Chronic insulin treatment amplifies PDGF-induced motility in differentiated aortic smooth muscle cells by suppressing the expression and function of PTP1B

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Chronic insulin treatment amplifies PDGF-induced motility in differentiated aortic smooth muscle cells by suppressing the expression and function of PTP1B. Am J Physiol Heart Circ Physiol 295: H163–H173, 2008. First published May 2, 2008; doi:10.1152/ajpheart.01105.2007.—Hyperinsulinemia plays a major role in the pathogenesis of vascular disease. Restenosis occurs at an accelerated rate in hyperinsulinemia and is dependent on increased vascular smooth muscle cell movement from media to neointima. PDGF plays a critical role in mediating neointima formation in models of vascular injury. We have reported that PDGF increases the levels of protein tyrosine phosphatase PTP1B and that PTP1B suppresses PDGF-induced motility in cultured cells and that it attenuates neointima formation in injured carotid arteries. Others have reported that insulin enhances the mitogenic and motogenic effects of PDGF in cultured smooth muscle cells and that hyperinsulinemia promotes vascular remodeling. In the present study, we tested the hypothesis that insulin amplifies PDGF-induced cell motility by suppressing the expression and function of PTP1B. We found that chronic but not acute treatment of cells with insulin enhances PDGF-induced motility in differentiated cultured primary rat aortic smooth muscle cells and that it suppresses PDGF-induced upregulation of PTP1B protein. Moreover, insulin suppresses PDGF-induced upregulation of PTP1B mRNA levels, PTP1B enzyme activity, and binding of PTP1B to the PDGF receptor-β, and it enhances PDGF-induced PDGF receptor phosphorylation. Treatment with insulin induces time-dependent upregulation of phosphatidylinositol 3-kinase (PI3-kinase)-δ and activation of Akt, an enzyme downstream of PI3-kinase. Finally, inhibition of PI3-kinase activity, or its function, by pharmacological or genetic means rescues PTP1B activity in insulin-treated cells. These observations uncover novel mechanisms that explain how insulin amplifies the motogenic capacity of the pivotal growth factor PDGF.

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effectiveness of PTP1B directed against PDGF by decreasing the expression and function of the tyrosine phosphatase, thereby amplifying the motogenic effect of PDGF.

MATERIALS AND METHODS

The studies were performed via a protocol approved by the Animal Care and Use Committee, University of Tennessee Health Sciences Center, in accordance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, NIH Publication No. 86-23, Revised 1996).

Materials. Male rats of the Sprague-Dawley strain were purchased from Charles River Laboratories (Wilmington, MA). Primaria tissue culture plates were from Falcon/Becton-Dickinson (Oxnard, CA); DMEM and DMEM:Ham’s F-12 (1:1) medium were from Cellgro Mediatech (Herndon, VA); porcine pancreatic elastase and clostridium histolyticum collagenase were from Worthington Biochemical (Lakewood, NJ); soybean trypsin inhibitor, PBS, and BSA (fraction V) were from Atlanta Biologicals (Lawrenceville, GA); bovine pancreatic insulin, protease inhibitor cocktail, isotype control mouse IgG2a, and p-nitrophenyl phosphate were from Sigma (St. Louis, MO); phosphatase inhibitor cocktail set I was from Calbiochem (La Jolla, CA); recombinant human PDGF-BB was from R&D Systems (Minneapolis, MN); LY294002 was from EMD Biosciences (San Diego, CA); wortmannin was from Biosource (Camarillo, CA); purified mouse anti-PTP1B monoclonal antibody was from BD Biosciences (San Jose, CA); antibody directed against p85 subunit of phosphatidylinositol 3-kinase (PI3-kinase) was from Upstate Biotechnology (Lake Placid, NY); protein G-Sepharose beads were from Amersham Pharmacia Biotechnology (Piscataway, NJ); goat polyclonal antibody against PDGFR-β and goat polyclonal phospho-specific antibody against PDGFR β(Y770) were from Santa Cruz Biotechnology (Santa Cruz, CA); and antibodies against PI3-kinase p85 was generously donated by Barry I. Posner (McGill University, Montreal, Canada). The deletion mutant of mutated p85 (Δ478–513) lacks the binding site for the catalytic unit p110 and has been demonstrated to function in dominant negative manner in cultured cells (31). It should also be noted that we have previously found that treatment with adenovirus at multiplicity of infection (MOI) values used in the present study does not induce cytotoxicity, as determined by the lack of trypan blue staining (data not shown).

Performance of cell culture. Vascular smooth muscle cells were isolated from thoracic aortas of Sprague-Dawley rats (weighing 100–120 g) and cultured in a manner that minimizes cellular dedifferentiation, as we have previously reported (3, 22). All experiments in the present study were performed using primary and confluent cultures that were serum starved for 24–48 h before experiments. Moreover, each individual experiment represents results from one such independent cell isolate.

Performance of Western blot analysis. To determine the levels of tyrosyl phosphorylated PDGFR-β, cells were subjected to lysis using RIPA buffer (150 mM NaCl, 50 mM Tris, 1% sodium deoxycholate, 1% SDS, and 1% Triton X-100, pH 7.2), supplemented with 2 mM sodium vanadate, 0.5 mM PMSF, 0.4 μM aprotinin, 10.5 μM leupeptin, 18 μM bestatin, 7.5 μM pepstatin A, and 7 μM E-64. Blots were probed using phospho-specific antibodies directed against phosphotyrosyl residue 770 in the PDGFR-β. After being blotted for Y770 phosphotyrosyl levels, blots were stripped and reprobed with antibody directed against PDGFR-β protein.

To determine PTP1B protein levels by direct Western blot analysis, cells were subjected to lysis using the following buffer: 188 mM Tris–HCl, 1 mM EDTA, 15% glycerol, and 3% SDS, pH 6.8, further supplemented with 1 mM sodium vanadate, 0.25 mM PMSF, 0.2 μM aprotinin, 5.25 μM leupeptin, 9 μM bestatin, 3.75 μM pepstatin A, and 3.5 μM E-64. Lysates were subjected to Western blot analysis, and band densities were measured by using National Institutes of Health Image software.

Measurement of cell motility. Aortic smooth muscle cell motility was measured by way of a monolayer wounded-culture assay via the use of National Institutes of Health Image 1.62 software, involving migration of cells into the breach, as described in detail in previous publications (4, 12, 50, 63). Motility experiments were performed in the presence of 5 mM hydroxyurea to prevent cell proliferation, as described previously (4). The motility index was expressed as distance migrated in 24 h.

Measurement of PTP1B mRNA levels via semiquantitative real-time PCR. Cells were pretreated with 100 nM insulin for 48 h and then treated without or with 20 ng/ml PDGF-BB for 8 h (in the continued absence or presence of insulin), followed by isolation of total RNA via a commercial kit (Aurum, total RNA sample pack) from Bio-Rad (Hercules, CA). Messenger RNA was transcribed into cDNA via Transcriptor First Strand cDNA Synthesis kit from Roche (Indianapolis, IN), followed by performance of real-time PCR for PTP1B or cyclophilin mRNA levels, using an LC 480 Real-Time PCR instrument (Roche Diagnostics). Cyclophilin was chosen to normalize mRNA levels on the basis of published work (14, 54). Real-time PCR was performed by using the following probes, derived via the Universal probe library software (unisalprobelibrary.com; Roche Applied Science): PTP1B sense primer: GGAAACGGTACCCGAGAT- GTCA (residues 247-267); PTP1B antisense primer: AGTCTATTTCTCTGTATGAATT (residues 291-315); cyclophilin sense primer: ACCTTCCACAGGGTCATCC (residues 306-324); and cyclophilin antisense primer: ACCTTCCAAGGGTCATCC (residues 306-324).
Measurement of PTP1B activity. Confluent aortic smooth muscle cells were exposed for 24 h to adenovirus expressing PTP1B (at MOI of 10 to 15), followed by rinsing to remove residual virus and further incubation for 48 h in the absence or presence of 100 nM insulin. Cells were then subjected to lysis using ice-cold HEPES buffer of the following composition: 50 mM HEPES, 150 mM NaCl, 2.5 mM EDTA, 1% Triton X-100, supplemented with protease inhibitor cocktail (0.5 mM PMSF, 0.4 μM aprotinin, 10.5 μM leupeptin, 18 μM bestatin, 7.5 μM pepstatin A, and 7 μM E-64) and serine/threonine phosphatase inhibitor cocktail (25 μM p-bromotetramisole oxalate, 5 μM cantharidin, and 5 nM microcystin). PTP1B was immunoprecipitated using anti-PTP1B for 3 h at 4°C, followed by incubation of immunocomplexes with protein G-Sepharose beads (50%) for 2 h at 4°C, followed by rinsing twice with ice-cold HEPES buffer (25 mM HEPES, 50 mM NaCl, 2.5 mM EDTA, 10 mM DTT and 5 mM β-mercaptoethanol, pH 7.2). To measure enzyme activity, immunoprecipitated PTP1B was incubated with 10 mM p-nitrophenyl phosphate as substrate for 10 min at 37°C. The reaction was terminated by the addition of 10 M NaOH followed by measurement of enzyme activity via the increase of absorbance at 405 nm, due to the generation of p-nitrophenol by enzymatic hydrolysis. The specificity of this method was verified in a previous study in which we showed that at least 80% of the immunoprecipitated phosphatase activity corresponds with PTP1B, as determined via an in-gel phosphatase assay (23).
Coimmunoprecipitation of PDGFR-β and catalytically inactive PTP1B. Cells were infected with control adenovirus expressing enhanced green fluorescent protein or with adenovirus expressing catalytically inactive PTP1B (C215S-PTP1B) at MOI values of 10–15 in the absence or presence of 100 nM insulin for 48 h. Cells were then rinsed to remove residual virus, followed by incubation for 24 h, in the continued absence or presence of 100 nM insulin. Cells were then subjected to lysis using RIPA buffer of the following composition: 150 mM NaCl, 50 mM Tris, 1% sodium deoxycholate, 1% SDS, and 1% Triton X-100, pH 7.2, further supplemented with 2 mM sodium vanadate, 0.5 mM PMSF, 0.4 µM aprotinin, 10.5 µM leupeptin, 18 µM bestatin, 7.5 µM pepstatin A, and 7 µM E-64, and the lysates were immunoprecipitated with anti-PTP1B or nonspecific IgG as control. Coimmunoprecipitated PDGFR-β levels were measured by Western blot analysis by using anti-PDGFR-β, whereas PTP1B protein levels were measured by using anti-PTP1B.

Statistical analysis. Statistical analyses of multiple comparisons were performed by ANOVA, followed by Fisher’s protected least significant difference test. Single comparisons were performed via unpaired Student’s t-test. Statistical significance was assumed for \( P < 0.05 \).

RESULTS

Insulin suppresses PDGF-induced PTP1B expression and amplifies PDGF-induced cell motility. Publications (27, 29) have reported that acute treatment with insulin attenuates PDGF-induced smooth muscle cell motility or proliferation, whereas other reports (19, 59) indicate that prolonged treatment with insulin enhances the mitogenic effect of PDGF. We have reported that PDGF increases PTP1B protein levels (6), that ectopic overexpression of PTP1B attenuates PDGF-induced motility in cultured smooth muscle cells (5), that vascular injury increases PTP1B levels (6), and that expression of dominant-negative PTP1B enhances neointima formation in injured rat carotid arteries (5). These studies support the existence of a negative regulatory feedback loop involving PTP1B, downstream of PDGFR activation in vascular smooth muscle cells. The purpose of the present study was to determine if pretreatment of cells with insulin would suppress PDGF-induced upregulation of PTP1B function, which would provide a mechanism that would explain amplification of PDGF-induced cell motility by insulin. We used highly differentiated primary cultured aortic smooth muscle cells obtained

![Graph](http://ajpheart.physiology.org/)
from adult rats to mimic the differentiation state of medial cells in blood vessels as closely as possible. As shown in Fig. 1, pretreatment of cells for 48 h with 100 nM insulin virtually abrogated the increase of PTP1B protein levels induced by PDGF. Data given in Fig. 2 show that treatment of cells with insulin at a concentration as low as 5 nM (with change of culture media twice a day to attenuate insulin degradation) also yielded virtually complete suppression of PDGF-induced PTP1B upregulation. Data provided in Fig. 3 indicate that the suppressive effect of insulin fails to occur without preincubation with insulin, indicating that chronic but not acute treatment has the capacity to suppress PDGF-induced elevation of PTP1B protein levels. Moreover, pretreatment of cells with insulin for 48 h abrogated the capacity of PDGF to induce upregulation of PTP1B mRNA levels.

Insulin attenuates the anti-motogenic effect of ectopically expressed PTP1B. We next tested the hypothesis that insulin would suppress the function of PTP1B in aortic smooth muscle cells, independently of its effect on PTP1B expression. To test this hypothesis, we induced ectopic expression of PTP1B via an adenoviral vector whereby expression of the enzyme was directed by the constitutively active cytomegalovirus enhancer/promoter, with the expectation that insulin would not have a significant effect on expression driven by this promoter. It should also be noted that we took care to perform these experiments using a relatively low concentration of PDGF (2 ng/ml) to avoid significant upregulation of endogenous PTP1B levels by PDGF.
As expected, insulin did not significantly alter the expression of ectopic PTP1B induced by infection with PTP1B-expressing adenovirus (Fig. 8A). As shown in Fig. 8B, treatment of cells with 2 ng/ml PDGF induced a twofold increase of cell motility. Moreover, overexpression of PTP1B blocked PDGF-induced motility in insulin-naive cells, confirming earlier results from our laboratory (5). However, pretreatment of cells with 100 (Fig. 8) or 5 nM insulin (data not shown) rescued the motogenic effect of PDGF in PTP1B-treated cells, without significantly altering baseline motility. In this regard, it should also be noted that insulin treatment alone does not alter cell number, as we have previously reported (12).

Insulin decreases the enzyme activity of ectopic or endogenous PTP1B. The aforementioned experiments are consistent with the notion that insulin may decrease the enzyme activity of PTP1B. However, the effect of insulin on PTP1B activity is controversial, with one study having reported increased enzyme activity, but several others having reported the opposite finding (8, 36, 37, 53). We therefore performed the next experiments to test the hypothesis that suppression of PTP1B function by insulin could be attributed, at least in part, to decreased enzyme activity. As shown in Fig. 9B, insulin induced a ~50% decrease of ectopically expressed PTP1B activity, uncovering an additional mechanism by which insulin induces suppression of the counter-regulatory activity of PTP1B. These data are further supported by Fig. 9A, indicating specificity of immunoprecipitation as well as equivalency of immunoprecipitated PTP1B levels in different treatment categories.

To verify the aforementioned findings in which we used ectopically expressed PTP1B, we also performed experiments to determine the effect of insulin on the enzyme activity of endogenous PTP1B. As shown in Fig. 10B, PDGF increased the activity of PTP1B, whereas insulin blocked the effect of PDGF. Insulin did not decrease baseline PTP1B activity, presumably due to the low levels of activity in cells not treated with PDGF. Data shown in Fig. 10A indicate equivalent levels of PTP1B in the specific immunoprecipitates, a result that was obtained by adding excess protein to immunobeads to induce equivalent immunoprecipitation in different treatment categories.

Insulin suppresses PTP1B-induced PDGFR-β phosphotyrosyl dephosphorylation. Recent publications (5, 20) have indicated that PTP1B has the capacity to decrease the levels of phosphotyrosyl in PDGFRs, an effect that is consistent with the antimotogenic influence of PTP1B directed against PDGF-induced motility. These findings prompted us to test the hypothesis that insulin would suppress PTP1B-induced phosphotyrosyl dephosphorylation of PDGFRs. As expected, treatment of control cells with PDGF-BB, at concentrations that do not elicit significant upregulation of endogenous PTP1B, induced a significant increase of phosphotyrosyl levels in residue Y770 of PDGFR-β in both the absence and presence of insulin (Fig. 11). Moreover, overexpression of PTP1B essentially abrogated PDGF-induced tyrosyl phosphorylation of PDGFR-β in insulin-naive cells, confirming previous findings (Fig. 11, A and B). However, in cells that were pretreated with insulin, the capacity of PTP1B to decrease phosphotyrosyl levels of PDGFR-β was markedly attenuated compared with that in insulin-naïve cells (Fig. 11, C and D).

Insulin antagonizes the association of PTP1B with PDGFR-β. The next experiments were performed to further probe the mechanism underlying insulin-induced inhibition of PTP1B function. We thus tested the hypothesis that insulin would attenuate the binding of PTP1B with its substrate, PDGFR-β. To perform this experiment, we used adenovirus expressing a substrate-trapping mutant of PTP1B, C215S-PTP1B, lacking catalytic activity, rather than wild-type PTP1B, on the basis of a report (17) indicating that wild-type PTP1B binds to substrates in a transient manner that is unlikely to be suitable for determination of interaction between enzyme and substrate in a biochemical assay.

![Fig. 11](http://ajpheart.physiology.org/)

**Fig. 11.** Pretreatment with insulin suppresses PTP1B-induced PDGFR-β phosphotyrosyl dephosphorylation. Cells were preincubated without (A and B) or with 100 nM insulin (C and D) for 48 h, followed by treatment with control adenovirus expressing enhanced green fluorescent protein or adenovirus expressing PTP1B, for 24 h in the continued absence or presence of insulin, followed by treatment with a range of PDGF-BB concentrations for 10 min. Levels of phospho-PDGFR-β and total PDGFR-β were measured by sequential Western blot analyses using anti-phospho-PDGFR-β (Y770) and anti-PDGFR. A and C: results from representative Western blots. B and D: summary of 3 experiments expressed as means ± SE of the densitometric ratio of phospho-PDGFR-β to PDGFR-β levels, normalized to control values (no insulin treatment and no overexpression of PTP1B). A and B: results in insulin-naive cells. C and D: results in cells treated with insulin. *P < 0.05, compared with values from cells treated with control virus. **P < 0.05, compared with values from cells treated with PTP1B-expressing adenovirus by analysis of variance, followed by Fisher’s PLSD test.
whereas catalytically inactive PTP1B has the capacity to bind substrate in a stable manner. To implement this aim, we measured the extent of specific coimmunoprecipitation of PDGFR-β with C215S-PTP1B (the latter being expressed via an adenoviral vector) in protein lysates from cells treated without or with insulin. As depicted in Fig. 12, treatment of cells with insulin essentially abrogated the capacity of C215S-PTP1B to induce coimmunoprecipitation of PDGFR-β.

Treatment with insulin enhances PI3-kinase-δ levels in a time-dependent manner. Several studies (40, 42, 43) have reported that hyperinsulinemia is associated with increased PI3-kinase activity in liver or skeletal muscle. Moreover, other recent studies found that hyperinsulinemia induces upregulation of PI3-kinase expression in rat aorta (55) and that PI3-kinase-δ levels are specifically upregulated in this model (30). These findings prompted us to investigate whether chronic, but not acute, insulin treatment would induce upregulation of PI3-kinase-δ in cultured primary aortic smooth muscle cells. As shown in Fig. 13, treatment of cells for 72 h with insulin increased the levels of PI3-kinase-δ but not the levels of PI3-kinase-α, −β, or −γ. Moreover, as shown in Figs. 13 and 14, PI3-kinase-δ levels increased in cells preincubated with insulin for 24 h to 72 h but not in cells not preincubated with insulin.

Insulin induces phosphorylation of Akt. It is well established that PI3-kinase is a pivotal mediator of insulin signaling (48). On the basis of this and the aforementioned findings, we tested the hypothesis that the PI3-kinase pathway may be activated by insulin treatment in cultured smooth muscle cells, as manifested by phosphorylation of Akt on amino acid residue seryl...
473, a well-accepted marker of Akt and PI3-kinase pathway activity (25). As shown in Fig. 15, insulin induced a marked increase of S473 phosphorylation level of Akt, indicative of PI3-kinase-Akt pathway activation.

Fig. 15. Induction of Akt phosphorylation by insulin treatment. Cells were treated without or with 100 nM insulin for 72 h. Cell lysates were then subjected to Western blot analysis of phosphorylated Akt (S473) via the use of a phosphospecific antibody (A, top). A separate Western blot was performed to document equivalent levels of total Akt (A, middle) and equivalent actin levels among treatment categories (A, bottom). B: summary of means ± SE of 3 experiments, expressed as the ratio of phospho-Akt/total Akt. *P < 0.05, compared with control values by Student’s t-test.

Fig. 16. PI3-kinase inhibitor LY294002 rescues PTP1B activity in insulin-treated cells. Cells were treated for 24 h with adenovirus expressing PTP1B, followed by infection with control adenovirus expressing EGFP or with adenovirus expressing dominant negative p85 (deletion mutant) adapter subunit (Δp85) for 48 h at MOI of 10–15. A: representative Western blot for expression levels of Δp85 (top) as well as PTP1B levels in immunoprecipitation experiments. B: summary of means ± SE of 3 independent experiments. *P < 0.05, compared with control virus treatment, followed by specific IP, by Student’s t-test.

Fig. 17. Dominant-negative p85 regulatory subunit of PI3-kinase rescues PTP1B activity in insulin-treated cells. Cells were treated for 24 h with adenovirus expressing PTP1B, followed by infection with control adenovirus expressing EGFP or with adenovirus expressing dominant negative p85 (deletion mutant) adapter subunit (Δp85) for 48 h at MOI of 10–15. A: representative Western blot for expression levels of Δp85 (top) as well as PTP1B levels in immunoprecipitation experiments. B: summary of means ± SE of 3 independent experiments. *P < 0.05, compared with control values by Student’s t-test.

Fig. 18. Schematic representation of interactions among PDGF, insulin, and PTP1B-mediated pathways. Arrows indicate stimulatory or positive effects whereas blocked lines indicate inhibitory or negative effects. Question mark refers to mechanism, the existence of which has been reported in other cells (Ref. 46) but is presently hypothetical in vascular smooth muscle cells.
insulin-induced inhibition of PTP1B activity. For this purpose, we used two well-established and relatively selective pharmacological antagonists of PI3-kinase, namely LY294002 and wortmannin (10). As shown in Fig. 16, LY294002 rescued the activity of PTP1B in insulin-treated cells and similar results were obtained via the use of wortmannin (data not shown). To confirm these findings, we utilized a genetic approach via the use of an adenovirus expressing a deletion mutant of the p85 adaptor subunit (Δ478–513) of PI3-kinase, reported to act in dominant negative manner against endogenous PI3-kinase (31). Figure 17A indicates expression levels of ectopic dominant negative p85 subunit of PI3-kinase as well as immuno-precipitated PTP1B levels. Furthermore, as shown in Fig. 17B, treatment of cells with adenovirus expressing dominant negative p85 regulatory subunit rescued insulin-suppressed PTP1B activity, supporting the results obtained via the use of pharmacological PI3-kinase antagonists.

DISCUSSION

Hyperinsulinemia is a well-established factor for the pathogenesis of cardiovascular disease (2, 11, 52, 56). Moreover, several studies (15, 21, 34, 38, 61) have reported that PDGF plays a major role in vascular remodeling that occurs in response to injury by activating the PDGFR-β isoform. Studies (44) have reported that PDGFR-β expression and phosphorylation are markedly increased during the peak of vascular smooth muscle cell migration into the neointima. In the present study, we report the novel findings that pretreatment of differentiated cultured rat aortic smooth muscle cells with insulin enhances PDGF-induced motility by decreasing the counter-regulatory effectiveness of PTP1B against the PDGFR via two distinct mechanisms: suppression of PTP1B mRNA and protein levels and decrease of PTP1B enzyme activity, the latter via a mechanism dependent on PI3-kinase function. Both functional and biochemical effects of insulin require chronic treatment, and they are manifested at a concentration of insulin as low as 5 nM, indicating that these effects are likely to be mediated via the insulin receptor, rather than the IGF-I receptor.

We reached our conclusions on the basis of findings indicating that pretreatment with insulin suppresses the capacity of PDGF to induce upregulation of PTP1B mRNA and protein levels. Furthermore, we found that insulin decreases PTP1B activity and that it attenuates substrate recognition by the enzyme, together with upregulation of Akt activity, indicating PI3-kinase activation. Activation of PI3-kinase by insulin is consistent with studies reporting association of hyperinsulinemia and increased PI3-kinase activity in rat liver and skeletal muscle (41–43). Moreover, recent studies (55) reported that hyperinsulinemia induced by insulin infusion induces specific upregulation of PI3-kinase-δ in rat aorta. We have now verified upregulation of PI3-kinase-δ, also confirming that PI3-kinase-α, -β, or -γ are not upregulated, and we find that this event occurs in time-dependent manner, implicating the δ-isoform in mediating the effect of chronic insulin treatment. These results are further supported by findings that pharmacological inhibitors of PI3-kinase, as well as a dominant negative allele of p85 targeted against PI3-kinase function, oppose insulin-induced attenuation of PTP1B activity.

Mechanism(s) mediating insulin-induced attenuation of PTP1B activity downstream of PI3-kinase have not yet been fully elucidated. However, we speculate that they may involve activation of Akt, followed by seryl phosphorylation of PTP1B, on the basis of our findings of Akt activation and a report (46) from another laboratory indicating that activated Akt induces seryl phosphorylation of PTP1B, leading to decreased PTP1B enzyme activity. A second potential mechanism that might explain suppression of PTP1B activity would be hydrogen peroxide-mediated oxidation of the catalytically essential cysteine residue in the active site of the enzyme, as has been reported to occur in a transient manner by several groups (36, 37). Although our data do not rule out redox regulation of PTP1B by long-term insulin treatment, the fact that the catalytic activity of PTP1B was found to be decreased in assays containing sulfhydryl agents (a treatment that would be expected to rescue reduced activity induced by oxidation of catalytic cysteine residue) and the finding that insulin treatment blocks the association of a mutant of PTP1B lacking the catalytic cysteine with the PDGFR indicate the existence of a novel, redox-independent, mechanism that can explain reduced PTP1B catalytic activity. These findings support a mechanism outlined in Fig. 18, summarizing the effects of insulin treatment on the signaling and function of PDGF via suppression of PTP1B.

The effects of insulin on cultured smooth muscle cell motility and proliferation appear to be diverse. Thus, acute treatment with insulin reportedly opposes PDGF-induced cell motility, at least in part, via a mechanism mediated by nitric oxide and cGMP (7, 27, 29). In the present study, insulin induced a modest increase (~10%) of motility. However, insulin virtually doubled the motogenic effect of PDGF. Because PDGF induces upregulation of PTP1B levels and because PTP1B induces suppression of PDGF-induced cell motility, as reported by our group (6) and as verified in the present study, the co-motogenic effect of insulin is directly attributable to its capacity to suppress the counter-regulatory influence of PTP1B. We have also reported that PTP1B levels are upregulated in vascular injury and that PTP1B suppresses the formation of neointima in injured carotid arteries. Thus the present findings provide a novel framework to explain how hyperinsulinemia enhances experimental neointima formation (19, 26, 45, 59) and vascular disease in humans with type 2 diabetes (2, 11, 52, 56).

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MECHANISMS OF ENHANCED CELL MOTILITY INDUCED BY INSULIN


