Insulin-Like Growth Factor Binding Protein-4 Expression Is Decreased by Angiotensin II and Thrombin in Rat Aortic Vascular Smooth Muscle Cells

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Abstract—Insulin-like growth factor-I (IGF-I) is a ubiquitous peptide that regulates cellular growth and differentiation and is involved in vascular proliferative responses. The effects of IGF-I are modulated by several IGF-I binding proteins (IGFBPs), including IGFBP-4, the main IGFBP produced by vascular smooth muscle cells (VSMCs). We have previously shown that angiotensin II (Ang II)–induced and thrombin-induced mitogenesis in VSMCs is dependent on autocrine IGF-I. In addition, we have demonstrated that IGF-I and IGFBP-4 mRNA levels are upregulated in the hypertensive aorta of abdominally coarcted rats, a high-renin hypertension model. To obtain further insight into the IGF-I system and to specifically study changes in IGFBP-4, a known inhibitor of IGF-I action, VSMCs were incubated with Ang II or thrombin. Compared with control, Ang II induced an 87% downregulation of IGFBP-4 mRNA levels at 24 hours, with a 61% decrease of IGFBP-4 levels, as determined by Western ligand blot analysis. Thrombin had the same depressor effects (87% for the mRNA levels and 61% for the protein levels). Ang II and thrombin coinubcation with 125I-IGFBP-4 in the conditioned media failed to reveal any increase in fragmentation, indicating that proteolytic cleavage of IGFBP-4 was not involved in the observed effects. Exogenous recombinant human IGFBP-4 decreased thrombin-induced DNA synthesis of human aortic VSMCs by 64%, whereas anti–IGFBP-4 antibody potentiated thrombin-induced DNA synthesis. These data suggest that downregulation of IGFBP-4 expression in VSMCs may play a critical role in vascular growth response to Ang II and thrombin in normal and diseased states, by increasing the bioavailability of IGF-I for its cell-surface receptor. (Arterioscler Thromb Vasc Biol. 2000;20:370-376.)

Key Words: insulin-like growth factor binding proteins ■ gene expression ■ vascular smooth muscle cells ■ angiotensin II ■ thrombin

Insulin-like growth factor-I (IGF-I) is a potent mitogen involved in normal developmental growth and in disease states. IGF-I exerts its effects through endocrine and autocrine/paracrine mechanisms.1 We have previously demonstrated IGF-I gene expression in cultured endothelial and vascular smooth muscle cells (VSMCs)2 and have hypothesized that this mitogen may play a key role in the intrinsic growth program in normal and diseased blood vessels. Thus, IGF-I is required for angiotensin II (Ang II)–induced and thrombin-induced mitogenesis,3–5 as demonstrated by experiments with anti–IGF-I antibodies or antisense oligonucleotides to downregulate IGF-I receptors. The expression of IGF-I is transiently induced in the medial smooth muscle cell layer of rat aorta after balloon angioplasty.6,7 The biological effects of IGF-I are mediated by well-characterized cell-surface receptors, and its bioavailability is also modulated by a family of specific high-affinity carrier proteins, termed insulin-like growth factor binding proteins (IGFBPs, reviewed in References 1 and 8). Rat aortic VSMCs produce predominantly IGFBP-4, a known inhibitor of IGF-I action, and lesser amounts of IGFBP-3 and a 31-kDa species, presumably IGFBP-2.

We have recently shown that abdominal aortic coarctation in the rat (a high-renin angiotensin model of hypertension) produces an increase in IGF-I mRNA levels in the hypertensive aorta,9 with an increase of IGFBP-4 mRNA levels in the same segment of the vasculature.10 In marked contrast, abdominal coarctation results in a decrease in IGFBP-4 expression in the abdominal (normotensive) aortas compared with normotensive aortas from sham-operated animals. We have postulated that this decrease in IGFBP-4 in the normotensive aorta could be humorally mediated by an increase in the circulating Ang II levels.

To obtain insight into the regulation of vascular IGFBP-4 expression, we performed experiments to characterize the potential regulation of IGFBP-4 by Ang II and by thrombin. Our findings demonstrate that these 2 mitogens induce a sustained decrease of IGFBP-4 expression in cultured rat aortic smooth muscle cells (RASMs) through a transcriptionally mediated mechanism. Furthermore, the addition of exogenous recombinant IGFBP-4 to human aortic VSMCs inhibited thrombin-induced DNA synthesis, whereas blocking IGFBP-4 by use of specific antibodies increased DNA synthesis in response to thrombin.
In light of our previous finding of increased IGFBP-4 mRNA levels in the hypertensive aortas of high-renin hypertensive rats, our present data suggest that several levels of regulation exist in vivo for IGFBP-4. Mechanical factors, such as high blood pressure (or shear stress), could counterbalance the inhibitory effects of Ang II on IGFBP-4 expression. Furthermore, the increase in free IGF-I resulting from a decrease in the IGFBP-4 levels could be an important mediator of Ang II–induced and thrombin-induced mitogenesis in vivo.

Methods

Cell Culture

Rat aortic VSMCs were isolated by digestion from rat thoracic aorta as described previously by Alexander et al. 11 Cells were plated on 100-mm dishes and were grown according to a well-established method in our laboratories. In brief, VSMCs were grown to 75% to 80% confluence in DMEM with 10% calf serum. Cells were used at passages 6 to 10. Cells were made quiescent in DMEM/Ham F-12 (1:1) with insulin, ascorbate, and transferrin for 24 hours. Subsequently, cells were washed once with PBS, fresh medium was added, and cells were treated with either 100 nmol/L Ang II or 1 U/mL thrombin. These concentrations of thrombin and Ang II were determined previously to induce robust changes in DNA synthesis, IGF-I, and IGF-1 receptor expression. 3,4 Total RNA and the conditioned media were harvested at different time points. To determine the effects of RNA synthesis inhibition, quiescent cells were incubated in the presence or absence of those mitogens with or without actinomycin D (10 μg/mL). Cells were preexposed to actinomycin D for 30 minutes before exposure to agonists.

Human aortic VSMCs were obtained from Clonetics and grown according to the manufacturer’s guidelines. In brief, cells were grown to 80% confluence in SmGM medium and then made quiescent in SmBM (serum-free medium) with insulin, ascorbate, and transferrin for 24 hours. Cells were then stimulated with agonists. Cell passages were from 6 to 10.

Northern Analysis

Quantification of IGFBP-4 mRNA transcripts was carried out by Northern analysis. A minimum of 3 or 4 groups with controls was used at each time point. RNA was prepared by using the TRI Reagent kit (Molecular Research Center) and was used only when the RNA was further verified by agarose–formaldehyde gel electrophoresis and ethidium bromide staining. For Northern analysis, 20-μg samples of total RNA were size-fractionated by agarose–formaldehyde gel electrophoresis before transfer to nylon membranes (Genescreen Plus, New England Nuclear). RNA loading and transfer efficiency were verified by methylene blue staining of membranes. Membranes were prehybridized for 3 hours and then hybridized overnight at 42°C in a solution containing 50% formamide, 5× SSPE, 5× Denhardt’s solution, 1% SDS, 10% dextran sulfate (molecular weight 500 000), 100 μg/mL denatured herring sperm DNA, and 5×105 cpm/mL of [32P]-labeled cDNA probes (IGFBP-4 and GAPDH).

The IGFBP-4 cDNAs were kindly provided by Dr S. Shimasaki (Whittier Institute for Diabetes and Endocrinology, Scripps Memorial Hospital, Whittier, Calif.). The IGFBP-4 cDNA clone is a rat 444-bp fragment including the entire coding sequence for IGFBP-4. The cDNAs were labeled by random priming with [32P]dCTP and the Prime-IT II kit from Stratagene. After hybridization, filters were washed with 2× SSPE at room temperature 2 times, then with 2× SSPE and 2% SDS at 60°C for 30 minutes, and then for 15 minutes in 0.1× SSPE before autoradiography. The film was then scanned by tomodensitometry with an LKB scanner (Ultrascan XL), and absorbance curves were integrated and compared. The molecular sizes of the detected RNA species were determined from the migration pattern of a 0.24- to 9.5-kb RNA ladder (GIBCO-BRL, Life Technologies, Inc.). As an additional control for loading and transfer of RNA samples, filters were cohybridized with a GAPDH cDNA probe. 12 The cDNA was kindly provided by Dr J.M. Blanchard (Université des Sciences et Techniques du Languedoc, Montpellier, France). All densitometric data were normalized for the GAPDH signal.

Western Ligand Blotting

Conditioned media were first acidified with an equal volume of 2N acetic acid, incubated at room temperature for 30 minutes, and then concentrated by Centocor columns with the use of a standard protocol in our laboratories. The samples were lyophilized and resuspended in sodium phosphate buffer. Western ligand blotting was performed according to the method of Hossenlop et al. 13 Briefly, the samples were loaded on a 12% SDS polyacrylamide gel and electrophoresed for 1 hour at 200 V. After electrophoresis, proteins were transferred to nitrocellulose, and the membrane was dried and sequentially blocked with 3% Nonidet P-40, 1% BSA, and 0.1% Tween 20 in Tris-buffered saline before incubation with 4×107 cpm/mL of [125I]-IGF-I for 16 to 18 hours. Blotted membranes were dried and exposed to film for 1 to 3 days, and band intensity was quantified with an Ultrascan XL laser tomodensitometer. Prestained molecular weight markers (Bio-Rad) were used for sizing.

Measurements of IGFBP-4 Protease Activity

Conditioned media from control, Ang II–treated, or thrombin-treated cells at each time point were split in equal volumes. IGF-II (10 nmol/L) was added in 1 of the samples and incubated at 37°C, either for 6 hours or overnight. The addition of IGF-I or IGF-II has been shown to activate IGFBP-4 protease. 14 The samples were then treated, and Western ligand blotting was performed as described above. These experiments were performed at least 3 times. To better detect any contribution of the IGFBP-4 protease to the observed reduction of IGFBP-4, an independent set of experiments was performed in identical conditions but with the addition of 10 000 cpm of [125I]-IGFBP-4 and 10 nmol/L IGF-II in the conditioned media and 6 hours of incubation. The samples were then concentrated and subjected to SDS-PAGE. Direct cell-dependent proteolytic degradation of IGFBP-4 was assayed by exposing quiescent RASMs in 60-mm dishes to Ang II or thrombin in the presence of 10 000 cpm of [125I]-IGFBP-4 for 24 hours. The media were collected, concentrated, and run on a 4% to 15% gradient SDS-polyacrylamide gel. The gels were subsequently dried and exposed to film for 1 to 3 days.

[3H]Thymidine Incorporation

VSMCs were plated in 24-well plates and grown to 80% confluence. After 2 rinses with PBS, cells were made quiescent for 48 hours in serum-free medium containing insulin, ascorbate, and transferrin. After an additional wash, cells were incubated for 24 hours in serum-free media with [3H]thymidine (1 μCi/mL) in the presence or absence of growth factors (thrombin or Ang II at 1 U/mL and 100 nmol/L, respectively). Cells were washed 3 times with ice-cold PBS and incubated on ice for 15 minutes with 10% trichloroacetic acid, and after 2 washes in ice-cold 95% ethanol, radioactivity was extracted with 0.2 N NaOH for assay by liquid scintillation spectrophotometry.

To determine the effects of IGFBP-4 on thrombin-induced or Ang II–induced DNA synthesis, experiments were performed in which cells were exposed to 2 to 8 μg/mL exogenous recombinant human IGFBP-4 (rIGFBP-4, Austral Biologicals) in the presence or absence of agonists. To determine the effects of anti–IGFBP-4 antibody on Ang II–induced or thrombin-induced DNA synthesis, cells were incubated with an anti–IGFBP-4 monoclonal antibody (Austral Biologicals) at 1:100 dilution in the presence or absence of agonists. Nonspecific polyclonal IgGs were used at dilutions similar to those used for internal controls. A minimum of 4 independent experiments was performed for each condition.

Statistical Analysis

Data are presented as mean±SEM. Statistical analysis was performed by 1-way or 2-way ANOVA when appropriate. A value of P<0.05 was considered significant.
Results

Regulation of IGFBP-4 mRNA by Ang II
As indicated in Figure 1, Northern analysis of total RNA from RASMs with the use of an IGFBP-4 cDNA probe yielded the expected 2.3-kb transcript. There was a rapid decrease in IGFBP-4 mRNA levels in the Ang II–treated group compared with the control group at various time points. Results from 4 independent experiments are shown in Figure 2. In each case, the IGFBP-4 mRNA signal obtained from Ang II–treated cells was corrected for the GAPDH signal (1.4-kb transcript) and compared with the levels (corrected for GAPDH) from control cells. The rapid decrease in IGFBP-4 mRNA was already evident at 3 hours and became statistically significant at 24 hours.

Regulation of IGFBP-4 mRNA by Thrombin
Quiescent RASMs exposed to thrombin similarly showed a rapid decrease in IGFBP-4 mRNA levels (Figures 1 and 2).

Western Ligand Blot Analysis of IGFBPs in Conditioned Media
Analysis of conditioned media by Western ligand blotting with 125I-IGF-I has previously been shown to detect 4 bands.15 The 28-kDa and 24-kDa doublet are the glycosylated and nonglycosylated variants of IGFBP-4 and are the main binding proteins secreted by rat VSMCs. IGFBP-3 (38- to 43-kDa doublet) and a 32-kDa IGFBP (likely IGFBP-2) are present in trace amounts. Densitometric analysis of 4 independent experiments (Figure 3) demonstrated a significant decrease of IGFBP-4 levels in response to Ang II and also to thrombin at 24 hours.

Protease Experiments
As shown in Figure 4, Western ligand blot analysis of conditioned media from control cells or cells exposed to Ang II or thrombin, incubated with or without IGF-II for 6 hours or overnight, failed to reveal any fragmentation suggestive of proteolytic degradation of IGFBP-4. Likewise, when 125I-IGFBP-4 was coincubated with conditioned medium from control or agonist-treated cells, there was no degradation, even in the presence of IGF-II (Figure 5). Furthermore, we
performed experiments in which $^{125}$I-IGFBP-4 was directly added to the cells in the absence or presence of agonists. SDS-PAGE of conditioned medium from these cells also failed to reveal agonist-induced degradation of IGFBP-4 (not shown).

**Actinomycin D Experiments**

To determine whether the agonist-induced downregulation of IGFBP-4 was transcriptionally or nontranscriptionally mediated, we incubated cells in the absence or presence of Ang II or thrombin with or without actinomycin D. As shown in Figures 6 and 7, actinomycin D increased basal IGFBP-4 mRNA levels but completely inhibited the ability of Ang II and thrombin to decrease IGFBP-4 mRNA and protein levels. It should be noted that actinomycin D decreased GAPDH mRNA levels in VSMCs. These data are consistent with transcriptional downregulation of IGFBP-4 expression by Ang II and thrombin.

**[$^{3}$H]Thymidine Incorporation**

To address the central issue of the role of IGFBP-4 in thrombin-induced and Ang II–induced mitogenesis, exogenous IGFBP-4 was added to the conditioned media to attempt to inhibit Ang II–induced and thrombin-induced DNA synthesis, with the hypothesis being that IGFBP-4 would quench free IGF-I. Thrombin and Ang II increased DNA synthesis by 90$^{\pm5}$% and 129$^{\pm7}$%, respectively, compared with control ($P<0.05$) in rat VSMCs. However, we were unable to reduce Ang II–induced and thrombin-induced DNA synthesis by recombinant human IGFBP-4 (2 $\mu$g/mL to 8 $\mu$g/mL) in our model of rat aortic VSMCs, because human IGFBP-4 does not bind to rat IGF-I (data on file at Austral Biologicals).

To circumvent this problem, because no recombinant rat IGFBP-4 is available commercially, we used human aortic VSMCs. In this cell system (Figure 8), the addition of 8 $\mu$g/mL IGFBP-4 produced a significant (64%) decrease in thrombin-induced DNA synthesis compared with control. On the contrary, blocking IGFBP-4 by the addition of specific anti–IGFBP-4 antibodies (Figure 9) increased thrombin-induced thymidine incorporation by 54% compared with control, presumably by increasing the bioavailability of free IGF-I. In contrast to rat VSMCs, human VSMCs failed to exhibit an increase in DNA synthesis in response to Ang II.
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hours, [3H]thymidine incorporation was determined. Values are specific human polyclonal IgG (IgG, dilution of 1:100). After 24 hours, [3H]thymidine incorporation was determined. The results are expressed as percentage of increase in DNA synthesis induced by thrombin over control. Values are the mean±SEM of 4 independent experiments. Each experiment was performed in quadruplicate.

Discussion

In the present study, we demonstrate a sustained decrease of IGFBP-4 mRNA and protein expression in response to Ang II and thrombin in RASMs. Because IGFBP-4 inhibits the effects of IGF-I, this downregulation could play a critical role in enhancing mitogenic responses in blood vessels by increasing the availability of IGF-I for its receptor. Compared with control, these 2 growth factors downregulate IGFBP-4 mRNA levels as early as 3 hours after exposure. IGFBP-4 in the conditioned media, however, was significantly decreased only at 24 hours. Because proteolysis of the IGFBPs is an important mechanism in the regulation of the actions of IGF,16 we explored whether such posttranslational regulation existed. Our data demonstrate that exposure of VSMCs to Ang II or thrombin does not induce significant IGFBP-4 protease activity. Indeed, there appears to be little basal IGFBP-4 protease activity in rat aortic VSMC–conditioned media. Thus, the decrease of IGFBP-4 in the conditioned media is likely transcriptionally related, as confirmed by the experiments with actinomycin D.

It is important to note that in other cell systems, such as human dermal fibroblasts17,18 or porcine vascular smooth muscle cells,14 IGFBP-4 proteolysis is an important regulator of the bioavailability of this protein. Thus, a protease-resistant IGFBP-4 has been shown to blunt IGF-I–induced DNA synthesis of porcine VSMCs.19

VSMC proliferation is involved in normal vascular wound healing and is an important feature in the development of various abnormal vascular growth responses, such as in atherosclerosis and vascular remodeling.20 Several growth factors are involved in this process. There is strong evidence suggesting that the IGF-I autocrine system acts as a critical determinant of vascular growth because it is required for the growth effects of several other cytokines (reviewed in Reference 8). We have previously shown a sustained induction of IGF-I mRNA transcription levels in the thoracic aorta in abdominally coarcted rats,9 a high-renin model of hypertension. In these aortas, there is a parallel decrease in IGF-I receptor transcription levels that is consistent with ligand-induced receptor downregulation.21 Because IGF-I binds to specific high-affinity carrier protein (IGFBPs), knowledge of the regulation of these carrier proteins is fundamental to the understanding of the actions of IGF-I. IGFBP-4 is the main IGFBP produced in vitro by rat aortic VSMCs.8 Our subsequent work revealed a sustained induction of IGFBP-4 mRNA levels in only the hypertensive segment of the aorta in abdominally coarcted rats.10 This induction was absent in the liver and in the normotensive vascular bed, namely, the infrarenal abdominal aorta of the coarcted animals. In fact, there was a trend toward a decrease of IGFBP-4 expression in those tissues, and in particular, there was a decrease in IGFBP-4 mRNA in normotensive aortas in coarcted (high levels of Ang II) animals compared with normotensive aortas from sham-operated (normal levels of Ang II) animals. Therefore, we postulated that mechanical factors led to IGFBP-4 upregulation in the hypertensive aorta and that humoral factor(s) could downregulate IGFBP-4 in normotensive tissues. Our present data are consistent with the hypothesis that Ang II downregulates IGFBP-4 expression in the normotensive tissue in abdominally coarcted rats and support the concept that hemodynamics factors, namely, increased pressure, are responsible for the increase in IGFBP-4 mRNA levels in hypertensive aortas. Upregulation of vascular IGFBP-4 expression could provide an important homeostatic mechanism, because it can potentially counterbalance IGF-I mitogenic activity in hypertensive or diseased vessels.

Although previous studies have shown that IGFBPs can stimulate22–24 and inhibit23 the metabolic and mitogenic effects of IGF-I, it has been convincingly demonstrated that IGFBP-4 is an inhibitory IGFBP25 (reviewed in Reference 16). Our data demonstrate that IGFBP-4 is an inhibitory IGFBP and is involved in modulating the final mitogenic response to growth factors such as thrombin and, likely, Ang II. Thus, exogenous human recombinant IGFBP-4 blunted thrombin-induced DNA synthesis in human aortic VSMCs. On the contrary, incubation with antibodies directed against IGFBP-4 significantly increased DNA synthesis in response to thrombin, presumably by decreasing the amount of this inhibitory protein available to bind free IGF-I. Attempts to directly measure free IGF-I in RASM-conditioned media with the currently available Diagnostic Systems Laboratories kit were unsuccessful because of lack of cross-reactivity of the anti-human IGF-I antibody.
The inhibitory nature of IGFBP-4 has been further confirmed by the use of transgenic models. Wang et al.\(^2\) have reported that overexpression of IGFBP-4 in smooth muscle cells in transgenic mice induces smooth muscle hypoplasia. Double transgenic mice overexpressing IGF-I and IGFBP-4 showed only a modest weight gain in VSMC-rich organs compared with transgenic mice overexpressing only IGF-I. In a different model, overexpression of IGFBP-4 in a malignant M12 epithelial cell line of prostate tumor significantly inhibited cell growth. This effect was lost with the addition of des(1–3) IGF-I, an analogue of IGF-I with very low affinity for IGFBPs. These data further strengthen the concept that IGFBP-4 is a powerful functional antagonist of IGF-I action in vivo by presumably decreasing IGF-I availability for its receptor.

Several growth factors have been shown to regulate IGFBP-4 transcript and protein levels. IGF-I itself has been reported to produce no effects or a small decrease in IGFBP-4 mRNA levels in several cell lines. Other growth factors, such as platelet-derived growth factor\(^2\) and fibroblast growth factor, have been shown to increase IGFBP-4 production in RASMs. Cochik et al.\(^3\) have shown that epidermal growth factor or transforming growth factor-\(\beta\) have no effects on IGFBP-4 production in porcine VSMCs. In another study, McCusker and Clemmons\(^4\) have demonstrated that IGFBP-4 and IGFBP-5 secretion was decreased by thrombin and transforming growth factor-\(\beta\). To our knowledge, the present data are the first documentation that growth factors and, specifically, G-protein–linked receptor agonists may markedly downregulate IGFBP-4 expression in VSMCs.

Ang II is produced locally and is available systematically to the vessel wall by the actions of renin and angiotensin-converting enzyme. Besides being a potent vasoconstrictor, it has been demonstrated to have multiple other effects on the cardiovascular system, such as promotion of VSMC growth, induction of reactive oxygen species production through stimulation of NADH/NADPH oxidase, and activation of proto-oncogenes, such as c-fos. Thus, Ang II plays an important role in the pathogenesis of cardiovascular disorders such as hypertension, atherosclerosis, and restenosis after interventional procedures. We have shown that Ang II–induced mitogenesis of VSMCs is critically dependent on autocrine activation of the IGF-I system.\(^5\) The present data strongly suggest that Ang II, by downregulating IGFBP-4 expression, leads to a marked increase of free IGF-I, which is thus available for stimulation of its receptor. Indeed, we have previously shown that Ang II causes a small increase in the total IGF-I secreted by VSMCs.\(^5\)

Thrombin has a well-established role in the coagulation cascade and has proinflammatory effects as well as mitogenic activity.\(^6\) Thus, it has been suggested that thrombin may have an important role in vascular proliferative responses. After activation of its 7-transmembrane G-protein–coupled cell surface receptor-1, thrombin induces the secretion of various autocrine growth factors, such as platelet-derived growth factor-AA, basic fibroblast growth factor, heparin binding epidermal growth factor, and transforming growth factor-\(\beta\) (reviewed in Reference 40). Hence, thrombin-stimulated VSMC proliferation is delayed and requires the de novo expression of \(\geq 1\) of these autocrine factors.\(^7\) Similar to Ang II,\(^3\) there is evidence that activation of the IGF-I receptor is required for thrombin-induced mitogenesis.\(^4\) Thus, an anti-IGF-I antisemur markedly reduced thrombin-induced DNA synthesis of RASMs, whereas nonimmune serum or an anti–fibroblast growth factor antibody were without effect. Downregulation of IGF-I receptors by antisense phosphorothioate oligonucleotides likewise markedly inhibited the mitogenic effects of thrombin.\(^4\) These data demonstrate that a functional IGF-I and IGF-I receptor pathway is essential for thrombin signaling. Thus, thrombin-induced downregulation of IGFBP-4 may also be critically important in increasing free IGF-I availability and promoting mitogenesis.

In summary, our present data indicate that Ang II and thrombin markedly reduce IGFBP-4 protein and mRNA levels in RASMs. The effects are transcriptionally mediated, and we could not demonstrate a proteolytic mechanism involved in the downregulation of this inhibitory binding protein. Because IGFBP-4 is the most abundant IGFBP secreted by VSMCs, these changes could drastically affect the bioavailability of free IGF-I for binding to its receptor. Thus, exogenous IGFBP-4 blunted the mitogenic effects of thrombin, and an anti–IGFBP-4 antibody potentiated these effects. Regulation of IGF-I, its receptor, and IGFBP-4 by growth factors/cytokines likely plays a critical role in modulating the final vascular growth response to an initial vascular insult. The present study supports the concept of a local vascular IGF-I system that participates in vascular remodeling.

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References


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