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Insulin Potentiates Platelet-Derived Growth Factor Action in Vascular Smooth Muscle Cells*

MARC L. GOALSTONE, RAMA NATARAJAN, PAUL R. STANDLEY, MARY F. WALSH, J. WAYNE LEITNER, KIRSTEN CAREL, STEVEN SCOTT, JERRY NADLER, JAMES R. SOWERS, AND BORIS DRAZNIN

The Department of Diabetes, Endocrinology and Metabolism (R.N., S.S., J.N.), City of Hope Medical Center, Duarte, California 91010; Research Service Detroit VA Medical Center and Department of Medicine (P.R.S., M.F.W., J.R.S.), Wayne State University, 48202; Research Service (M.L.G., J.W.L., K.C., B.D.), Denver VA Medical Center and Department of Medicine University of Colorado Health Sciences Center, Denver, Colorado 80220

ABSTRACT

Correlative studies have indicated that hyperinsulinemia is present in many individuals with atherosclerosis. Insulin resistance has also been linked to cardiovascular disease. It has proved to be difficult to decipher whether hyperinsulinemia or insulin resistance plays the most important role in the pathogenesis of atherosclerosis and coronary artery disease.

In this study, we demonstrate that insulin increases the amount of farnesylated p21Ras in vascular smooth muscle cells (VSMC), thereby augmenting the pool of cellular Ras available for activation by platelet-derived growth factor (PDGF). In VSMC incubated with insulin for 24 h, PDGF's influence on GTP-loading of Ras was significantly increased. Furthermore, in cells preincubated with insulin, PDGF increased thymidine incorporation by 96% as compared with a 44% increase in control cells (a 2-fold increment). Similarly, preincubation of VSMC with insulin increased the ability of PDGF to stimulate gene expression of vascular endothelial growth factor 5- to 8-fold. The potentiating influence of insulin on PDGF action was abrogated in the presence of a farnesyltransferase inhibitor. Thus, the detrimental influence of hyperinsulinemia on the arterial wall may be related to the ability of insulin to augment farnesyltransferase activity and provide greater amounts of farnesylated p21Ras for stimulation by various growth promoting agents. (*Endocrinology* **139:** 4067–4072, 1998)

OVER A QUARTER of a century ago, Stout and Vallance-Owen suggested that insulin may be involved in the pathogenesis of atherosclerosis (1). Since then, clinical and epidemiological evidence in favor of this hypothesis has grown significantly (reviewed in Ref. 2). Association studies have clearly indicated that hyperinsulinemia is present in many individuals with ischemic heart disease, cerebrovascular, and peripheral vascular disease. Four large prospective investigations have shown that hyperinsulinemia is a predictor of coronary artery disease (CAD)[1] (3–6), though a few did not support such a relationship (7, 8). The greatest association of hyperinsulinemia with CAD has been found in Finland, a population with a very high frequency of CAD (3). A recent prospective (6) study of 2103 men from Québec clearly showed that high fasting insulin concentrations are an independent predictor of CAD. This important study used an insulin assay without cross-reactivity with proinsulin, thus avoiding that confounding influence. Several investigators have reported a relationship between carotid wall atherosclerotic lesions and insulin levels and/or insulin resistance (9–13), whereas others have demonstrated a relationship between elevated plasma insulin and vasospastic angina (14). Thus, hyperinsulinemia does appear to be a predictor for the development of CAD and stroke.

On the other hand, patients with diabetes mellitus have a substantially increased risk of developing and dying of cardiovascular disease. Eight prospective studies with patient numbers ranging from 121 in the Whitehall Study to over 5,000 in the Multiple Risk Factor Intervention Trial (MRFIT), have concluded that patients with diabetes exhibit greatly increased cardiovascular morbidity and mortality (5, 15–19). High levels of insulin have also been found to be associated with large vessel disease in both diabetic and nondiabetic individuals (20–23).

In most instances, endogenous hyperinsulinemia occurs in states of insulin resistance (24, 25). In fact, hyperinsulinemia is a hallmark of the insulin resistance, manifested otherwise by normal or high levels of glucose in the face of hyperinsulinemia. Insulin resistance has also been linked to cardiovascular disease, although it has been extremely difficult to decipher whether hyperinsulinemia or insulin resistance plays the most important role in the pathogenesis of atherosclerosis and CAD (1, 24, 26). The question of whether or not insulin has a direct effect on the vascular wall lies at the center of this controversy.

We have recently shown that insulin, a mild mitogen on its own, potentiates the influence of other growth factors on the Ras pathway in 3T3 L1 fibroblasts and adipocytes (27–29).

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Address all correspondence and requests for reprints to: Boris Draznin, M.D., Ph.D., VA Hospital (151), 1055 Clermont Street, Denver, Colorado 80220. E-mail: bdraznin@sembilan.uchsc.edu.

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The mechanism of this potentiating influence of insulin appears to involve an activation of farnesyltransferase (FTase), an enzyme responsible for isoprenylation (farnesylation) of Ras proteins (30). Farnesylation is a posttranslational modification of Ras, required for its association with the plasma membrane (31). Because only farnesylated p21Ras is activated by GTP loading, regulation of farnesylation of Ras by insulin becomes critical for subsequent activation of the Ras pathway. Thus, via its stimulatory effect on FTase, insulin significantly augments the amounts of farnesylated and plasma membrane-associated p21Ras, thereby increasing the pool of cellular Ras available for activation by various growth factors. In the present study, we demonstrate that in porcine and rat vascular smooth muscle cells (VSMC), hyperinsulinemia significantly potentiates the mitogenic properties of platelet-derived growth factor (PDGF). In cells preincubated with insulin, PDGF caused greater activation of p21Ras, thymidine incorporation, and vascular endothelial growth factor (VEGF) gene expression. The influence of hyperinsulinemia was abrogated in the presence of a farnesyltransferase inhibitor, supporting our hypothesis that insulin's effect is mediated by an increased activity of FTase and resultant increases in the size of the cellular pool of farnesylated p21Ras (28).

Materials and Methods

Materials

Cell culture media and supplies were from Life Technologies, Inc. (Gaithersburg, MD) and Gemini Bioproducts (Calabasas, CA), radioisotopes from DuPont NEN (Boston, MA), all standard chemicals were from Sigma (St. Louis, MO), anti-Ras monoclonal antibody (Y13–259) was from Transduction Laboratories (Lexington, KY) and protein G-PLUS/Protein A agarose from Oncogene Research Products, Inc. (Cambridge, MA). The farnesyltransferase inhibitor, α-hydroxyfarnesylphosphonic acid (α-HFPA) was from Biomol (Plymouth Meeting, PA), all supplies and reagents for SDS-PAGE were from Bio-Rad (Hercules, CA), and the enhanced chemiluminescence kit (ECL) was from Amersham (Arlington Heights, IL).

Culture of rat and porcine VSMC

Freshly disassociated VSMC were isolated from rat aorta and tail arteries Wistar, Charles River Laboratories (Wilmington, MA) using techniques previously described (32). Porcine VSMC were cultured from porcine smooth muscle explants and used from passages 2–6 as described earlier (33). Cells were cultured in DMEM containing 100 mg/dl glucose and 10% FCS.

Separation of farnesylated and unfarnesylated p21Ras

VSMC were serum-starved overnight and incubated with or without insulin (10 nM) for the indicated times. Equal volumes of cell lystate and 4% Triton X-114 were combined in a borosilicate glass tube, vortexed and incubated at 37 C for three minutes. Solutions were kept at room temperature until phases had separated. Equal samples from each phase were placed in separate 1.5 ml Eppendorf tubes, and p21Ras was immunoprecipitated using a monoclonal antibody (Y13–259). Relative amounts of p21Ras were determined by Western blotting followed by densitometry (27, 28).

Insulin-mediated p21 Ras·GTP formation

Confluent VSMC were serum and phosphate starved for 24 h and labeled with ^{32}P-orthophosphate (250 μCi) overnight. Cells were then incubated with or without insulin (10 nM) for 24 or 48 h as indicated. In some experiments, cells were also incubated with the farnesyltransferase inhibitor αHFPA (1 μM). Cells were then challenged with PDGF for 10 min after preincubations with insulin. Precleared cell lysates were immunoprecipitated with anti-Ras antibody (Y13–259), and the nucleotides were separated by thin layer chromatography. GTP and GDP were visualized by autoradiography and using acid molybdate reagent, cut, and quantified by liquid scintillation counting (28).

Thymidine incorporation

Rat aortic VSMC (passages 6–8) were plated onto 6-well plates and allowed to reach 80% confluence. At that time, the medium was aspirated and replaced with serum-free D-MEM/F-12 (Gibco BRL, Gaithersburg, MD) with and without the FTase inhibitor (1 μM). After 36 h, insulin (10 nM) was added to half the wells and incubation continued before addition of PDGF-BB (human recombinant PDGF from Gibco BRL). Cells were incubated for a further 6 h before ^{3}H-thymidine (1 μCi/dish) incorporation for 1 h at 37 C. After washing with cold PBS the cells were precipitated with 10% TCA and solubilized with 0.4 N NaOH. Samples were dissolved in Opti-Fluor before scintillation counting (Tri Carb 2500 TR) Packard (Downers Grove, IL). Aliquots of each sample were reserved for protein determination Bio-Rad Laboratories (Hercules, CA) and thymidine incorporation corrected for protein content.

Northern blotting to detect VEGF messenger RNA (mRNA)

Nearly confluent porcine VSMC in 100-mm dishes were made quiescent by placing in medium containing 0.4% FCS and 0.2% BSA for 24 h. Cells were then rinsed with PBS and placed in fresh medium containing 0.2% BSA alone. Insulin (10 nM) was added 24 h later to indicated dishes. After a further 24 h period, PDGF-BB was added to some dishes and the cells incubated for another 3.5 h. At the end of this period, the plates were cooled on ice and RNA was extracted using RNA-STAT 60 (Teltest, Friendswood, TX). Total RNA (20 μg) from each of the samples was size fractionated on 1.2% agarose gels containing 0.5% formaldehyde. The denatured RNA was then transferred to positively charged nylon membranes and hybridized to the specific ^{32}P-labeled VEGF complementary DNA (cDNA) probe. The plasmid containing a 930-bp fragment of the human VEGF cDNA was a gift from Genentech (San Francisco, CA). The cDNA probe was radiolabeled with α-^{32}P-dCTP using a random primer labeling system. Hybridization was performed in 50% formamide containing 4 × SSPE, 5 × Denhardt's solution, 3% SDS and 0.5 mg/ml salmon sperm DNA at 42 C overnight. Washing conditions were 2 × SSPE/0.1% SDS at room temperature for 15 min, 1 × SSPE/0.1% SDS at 37 C for 15 min, and 0.5 × SSPE/0.1% SDS at 53 C for 15 min. Blots were exposed to KODAK film for 24 h. Control for RNA quantity and loading efficiency was determined from ethidium bromide stains of 18S and 28S RNA as well as by the measurements of the levels of mRNA of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a housekeeping gene. The autoradiograms and gels were analyzed by densitometry to quantitate the ratio of VEGF to 18S and 28S or to GAPDH mRNA.

Results

In initial experiments, we established that insulin augments the amount of farnesylated p21Ras in VSMC. Rat (Fig. 1, A and B) or porcine (Fig. 1, C and D) VSMC were grown to 80% confluence and, after 24 h of serum starvation, cells were exposed to insulin (10 nM) for an additional 24 h. Farnesylated p21Ras was extracted from the cell lysate with Triton X-114 and determined by Western blotting (as described in *Materials and Methods*). Insulin significantly ($P < 0.05$) increased the amount of farnesylated p21Ras in both types of VSMC, similarly to that we observed previously in 3T3-L1 fibroblasts and adipocytes (27–29).

If larger amounts of farnesylated p21Ras are available for activation in VSMC, responses of these cells to various growth factors might be enhanced. We examined this possibility in experiments with PDGF. PDGF significantly stimulated activation of p21Ras in porcine VSMC as determined

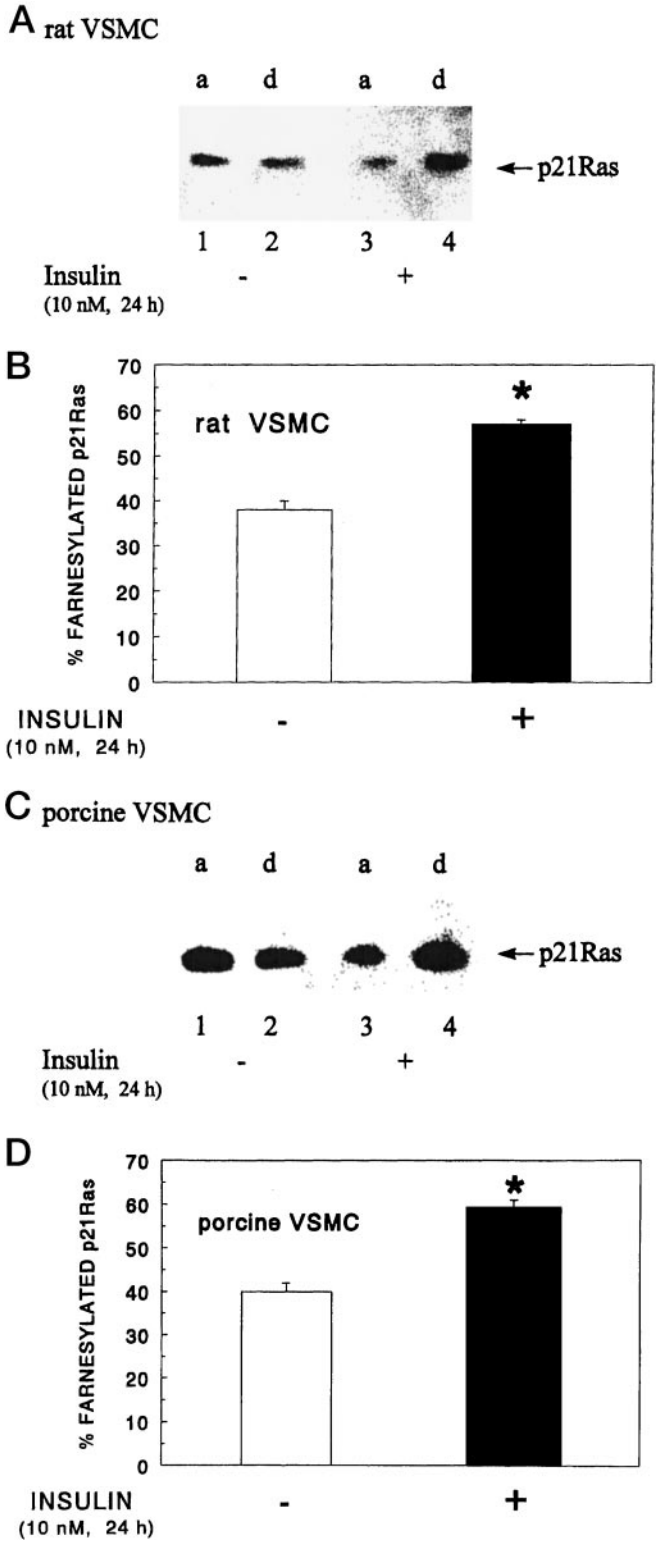

FIG. 1. Effect of insulin (10 nM for 24 h) upon the amount of farnesylated p21 Ras in rat (A and B) and porcine (C and D) VSMC. A and C, Representative Western blot analyses of Ras proteins detected in either aqueous (a) or detergent (d) phase of cell lysates. B and D, Summaries of three independent experiments for each tissue, representing the amount of farnesylated p21 Ras (recovered in the detergent phase) as a percent of the total cellular Ras. Results represent mean ± SEM. *, $P < 0.05$.

by p21Ras GTP loading (Fig. 2). This influence of PDGF was further increased by preincubation of these cells with insulin (10 nM) for 24 h. The presence of the FTase inhibitor, α-HFPA (1 μM), completely inhibited the potentiating influence of insulin. The effect of α-HFPA was apparent within 30 min of incubation, and had no detrimental influence on cell viability, as assayed by DNA synthesis.

Activation of p21Ras has been shown to mediate nuclear effects of growth factors, including PDGF (34). Indeed, PDGF is a potent promoter of both DNA synthesis (as measured by thymidine incorporation) and VEGF expression (35, 36). We therefore assessed the potentiating influence of insulinemia on these actions of PDGF. Preincubation of rat VSMC with insulin (10 nM) for 24 h significantly increased the ability of submaximally effective doses of PDGF to stimulate thymidine incorporation (Fig. 3). In control cells, PDGF increased thymidine incorporation by 44% above basal and in cell preincubated with insulin by 96% ($P < 0.05$), a 2-fold increment. This potentiating effect of insulin was again abolished by the FTase inhibitor, α-HFPA.

Similarly, preincubation of VSMC with insulin (10 nM for 24 h) significantly increased the ability of submaximal doses of PDGF to stimulate VEGF gene expression in these cells (Fig. 4). Results of hybridization of the blot with a specific VEGF cDNA probe are seen in the *upper panels* along with a GAPDH probe in the *lowermost panel*. Ethidium bromide staining of 18S and 28S RNA are seen in the *middle panels*. VEGF mRNA is seen as a strong band around 3.6 kb. The blot on the *left* depicts the results obtained with 1 pM PDGF, whereas the blot on the *right* with 5 pM PDGF, both in the absence and presence of insulin. The *bar graphs* below each demonstrate the densitometric quantitation of the blots. The influence of PDGF on VEGF mRNA was enhanced 5- to 8-fold by preincuabation with insulin.

Discussion

This study demonstrates a novel aspect of insulin action in VSMC: insulin as a strong potentiator of the nuclear effects of PDGF mediated via the Ras pathway. Preincubation of VSMC with insulin for 24 h doubled the effect of PDGF on thymidine incorporation and increased its effect on VEGF

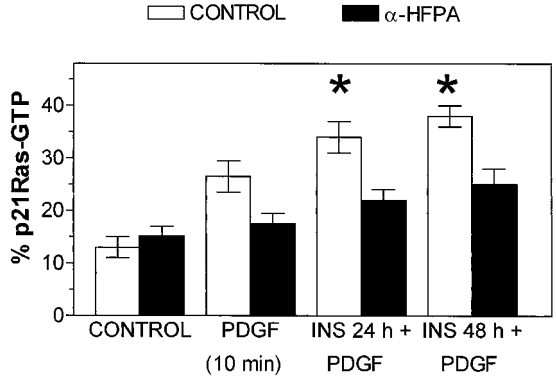

FIG. 2. Influence of preincubation with insulin for 24 or 48 h on the ability of PDGF to activate p21Ras in porcine VSMC. The presence of α-HFPA (*closed bars*) blocked the ability of insulin to potentiate the effect of PDGF. Results represent mean ± SEM of four independent experiments. *, $P < 0.05$ *vs*. PDGF alone.

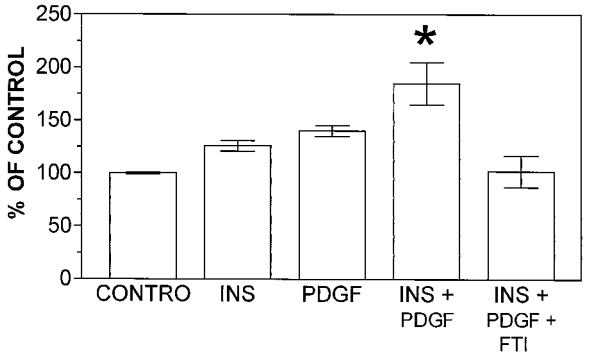

FIG. 3. Influence of preincubation with insulin (24 h) on the ability of PDGF to stimulate ^{3}H-thymidine incorporation in rat VSMC. The presence of α-HFPA (designated here FTI - a farnesyltransferase inhibitor) blocked the potentiating effect of insulin. Results represent mean ± SEM of four independent experiments. *, $P < 0.05$ *vs.* PDGF alone.

gene expression 5- to 8-fold. The potentiating influence of insulin on the activation of p21Ras and thymidine incorporation was completely blocked by an inhibitor of FTase, supporting the hypothesis that the mechanism of the insulin-induced potentiation involves its effect on FTase. We have previously demonstrated that in 3T3-L1 fibroblasts and adipocytes, insulin promotes the phosphorylation and activation of FTase, an enzyme responsible for isoprenylation of p21Ras (27–29). Farnesylation of p21Ras results in the anchoring of Ras at the plasma membrane where it can be activated by various growth factors (30). Thus, hyperinsulinemia via its effect on FTase can increase the cellular pools of farnesylated p21Ras and thereby augment cellular mitogenic responses to a variety of growth factors (28).

Although insulin is a relatively weak mitogen, the detrimental influence of hyperinsulinemia on the arterial wall may be related to its ability to potentiate the mitogenic action of other growth factors. We now demonstrate that hyperinsulinemia augments the magnitude of the nuclear action of PDGF in the VSMC, presumably via its effect on FTase and the size of the cellular pool of farnesylated p21Ras (Fig. 1). These observations provide additional strong experimental evidence that hyperinsulinemia may directly affect the vascular wall by enhancing cellular responses to growth factors and other substances working via the Ras pathway. Among these substances are adrenergic agonists, cytokines, and advanced glycosylation end-products (37, 38). The latter have been recently shown to activate the Ras pathway in VSMC (38).

Insulin increases mitogenic signaling pathways and increases thymidine incorporation into DNA in vascular endothelial and smooth muscle cells (26). Many of the effects of insulin on vascular growth and remodeling are likely to be mediated through an insulin-like growth factor (IGF-1) receptor in endothelial and VSMC (39–41) or indirectly by stimulating IGF-1 synthesis by VSMC (41). Insulin and IGF-1 are structurally related, share receptors, and have similar postreceptor actions. Unlike insulin, which must traverse the endothelium before acting on VSMCs *in vivo*, IGF-1 is synthesized by VSMCs and is more likely to act in autocrine and paracrine processes. In addition to stimulating vascular cell

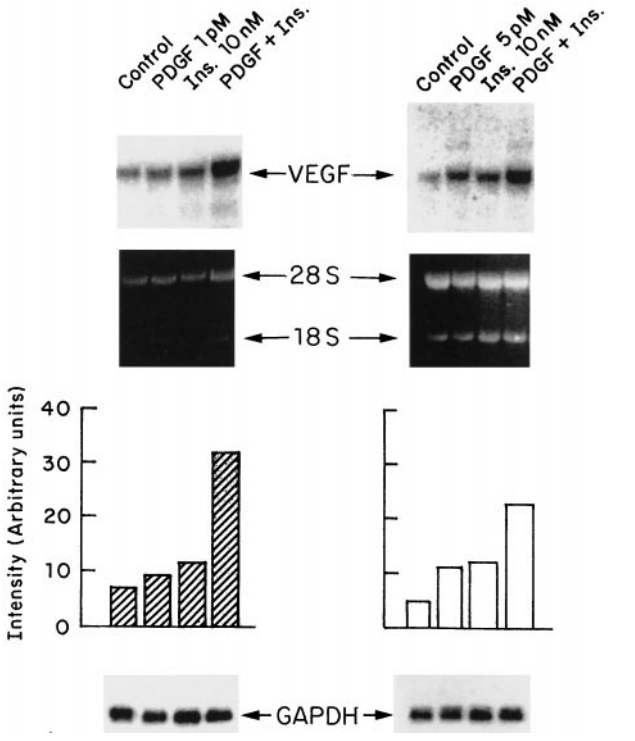

FIG. 4. Influence of preincubation with insulin (10 nM) for 24 h on the ability of PDGF to stimulate VEGF gene expression in porcine VSMC. A representative experiment (from two independent experiments) with either 1 pM PDGF (*left panel*) or 5 pM PDGF (*right panel*) is shown. Intensity of the mRNA signal is depicted in the *bar graph* below.

mitogenesis and growth, IGF-1 enhances proteoglycan synthesis by microvascular and macrovascular endothelial cells (41). However, activation of FTase, with resultant increases in the amount of farnesylated p21Ras, appears to be specific for insulin but not IGF-1 (unpublished observations). At the same time, we have demonstrated that hyperinsulinemia potentiates activation of p21Ras by IGF-1 at least in 3T3-L1 fibroblasts (28). Whether similar potentiation of IGF-1 action exists in VSMC remains to be examined.

PDGF is secreted by platelets and vascular cells and appears to play a key autocrine/paracrine role in the development of diabetic vascular complications and atherosclerosis (42–45). In the present study, we assessed two nuclear actions of PDGF: thymidine incorporation and VEGF expression. Both effects were significantly potentiated by hyperinsulinemia (Figs. 3 and 4). VEGF is a potent angiogenic and vascular permeability factor and can induce monocyte migration through the endothelium (46–48). These events are essential early steps in the atherosclerotic process that, in turn, are mediated by the actions of multiple growth factors, lipids and cytokines (49). Growth factors such as angiotensin II and PDGF induce VEGF in VSMC (35, 36, 50). Thus, VEGF is an attractive candidate for the pathological neovascularization and endothelial permeability observed in atherosclerosis (51). The present results show that hyperinsulinemia can augment PDGF-induced VEGF expression, and provide new evidence for the role of insulin on the development of atherosclerotic complications.

Although this study does not directly address the possible influence of hyperinsulinemia on the development of atherosclerosis, it provides strong evidence that it can influence this process. Hyperinsulinemia may create a new background in the signal transduction machinery, on which the development of atherosclerosis is significantly accelerated. In our previous studies, we demonstrated that insulin augments the ability of other growth factors to activate p21Ras (28). The novel aspect of the present investigation extends beyond this step and points out that insulin also augments nuclear responses to growth factors, in this case to PDGF. This study offers new evidence that hyperinsulinemia *per se*, without insulin resistance, may engender an atherogenic milieu in the cells of the vascular wall. Additional studies are needed to determine whether insulin's effect on the amounts of farnesylated p21Ras directly promotes the development of atherosclerosis. If this is the case, inhibitors of FTase can play an extremely important role as potential therapeutic agents to retard the progression of atherosclerosis in hyperinsulinemic, insulin-resistant individuals.

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References

1. **Stout RW, Vallance-Owen J** 1969 Insulin and atheroma. Lancet 1:1078–1080
2. **Stout RW** 1996 Hyperinsulinemia and atherosclerosis. Diabetes 45:45–46
3. **Pyorala K** 1979 Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: Results from two populations in Finland. Diabetes Care 2:131–141
4. **Welborn TA, Wearne K** 1979 Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diabetes Care 2:154–160
5. **Fontbonne A, Charles MS, Thibult N, Richard JL, Claude JR, Warnet JM, Rosselin GE, Eschwége E** 1991 Hyperinsulinemia as a predictor of coronary heart disease mortality in a healthy population: The Paris Prospective Study, 15-year follow-up. Diabetologia 34:356–361
6. **Després J-P, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ** 1996 Hyperinsulinemia as an independent risk factor of ischemic heart disease. N Engl J Med 334:952–957
7. **Welin L, Eriksson H, Larsson B, Ohlson LO, Svärdsudd K, Tibblin G** 1992 Hyperinsulinemia is not a major coronary risk factor in elderly men: the study of men born in 1913. Diabetologia 35:766–770
8. **Hargreaves AD, Logan RL, Elton RA, Buchanan KD, Oliver MF, Riemersmaa RA** 1992 Glucose intolerance, plasma insulin, HDL cholesterol and obesity: 12-year follow-up and development of coronary heart disease in Edinburgh men. Atherosclerosis 94:61–69
9. **Ferrara LA, Mancini M, Celentano A, Galderisi M, Iannuzzi R, Marotta T, Gaeta I** 1994 Early changes of the arterial carotid wall in uncomplicated primary hypertensive patients. Arterioscler Thromb 14:1290–1296
10. **Folsom AR, Eckefeldt JH, Weitzman S, Chambless LE, Barnes RW, Cram KB, Hutchinson RG** 1994 Atherosclerosis Risk in Communities (ARIC) Study investigators: relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Stroke 25:66–73
11. **Salomaa V, Riley W, Kark JK, Nardo C, Folsom AR** 1995 Non-insulin dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: the ARIC study. Circulation 91:1432–1443
12. **Agewall S, Fageberg B, Attvall S, Wendelhag I, Urbanavicius V, Wikstrand J** 1995 Carotid artery wall intima-media thickness is associated with insulin-mediated glucose disposal in men at high and low coronary risk. 1995. Stroke 26:956–960
13. **Shinozaki K, Naritomi H, Shimizu T, Suzuki M, Ikebuchi M, Sawada T, Harano Y** 1996 Role of insulin resistance associated with compensatory hyperinsulinemia in ischemic stroke. Stroke 27:37–43
14. **Shinozaki K, Suzuki M, Ikebuchi M, Takaki H, Hara Y, Tsushima M, Harano Y** 1995 Insulin resistance associated with compensatory hyperinsulinemia as an independent risk factor for vasospastic angina. Circulation 92:1749–1757
15. **Laakso M, Lehto S, Penttila I, Pyorala K** 1993 Lipids and lipoproteins predicting coronary heart disease mortality and morbidity in patients with non-insulin dependent diabetes.Circulation 88:1421–1430
16. **Kannel WB, McGee DL** 1979 Diabetes and cardiovascular risk factors: The Framingham Study. Circulation 1:8–13
17. **Pan WH, Cedres LB, Liu K, Dyer A, SchoenbergerJA, Shekelle RB, Stamler R, Smith D, Collette P, Stamler J** 1986 Relationship of clinical diabetes and asymptomatic hyperglycemia to risk of coronary heart disease mortality in men and women. Am J Epidemiol 123:504–516
18. **Stamler J, Vaccaro O, Neaton JD, Wentworth D, for the Multiple Risk Factor Intervention Trial Group** 1993 Diabetes, other risk factors and 12-year cardiovascular mortality for men screened in the multiple risk factor intervention trial. Diabetes Care 16:434–444
19. **Janka HU, Ziegler AG, Standl E, Mehnert H** 1987 Daily insulin dose as a predictor of macrovascular disease in insulin-treated non-insulin dependent diabetics. Diabetes Metab 13:359–364
20. **Stolar MW** 1988 Atherosclerosis in diabetes: the role of hyperinsulinemia. Metab Clin Exp [Suppl 1] 2:1–9
21. **Knight TM, Smith Z, Whittles A, Sahota P, Lockton JA, Hogg G, Bedford A, Toop M, Kernohan EEM, Baker MR** 1992 Insulin resistance, diabetes, and risk markers for ischemic heart disease in Asian men and non-Asian men in Bradford. Br Heart J 67:343–350
22. **Ronnemaa T, Laakson M, Pyorala K, Kallio V, Puukka P** 1991 High fasting plasma insulin is an indicator of coronary heart disease in non-insulin dependent diabetic patients and non-diabetic subjects. Arterioscler Thromb 11:80–90
23. **Uusitupa MIJ, Niskanen LK, Siitonen O, Voutilainen E, Pyorala K** 1990 5-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin dependent diabetic and non-diabetic subjects. Circulation 82:27–36
24. **Reaven GM** 1988 Role of insulin resistance in human disease. Diabetes 37:1595–1607
25. **DeFronzo RA, Ferrannini E** 1991 Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194
26. **Sowers JR, Sowers PS, Peuler JD** 1994 Role of insulin resistance and hyperinsulinemia in development of hypertension and atherosclerosis. J Lab Clin Med 123:647–652
27. **Goalstone ML, Draznin B** 1996 Effect of insulin on farnesyltranserase activity in 3T3–L1 adipocytes. J Biol Chem 271:27585–27589
28. **Leitner JW, Kline T, Carel K, Goalstone M, Draznin B** 1997 Hyperinsulinemia potentiates activation of p21Ras by growth factors. Endocrinol 138:2211–2214
29. **Goalstone M, Carel K, Leitner JW, Draznin B** 1997 Insulin stimulates the phosphorylation and activity of farnesyltransferase via the Ras-MAP kinase pathway. Endocrinology 138:5119–5124
30. **Zhang FL, Casey RJ** 1996 Protein prenylation: molecular mechanisms and functional consequences. Ann Rev Biochem 65:241–269
31. **Kikuchi A, Williams LT** 1994 The post-translational modification of Ras p21 is important for Raf-1 function. J Biol Chem 269:20054–20059
32. **Dominguez LJ, Davidoff AJ, SrinivasPR, Standley PR, Walsh MF, Sowers JR** 1996 Effects of metformin on tyrosine kinase activity, glucose transport, and intracellular calcium in rat vascular smooth muscle. Endocrinology 137:113–121
33. **Natarajan R, Gu JL, Rossi J, Gonzales N, Lanting L, Xu L, Nadler JL** 1993 Elevated glucose and angiotensin II increase 12-lipoxygenase activity and expression in porcine aortic smooth muscle cells. Proc Natl Acad Sci USA 90:4947–4951
34. **Burgering BMT, Boss J** 1995 Regulation of Ras-mediated signaling: more than one way to skin a cat. Trends Biochem Sci 20:18–22
35. **Stavri GT, Hong Y, Zachary IC, Breier G, Baskerville PA, Yia-Herttuala S, Risau W, Martin JF, Erusalimsky JD** 1995 Hypoxia and *platelet-derived growth factor-BB* synergistically upregulate the express of vascular endotheial growth factor in vascular smooth muscle cells. FEBS Lett 358:311–315
36. **Williams B, Quinn-Baker A, Gallacher B** 1995 Serum and platelet-derived growth factor-induced expression of vascular permeability factor mRNA by human vascular smooth muscle cells *in vitro*. Clin Sci 88:141–147
37. **Macara IG, Lounsburg KM, Richards SA, McKierman C, Bar-Sagi D** 1996 The Ras superfamily of GTPases. FASEB J 10:625–630
38. **Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM** 1997 Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. J Biol Chem 272:17810–17814
39. **King GL, Goodman AD, Buzney S, Moses A** 1985 Receptors and insulin-like growth factors on cells from bovine retinal capillaries and aorta. J Clin Invest 75:1028–1036
40. **Barkis GL, Plant CE, Walsh MF, Sowers JR** 1996 Analogy between endothelial/mesangial cell and endothelial/vascular smooth muscle cell interactions: role of growth factors and mechanotransduction. In: Sowers JR (ed), Totowa NJ, Endocrinology of the vasculature. Humana Press, pp 341–355
41. **Sowers JR** 1997 Insulin and insulin-like growth factor in normal and pathological cardiovascular physiology. Hypertension 29:691–699
42. **Inaba T, Ishibashi S, Gotoda T, Kawamura M, Morino N, Nojima Y, Kawakami M, Yazaki Y, Yamada N** 1996 Enhanced expression of platelet-derived growth factor-beta receptor by high glucose: involvement of platelet-derived growth factor in diabetes angiopathy. Diabetes 45:507–512

43. **Williams LT** 1989 Signal transduction by the platelet-derived growth factor receptor. Science 243:1564–1570
44. **Jawien A., Bowen-Pope DF, Lindner V, Schwartz SM, Clowes AW** 1993 Platelet-derived growth factor promotes smooth muscle migration and intimal thickening in a rat model of balloon angioplasty. J Clin Invest 89:507–511
45. **Ross R, Raines WE, Bowen-Pope DF** 1986 The biology of platelet-derived growth factor. Cell 46:155–169
46. **Leung DW, Cachianes G, Knang WJ, Goeddel DW, Ferrara N** 1989 Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246:1306–1309
47. **Clauss MM, Gerlach H, Brett J, Wang F, Familletti PC, Pan YCE, Olander JV, Connolly DT, Stern D** 1990 Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. J Ext Med 172:1535–1545
48. **Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimbruber RM, Feder J** 1989 Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 84:1470–1478
49. **Ross R** 1993 The pathogenesis of arherosclerosis: a perspective for the 1990s. Nature 362:801–809
50. **Williams B, Baker AQ, Gallacher B, Lodwick D** 1995 Angiotensin II increases vascular permeability factor gene expression by human vascular smooth muscle cells. Hypertension 25:913–917
51. **Kuzuya M, Satake S, Esaki T, Yamada K, Hayashi T, Naito M, Asai K, Iguchi A** 1995 Induction of angiogenesis by smooth muscle cell-derived factor: possible role in neovascularization in atherosclerotic plaque. J Cell Physiol 164:658–667
52. **Gibbs JB, Pompliano DL, Mosser SD, Rands E, Lingham RB, Singh SB, Scolnick EM, Kohl N, Oliff A** 1993 Selective inhibition of farnesyl-protein transferase blocks ras processing *in vivo*. J Biol Chem 268:7617–7620