Effect of Insulin on Farnesyltransferase

SPECIFICITY OF INSULIN ACTION AND POTENTIATION OF NUCLEAR EFFECTS OF INSULIN-LIKE GROWTH FACTOR-1, EPIDERMAL GROWTH FACTOR, AND PLATELET-DERIVED GROWTH FACTOR*

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We have previously demonstrated that insulin activates farnesyltransferase (FTase) and augments the amounts of farnesylated p21\textsuperscript{ras} (Goalstone, M. L., and Draznin, B. (1996) J. Biol. Chem. 271, 27585-27589). We postulated that this aspect of insulin action might explain the “priming effect” of insulin on the cellular response to other growth factors. In the present study, we show the specificity of the effect of insulin on FTase. Insulin, but not insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), or platelet-derived growth factor (PDGF), stimulated the phosphorylation of the \(\alpha\)-subunit of FTase and the amounts of farnesylated p21\textsuperscript{ras}. Even though all four growth factors utilized the Ras pathway to stimulate DNA synthesis, only insulin used this pathway to influence FTase. Insulin failed to stimulate FTase in cells expressing the chimeric insulin/IGF-1 receptor and in cells derived from the insulin receptor knock-out animals. Insulin potentiated the effects of IGF-1, EGF, and PDGF on DNA synthesis in cells expressing the wild type insulin receptor, but this potentiation was inhibited in the presence of the FTase inhibitor, \(\alpha\)-hydroxyfarnesylphosphonic acid. We conclude that the effect of insulin on FTase is specific, requires the presence of an intact insulin receptor, and serves as a conduit for the “priming” influence of insulin on the nuclear effects of other growth factors.

Insulin, a major metabolic hormone and a mild mitogen, recently has been shown to augment the ability of other growth factors to activate p21\textsuperscript{ras} (1, 2). This aspect of insulin action has been attributed to the stimulatory influence of insulin on farnesyltransferase (FTase)

activity (3). FTase is a ubiquitous enzyme that catalyzes the attachment of farnesyl to p21\textsuperscript{ras} (4, 5). Newly farnesylated p21\textsuperscript{ras} is translocated to the plasma membrane where it can be activated by GTP loading in response to other growth factors and growth-promoting agents (1, 2, 6). Thus, by its stimulatory influence of FTase, insulin increases the size of the cellular pool of farnesylated p21\textsuperscript{ras} that is available for activation. The effects of insulin on the size of the cellular pool of farnesylated p21\textsuperscript{ras} as well as its ability to potentiate the activation of p21\textsuperscript{ras} by other growth factors are completely blocked by inhibitors of FTase (1–3, 7).

Insulin signaling to FTase appears to flow via the Ras-MAP kinase pathway in a positive feedback fashion (8). Insulin stimulates the activation of p21\textsuperscript{ras} and increases the activity of MAP kinase (9, 10). The latter promotes the phosphorylation and activation of FTase, which farnesylates more p21\textsuperscript{ras}, thus preparing p21\textsuperscript{ras} for subsequent activation. Because many other growth factors also activate the Ras-MAP kinase pathway (11), it was important to determine whether these other growth factors signal to FTase in a manner similar to that of insulin. In other words, the question of the specificity of insulin action on FTase remained to be resolved.

This study was designed to compare the influence of insulin on the farnesylation of p21\textsuperscript{ras} with that of insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF). Additionally, we determined whether the effect of insulin was mediated via its own cell surface receptor or the closely related IGF-1 receptor. Finally, because insulin appears to stimulate FTase activity, resulting in increased amounts of farnesylated p21\textsuperscript{ras} that are available for activation, and may regulate the cellular responsiveness to other growth factors, we examined whether the potentiating effects of insulin on the cellular nuclear responses to IGF-1, EGF, and PDGF were mediated by the effect of insulin on FTase.

EXPERIMENTAL PROCEDURES

Materials—Tissue culture media, gentamicin, methotrexate, and phosphate-free Dulbecco’s medium were from Life Technologies, Inc. Fetal calf serum and Fungizone were from Gemini Bio-Products, Inc. (Calabasas, CA). Bovine serum albumin and other biochemicals were from Sigma. The anti-p21\textsuperscript{ras} rat monoclonal IgG (Y13-259) and Protein G-PLUS/Protein A agarose immunoprecipitation reagents were from Oncogene Research Products (Cambridge, MA). [\textsuperscript{32}P]Orthophosphate was from NEN Life Science Products. The enhanced chemiluminescence (ECL) kit is a product of Amersham Pharmacia Biotech. The FTase \(\alpha\) subunit antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). SDS-PAGE supplies were from Bio-Rad and the \(\alpha\)-hydroxyfarnesylphosphonic acid (\(\alpha\)-HFPA) was from Biomol (Plymouth Meeting, PA). Fibroblasts expressing the wild type insulin receptor (3T3-IR7), wild type IGF-1 receptor (3T3-NWTh3), and the chimeric receptors, N-terminal insulin/C-terminal IGF-1 receptor (3T3-Rch1), and the N-terminal IGF-UC-terminal insulin receptor (3T3-Chi-1) were a gift from Dr. Derek LeRoith (National Institutes of Health). Hepatocytes from control (hybrids of C57b16 and 129/Sv strains) and transgenic mice lacking the insulin receptor gene were developed in the laboratory of Dr. D. Accili (NIH).

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§ The abbreviations used are: FTase, farnesyltransferase; IGF-1, insulin-like growth factor-1, EGF, epidermal growth factor; PDGF, platelet-derived growth factor; Ins, insulin; MAP, mitogen-activated protein; MEK, MAP kinase kinase; PAGE, polyacrylamide gel electrophoresis; \(\alpha\)-HFPA, \(\alpha\)-hydroxyfarnesylphosphonic acid; BrdUr, bromodeoxyuridine.

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Influence of Growth factors on FTase and BrdUrd Incorporation in 3T3-L1 Fibroblasts—In initial experiments, we compared the effects of insulin on the amounts of farnesylated \( p21^{ras} \) in 3T3-L1 fibroblasts with the effects of IGF-1, EGF, and PDGF, three growth-modulating hormones that utilize the Ras pathway. Only insulin was capable of significantly increasing the amounts of farnesylated \( p21^{ras} \) at 1 and 24 h of incubation (Fig. 1). Because insulin-induced activation of FTase requires phosphorylation of the FTase \( \alpha \)-subunit (8), we examined the influence of all four growth factors on the phosphorylation of the \( \alpha \)-subunit of FTase in these cells. In contrast to insulin, which increased the phosphorylation of the \( \alpha \)-subunit several-fold, other growth factors (13 nM IGF-1, 10 pm PDGF, and 20 nm EGF) were without effect (Fig. 2).

Even though IGF-1, EGF, and PDGF failed to phosphorylate and activate FTase, they actively promoted BrdUrd incorporation in the same cells. PDGF was the most potent among these growth factors. The 50% maximal stimulation was achieved with 0.01 nm PDGF, 0.1 nm IGF-1, 3 nm insulin, and 10 nm EGF. The inhibitor of MEK kinase (PD98059) completely
Insulin activates farnesyltransferase

**Fig. 4.** Effect of insulin and IGF-1 on the amount of farnesylated p21ras in cells expressing wild type insulin or IGF-1 receptors or chimeric Ins/IGF-1 or IGF-1/Ins receptors. Fibroblasts expressing receptors, as described in Fig. 3, were incubated in insulin (100 nM) or IGF-1 (13 nM) for 1 h. Relative amounts of farnesylated p21ras were determined as described under “Experimental Procedures.” Results are expressed as mean ± S.E. of farnesylated p21ras as a percent of total cellular p21ras. (n = 5; * p < 0.05).

blocked the influence of all four growth factors on BrdUrd incorporation (not shown), indicating an involvement of the Ras-MEK-MAP kinase signaling pathway in this process. Thus, while these four hormones utilize the Ras signaling pathway to stimulate the DNA synthesis, only insulin uses the same pathway (8) to activate FTase.

**Effect of Insulin on FTase Is Mediated via the Intact Insulin Receptor**—To further explore the differences between insulin and other growth factors, we compared the effects of insulin and IGF-1 in cells expressing the corresponding wild type or chimeric insulin/IGF-1 receptors. The latter were created in two ways: outside insulin receptor-inside IGF-1 receptor and vice versa (12). IGF-1 potently increased BrdUrd incorporation in cells expressing either the IGF-1 wild type (up to 250% above basal) or insulin/IGF-1 chimeric (up to 160% above basal) receptors (Fig. 3A). Despite its significant effect on BrdUrd incorporation, IGF-1 had no effect on the amounts of farnesylated p21ras (Fig. 4A) in any of the cell lines, suggesting that the mitogenic effect of IGF-1 is not accompanied by changes in FTase activity.

Insulin stimulated BrdUrd incorporation in all four cell lines, with a maximal increase of up to 250% in cells expressing the wild type insulin receptor (Fig. 3B). In contrast to its ability to increase the incorporation of BrdUrd in the four cell lines, insulin increased the amounts of farnesylated p21ras only in those cells that expressed the wild type insulin receptor (Fig. 4B). Insulin failed to increase the amounts of farnesylated p21ras in cells that expressed the chimeric insulin/IGF-1 receptor, suggesting that the intact insulin receptor is needed to maintain insulin signaling to FTase. In agreement with these observations, insulin also failed to significantly stimulate the phosphorylation of the α-subunit of FTase in cells expressing the chimeric insulin/IGF-1 receptor (Fig. 5), while being very potent in cells expressing the wild type insulin receptor. IGF-1 did not promote the phosphorylation of the α-subunit in the IGF-1/insulin chimeric cells (not shown). Thus, taken together, these data support the notion that both the N- and C-terminal domains of the insulin receptor are required for insulin signaling to FTase to increase FTase phosphorylation and activity.

To further confirm that insulin requires the presence of its receptor, we measured the amounts of insulin-stimulated farnesylated p21ras in cells expressing or lacking insulin receptors. Cells expressing wild type insulin receptors (IR+/+, WT) or lacking insulin receptors (IR−/−, KO) were incubated in the presence or absence of insulin (100 nM) for 1 h. Relative amounts of farnesylated p21ras were determined as described under “Experimental Procedures.” Results are expressed as mean ± S.E. of farnesylated p21ras as a percent of total cellular p21ras (n = 5; * p < 0.05).

**Insulin Potentiates Nuclear Action of Other Growth Factors via Its Effect on FTase**—Although insulin is a relatively weak mitogen, it can potentiate the magnitude of activation of the...
Ras pathway by other growth factors (1, 2). We have previously demonstrated that the preincubation of 3T3-L1 fibroblasts with insulin for 24 or 48 h resulted in greater amounts of farnesylated p21\textsuperscript{ras} loaded with GTP in response to IGF-1 and EGF (1). In the present study, we examined whether the nuclear effects of IGF-1, EGF, and PDGF that lie downstream from the activation of the Ras pathway are also augmented by insulin. We found that insulin in a dose-dependent manner potentiates the effects of IGF-1, EGF, and PDGF on BrdUrd incorporation (Fig. 7), thus enhancing the mitogenic influence of these growth factors. Preincubation of 3T3-L1 fibroblasts with increasing doses of insulin for 24 h progressively increased the ability of EGF, PDGF, and IGF-1 to stimulate BrdUrd incorporation.

If the potentiating influence of insulin reflects its ability to increase the amounts of farnesylated p21\textsuperscript{ras} available for activation by other growth factors, then inhibitors of FTase should block the observed potentiation. Indeed, we found that the presence of 1 \textmu M \alpha-HFPA (a potent inhibitor of FTase) completely blocked the ability of insulin to potentiate the effects of IGF-1, EGF, and PDGF on BrdUrd incorporation (Fig. 8). Moreover, because insulin increased the amounts of farnesylated p21\textsuperscript{ras} only in cells that expressed the wild type insulin receptor (Fig. 4), we compared the ability of insulin to potentiate the effect of IGF-1 in these cells with that seen in cells that expressed a chimeric insulin/IGF-1 receptor (Fig. 9). This was in agreement with the inability of insulin to promote the phosphorylation of the FTase \alpha-subunit (Fig. 5) and increase the amounts of farnesylated p21\textsuperscript{ras} (Fig. 4) in cells expressing the chimeric insulin/IGF-1 receptor.

**DISCUSSION**

The present study strongly indicates that the effect of insulin on the farnesylation of p21\textsuperscript{ras} is specific for this hormone and is not mimicked by IGF-1, EGF, or PDGF, even though all four...
growth factors activate the Ras-MAP kinase pathway (Fig. 10). All four growth factors utilize this pathway to promote DNA synthesis as measured by the incorporation of BrdUrd. However, only insulin uses the same pathway to promote phosphorylation and activation of FTase (8).

We have previously demonstrated that insulin’s signal to FTase flows via the Ras-MAP kinase in a positive feedback fashion (8). Insulin rapidly increases Ras-GTP loading and activates the serine-threonine kinases, such as Raf-1, MEK, and MAP, which in turn leads to the phosphorylation of the α-subunit of FTase and increments in its enzymatic activity (8). Why activation of the Ras pathway by IGF-1, EGF, and PDGF is insufficient to activate FTase is still unknown. However, the concept of insulin action specificity is not unique. For example, numerous growth-promoting agents, including insulin, stimulate phosphatidylinositol 3-kinase, but only insulin promotes the associated increases in glucose uptake (14). Subcellular localization of the insulin-sensitive phosphatidylinositol 3-kinase is believed to be responsible for this phenomenon (15). Whether or not cellular distribution of the MAP kinase influences the effect of insulin on FTase remains to be elucidated. A possibility is that the temporal relationship of MAP kinase activation by various growth factors is involved in the regulation of FTase phosphorylation and activation should also be examined in future studies.

An alternative explanation of the specificity of insulin action on FTase is based on the fact that even with insulin, the mere activation of the Ras-MAP kinase pathway may not be sufficient to phosphorylate and activate FTase. Cells that express the chimeric insulin/IGF-1 receptor and signal via the Ras pathway for DNA synthesis in response to insulin fail to phosphorylate and activate FTase (Figs. 4 and 5). These observations indicate that the activation of FTase is not only specific for insulin but also requires the presence of a competent insulin receptor: one with the insulin C-terminal domain (Fig. 10). The presence of an intact insulin receptor appears to be necessary to generate an important part of the insulin signaling to FTase. This component of the signal, in concert with the signal traveling through the Ras-MAP kinase pathway, is responsible for the activation of FTase. It appears that although in the absence of the C-terminal domain of the insulin receptor insulin can still promote DNA synthesis, it loses its ability to activate FTase, increase the amounts of farnesylated p21ras, and potentiates the nuclear effects of the other growth factors. Conceivably, the number of chimeric receptors per cell can influence the cellular responsiveness to either IGF-1 or insulin. Further studies are required to identify the nature of the signaling component that connects the C-terminal domain of the insulin receptor with the FTase molecule.

FTase, a ubiquitous enzyme, catalyzes the attachment of the farnesyl moiety to p21ras proteins (4, 5). Farnesylation of Ras proteins is a mandatory step in the post-translational modification of p21ras and allows p21ras to anchor at the plasma membrane and be activated by GTP loading (16). FTase consists of two subunits, the β-subunit, which binds both the Ras protein and the farnesyl lipid, and the α-subunit whose role remains incompletely understood (17). Interestingly, in the absence of the α-subunit, the β-subunit neither displays its catalytic activity nor is stable (18). We have recently shown that insulin promotes the phosphorylation of the α-subunit, accompanied by an increase in the enzymatic activity of FTase (3, 8). Activation of FTase by insulin results in increased amounts of farnesylated p21ras available for activation by other growth factors (1, 2). This facet of insulin action constitutes the basis for the ability of insulin to potentiate the nuclear effects of IGF-1, EGF, and PDGF demonstrated in the present study. Thus, although insulin is a mild mitogen in its own rights, it creates a background for the cellular responsiveness to other growth factors.

This “priming” aspect of insulin action may play an exceptionally important role in the pathogenesis of certain diabetic complications that are associated with hyperinsulinemia, such as the progression of atherosclerosis (19). We have recently demonstrated that hyperinsulinemia potentiates the influence of PDGF on the mRNA levels of vascular endothelial growth factor in rat and porcine vascular smooth muscle cells (2). Similarly to the results described in this work, the potentiating influence of insulin on PDGF action was abated in the presence of the FTase inhibitor, α-HFPA, supporting our hypothesis that insulin modulates the cellular responsiveness to growth factors via its stimulatory effects on FTase.

In accordance with our hypothesis, the IR−/− cells failed to increase the levels of farnesylated p21ras in response to insulin. However, the fact that approximately 40% of cellular Ras is farnesylated even in cells lacking insulin receptors (IR−/−) suggests that there is a certain rate of farnesylation that is independent of insulin action. Even though insulin may not govern the basal level of isoprenylation, it is capable of increasing the amounts of farnesylated p21ras that are available for activation by various growth-promotion agents, thereby regulating cellular responsiveness to these agents. In cases of hyperinsulinemia, the availability of greater amounts of farnesylated p21ras may determine the rates of mitogenic and proliferative cellular responses to growth factors that activate p21ras.

In summary, the present data indicate for the first time that insulin substantially potentiates the nuclear action of IGF-1, EGF, and PDGF, and the mechanism of this potentiation involves insulin-induced increases in the amounts of farnesylated p21ras. Interestingly, this effect is specific for insulin and requires the presence of the intact insulin receptor.

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REFERENCES

1. Leitner, J. W., Kline, T., Carel, K., Goalstone, M., and Draznin, B. (1997) Endocrinology 138, 2211–2214
2. Goalstone, M. L., Natafaran, R., Standley, P. R., Walsh, M. F., Leitner, J. W., Carel, K., Scott, S., Nadler, J., Sowers, J. R., and Draznin, B. (1998) Endocrinology, in press
3. Goalstone, M. L., and Draznin, B. (1996) J. Biol. Chem. 271, 27585–27589
4. Moores, S. L., Schaper, M. D., Messer, S. D., Randis, E., O’Hara, M. B., Garsky, V. M., Marshall, M. S., Pompiano, D. L., and Gibbs, J. B. (1991) J. Biol. Chem. 266, 14603–14610
5. Zhang, F. L., and Casey, P. J. (1996) Annu. Rev. Biochem. 65, 241–269
6. Goalstone, M., Leitner, J. W., and Draznin, B. (1997) Biochem. Biophys. Res. Commun. 239, 42–45
7. Vogt, A., Qian, Y., Blaskovich, M. A., Fossum, R. D., Hamilton, A. D., and Sebti, S. M. (1995) J. Biol. Chem. 270, 660–664
8. Goalstone, M., Carel, K., Leitner, J. W., and Draznin, B. (1997) Endocrinology 138, 5119–5124
9. Olefsky, J. M. (1990) Diabetes 39, 1009–1016
10. Cheatham, B., and Kahn, C. R. (1995) Endocrinology 131, 1165–1177
11. Rother, K. I., Imai, Y., Caruso, M., Beguinot, F., Formisano, P., and Accili, D. (1998) J. Biol. Chem. 273, 17491–17497
12. Wiese, R. J., Mastick, C. C., Laar, D. F., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 1383–1390
13. Stout, R. W. (1996) Diabetes 45, 45–46