Insulin Affects Vascular Smooth Muscle Cell Phenotype and Migration Via Distinct Signaling Pathways

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Insulin maintains vascular smooth muscle cell (VSMC) quiescence yet can also promote VSMC migration. The mechanisms by which insulin exerts these contrasting effects were examined using α-smooth muscle actin (α-SMA) as a marker of VSMC phenotype because α-SMA is highly expressed in quiescent but not migratory VSMC. Insulin alone maintained VSMC quiescence and modestly stimulated VSMC migration. Wortmannin, a phosphatidylinositol 3-kinase (PI3K) inhibitor, decreased insulin-stimulated expression of α-SMA mRNA by 26% and protein by 48% but had no effect on VSMC migration. PD98059, a mitogen-activated protein kinase (MAPK) kinase inhibitor, decreased insulin-induced VSMC migration by 52% but did not affect α-SMA levels. Platelet-derived growth factor (PDGF) promoted dedifferentiation of VSMC, and insulin counteracted this effect. Furthermore, insulin increased α-SMA mRNA and protein levels to 111 and 118%, respectively, after PDGF-induced dedifferentiation, an effect inhibited by wortmannin. In conclusion, insulin's ability to maintain VSMC quiescence and reverse the dedifferentiating influence of PDGF is mediated via the PI3K pathway, whereas insulin promotes VSMC migration via the MAPK pathway. Thus, with impaired PI 3-kinase signaling and intact MAPK signaling, as seen in insulin resistance, insulin may lose its ability to maintain VSMC quiescence and instead promote VSMC migration.
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Atherosclerosis is a major cause of morbidity and mortality in patients with insulin resistance (1–3). Vascular smooth muscle cells (VSMCs) respond to arterial wall injury by intimal proliferation and play a key role in atherogenesis by proliferating and migrating excessively in response to repeated injury, such as smoking and hypertension (4,5). In contrast, fully differentiated, quiescent VSMCs allow for arterial vasodilation and vasoconstriction. These disparate roles of VSMC are possible because two VSMC phenotypes exist, representing extremes along a continuous spectrum. The quiescent, fully differentiated phenotype characterizes the usual state in the vascular wall responsible for vascular contraction and dilation. The migratory or proliferative phenotype is present during response to injury (6). Maintenance of the quiescent phenotype requires the presence of factors that include insulin and cAMP response-element binding protein, whereas platelet-derived growth factor (PDGF) promotes VSMC proliferation and migration (7–9).

α-Smooth muscle actin (α-SMA) is a protein expressed early in VSMC differentiation (6). As VSMCs differentiate toward the quiescent state, levels of α-SMA increase (10). When VSMCs undergo a phenotypic switch to a migratory or proliferative phenotype in response to injury, levels of α-SMA decrease (10). We used α-SMA protein and mRNA levels as markers for VSMC phenotype to examine the pathways of insulin signaling in VSMCs.

A cardinal feature of insulin resistance is impairment of insulin signaling along intracellular pathways regulating the metabolic effects of insulin. The major pathway responsible for these effects involves the tyrosine phosphorylation of insulin receptor substrate-1 and -2 (IRS-1, IRS-2) with activation of phosphatidylinositol 3-kinase (PI3K) and Akt (protein kinase B) (11–13). Whereas signaling along this pathway is impaired in virtually all states of insulin resistance, insulin signaling along the pathway leading to mitogenic effects of insulin remains intact in insulin resistant states and responds normally to insulin (14–16). This pathway involves the sequential activation of Shc, Ras, Raf, and extracellular signal-regulated kinase mitogen-activated protein kinase (MAPK) (17–20). The differential responsiveness of these two major signaling branches of insulin action has been convincingly shown in VSMCs, endothelial cells, and skeletal muscle cells (14–16). Because insulin resistance is invariably accompanied by compensatory hyperinsulinemia, the preferential signaling along the mitogenic pathway of insulin action in response to hyperinsulinemia may contribute to the progression of atherosclerosis (16,21).

The present study demonstrates that insulin contributes to the maintenance of the quiescent phenotype of VSMC and that this aspect of its action is mediated via the PI3K-dependent pathway. Insulin also uses the PI3K-dependent pathway to counteract the dedifferentiating influence of PDGF. In contrast, insulin effect on VSMC migration seems to be mediated through the MAPK-dependent pathway.

RESEARCH DESIGN AND METHODS

Materials. FBS was purchased from Gemini Bio-Products (Woodland, CA). Cell culture media and Northern blotting reagents were from Invitrogen

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α-SMA, α-smooth muscle actin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

(Carlsbad, CA). PD98059 was from Calbiochem (San Diego, CA). Northern blotting reagents and supplies were from Ambion (Austin, TX), Amersham (Piscataway, NJ), Stratagene (La Jolla, CA), Schleicher and Schuell (Keene, NH), and Tel-Test "B" (Friendswood, TX). The pGEM4Z plasmid containing the α-SMA rat cDNA probe was a gift from Dr. Gary K. Owens (University of Virginia Health Sciences Center). All other reagents were from Sigma (St. Louis, MO).

Isolation, characterization, and growth of VSMCs. Bovine aortic VSMCs were isolated by media explant from adult animals. These VSMCs were a gift from Dr. Peter Watson (University of Colorado Health Sciences Center). Cells were passaged from explant cultures into tissue culture flasks after brief treatment with trypsin-EDTA. The VSMCs were grown in a medium consisting of minimum essential medium with Earle's salts, L-glutamine, and sodium bicarbonate supplemented with 88 units/ml penicillin, 88 μg/ml streptomycin, 2 mmol/l L-glutamine, 10% FBS, and 0.1 mmol/l nonessential amino acids. Cells were used between passages 3 and 6. For all experiments, cells were switched to serum-reduced medium (growth medium above except with 0.1% FBS) and maintained for 24 h before further treatments, to maintain quiescence.

Northern blotting and Western blotting. In the first set of in vitro RNA experiments, VSMCs were serum-starved for 24 h, then incubated for 24 h (RNA) or 60 h (protein) with 10 nmol/l insulin, 50–100 nmol/l wortmannin (a PI3K inhibitor), 30 pmol/l PDGF, and/or 20 μmol/l PD98059 (a specific MAPK inhibitor). Dose-response studies were performed for 1–1,000 nmol/l insulin and for 10 nmol/l insulin in the presence of 1–100 nmol/l wortmannin. In a separate set of experiments, VSMCs were serum-starved for 24 h, pretreated with PDGF for 48–60 h to induce dedifferentiation, then incubated for 24 h (RNA) or 60 h (protein) with insulin, wortmannin, PDGF, and/or PD98059. VSMCs in the control group were serum-starved for the duration of the experiment. Medium and treatments were refreshed every 24–48 h.

For Northern blotting, total RNA was isolated from VSMCs using a suspension of guanidinium thiocyanate and phenol with chloroform extraction and isopropanol precipitation. RNA samples were electrophoresed through a 1.2% agarose denaturing gel containing 1× MOPS and 6% formaldehyde, then transferred to a Nytran N membrane. Probes were labeled with [α-^{32}P]dATP or [α-^{32}P]dCTP using random 9-mer primers then column-purified. Blots were hybridized with the α-SMA cDNA probe at 1×10^6 cpm/ml overnight, washed, and visualized by autoradiography. Densitometry was performed using Quantity One version 4.1.1 software on a Bio-Rad Fluor-S MultiImager (Bio-Rad, Hercules, CA). Blots were then stripped and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or ubiquitin cDNA control probe in the same manner as described above.

For Western blotting, cells were lysed and sonicated. Ten to 30 μg of each sample was dried using a Speedvac Concentrator (Savant, Holbrook, NY), resuspended in Laemmle, and stored at −20°C until used. Samples were then boiled for 5–10 min, and 2.5–7.5 μg of total protein per lane was resolved by SDS-PAGE before transfer to polyvinylidene fluoride membranes. Membranes were probed with a mouse monoclonal α-SMA antibody (Sigma). Blots were then incubated with rabbit anti-mouse polyclonal antibody conjugated with horseradish peroxidase and α-SMA protein detected by chemiluminescence. Densitometry was performed on a Bio-Rad Fluor-S MultiImager.

Migration assays. The modified Boyden Chamber technique was used to quantify VSMC chemokinesis in response to 24-h incubation with insulin, wortmannin, and PD98059. A 24-well Transwell apparatus (Costar, Corning, NY) was used, with each well containing a 6.5-mm polycarbonate membrane with 8-μm pores, coated with Type I collagen. VSMCs were incubated in serum-reduced medium for 24 h, then with insulin, wortmannin, and/or PD98059 for another 24 h. The cells were trypsinized, resuspended in serum-free medium, and plated at a density of 30,000 per membrane. After cell attachment for 15 min, PDGF was added to the bottom chamber of each well. Cells migrating through the membrane to the underside of the apparatus for 1.5 h were then fixed and stained. After overnight air drying, cells on the plating surface were removed. The migrated cells were quantified as number of cells migrating per low-power microscopic field.

Data analysis. Between 3 and 11 individual experiments, each in duplicate, were performed for every experimental design. The results are expressed as mean ± SE. The Student's t test was used to analyze all data, with $P \leq 0.05$ considered statistically significant. For Northern blots, α-SMA optical densities were corrected according to the intensity of corresponding GAPDH or ubiquitin mRNA signals. All results are expressed as percentage of control.

RESULTS

Insulin mediates VSMC quiescence via the PI3K signaling pathway. We set out to determine the role of insulin in maintenance of the quiescent (differentiated) state of VSMCs. Because α-SMA expression reflects the state of VSMC differentiation, we measured levels of α-SMA protein and mRNA to assess the effect of insulin on VSMC differentiation and quiescence. After 60-h incubation with insulin, α-SMA protein levels increased to 123% of control ($P < 0.001$; Fig. 1*A* and *B*). It is interesting that treatment of VSMCs with insulin in the presence of wortmannin resulted in a significant decrease in α-SMA levels to 74% of control ($P < 0.001$ vs. insulin alone), pointing to the importance of the PI3K-dependent signaling pathway in mediating insulin effect on VSMC differentiation. In contrast, the presence of the MAPK pathway inhibitor PD98059 had no effect on the ability of insulin to maintain cell quiescence. Neither wortmannin nor PD98059 had any independent effect on α-SMA protein levels.

We then assessed the influence of insulin on levels of α-SMA mRNA in VSMCs. Cells were incubated with insulin for 24 h in the presence or absence of either wortmannin or PD98059. Within this time frame, insulin maintained α-SMA mRNA at control levels (Fig. 1*C* and *D*). However, VSMCs incubated with insulin in the presence of wortmannin displayed a significant reduction in the levels of α-SMA mRNA to 75% of control. Conversely and in agreement with expression of α-SMA at the protein level, inhibition of the MAPK-dependent pathway had no effect on the ability of insulin to maintain the levels of α-SMA mRNA.

Insulin and wortmannin dose-response studies. To evaluate the effect of insulin and wortmannin on α-SMA protein and mRNA levels, we performed dose-response experiments. Physiologically, insulin concentrations in vivo may rise to ~1 nmol/l. In the presence of insulin resistance, serum insulin concentrations may exceed these levels. We performed insulin dose-response experiments to determine the effect of physiologic and supraphysiologic concentrations of insulin on α-SMA protein and mRNA levels in VSMCs. Insulin (1 nmol/l) had no effect on α-SMA protein levels but increased mRNA levels significantly (Fig. 2). The maximal effect of insulin was reached at 10 nmol/l on both α-SMA protein and mRNA levels. At markedly supraphysiologic insulin concentrations, there was a plateau in mRNA levels but no effect on α-SMA protein levels compared with control.

Wortmannin was described in 1992 as a myosin light-chain kinase inhibitor (22) at micromolar concentrations. It was later found that wortmannin is a specific PI3K inhibitor at nanomolar concentrations, with varying IC50 depending on the cell type studied (23–25). The 10- to 100-nmol/l doses of wortmannin have been used most commonly to inhibit PI3K activity in VSMCs (26–28). We performed dose-response studies of wortmannin in the presence of insulin to examine the effects on α-SMA protein and mRNA levels in bovine aortic VSMCs. At wortmannin doses ranging from 1 to 50 nmol/l, there was no significant effect on α-SMA protein levels (Fig. 3). However, there was a significant decrease in α-SMA protein with 100 nmol/l wortmannin in the presence of 10 nmol/l insulin. There was a significant decrease in α-SMA mRNA levels beginning with 10 nmol/l wortmannin in the presence of insulin. This was a threshold effect because 100 nmol/l did not decrease α-SMA mRNA levels further.

Insulin counteracts the effect of PDGF on VSMCs. Having determined that insulin maintains differentiation and quiescence of VSMCs, we explored its ability to

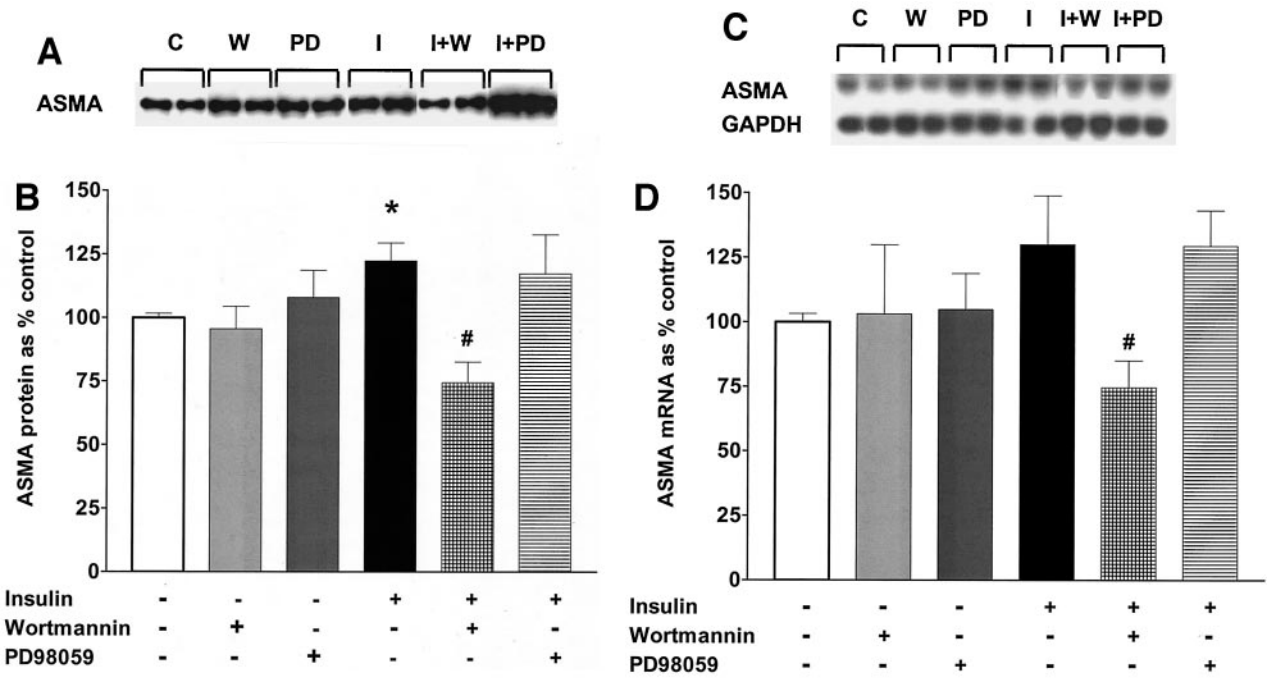

FIG. 1. Effect of insulin on α-SMA protein and mRNA levels in VSMCs. Cells were incubated either with 10 nmol/l insulin (I) or insulin and 100 nmol/l wortmannin (W) or insulin and 20 μmol/l PD98059 (PD). *A*: Representative Western blot of α-SMA protein. *B*: Results of Western blotting are expressed as percentage of control (C; VSMCs incubated in serum-reduced medium only) and plotted as mean ± SE of 10 or 11 experiments, each performed in duplicate. *$P < 0.001$ vs. control #$P < 0.001$ vs. insulin alone. *C*: Representative Northern blot of α-SMA mRNA. *D*: Amounts of α-SMA mRNA as determined by Northern blotting. Results are corrected for GAPDH or ubiquitin signal, expressed as percentage of control, and plotted as mean ± SE of six to nine experiments performed in duplicate. #$P < 0.05$ vs. insulin alone.

counteract the effects of PDGF, one of the most potent stimuli of VSMC proliferation. VSMCs were incubated for 60 h with either PDGF alone or PDGF and insulin administered simultaneously. As expected, PDGF decreased α-SMA protein levels to 78% of control (Fig. 4*A* and *B*), suggesting a shift in VSMC phenotype toward dedifferentiation and proliferation. Insulin effectively blocked PDGF action, restoring α-SMA to the control level. When the

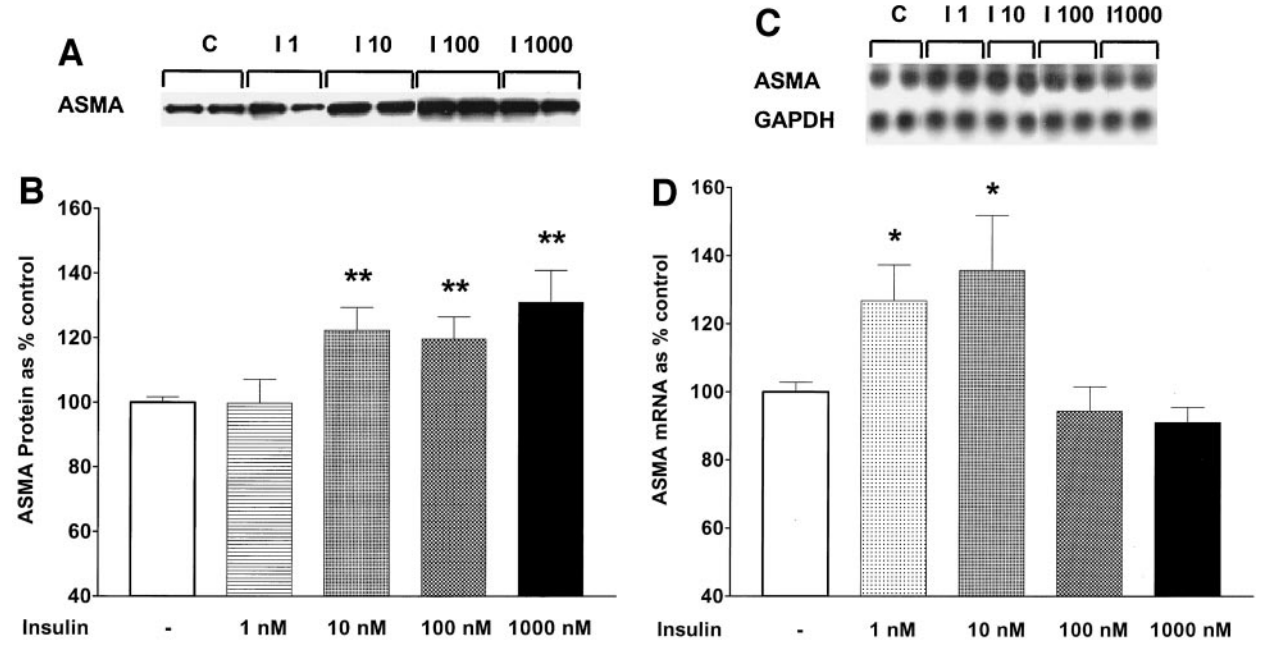

FIG. 2. Insulin dose-response curve for α-SMA protein and mRNA levels in VSMCs. Cells were incubated with 1, 10, 100 or 1,000 nmol/l insulin (*I*). *A*: Representative Western blot of α-SMA protein. *B*: Results of Western blotting are expressed as percentage of control (C) and plotted as mean ± SE of two experiments, each performed in duplicate. *C*: Representative Northern blot of α-SMA mRNA. *D*: Amounts of α-SMA mRNA as determined by Northern blotting. Results are corrected for GAPDH signal, expressed as percentage of control, and plotted as mean ± SE of four to six experiments performed in duplicate. *$P < 0.05$ vs. control; **$P < 0.001$ vs. control.

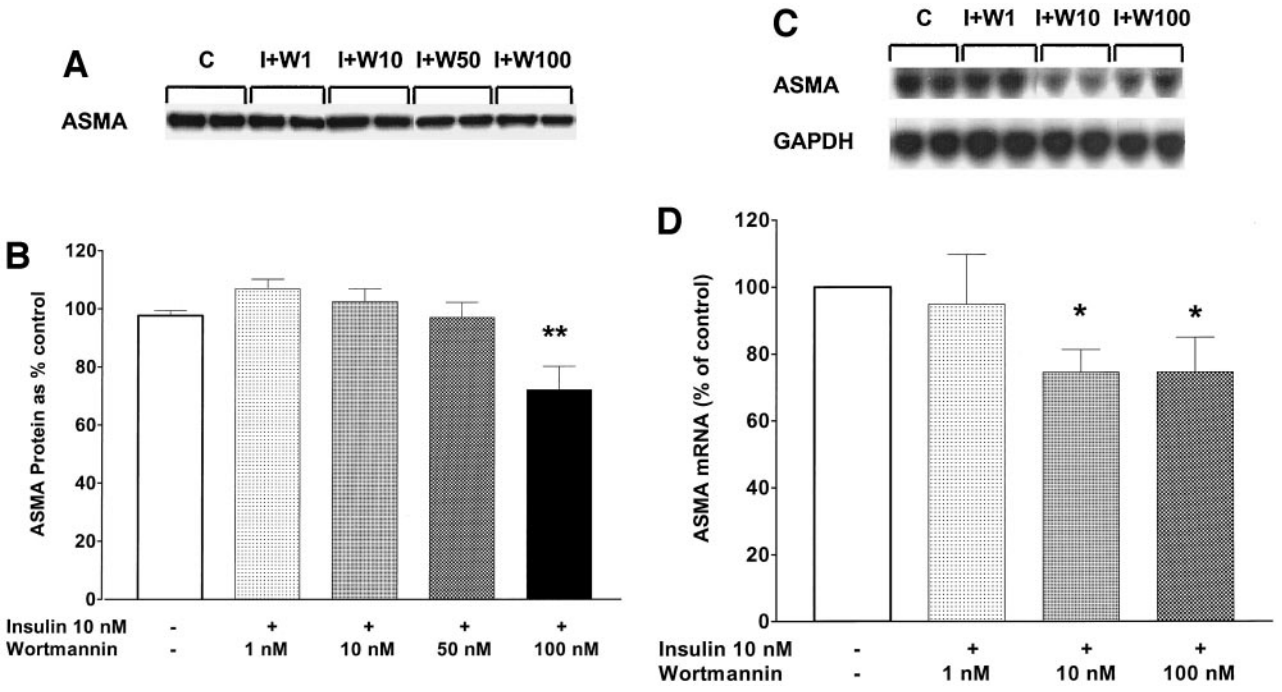

FIG. 3. Wortmannin dose-response curve in the presence of insulin, for α-SMA protein and mRNA levels in VSMCs. Cells were incubated with 10 nmol/l insulin (I) and 1, 10, 50, or 100 nmol/l wortmannin (W). *A*: Representative Western blot of α-SMA protein. *B*: Results are expressed as percentage of control (C; VSMCs incubated in serum-reduced medium only) and plotted as mean ± SE of two experiments, each performed in duplicate. *C*: Representative Northern blot for α-SMA mRNA. *D*: Results are corrected for GAPDH signal, expressed as percentage of control, and plotted as mean ± SE of four experiments, each performed in duplicate. *$P < 0.05$ vs. control; **$P < 0.001$ vs. control.

VSMCs were incubated with PDGF and insulin in the presence of wortmannin, this protective effect of insulin was lost, allowing PDGF to reduce levels of α-SMA protein to 68% of control ($P < 0.01$ vs. insulin plus PDGF). Wortmannin had no effect on PDGF action.

These results were mirrored at the mRNA level (Fig. 4*C*

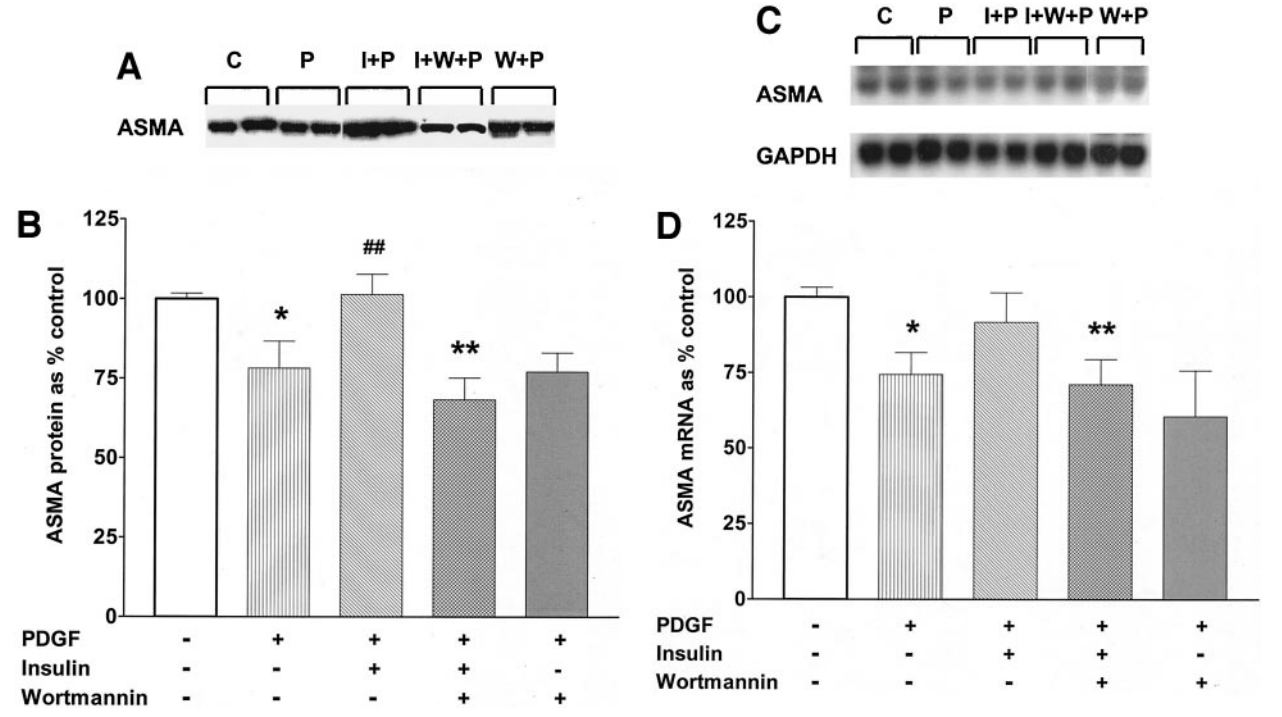

FIG. 4. Insulin counteracts the effect of PDGF on VSMC α-SMA protein and mRNA. Cells were incubated with 30 pmol/l PDGF (P) or with 10 nmol/l insulin (I) and PDGF, or insulin, PDGF, and 100 nmol/l wortmannin (W). *A*: Representative Western blot of α-SMA protein. *B*: Results are expressed as percentage of control (C) and plotted as mean ± SE of 7–10 experiments, each performed in duplicate. *$P < 0.001$ vs. control; ##$P < 0.05$ vs. PDGF; **$P < 0.01$ vs. insulin plus PDGF. *C*: Representative Northern blot for α-SMA mRNA. *D*: Results are corrected for GAPDH or ubiquitin signal, expressed as percentage of control, and plotted as mean ± SE of 6–11 experiments performed in duplicate. *$P < 0.01$ vs. control; **$P < 0.05$ vs. insulin plus PDGF.

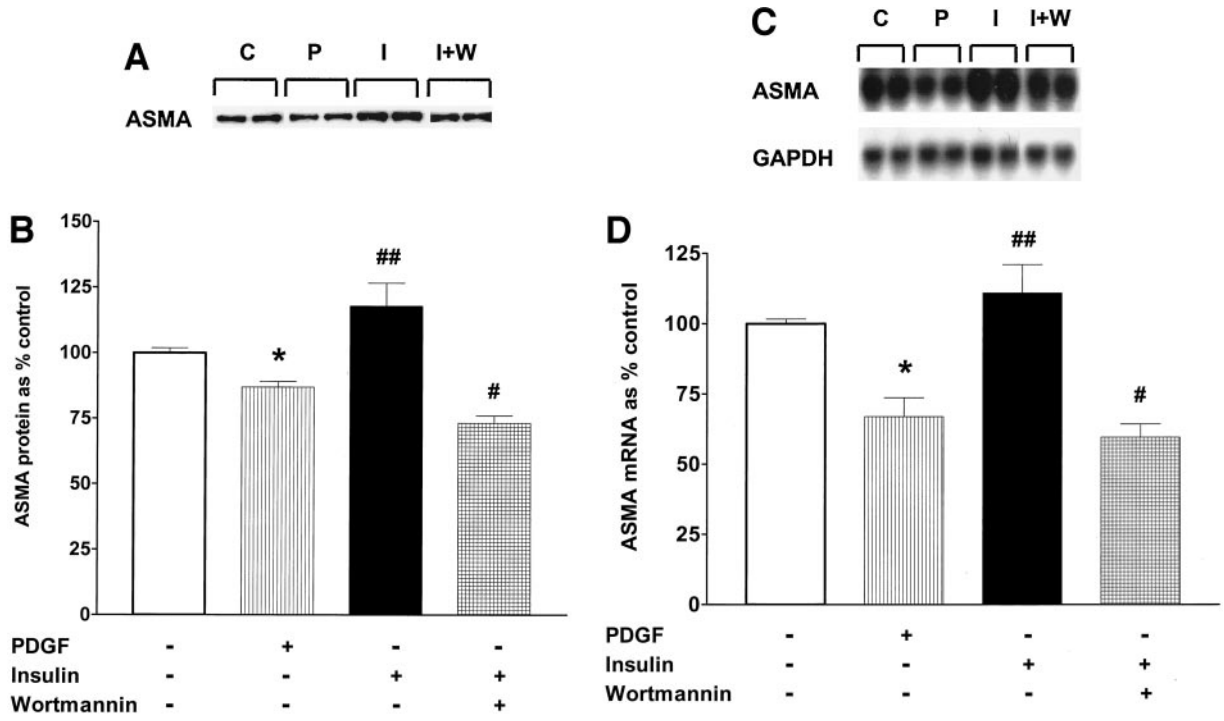

FIG. 5. Insulin restores α-SMA protein and mRNA levels after dedifferentiation by PDGF. Cells were preincubated with 30 pmol/l PDGF (P) to cause dedifferentiation, then incubated further with PDGF or with 10 nmol/l insulin (I) alone or with insulin and 100 nmol/l wortmannin (W). *A*: Representative Western blot of α-SMA protein. *B*: Results are expressed as percentage of control (C) and plotted as mean ± SE of four to seven experiments, each performed in duplicate. *C*: Representative Northern blot of α-SMA mRNA. *D*: Results are corrected for GAPDH signal, expressed as percentage of control, and plotted as mean ± SE of six or seven experiments performed in duplicate. *$P < 0.001$ vs. control; ##$P < 0.01$ vs. PDGF; #$P < 0.01$ vs. insulin alone.

and *D*). After 24 h of incubation, PDGF significantly reduced the levels of α-SMA mRNA to 76% of control ($P < 0.01$), and insulin attenuated this effect of PDGF. The ability of insulin to counteract the effect of PDGF was completely lost in the presence of wortmannin. Under these conditions, PDGF reduced the levels of α-SMA mRNA to 55% of control.

We then asked whether insulin could restore the differentiated quiescent phenotype of VSMCs after they were propelled to dedifferentiate with PDGF. VSMCs were first incubated with PDGF for 48–60 h in serum-reduced medium that was then changed to one containing either insulin alone or insulin with wortmannin for an additional 60-h incubation period. Media and treatments were refreshed every 24 h. In these experiments, PDGF reduced levels of α-SMA protein to 87% of control ($P < 0.001$; Fig. 5*A* and *B*). Subsequent incubation with insulin increased levels of α-SMA to 118% of control ($P < 0.01$). However, the presence of wortmannin completely blocked the ability of insulin to restore levels of α-SMA protein (73% of control).

Similar data were obtained at the mRNA level (Fig. 5*C* and *D*). Whereas further incubation with PDGF significantly reduced the levels of α-SMA mRNA to 67% of control, incubation with insulin was able to restore mRNA levels to 111% of control, indicating complete reversal to a quiescent phenotype. This effect of insulin was significantly attenuated in the presence of wortmannin.

Insulin mediates VSMC migration via the MAPK-dependent pathway. Having established the role of the PI3K-dependent signaling in the mechanism of insulin effect on VSMC differentiation and quiescence, we turned our attention to a moderate but significant effect of insulin on VSMC migration. Insulin, a mild mitogen, increased VSMC migration after 24 h of incubation to 133% of control (Fig. 6). Although wortmannin's effect on VSMC migration was not significant, the presence of PD98059 significantly blocked the effect of insulin on cell migration, suggesting

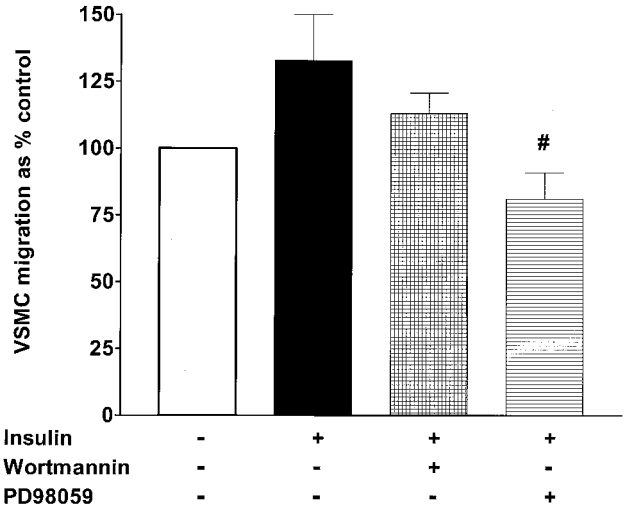

FIG. 6. Effect of insulin on VSMC migration. Cells were incubated with 10 nmol/l insulin, insulin plus 100 nmol/l wortmannin, or insulin plus 20 μmol/l PD98059. VSMC migration was quantified using the Boyden chamber technique. Results are expressed as percentage of control and plotted as mean ± SE of six experiments performed in duplicate. #$P < 0.05$ vs. insulin alone.

the important role of the MAPK signaling pathway in mediating this aspect of insulin action.

DISCUSSION

This work demonstrates two salient points. First, insulin maintains VSMC quiescence and differentiation via PI3K-dependent and not MAPK-dependent signaling, whereas its effect on migration is mediated mainly via the MAPK pathway. Second, insulin is able to counteract the effects of PDGF on dedifferentiation of VSMCs, and this aspect of insulin action is also mediated via PI3K-dependent signaling. Both observations are critical for a better understanding of the role of insulin in vascular biology and the potential role of hyperinsulinemia in atherosclerosis in patients with insulin resistance.

Insulin resistance is a clinically defined phenomenon in which higher-than-normal concentrations of insulin are required to maintain normoglycemia (29). The sine qua non of insulin resistance is a compensatory hyperinsulinemia that arises to promote glucose uptake by adipose tissue and skeletal muscle and glucose utilization by all insulin target tissues. Despite a well-recognized tight association of insulin resistance with development and progression of atherosclerosis (1–3), the molecular mechanism of this association remains largely unknown. A central unresolved question is whether compensatory hyperinsulinemia has any independent detrimental action or is simply a marker of insulin resistance, with insulin resistance exerting its proatherogenic action in an undefined manner. Even though several retrospective clinical studies support the hypothesis that hyperinsulinemia per se is detrimental, increasing the risk for cardiovascular and other diseases (30–34), an almost equal number of studies do not support the idea that hyperinsulinemia is an independent cardiovascular risk factor (35,36). A better understanding of the mechanism of insulin signaling in VSMCs can clarify the role of compensatory hyperinsulinemia in the pathogenesis of progression of atherosclerosis in patients with insulin resistance.

Recent studies have demonstrated the existence of two major signaling pathways that mediate insulin action (37). Signal transduction along both pathways is initiated by insulin binding to the insulin receptor. Transmission of this signal through the pathway that involves IRS proteins, PI3K, and Akt is responsible for most, if not all, of the metabolic aspects of insulin action (11–13). The second signaling pathway, involving Ras, Raf, and MAPK, is responsible for the mitogenic aspects of insulin action and is not involved in the mechanisms of metabolic insulin action (17–20). Several investigators have also observed in experimental in vitro systems, in animal models of insulin resistance, and in insulin-resistant humans that in the presence of metabolic insulin resistance, many insulin target tissues, including vasculature, display a significant defect of PI3K signaling but retain normal sensitivity to insulin via the MAPK signaling pathway (14–16,38).

Under physiologic conditions, insulin maintains VSMC quiescence and supports the differentiated state. We have now demonstrated that this action of insulin is mediated via the PI3K pathway. Our results are in agreement with those of Hayashi et al. (8), who determined that IGF-1 and, to a lesser extent, insulin maintain VSMC differentiation via the PI3K signaling pathway. Under normal circumstances, insulin plays an important antiatherogenic role in both VSMCs and endothelial cells. It maintains the quiescent or differentiated phenotype of VSMCs, counteracts the proatherogenic effects of PDGF in VSMC, and also counteracts the proatherogenic effects of vascular endothelial growth factor (VEGF) in endothelial cells (16,39). However, because these aspects of insulin action are mediated via the PI3K signaling pathway, insulin loses its antiatherogenic protective effects in insulin-resistant states, when signaling along the PI3K-dependent pathway is ineffective (21). We have previously shown that endothelial cells with inhibited signaling along the PI3K-dependent pathway lose their ability to antagonize the effect of VEGF on expression of intracellular adhesion molecule and E-selectin in the presence of insulin (16). The present data indicate that in VSMCs with inhibited PI3K signaling, insulin loses its ability to counteract the effects of PDGF on VSMC dedifferentiation.

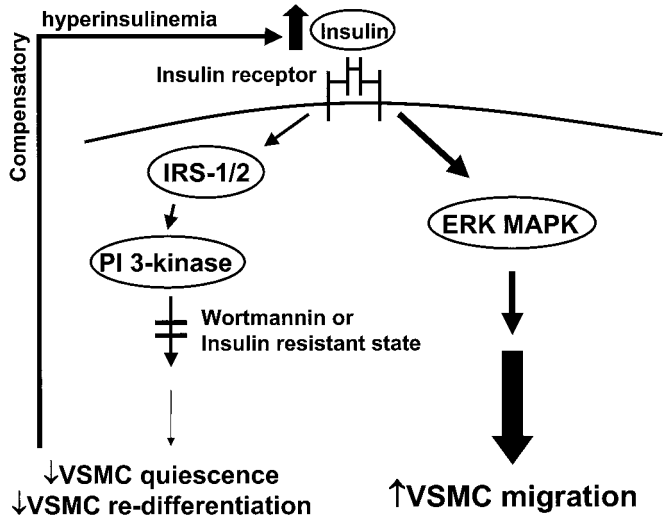

FIG. 7. Diagram of putative effects of insulin and PI3K-dependent pathway impairment on VSMCs. With normal PI3K signaling, insulin maintains VSMC quiescence, counteracts PDGF-induced dedifferentiation, restores quiescence after PDGF-induced dedifferentiation, and has a modest effect of stimulating VSMC migration. In the presence of PI3K-dependent pathway impairment, insulin loses its antiatherogenic effects on VSMC phenotype and may promote VSMC migration, thus exerting proatherogenic effects.

In contrast to maintenance of VSMC differentiation, intracellular signals responsible for VSMC migration and proliferation seem to involve the Ras-MAPK–dependent pathway (40–42). Preservation of normal responsiveness of MAPK-dependent signaling in the presence of a diminution in the strength of PI3K-dependent pathway has enormous implications for the role of compensatory hyperinsulinemia in the pathogenesis of the progression of atherosclerosis in patients with insulin resistance. For example, in endothelial cells, insulin in the presence of wortmannin blocked insulin stimulation of endothelial nitric oxide synthase expression (16). Whereas the antiatherogenic effectiveness of insulin is lost in the presence of metabolic insulin resistance, its proatherogenic potential, mediated largely via MAPK-dependent signaling, may be enhanced (Fig. 7).

Our experimental observations support the concept that

the antiatherogenic actions of insulin are mediated via the PI3K signaling pathway, whereas its proatherogenic influence is mediated via the MAPK pathway. This proatherogenic influence of insulin becomes evident in metabolic insulin resistance and in the presence of compensatory hyperinsulinemia. The compensatory hyperinsulinemia that invariably accompanies metabolic insulin resistance may contribute to progression of atherosclerosis through unimpaired signaling along the MAPK pathway (Fig. 7).

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