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Augmentation of synthesis of plasminogen activator inhibitor type 1 by insulin and insulin-like growth factor type I: Implications for vascular disease in hyperinsulinemic states

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ABSTRACT **Accelerated atherosclerosis accompanying diabetes mellitus, obesity, and some types of hypertension has been associated with hyperinsulinemia, augmented plasma plasminogen activator inhibitor type 1 (PAI-1), or both. We hypothesized that insulin and insulin-like growth factor type I (IGF-I) can influence synthesis of PAI-1, thereby potentially attenuating fibrinolysis. In HepG2 cells used as a model system, concentrations of insulin and IGF-I consistent with those seen in plasma independently stimulated PAI-1 synthesis. Accumulation of PAI-1 protein in conditioned medium over 24 hr was stimulated more with insulin alone than with the combination. Synergistic increases were evident, however, in the accumulation of PAI-1 protein over 48 hr with a concomitant increase in PAI-1 mRNA. A 10- to 20-fold increase in IGF binding protein I mRNA was seen 16–48 hr after exposure of the HepG2 cells to insulin and IGF-I, an increase abolished by cycloheximide. The results obtained are consistent with the hypothesis that hyperinsulinemia coupled with physiologic concentrations of IGF-I may attenuate fibrinolytic activity *in vivo*, thereby contributing to accelerated atherosclerosis.**

Despite the profound benefits conferred by insulin to patients with diabetes mellitus, accelerated atherosclerosis is not abated and remains the leading cause of death (1). Growth factor-deficient dwarfs do not exhibit markedly accelerated atherosclerosis despite intense and sustained hyperglycemia (2). Furthermore, the severity of macrovascular complications in patients with diabetes mellitus does not correlate closely with the apparent severity of hyperglycemia (3) nor does stringent control of hyperglycemia translate into reduction of the incidence of fatal cardiovascular events (4).

Administration of insulin frequently leads to insulin resistance and hyperinsulinemia (5). Long before type II diabetes becomes manifest, reduced clearance of glucose with compensatory hyperinsulinemia is detectable (6). Obesity (especially central or android type) is accompanied by abnormal clearance of glucose, with normoglycemia maintained at the expense of both fasting and postprandial hyperinsulinemia (7). Some patients with hypertension exhibit insulin resistance and analogous hyperinsulinemia (8).

Because of homologies between insulin and insulin-like growth factor type I (IGF-I) (9, 10), they might synergistically influence synthesis of plasminogen activator inhibitor type 1 (PAI-1). IGF-I binding protein (IGFBP-I), an insulin-responsive carrier (11), may do so as well.

PAI-1, a serine protease inhibitor (12) in platelets, endothelial cells, hepatocytes, and plasma (13–16), decreases fibrinolytic activity *in vitro* and *in vivo* (16, 17). In patients with acute myocardial infarction, increases in PAI-1 potentiate thrombosis (17). Increased plasma insulin has been associated with increased PAI-1 (18–20). The present study was performed to define the effects of insulin, IGF-I, and a combination of the two on the synthesis and elaboration of PAI-1.

METHODS

Cell Culture. HepG2 cells obtained from the American Tissue Culture Center were grown to confluence in minimal essential medium (GIBCO) supplemented with penicillin (30 units/ml), streptomycin (30 μg/ml) (GIBCO), 2 mM L-glutamine, and 10% Nu-Serum (Collaborative Research). After reaching confluence, the cells were maintained in serum-free conditions for 16 ± 0.5 hr in Dulbecco's modified Eagle's medium with Ham's nutrient mixture F-12 (DMEM) (GIBCO) without supplement. Subsequently, bovine insulin (Sigma) resuspended in DMEM with 10% fetal bovine serum (FCS) (Collaborative Research) and/or human recombinant IGF-I (CalBiochem) resuspended in 10 mM acetic acid were added in the concentrations described for specific experiments. No more than 5 μl of FCS had to be added (<0.25%). Controls with the maximal amounts of vehicles for both insulin and IGF-I were performed in each case.

Quantification of PAI-1 Protein. Supernatant fraction of conditioned medium supplemented with 0.01% Tween 80 was stored at −20°C until assay. PAI-1 protein was assayed by ELISA (Tint Elise, Biopool, Umea, Sweden), which detects PAI-1 whether latent, active, or complexed with tissue-type plasminogen activator. Functional activity of PAI-1 was measured spectrophotometrically with a chromogenic substrate (S-2251; KabiVitrum, Stockholm) (21).

Determination of Rates of Degradation of PAI-1 in Conditioned Medium. PAI-1 produced by Bowes melanoma cells (American Diagnostica, Greenwich, CT) was radiolabeled with $Na^{125}I$ (ICN) with chloramine T (Sigma). Free ^{125}I was removed by Sephadex G-25 chromatography resulting in a protein bound/total ratio (protein bound plus free ^{125}I) of 93%. Protein-bound radioactivity was determined in trichloroacetic acid (10%; wt/vol) precipitates of conditioned medium to which ^{125}I-labeled PAI-1 was added at time 0.

Cellular Content of PAI-1 mRNA. Total cellular RNA was isolated by chloroform/phenol purification and 2-propanol precipitation of RNA (RNAzol; cinna Biotecx, Friendswood, TX). RNA (10 μg) was size fractionated with 1.5% formaldehyde agarose gels and assayed by Northern blotting (22). Prehybridization with 50% formamide/10% Denhardt's solution/0.5 M Tris·HCl/1.0 M NaCl/0.1% sodium pyrophosphate/1% SDS/10% dextran sulfate/denatured calf thymus DNA (100 μg/ml) was performed for 6 hr at 42°C. Hybridization was performed for 20–24 hr at 42°C with a 0.9-kilobase (kb) cDNA probe for PAI-1 generated by digestion of PAI-1 cDNA with *Eco*RI and *Sal* I and isolated by batch affinity

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Abbreviations: PAI-1, plasminogen activator inhibitor type 1; IGF-I, insulin-like growth factor type I; IRI, immunoreactive insulin; IGFBP-I, insulin-like growth factor binding protein type I.

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absorption with NaI glass beads (Geneclene, Bio 101, La Jolla, CA) and with a 0.6-kb probe for glyceraldehyde-3-phosphate dehydrogenase (*Xba* I/*Hin*dIII digestion) random prime labeled with [α-^{32}P]dCTP (Amersham). Subsequently, the membranes were stripped of probe. They were again prehybridized and hybridized with an IGFBP-I cDNA probe (0.3 kb; *Bam*HI/*Pst* I digestion). Total radioactivity of hybridized bands was quantified by radioisotopic scanning (Ambis Scanner, Automated Microbiology Systems, San Diego). Autoradiograms were obtained at −70°C conventionally.

Inhibition of Protein Synthesis. Cycloheximide (Sigma) was added to the medium (25 μg/ml) of serum-starved confluent cells. Insulin and/or IGF-I were added 30 min later. This amount of cycloheximide inhibits incorporation of [^{35}S]methionine into protein by 93% (23).

Analysis of Data. The impact of the combination of insulin and IGF-I on accumulation of PAI-1 protein in conditioned medium was delineated in terms of antagonism, noninteraction, or synergy with two mathematical models—the isobol (24) and the median effect model (25). With the isobol analysis, synergy, noninteraction, and antagonism are defined for two agents, a and b, as $a/A_e + b/B_e = r$, where a is the dose of a used in combination with b, A_e is the equieffective dose of a alone, b is the dose of b in combination with a, B_e is the equieffective dose of b alone, and r is the sum of the ratios. ($r < 1$ identifies synergy, $r = 1$ identifies noninteraction, and $r > 1$ identifies antagonism.)

With the median effect model the nature of an interaction for two agents (1 and 2) is defined as follows:

$$\frac{D_1}{DM_1} + \frac{D_2}{DM_2} + \frac{D_1}{DM_1} \times \frac{D_2}{DM_2} = r,$$

where D is the dose of either agent 1 or 2 necessary to produce 50% of the maximum effect (ED_{50}) in a combination of a fixed molar ratio of both agents, and DM is the dose of either agent 1 or 2 required to produce the same ED_{50} when the agent is used alone. The two agents used in combination must be combined in a fixed molar ratio over a range of concentrations to define the ED_{50} for the combination. r is defined as the sum of the ratios. The median effect model is applicable to nonmutually exclusive agents that exhibit sigmoidal dose–response relationships individually (25).

Data are expressed as means ± SE. Univariate and multivariate analyses were used to compare results in control cells with those in cells exposed to insulin, IGF-I, or both. One-tailed Student's t tests were used to assess significance with the isobol and median effect methods (defined as $P < 0.05$).

RESULTS

Effects of Insulin and IGF-I Alone and in Combination on Accumulation of PAI-1 Protein in Conditioned Medium. Insulin, IGF-I, or a combination of insulin and IGF-I in a molar ratio of 1:20 was added to serum-starved cells (time 0). Conditioned medium from 9.6-cm^2 tissue culture plates (n = 3 for each set of conditions) was harvested after either 24 (Fig. 1) or 48 (Fig. 2) hr and assayed for PAI-1 functional activity and for total PAI-1 protein by ELISA. When newly synthesized proteins are labeled with [^{35}S]methionine, the increase in intracellular PAI-1 protein is followed by an increase in labeled PAI-1 in the extracellular matrix (maximal after 9 hr) and a subsequent increase in labeled PAI-1 in the conditioned medium (maximal after 24 hr) (26).

As shown in Fig. 1, insulin increased the accumulation of PAI-1 by ≈8-fold from 55 ± 5.9 to 444 ± 30.6 ng/ml ($P < 0.01$) when its concentration was increased from 0 to 7.3 nM. IGF-I increased the accumulation of PAI-1 protein 4- to 5-fold from 55 ± 5.9 to 253 ± 1.7 ng/ml ($P < 0.001$) when its concentration was increased from 0 to 212 nM. The combination of insulin and IGF-I increased the accumulation of PAI-1 protein over 24 hr to an extent similar to that seen with IGF-I alone. Changes in PAI-1 activity paralleled changes in the concentration of PAI-1 protein with each stimulus (Fig. 1). No difference was observed in the rate of degradation of ^{125}I-labeled PAI-1 over 24 hr in cells exposed to insulin and/or IGF-I compared with controls. Immunoreactive insulin (IRI) concentrations in the 24-hr conditioned medium were not significantly different in medium from culture plates from cells treated with insulin alone and those from cells treated with the combination of insulin and IGF-I. Greater than 93% of insulin had been catabolized in both.

Analysis of the effects of insulin and IGF-I, alone and in combination, by the isobol and by the median effect methods demonstrated antagonistic effects between insulin and IGF-I on the accumulation of PAI-1 protein over 24 hr (isobol: r = 10.2 ± 2.9, $P < 0.10$ compared with an r value of 1; median effect: $r > 4000$, $P < 0.01$ compared with an r value of 1). However, when PAI-1 was assayed in conditioned medium 48 hr after addition of insulin, IGF-I, or both, results were quite different. As shown in Fig. 2, the effect on functional activity paralleled the effect on total protein. The decrement in activity of PAI-1 in conditioned medium from insulin-treated cells compared with the activity in the 24-hr samples is the result of inactivation of PAI-1 over time. Insulin alone induced minimal increases beyond those observed after 24 hr with a maximal increase of 3-fold [594 ± 50.2 ng/ml with insulin (10.6 nM) compared with control value of 176 ± 10.3 ng/ml; $P < 0.01$]. IGF-I (212 nM) elicited an ≈4-fold increase in PAI-1 in 48 hr (856 ± 76 ng/ml compared with control value of 176 ± 10.3 ng/ml; $P < 0.05$). The combination of insulin

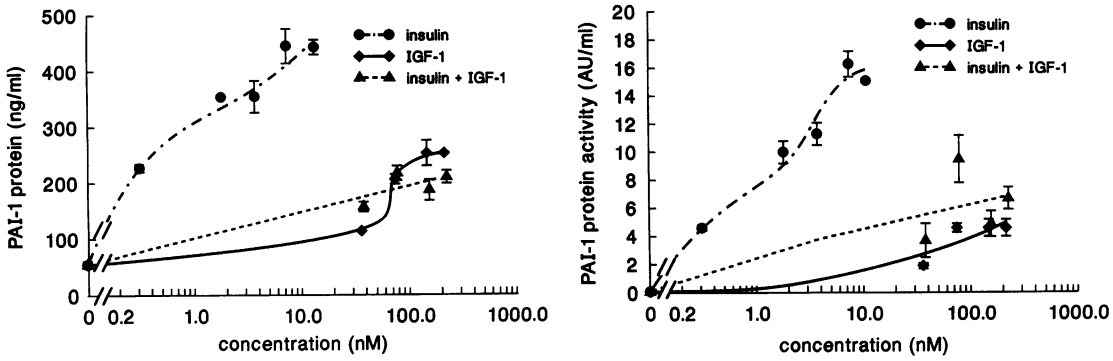

FIG. 1. PAI-1 protein in response to insulin and/or IGF-I. Total PAI-1 protein was assayed by ELISA (*Left*) and by activity (*Right*) (means ± SE). All increases were significant ($P < 0.05$).

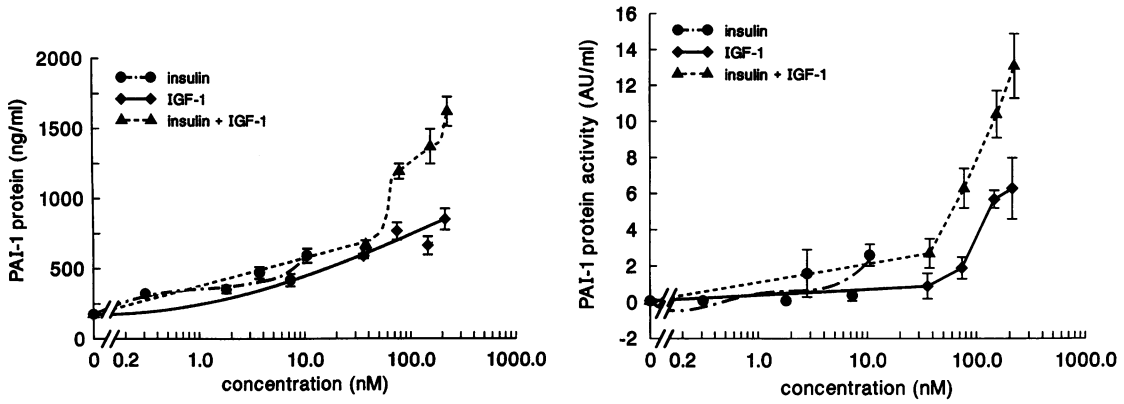

FIG. 2. PAI-1 protein (*Left*) and activity (*Right*) 48 hr after addition of insulin and/or IGF-I. All increases were significant ($P < 0.05$) except for those with the lowest concentration of IGF-I and with <10.6 nM insulin (*Right*).

and IGF-I led to a 9-fold increase in the accumulation of PAI-1 protein [control, 176 ± 10.3 ng/ml; 1628 ± 105.1 ng/ml with insulin (10.6 nM) plus IGF-I (212 nM); $P < 0.001$].

Once again, no difference was observed in the rate of degradation of ^{125}I-labeled PAI-1 48 hr after the addition of insulin and/or IGF-I. IRI levels were not significantly different in conditioned medium from cells exposed to insulin alone compared with cells exposed to insulin and IGF-I. Although there was a trend toward increased IRI concentrations in conditioned medium from cells exposed to insulin in combination with IGF-I, the residual amount of insulin was <3% of that initially present. Results with the isobol and median effect methods indicated synergy (isobol: $r = 0.20 \pm 0.18$, $P < 0.01$ compared with $r = 1$; median effect: $r = 0.24$, $P < 0.01$ compared with $r = 1$.

Effects of Insulin and IGF-I on the Accumulation of PAI-1 Protein and mRNA as a Function of Time. PAI-1 protein in conditioned medium and total cellular RNA from 28.3-cm^2 tissue culture plates were assayed 0, 8, 16, 24, 36, and 48 hr ($n = 4$ for each set of conditions) after addition of insulin, IGF-I, or both. The hybridized glyceraldehyde-3-phosphate dehydrogenase mRNA (control) generated a signal of constant intensity at each interval. Two species of PAI-1 mRNA (3.2 and 2.2 kb) were detected and no differential effect on the two species could be identified (27). Northern blots of RNA from control cells (treated with vehicle only) revealed no increase in PAI-1 mRNA over the 48-hr interval.

After the addition of 8.72 nM insulin (Fig. 3), PAI-1 mRNA increased to 2- to 3-fold after 16 and 24 hr ($P = 0.10$ and 0.07, respectively). The same stimulus led to accumulation of PAI-1 protein in the conditioned medium, which reached a plateau after 16 hr. IGF-I (84.4 nM) increased PAI-1 mRNA by 3- to 4-fold within 24 hr ($P < 0.02$) (Fig. 4). It led also to accumulation of PAI-1 protein in conditioned medium within 8 hr with further increases over 48 hr. The addition of 84.4 nM IGF-I combined with 8.72 nM insulin led to a marked increase in PAI-1 mRNA (Fig. 5). Contrary to the antagonistic effects of the two moieties with respect to accumulation of PAI-1 protein in the 24-hr interval, the combination led to a 12-fold increase in PAI-1 mRNA after 16 hr ($P < 0.001$) and a plateau in increased PAI-1 mRNA persisted over 48 hr. This increase was significantly greater ($P < 0.01$) than that accounted for by simple addition of the increases attributable to insulin alone (3-fold) and IGF-I alone (3- to 4-fold).

Inhibition of PAI-1 protein synthesis by cycloheximide was documented in both control and stimulated HepG2 cells throughout the 48-hr interval. Continued cell viability was demonstrated by return of PAI-1 protein and mRNA accumulation when media were replaced. The 2.2-kb mRNA did not increase above basal levels with cycloheximide alone or when insulin, IGF-I, or a combination of insulin and IGF-I with cycloheximide ($n = 3$ for each) was present. Cycloheximide alone increased the 3.2-kb mRNA beginning 16 hr after its addition with the increase continuing for 48 hr. Nevertheless, the increase in the 3.2-kb mRNA 8 and 16 hr after addition of insulin and IGF-I was significantly attenuated ($P < 0.05$ and 0.01) with cycloheximide (Fig. 6).

When the Northern blots were analyzed for IGFBP-I mRNA ($n = 3$ for each set of conditions), IGFBP-I was detected as two bands. The primary band (1.6 kb) was consistent with the full-length transcript. The secondary band (at ≈4.5 kb) is thought to be a primary unprocessed transcript (11). IGFBP-I was quantified as the 1.6-kb band. The 4.5-kb band exhibited parallel responses under various conditions.

Hybridization of RNA from control cells (treated with vehicle only) demonstrated a 2-fold increase in IGFBP-I mRNA after 48 hr. Addition of 84.4 nM IGF-I led to a 2-fold increase in IGFBP-I (P not significant vs. control) after 24 hr (Fig. 4). IGFBP-I continued to increase through 48 hr (cpm at time 0 = 47.6 ± 11.1; cpm after 48 hr = 207.7 ± 37.3; cpm after 48-hr control = 126.6 ± 29.1; $P = 0.07$) (Fig. 4). Insulin increased IGFBP-I 6-fold 24–48 hr ($P < 0.02$) after the addition of 8.72 nM (Fig. 3). The combination of insulin (8.72 nM) and IGF-I (84.4 nM) resulted in a 10-fold increase ($P <$

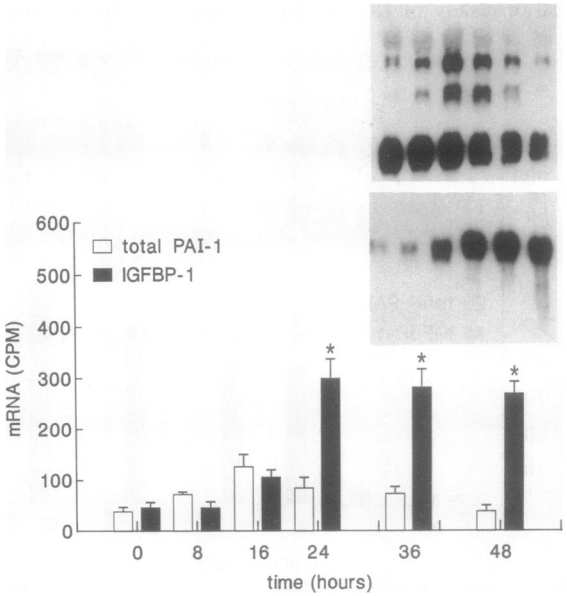

FIG. 3. PAI-1 and IGFBP-I mRNA after addition of 8.72 nM insulin. (*Inset*) Representative autoradiogram. The top two bands are 3.2- and 2.2-kb PAI-1 mRNA (summed for quantification), the third band is glyceraldehyde-3-phosphate dehydrogenase mRNA, and the bottom band is 1.65-kb IGFBP-I mRNA. *, $P < 0.02$.

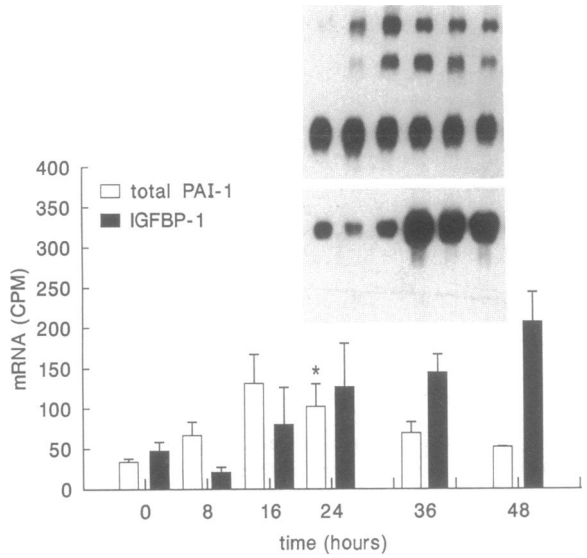

FIG. 4. PAI-1 and IGFBP-I mRNA after addition of 84.4 nM IGF-I. (*Inset*) Representative autoradiogram. The top two bands are 3.2- and 2.2-kb PAI-1 mRNA (summed for quantification), the third band is glyceraldehyde-3-phosphate dehydrogenase mRNA, and the bottom band is 1.65-kb IGFBP-I mRNA. *, $P < 0.02$.

0.02) after 16 hr and a further increase to nearly 20-fold ($P < 0.001$) after 48 hr (Fig. 5). Northern blots from cells pretreated with cycloheximide demonstrated a decrease in IGFBP-I of >75% throughout under all conditions.

Effects of Insulin in Concentrations Seen in Plasma from Hyperinsulinemic Patients Plus Physiologic Concentrations of IGF-I on Accumulation of PAI-1 Protein and mRNA. To identify potential effects of a single bolus of insulin on production of PAI-1 in association with a single bolus of IGF-I consistent with concentrations in plasma (28), conditioned medium and cellular RNA were harvested 48 hr after addition of IGF-I (84.4 nM) and selected, incremental concentrations of insulin ($n = 3$ for each set of conditions). The range of concentrations of insulin included values approximating normal fasting concentrations of insulin in plasma (based on assay of IRI) in lean subjects (10 microunits/ml,

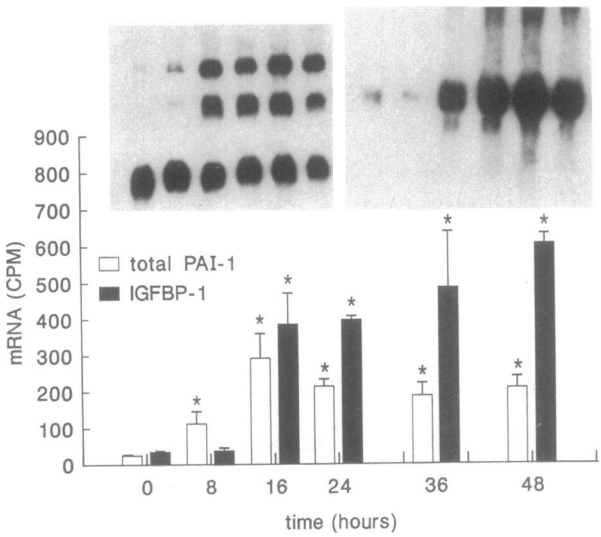

FIG. 5. PAI-1 and IGFBP-I mRNA after addition of 8.72 nM insulin and 84.4 nM IGF-I. (*Inset*) Representative Northern blots. The top two bands on the left are 3.2- and 2.2-kb PAI-1 mRNA (summed for quantification), the third band on the left is glyceraldehyde-3-phosphate dehydrogenase mRNA, and the prominent band on the right is 1.65-kb IGFBP-I mRNA. *, $P < 0.02$.

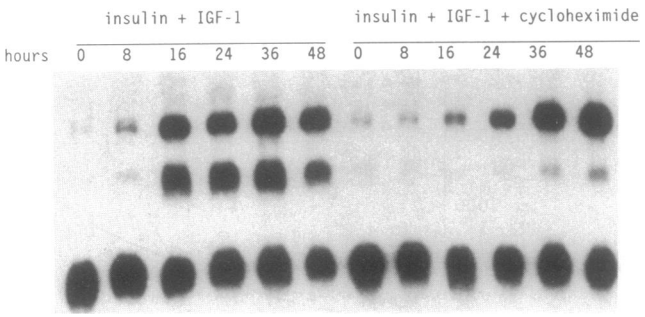

FIG. 6. Representative Northern blot demonstrating the effect of insulin (8.72 nM) plus IGF-I (84.4 nM) on PAI-1 mRNA without (left six lanes) and with (right six lanes) pretreatment of cells with cycloheximide (25 μg/ml). The top band is 3.2-kb PAI-1 mRNA, the middle band is 2.2-kb PAI-1 mRNA, and the bottom band is glyceraldehyde-3-phosphate dehydrogenase.

equivalent to 0.07 nM), fasting insulin levels in obese patients (40 microunits/ml, equivalent to 0.29 nM), and the average serum IRI in diabetic patients treated with exogenous insulin for >2 years (500 microunits/ml, equivalent to 3.63 nM) (5). Accumulation of PAI-1 protein in the conditioned medium increased 2-fold (from 201 ± 8.3 to 317 ± 47.7 ng/ml; $P < 0.05$) when the concentration of insulin was increased from 0.07 to 3.63 nM (Fig. 7). PAI-1 mRNA increased 3- to 4-fold (from 203 ± 33 cpm to 725 ± 19.9 cpm; $P < 0.01$) with the same increase in the concentration of insulin. PAI-1 mRNA increased 16-fold (from 52 ± 0.7 to 725 ± 19.9 cpm; $P < 0.001$) with 84.4 nM IGF-I plus 3.63 nM insulin compared with controls. IGFBP-I mRNA increased 10-fold from 126 ± 29.1 in control cells to 1061 ± 108 cpm in cells exposed to 3.63 nM insulin plus 84.4 nM IGF-I ($P < 0.001$).

DISCUSSION

Because both functional activity and immunodetectable PAI-1 protein increased in parallel, because the synergistic increase in PAI-1 protein was preceded by a synergistic increase in PAI-1 mRNA, and because neither insulin nor IGF-I (alone or together) altered the rate of degradation of ^{125}I-labeled PAI-1, the results indicate that the combination of insulin plus IGF-I increases synthesis of functionally active PAI-1 protein. Although HepG2 cells exhibit many characteristics of human hepatocytes including receptor-mediated endocytosis of tissue-type plasminogen activator (29) and synthesis of coagu-

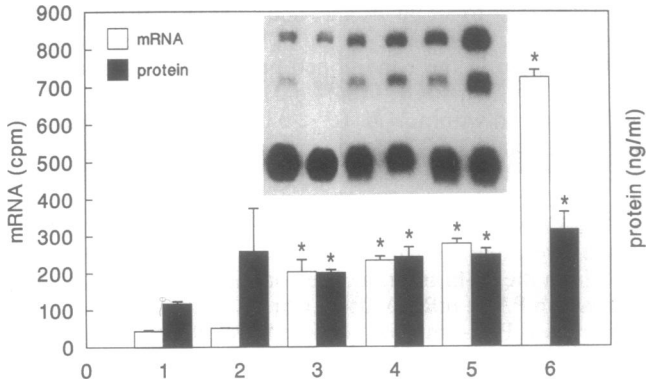

FIG. 7. Effects of IGF-I and insulin on PAI-1. Protein and mRNA values are plotted on the same scale. (*Inset*) Representative Northern blot. The top two bands are 3.2- and 2.2-kb PAI-1 (summed for quantification), and the bottom band is glyceraldehyde-3-phosphate dehydrogenase mRNA. Each combination of insulin and IGF-I significantly increased PAI-1 mRNA and protein. Bars: 1, control; 2, 84.4 nM IGF-I; 3, 84.4 nM IGF-I and 0.07 nM insulin; 4, 84.4 nM IGF-I and 0.29 nM insulin; 5, 84.4 nM IGF-I and 1.45 nM insulin; 6, 84.4 nM IGF-I and 3.60 nM insulin. *, $P < 0.05$.

lation factors and acute-phase reactants (30) extrapolation of results to arterial endothelial cells or adult hepatocytes cannot be assumed without direct confirmation. However, preliminary results with swine aortic endothelial cells in primary culture show similar increases (data not shown).

A single bolus of insulin induced a maximal increase in PAI-1 mRNA within 8–16 hr and in PAI-1 protein within 16–24 hr. Other investigators have shown an increase in PAI-1 protein in response to insulin in HepG2 cells (31, 32) as well as an increase in PAI-1 protein and mRNA in cultured human hepatocytes (32). However, the previously observed increases in PAI-1 were either in response to 10-fold higher concentrations of insulin or much less marked than those we observed with insulin and IGF-I. Our observations are consistent with increased synthesis mediated at the level of transcription in response to concentrations of insulin, consistent with those in plasma in obese subjects and in diabetics who have been treated with insulin (5, 6). Although insulin complexed to neutralizing antibody does not stimulate PAI-1 production by HepG2 cells (data not shown), insulin–antibody complexes provide a circulating depot from which free insulin is elaborated *in vivo* (5). The impact of sustained hyperinsulinemia on production of PAI-1 in insulin-resistant cells with altered insulin receptor number and/or affinity cannot be inferred from these results.

Compared with insulin, IGF-I stimulated greater expression of PAI-1 mRNA and less accumulation of PAI-1 protein. The concentration range over which the effect of IGF-I was seen is consistent with physiologic concentrations of IGF-I in plasma (28). *In vivo*, even more marked effects may be anticipated because of the association of IGF-I with its circulating carrier protein, IGFBP-III, leading to prolongation of half-life and augmentation of efficacy (11). In addition, local concentrations of IGF-I may be high in the vascular wall because of synthesis by component cells, particularly when hypertension induces arterial smooth muscle production of IGF-I (33).

The combination of IGF-I and insulin exhibited complex effects. In the first 24 hr, antagonistic effects on accumulation of PAI-1 protein were demonstrated with both the isobol and median effect methods of analysis. In contrast, synergy was evident with respect to PAI-1 mRNA even after 16 hr. These divergent effects may reflect, in part, secretion of PAI-1 protein into the extracellular matrix in the first 24 hr with sequestration from conditioned medium. This interpretation is consistent with the synergistic increases in PAI-1 protein seen in the conditioned medium over the 48-hr interval.

IGF-I inhibits degradation of insulin in HepG2 cells (34) particularly with low concentrations of insulin (<1.7 nM) and high concentrations of IGF-I (130 nM) (34). More than 97% of the exogenous insulin was catabolized in 24 hr, and synergistic increases in PAI-1 mRNA and protein could not be attributed to altered degradation of insulin.

The expression of IGFBP-I mRNA significantly increased 16 hr after exposure of HepG2 cells to insulin. It was further augmented when IGF-I was added with insulin. Pretreatment with cycloheximide abolished the increase, implicating a second message. The increased production of IGFBP-I may contribute to the increase in PAI-1 expression. Although IGFBP-I may increase the penetration into and direct effects in cells of IGF-I, conversely it could inhibit penetration and effects of IGF-I thereby decreasing inhibition of insulin by IGF-I. The lack of increase in IGFBP-I 8 hr after the addition of insulin plus IGF-I confirms that IGFBP-I does not account for the early increase in PAI-1. However, IGFBP-I may contribute to the plateau of increase in PAI-1 mRNA 24–48 hr after the addition of insulin plus IGF-I.

Our results are consistent with the possibility that accelerated vascular disease in hyperinsulinemic subjects may be mediated, in part, by increased synthesis of PAI-1 and attenuation of fibrinolytic activity at the site of arterial lesions and in plasma. Our results may account also, at least in part, for the minimal vascular disease despite significant hyperglycemia in growth hormone-deficient dwarfs, who have low circulating IRI levels and lack the capacity for production of IGF-I (2). Further elucidation of the role of PAI-1 synthesis in response to insulin and IGF-I in the pathogenesis of vascular disease in diabetics and other hyperinsulinemic subjects may contribute ultimately to development of innovative and effective prophylactic interventions.

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