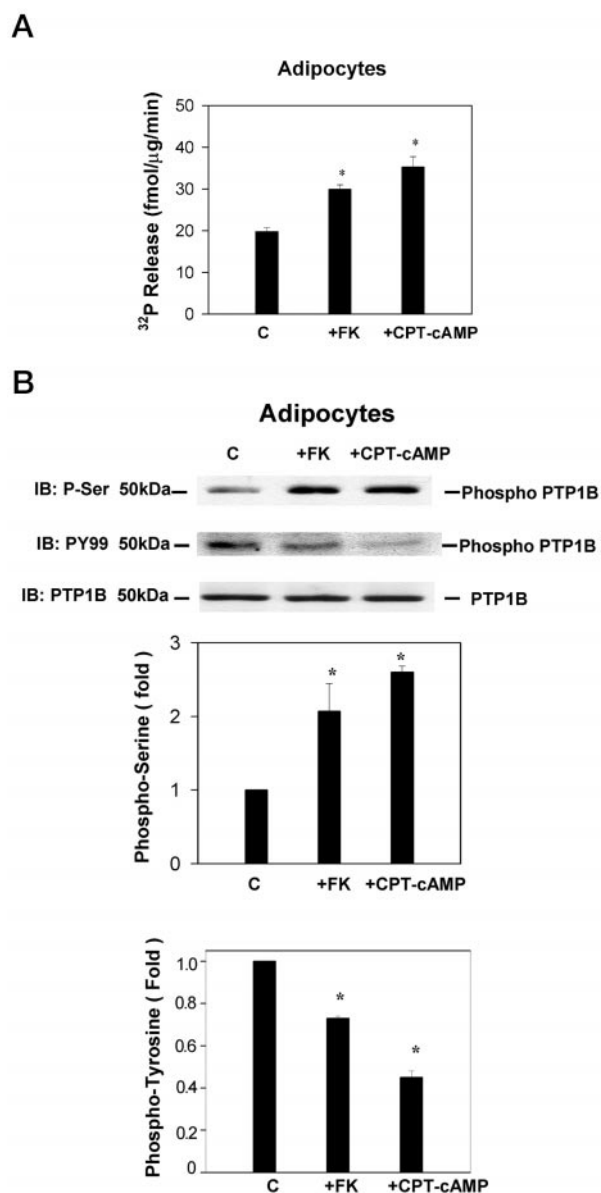


FIG. 6. Activity and content of phosphoserine of protein-tyrosine phosphatase 1B is increased transiently while phosphotyrosine content declines in adipocytes treated with forskolin or cyclic AMP. Mouse adipocytes were challenged with either forskolin (*FK*) or CPT-cyclic AMP for 15 min *in vitro*. Aliquots of the adipocytes were sampled at the 15-min point. Whole-cell extracts of adipocytes were prepared, and immunoprecipitates of PTP1B were subjected to direct measurement of PTP1B activity using the labeled Raytide as a substrate (A) or to immunoblotting (B). The blots were stained with antibodies against PTP1B as well as with anti-phosphotyrosine antibodies (PY99) and anti-phosphoserine antibodies (P-Ser). The immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography of Kodak X-Omat film. Shown are the results activities from four separate adipocytes preparation and the results of a typical immunoblot as well as a compilation of the relative changes in phosphotyrosine content (-fold over control) obtained from four blots prepared from as many separate adipocytes preparations (*bar graphs*). The data in the *bar graphs* are mean values \pm S.E.

ing a challenge with insulin, and PTP1B activity was measured using the radiolabeled, phosphorylated Raytide substrate, highly specific for PTP1B (Fig. 1). Insulin administration *in vivo* stimulated a sharp, transient decline in the activity of fat PTP1B (Fig. 1A). The activity of PTP1B declined by 60–70% in fat tissue within 15 min of administration and recovered to normal, basal levels within 60 min of the challenge with insu-

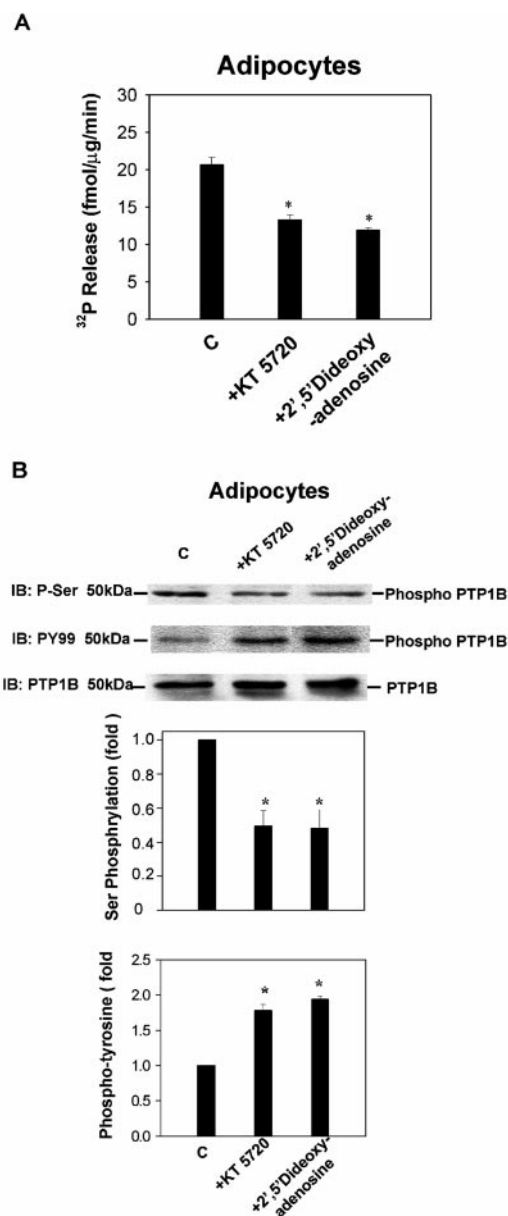
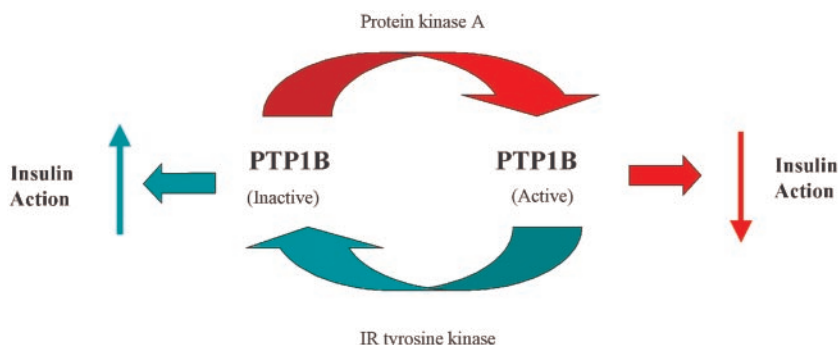


FIG. 7. Activity and content of phosphoserine of protein-tyrosine phosphatase 1B is decreased while phosphotyrosine content increases in adipocytes treated with either the protein kinase A inhibitor KT5720 or 2',5'-dideoxyadenosine. Mouse adipocytes were challenged with either KT5720 (5 μM) or 2',5'-dideoxyadenosine (1 mM) for 15 min *in vitro*. Aliquots of the adipocytes were sampled at the 15-min point. Whole-cell extracts of adipocytes were prepared, and immunoprecipitates of PTP1B were subjected to direct assay of PTP1B activity using the labeled Raytide for a substrate (A) or to immunoblotting (B). The blots were stained with antibodies against PTP1B as well as with anti-phosphotyrosine antibodies (PY99) and anti-phosphoserine antibodies (P-Ser). The immune complexes were detected using a horseradish peroxidase-conjugated secondary antibody, the chemiluminescence reagent, and brief autoradiography of Kodak X-Omat film. Shown are the results from four separate preparations of adipocytes used for assay of PTP1B activity (A) or of a typical immunoblot as well as a compilation of the relative changes in phosphotyrosine content (-fold over control) obtained from four blots prepared from as many separate adipocytes preparations (*bar graphs*, B). The data in the *bar graphs* are mean values \pm S.E.

lin. The decline in PTP1B was maximal at 15 min, as sampling at earlier times displayed a lesser decline (not shown). Analysis of the PTP1B regulation by insulin in skeletal, hind muscle yielded similar results, *i.e.* insulin stimulates a decline in PTP1B activity, albeit a more modest decline (Fig. 1B).

To address the nature of the change in PTP1B that accom-

FIG. 8. Schematic of the regulation of protein-tyrosine phosphatase 1B in response to insulin and elevated cyclic AMP accumulation *in vivo*. See the text for details.



panies the decline in activity in response to insulin, fat and skeletal muscle were sampled and the tissue extracts were used to examine the phosphotyrosine content of PTP1B via immunoblotting with anti-phosphotyrosine antibodies. In fat tissue extracts from insulin-treated mice, the amount of phosphotyrosine content in PTP1B increased about 2-fold (Fig. 2A). The increase in PTP1B tyrosine phosphorylation peaked at 15 min after challenge with insulin, declining thereafter to near normal, basal levels within 60 min. The amount of PTP1B protein itself was not influenced by insulin treatment *in vivo*, over this time period. The changes in PTP1B activity (decreases) and those of phosphotyrosine content (increases) appear to correlate (compare Figs. 1 and 2). Sampling of PTP1B was also performed in skeletal muscle from mice prior to and following administration of insulin *in vivo* (Fig. 2B). The time courses in skeletal muscle for tyrosine phosphorylation of PTP1B in response to insulin administration were very similar to those in fat tissue. The relative increase in the amount of phosphotyrosine content in the PTP1B in response to insulin was routinely observed to be greater in the skeletal muscle than in the fat tissue. In acutely prepared mouse adipocytes, treatment with the insulin receptor tyrosine kinase inhibitor tryphostin AG1024 resulted in a complete loss of insulin-stimulated inhibition of PTP1B activity (Fig. 3) and abolition of the insulin-stimulated increase in PTP1B phosphotyrosine content (data not shown).

Changes in serine phosphorylation have been reported to alter the activity of PTP1B (23). We probed if the action of insulin modified phosphoserine content as well as phosphotyrosine content of PTP1B. Using the peak response at 15 min following insulin administration *in vivo*, the phosphotyrosine and phosphoserine content of PTP1B was sampled in fat and skeletal muscle (Fig. 4). Phosphotyrosine content increased significantly in response to the challenge with insulin at 15 min. The amount of phosphoserine content of PTP1B declined <20% in the samples prepared from fat tissue (Fig. 4A). The same results were obtained in the skeletal, hind muscle of the animals following insulin administration (Fig. 4B). For insulin action *in vivo* it seems clear that the decline in PTP1B activity in response to insulin can be explained by the sharp increase in phosphotyrosine content coupled with a much more modest decrease in phosphoserine content in PTP1B.

Serine phosphorylation of PTP1B in cultured cells has been reported to lead to either no change or increased PTP1B activity, whereas no such studies have been conducted *in vivo*. The effects of elevating intracellular cyclic AMP on PTP1B activity and serine phosphorylation were examined *in vivo* (Fig. 5). The administration of the diterpene activator of adenylyl cyclase forskolin to mice resulted in a sharp increase in cyclic AMP accumulation (not shown). Activation of adenylyl cyclase with forskolin was accompanied by a sharp increase in phosphoserine content of PTP1B in fat (Fig. 5A) and skeletal muscle (Fig. 5B). The increased serine phosphorylation was accompa-

nied by an increase in the activity of PTP1B (Fig. 5C). Interestingly, forskolin treatment was found to reduce the amount of phosphotyrosine in fat and skeletal muscle PTP1B by ~50% (Fig. 5, A and B).

To complete the study of the regulation of PTP1B activity and phosphorylation, we made use of agents that can readily alter intracellular cyclic AMP levels and white fat adipocytes prepared acutely from these FVB mice (Fig. 6). Treating mouse adipocytes with either forskolin or with a water-soluble, hydrolysis-resistant form of cyclic AMP (cyclophenylthio-cyclic AMP, CPT-cyclic AMP) resulted in an increased PTP1B activity (Fig. 6A) and serine phosphorylation (Fig. 6B). Phosphoserine content of PTP1B increased more than 1-fold over basal levels in response to either forskolin or CPT-cyclic AMP at 15 min (Fig. 6A). The phosphotyrosine content of PTP1B, in contrast, declined in response to challenge with either agent by 25–40%. These data suggest that elevation of intracellular cyclic AMP levels, and presumably activation of protein kinase A, leads to increased serine phosphorylation of PTP1B, while catalyzing a decline in phosphotyrosine content.

We explored the effects of reducing input from cyclic AMP by inhibition of protein kinase A with KT5720 and suppression of cyclic AMP levels directly with 2',5'-dideoxyadenosine (Fig. 7). To test whether or not the effects on PTP1B phosphorylation were dependent upon activation of protein kinase A, we examined the effects of treatment with the protein kinase inhibitor KT5720 on the activity (Fig. 7A) and phosphorylation state (Fig. 7B) of PTP1B. KT5720 treatment for 15 min alone led to a decline in the activity and phosphoserine content of PTP1B, much like that obtained in response to 2',5'-dideoxyadenosine, a potent inhibitor of adenylyl cyclase. Experiments, in which cyclic AMP levels were reduced with 2',5'-dideoxyadenosine, demonstrated a decrease in PTP1B activity (Fig. 7A) and in the phosphoserine content of PTP1B, while phosphotyrosine content increased (Fig. 7B). As was noted with suppression of cyclic AMP levels with the adenosine analogue, PTP1B phosphotyrosine content declined while phosphoserine content increased. These data suggest that either via a suppression of cyclic AMP levels or by the inhibition of protein kinase A, the phosphoserine content of PTP1B is reduced by ~50%, while phosphotyrosine content increased 2-fold.

DISCUSSION

The protein-tyrosine phosphatase 1B has been implicated as a negative regulator on tyrosine kinase action (24), including insulin signaling (27). PTP1B levels are elevated in various genetic models of insulin resistance associated with diabetes (13, 16) and obesity (14), although declines in skeletal muscle PTP1B have been reported in patients with diabetes with insulin resistance (12). It has been shown for mice with targeted deficiency of the heterotrimeric G-protein $G_{\alpha_{12}}$ (28) and frank insulin resistance that PTP1B expression is increased (29). Mice lacking the *PTP1B* gene (*PTP1B*^{-/-}) demonstrate in-

creased insulin sensitivity and resistance to obesity (30).

Little is known about the extent to which tyrosine kinases may regulate PTP1B expression or activity. PTP1B can be phosphorylated by the activated epidermal growth factor receptor (24). Studies using cells in culture clearly demonstrate that PTP1B is a substrate for phosphorylation on both serine (21) and tyrosine (24) residues. Whether or not PTP1B is a target for phosphorylation by protein kinases *in vivo* was the hypothesis tested in the current work. Challenging mice with insulin leads to a rapid and marked decline in PTP1B activity in two insulin-sensitive tissues, fat and skeletal muscle. Treatment with AG1024 blocks insulin-stimulated inhibition of PTP1B and PTP1B phosphotyrosine content. Insulin stimulation does not alter the abundance of this protein-tyrosine phosphatase.

The decline in PTP1B activity is accompanied by an increase in the tyrosine phosphorylation of PTP1B in response to insulin stimulation *in vivo*. Insulin stimulates a sharp increase in the amount of PTP1B phosphotyrosine, whereas the phosphoserine content of PTP1B displayed a modest decline. Although the tyrosine residue(s) on PTP1B phosphorylated in response to insulin have not been defined, an FENSYMCM motif in the C-terminal domain of the molecule, in close proximity to the active site, seems a likely candidate.

Increased phosphoserine content of PTP1B was stimulated in response to the elevation of cyclic AMP *in vivo*. This confirms earlier studies on the serine phosphorylation of PTP1B in HeLa cells in response to elevated cyclic AMP and a study showing that phosphorylation of PTP1B regulates its activity (23). The increase in the amount of phosphoserine in PTP1B assayed *in vivo* was accompanied by an increase in the level of PTP1B activity. When examined in adipocytes acutely prepared from mice, PTP1B activity also was found to be increased by elevation of cyclic AMP levels by treatment with the diterpene activator of adenylyl cyclase forskolin as well as by addition of CPT-cyclic AMP directly to the adipocytes medium. The increases in phosphoserine content in PTP1B in response to elevated cyclic AMP was accompanied by a modest decline in phosphotyrosine content.

PTP1B serine phosphorylation and activity of PTP1B was reduced by inhibition of protein kinase A, suggesting a critical role for not only insulin, but also protein kinase A in the regulation of PTP1B (23). Phosphorylation of PTP1B by protein kinase C has been reported (23, 31), although the effects of this serine/threonine phosphorylation on activity remains controversial (31). Reducing the level of intracellular cyclic AMP with 2',5'-dideoxyadenosine resulted in a decline in PTP1B activity. PTP1B displays a canonical site (LRRLSTK) for protein kinase A, which appears to allosterically increase enzymatic activity when phosphorylated. The active site of the PTP1B is contained in the C-terminal region of the molecule, within residues 229–241 (IHCSAGCGRTGAI) (32, 33). In addition, protein kinase A attenuates insulin receptor kinase activity (34). The decline in phosphotyrosine content of PTP1B in response to elevated cyclic AMP noted here may reflect protein kinase A-stimulated loss of insulin receptor kinase activity.

Declines in cyclic AMP levels provoke a decline in PTP1B activity and would be expected to enhance insulin signaling. The PTP1B-deficient mice display enhanced insulin signaling (30). $G\alpha_{12}$ inhibits adenylyl cyclase, lowers intracellular cyclic AMP, and would be expected to reduce PTP1B and thereby enhance insulin signaling. Expression of a GTPase-deficient, constitutively active mutant of $G\alpha_{12}$ does, in fact, lead to reduced cyclic AMP levels and enhanced glucose tolerance in transgenic mice (35). The increased levels of cyclic AMP observed in transgenic mice with targeted loss of $G\alpha_{12}$ in fat and skeletal muscle (36), likewise, lead to elevated levels of PTP1B

and insulin resistance (29). The disruption of the PTP1B gene, in contrast, leads to enhanced insulin sensitivity and resistance to obesity. Thus, elevation of cyclic AMP provokes increased serine phosphorylation and decreased tyrosine phosphorylation of PTP1B and consequent increased activity, whereas treatment with insulin leads to an increased phosphotyrosine content of PTP1B and reduced activity.

A simple model for the regulation of PTP1B is shown (Fig. 8). According to the model, activation of protein kinase A leads to increased serine phosphorylation and activity of PTP1B, which counter-regulates insulin signaling. Stimulation of insulin, in contrast, leads to increased tyrosine phosphorylation and inhibition of PTP1B, which enhances insulin signaling. PTP1B activity appears to be a critical point in reinforcing insulin responses at a point downstream of the insulin receptor tyrosine kinase. PTP1B acts as a point of counter-regulation of insulin action by agents such as catecholamines, which elevate cyclic AMP and activate both protein kinase A and PTP1B.

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REFERENCES

- Schlessinger, J. (2000) *Cell* **103**, 211–225
- Hunter, T. (1998) *Harvey Lect.* **94**, 81–119
- Hunter, T. (2000) *Cell* **100**, 113–127
- Virkamakki, A., Ueki, K., and Kahn, C. R. (1999) *J. Clin. Invest.* **103**, 931–943
- Pessin, J. E., and Saltiel, A. R. (2000) *J. Clin. Invest.* **106**, 165–169
- Tonks, N. K., Diltz, C. D., and Fischer, E. H. (1988) *J. Biol. Chem.* **263**, 6731–6737
- Brown-Shimer, S., Johnson, K. A., Hill, D. E., and Bruskin, A. M. (1992) *Cancer Res.* **52**, 478–482
- van der Sar, A. M., de Fockert, J., Betist, M., Zivkovic, D., and den Hertog, J. (1999) *Int. J. Dev. Biol.* **43**, 785–794
- Cicirelli, M. F., Tonks, N. K., Diltz, C. D., Weiel, J. E., Fischer, E. H., and Krebs, E. G. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 5514–5518
- Worm, D., Vinten, J., and Beck-Nielsen, H. (1999) *Diabetologia* **42**, 1146–1149
- Kenner, K. A., Anyanwu, E., Olefsky, J. M., and Kusari, J. (1996) *J. Biol. Chem.* **271**, 19810–19816
- Byon, J. C., Kusari, A. B., and Kusari, J. (1998) *Mol. Cell Biochem.* **182**, 101–108
- Kusari, J., Kenner, K. A., Suh, K. I., Hill, D. E., and Henry, R. R. (1994) *J. Clin. Invest.* **93**, 1156–1162
- Ahmad, F., and Goldstein, B. J. (1995) *Am. J. Physiol.* **268**, E932–E940
- Ahmad, F., and Goldstein, B. J. (1995) *Metabolism* **44**, 1175–1184
- Worm, D., Vinten, J., and Beck-Nielsen, H. (1999) *Diabetologia* **42**, 1146–1149
- Kennedy, B. P., and Ramachandran, C. (2000) *Biochem. Pharmacol.* **60**, 877–883
- Tonks, N. K., Diltz, C. D., and Fischer, E. H. (1988) *J. Biol. Chem.* **263**, 6722–6730
- Brown-Shimer, S., Johnson, K. A., Lawrence, J. B., Johnson, C., Bruskin, A., Green, N. R., and Hill, D. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 5148–5152
- Barford, D., Flint, A. J., and Tonks, N. K. (1994) *Science* **263**, 1397–1404
- Sarmiento, M., Zhao, Y., Gordon, S. J., and Zhang, Z. Y. (1998) *J. Biol. Chem.* **273**, 26368–26374
- Schievella, A. R., Paige, L. A., Johnson, K. A., Hill, D. E., and Erikson, R. L. (1993) *Cell Growth Differ.* **4**, 239–246
- Shifrin, V. I., Davis, R. J., and Neel, B. G. (1997) *J. Biol. Chem.* **272**, 2957–2962
- Brautigan, D. L., and Pinault, F. M. (1993) *Mol. Cell. Biochem.* **127–128**, 121–129
- Liu, F., and Chernoff, J. (1997) *Biochem. J.* **327**, 139–145
- Moxham, C. M., Tabrizchi, A., Davis, R. J., and Malbon, C. C. (1996) *J. Biol. Chem.* **271**, 30765–30773
- Streuli, M., Krueger, N. X., Tsai, A. Y., and Saito, H. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 8698–8702
- Egawa, K., Maegawa, H., Shimizu, S., Morino, K., Nishio, Y., Bryer-Ash, M., Cheung, A. T., Kolls, J. K., Kikkawa, R., and Kashiwagi, A. (2001) *J. Biol. Chem.* **276**, 10207–10211
- Moxham, C. M., Hod, Y., and Malbon, C. C. (1993) *Science* **260**, 991–995
- Moxham, C. M., and Malbon, C. C. (1996) *Nature* **379**, 840–844
- Elchebly, M., Payette, P., Michaliszyn, E., Cromlish, W., Collins, S., Loy, A. L., Normandin, D., Cheng, A., Himms-Hagen, J., Chan, C. C., Ramachandran, C., Gresser, M. J., Tremblay, M. L., and Kennedy, B. P. (1999) *Science* **283**, 1544–1548
- Flint, A. J., Gebbink, M. F., Franza, B. R., Hill, D. E., and Tonks, N. K. (1993) *EMBO J.* **12**, 1937–1946
- Hoppe, E., Berne, P. F., Stock, D., Rasmussen, J. S., Moller, N. P., Ullrich, A., and Huber, R. (1994) *Eur. J. Biochem.* **223**, 1069–1077
- Su, X. D., Taddei, N., Stefani, M., Ramponi, G., and Nordlund, P. (1994) *Nature* **370**, 575–578
- Stadtmauer, L., and Rosen, O. M. (1986) *J. Biol. Chem.* **261**, 3402–3407
- Chen, J. F., Guo, J. H., Moxham, C. M., Wang, H. Y., and Malbon, C. C. (1997) *J. Mol. Med.* **75**, 283–289
- Moxham, C. M., Hod, Y., and Malbon, C. C. (1993) *Dev. Genet.* **14**, 266–273