Insulin-like growth factor-I (IGF-I) is a potent mitogen involved in normal growth and development. It exerts its effects through both endocrine and autocrine/paracrine mechanisms. This peptide functions essentially as a progression factor, ie, allowing cells rendered competent by exposure to mitogens such as platelet-derived growth factor (PDGF) to progress through G1 to S phase. We have previously demonstrated IGF-I gene expression in cultured endothelial and vascular smooth muscle cells (VSMCs) 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 expression is transiently induced in the medial smooth muscle cell layer of rat aorta after balloon angioplasty. Recently, we have shown that in a high-renin model of hypertension in the rat, IGF-I mRNA increases in the hypertensive aorta. This increase may be mediated by both hemodynamic and humoral factors. Thus, angiotensin II transcriptionally regulates IGF-I expression in VSMCs, and IGF-I is required for angiotensin II to exert its mitogenic effects.

The effects of IGF-I are modulated by a family of specific high-affinity carrier proteins, termed insulin-like growth factor binding proteins (IGFBPs) (reviewed in reference 8). Six IGFBPs have been isolated and their cDNAs cloned. IGFBPs in the pericellular environment have the potential capacity to modulate the local as well as systemic metabolic and mitogenic activities of IGF. They have been described to have both inhibitory and/or stimulatory effects. Porcine VSMCs in culture have been shown to secrete IGFBP-4 and IGFBP-2. Recently, we have demonstrated that rat aortic smooth muscle cells in culture secrete primarily IGFBP-4 and low amounts of IGFBP-3 and a 31-kD IGFBP. The postulated central role of IGFBPs in modulating IGF-I action led us to hypothesize that IGFBPs, like IGF-I, might be regulated in hypertension. The aim of this study was to determine the consequence of the establishment of high-renin hypertension in the rat on IGFBP-4 and IGFBP-3 expression in vascular and extravascular tissues.

Our findings demonstrate a sustained increase in IGFBP-4 mRNA levels in the hypertensive aorta. In marked contrast, abdominal coarctation resulted in a small decrease in IGFBP-4 expression in the abdominal (normotensive) aorta and transiently suppressed IGFBP-4 and IGFBP-3 expression in hepatic tissue. These data indicate that mechanical factors upregulate IGFBP-4 mRNA levels in the vasculature and suggest a unique mechanism whereby induction of this inhibitory
binding protein may modulate the final magnitude of the mitogenic response to IGF-I.

Methods
Experimental Animal Model
Abdominal coarctation was carried out as previously described.6 Male Sprague-Dawley rats (200 to 250 g; Harlan Sprague Dawley Inc, Indianapolis, Ind) were anesthetized with a 1:5 mixture of ketamine and xylazine (20 and 100 mg/kg, respectively). The abdominal aorta was exposed through a left abdominal incision, and the aorta was constricted immediately proximal to the left renal artery with a 4-0 silk suture. The suture was tied around the aorta over a 0.4-mm diameter wire, and the wire was removed. The wound was closed, and all animals received penicillin G as prophylaxis. For sham-operated animals, the aorta was exposed in an identical manner and lifted free from the surrounding tissue proximal to the left renal artery, and a suture was passed around the aorta but not tied. The rats were allowed to recover from anesthesia and were returned to cages for 1, 3, 7, and 14 days. Nonoperated littersmates served as control animals at day 0. Animal experimentation was done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Emory Animal Care and Use Committee.

Hemodynamic Studies and Tissue Processing
Baseline blood pressures were obtained by introducing catheters (polyethylene tubing of 0.038-inch outer diameter; Intramedic) into the right carotid and right femoral arteries in a group of control animals (nonoperated) matched for sex, age, and weight. After 1, 3, 7, and 14 days, the operated animals were evaluated in a similar manner after adequate anesthesia. Pressure was recorded with an RM Dynograph type 511 (Beckman Instruments Inc, Bioanalytical Systems Group). The rats were then killed, and tissue was rapidly removed and frozen in liquid nitrogen. The thoracic aorta was excised from the arch to 1 cm above the coarctation. The abdominal aorta was removed from 0.5 cm below the ligature to 0.5 cm distal to its bifurcation.

Northern Analysis
Quantification of IGFBP mRNA transcripts in normotensive and hypertensive rats was carried out by Northern analysis. Four groups of sham-operated and coarcted rats (four rats per group) were used at each time point. RNA was prepared from pooled flash-frozen tissue using the guanidium isothiocyanate/cesium chloride method.22 RNA was measured by spectrophotometry, and only RNA with a 260/280 optical density ratio of 1.8 to 2.0 was used. RNA purity was further verified by agarose-formaldehyde gel electrophoresis and ethidium bromide staining. For Northern analysis, 10-μg samples of total RNA were size-fractionated by agarose-formaldehyde gel electrophoresis and ethidium bromide staining. For Northern analysis, 10-μg samples of total RNA were size-fractionated by agarose-formaldehyde gel electrophoresis and ethidium bromide staining. For Northern analysis, 10-μg samples of total RNA were size-fractionated by agarose-formaldehyde gel electrophoresis and ethidium bromide staining. For Northern analysis, 10-μg samples of total RNA were size-fractionated by agarose-formaldehyde gel electrophoresis and ethidium bromide staining.

IGF-I Radioimmunoassay and Serum Creatinine Levels
Blood samples from sham-operated and coarcted animals were collected in prechilled tubes and maintained on ice for 2 to 3 hours until coagulation occurred. Serum was collected by centrifugation (1700g, 30 minutes, 4°C). The serum was stored until analysis at −80°C. An acid-ethanol cryoprecipitation method was used to separate IGFBP s.24 The recovery of 125I-labeled IGF-I using this method was 46±1% (n=4). Extracted sera were assayed for IGF-I immunoreactivity with a polyclonal anti-IGF-I rabbit antiserum kindly provided by the National Hormone and Pituitary program of the National Institute of Diabetes and Digestive and Kidney Diseases. A double-antibody technique was used to precipitate bound counts using goat anti-rabbit antiserum and normal rabbit serum as a carrier. Standard curves were generated using human recombinant IGF-I (Amgen Biologicals). IGF-I immunoreactivity was expressed as micrograms per liter. Sera from three separate groups of sham-operated and coarcted rats (four rats per group) obtained at 7 and 14 days postoperatively were assayed for creatinine levels. The colorimetric assays were performed with an autoanalyzer by the Division of Animal Resources, Emory University.

Western Ligand Blotting
Sera from sham-operated and coarcted rats were first acidified with an equal volume of 2N acetic acid, incubated at room temperature for 30 minutes, then neutralized by addition of 2N NaOH to obtain a final pH of 6.5 before being mixed with SDS sample buffer and boiled. Western ligand blotting was done according to the method of Hossenlopp et al25 as we have previously described.21 Briefly, the samples were loaded onto a 4% to 15% gradient SDS-polyacrylamide minigel and electrophoresed for 1 hour at 200 V. For some experiments, 16X20-cm 10% polyacrylamide-SDS gels were used (electrophoresed for 1 hour at 16 mA, then 4 hours at 24 mA). After electrophoresis, proteins were transferred to nitrocellulose, and the membrane was dried and sequentially blocked with 3% Nonidet (NP 40), 1% bovine serum albumin, and 0.1% Tween 20 in Tris-buffered saline before incubation with 4X105 cpm/mL of 125I-labeled IGF-I for 16 to 18 hours. Rinsed 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.

Statistical Analysis
All data represent a minimum of three separate experiments and are expressed as mean±SEM. ANOVA was used for time course analysis, and comparisons between groups were performed with a paired Student’s t test when appropriate.

Materials
[α-32P]dCTP (3000 Ci/mmol) and 125I-IGF-I (1630 Ci/mmol) were obtained from Du Pont—New England Nuclear. SDS, NP 40, bovine serum albumin, and Tween 20 were purchased from Sigma Chemical Co. Formamide and formaldehyde were purchased from Sigma Chemical Co. 

Hypertension Vol 24, No 6 December 1994

680

Hypertension Vol 24, No 6 December 1994

680
Hemodynamic Measurements
Abdominal interrenal aortic coarctation resulted in a rapid hypertensive response. Fig 1 summarizes mean arterial pressures determined by simultaneous recording of carotid and femoral pressures. Mean carotid arterial pressure in coarcted rats increased by 12±10% at day 1, 28±10% at day 3, and 55±3% at day 14 compared with sham. Femoral arterial pressures decreased acutely after coarctation and then returned gradually toward baseline. At 14 days, femoral pressure in the coarcted animals was similar to carotid pressure in the sham-operated animals. Sham-operated paired littermates had no significant change in blood pressure as measured up to 14 days postoperatively. Carotid and femoral pressures were not significantly different in sham-operated animals, and these pressures did not differ significantly from those of control (nonoperated) animals.

Regulation of Vascular IGFBP-4 and IGFBP-3 mRNA Levels
As indicated in Fig 2, top, Northern analysis of total RNA from rat aorta using an IGFBP-4 cDNA probe yielded the expected 2.3-kb transcript. Compared with sham-operated thoracic aortas, IGFBP-4 mRNA levels increased rapidly in thoracic aortas from banded rats. Fig 2, bottom, shows results from four independent experiments. In each case, the IGFBP-4 mRNA signal obtained from thoracic hypertensive aortas was corrected for the GAPDH signal and compared with IGFBP-4 mRNA levels (corrected for GAPDH) from identical aortic segments from sham-operated rats. The rapid increase in IGFBP-4 mRNA in hypertensive thoracic aortas reached a plateau by 3 days (2.5-fold increase) and persisted for at least 14 days. In striking contrast, aortic constriction did not significantly elevate IGFBP-4 mRNA levels in the abdominal aorta; rather, there was a gradual small decrease compared with sham-operated abdominal aortas. IGFBP-3 mRNA levels did not change significantly in the thoracic or abdominal aorta after aortic constriction (Fig 3).

Regulation of Hepatic IGFBP-4 and IGFBP-3 mRNA Levels
To determine the tissue specificity of the observed induction of IGFBP-4 expression in hypertensive aortic segments from coarcted rats, we measured IGFBP-4 and IGFBP-3 mRNA levels in livers from sham-operated and coarcted animals. As shown in Fig 4, top, hepatic IGFBP-4 mRNA levels decreased transiently in banded animals, with a maximal decrease at 7 days and a return toward baseline at 14 days. Similarly, hepatic IGFBP-4 mRNA levels decreased transiently in banded animals. The
results from four independent experiments are shown in Fig 4, bottom, in which the percent change in hepatic IGFBP-4 and IGFBP-3 mRNA levels (compared with sham-operated rats) is shown. As is evident, aortic coarctation suppressed hepatic IGFBP-3 and IGFBP-4 mRNA levels by approximately 50% at day 7 compared with sham-operated rats, and this effect had reversed by 14 days.

**Western Ligand Analysis of Circulating IGFBPs**

Analysis of rat serum by Western ligand blotting using $^{125}$I-IGF-I detected multiple bands (Fig 5). A triplet at 40 to 50 kD represents IGFBP-3, which we have confirmed by immunoblotting using anti-human IGFBP-3 antiserum (Upstate Biotechnology Inc, results not shown). There is a weak 32-kD band, a stronger 30-kD band consistent with IGFBP-2 as identified by Gargosky et al in rat serum, and a 24-kD IGFBP consistent with IGFBP-4. Densitometric analysis of four independent experiments at 1, 3, 7, and 14 days (results not shown) demonstrated no significant variation in circulating IGFBPs after aortic coarctation.

**Serum IGF-I and Creatinine Levels**

Measurements of circulating IGF-I (Fig 6) indicated that there was a small decrease in coarcted compared with sham rats that was of borderline statistical significance ($P=.08$, $n=4$). Serum creatinine levels were not significantly different between coarcted or sham-operated animals at 7 days (coarcted, 0.9±0.0 mg/dL; sham, 0.73±0.03 mg/dL) or at 14 days (coarcted, 0.67±0.07 mg/dL; sham, 0.67±0.07 mg/dL).

**Discussion**

Hypertension is a major risk factor for cardiovascular disease, leading to a substantial rate of morbidity and mortality. Although several peptide growth factors have been postulated to be important in the initiation and progression of vascular growth and remodeling in hypertension, there is little information on the involvement of growth factors in hypertensive VSMC growth in vivo. IGF-I is mitogenic for many cell types and plays a crucial role in mammalian growth. We and others have demonstrated an important role for this peptide in VSMC proliferative responses in vitro. This increase in IGF-I mRNA is followed by a decrease in IGF-I receptor transcript levels, consistent with ligand-induced receptor down-

![Fig 4. Northern blot and line graph show hepatic insulin-like growth factor binding protein-3 and -4 (IGFBP-3 and IGFBP-4) mRNA levels after aortic coarctation. Top, Representative Northern analysis. Total RNA (20 μg per lane) from livers of coarcted (B) and sham-operated (S) rats at different time points was cohybridized to an IGFBP-3 cDNA probe and GAPDH probe. Bottom, Summary of densitometric analysis of changes in hepatic IGFBP-3 and IGFBP-4 mRNA levels after aortic coarctation. Shown is the percent change in IGFBP-3 and IGFBP-4 mRNA levels in livers from coarcted compared with sham-operated rats at different time points (mean±SEM, $n=4$). *$P<.05$.](image)

![Fig 5. Western ligand blot shows analysis of rat serum. Serum from coarcted (B) and sham-operated (S) rats at 7 days was electrophoresed and subjected to Western ligand blotting as described in "Methods." Sizes of prestained molecular weight markers are indicated. 0.73±0.03 mg/dL or at 14 days (coarcted, 0.67±0.07 mg/dL; sham, 0.67±0.07 mg/dL).](image)

![Fig 6. Line graph shows serum insulin-like growth factor-I (IGF-I) levels. Serum IGF-I levels from sham and coarcted animals at different time points were measured as described in "Methods" (mean±SEM, $n=4$).](image)
As IGF-I is complexed to specific high-affinity carrier proteins within identical aortic segments from sham-operated or abdominally coarcted animals, this induction of IGF responsiveness at the cellular level. Thus, induction of IGF-I in the thoracic tissues of the abdominally coarcted rat is unlikely to account for the transient decrease in hepatic IGFBP-4 and IGFBP-3 mRNA levels in coarcted rats, consistent with prior studies demonstrating preserved overall renal function in this model. A potential explanation for the reduction in hepatic IGFBP mRNA levels in coarcted rats is that stimulation of the renin-angiotensin system resulted in inhibition of hepatic IGFBP-4 and IGFBP-3 expression. Indeed, we have previously determined that plasma renin activity increases maximally at 7 days after coarctation and normalizes by 14 days, thus paralleling the change in hepatic IGFBP-4 and IGFBP-3 mRNA levels. Furthermore, recent data from our laboratory indicate that angiotensin II decreases IGFBP-4 mRNA levels and IGFBP-3 secretion from cultured rat aortic smooth muscle cells. It is not likely that the transient decrease in hepatic IGFBP-3 and IGFBP-4 mRNA levels reflected nutritional changes because we have previously shown that aortic coarctation does not alter weight gain in those animals. Although the decrease in hepatic IGFBP mRNA levels in coarcted rats compared with shams was not reflected in changes in circulating IGFBPs, as determined by Western ligand blotting, this method is of limited reliability in quantitating IGFBPs. We have thus not ruled out potential effects of aortic coarctation on the translation of and/or posttranslational modification of IGFBPs nor on changes in the clearance of these proteins. Measurements of circulating IGF-I demonstrated a small decrease after coarctation that was of borderline statistical significance (P = .08).

Limited data are available on vascular expression of growth factors in animal models of hypertension. Thus, Sarzani et al, using a deoxycorticosterone acetate–salt model of hypertension in the rat, found no induction of PDGF-α-chain and β-chain, FGF, endothelial cell growth factor, IGF-I, and IGF-II but did demonstrate a significant increase in TGF-β transcripts in the hypertensive vascular wall. The same group showed an increase in PDGF-β receptor mRNA in this model. It is possible that the absence of changes in IGF-I transcript levels in this model was secondary to an inhibitory effect of the mineralocorticosterone. In the abdominal coarctation model of hypertension, IGF-I mRNA is clearly induced in the hypertensive aorta, as we have previously shown.

In summary, our present data indicate that abdominal coarctation in the rat is accompanied by a transient decrease in hepatic IGFBP-4 and IGFBP-3 expression, no change in circulating IGFBPs (as detected by Western ligand blotting), and a rapid and sustained increase in IGFBP-4 mRNA that is restricted to the hypertensive aorta. Further studies, currently underway, are needed to establish correlations between IGFBP-4 mRNA and protein levels in normotensive and hypertensive blood vessels and to characterize the distribution of IGFBP-4 mRNA transcripts in the vasculature. Our current findings support the concept of a local vascular IGF-I system that participates in hypertensive vascular remodeling.

Acknowledgments

This work was supported by National Institutes of Health grants HL-47035, HL-45317, and DK 45215; a grant from the American Heart Association, Georgia Affiliate (to P.D.); and a
References


Downloaded from http://hyper.ahajournals.org/ by guest on June 4, 2017


45. Lindner A, Reidy MA. Proliferation of smooth muscle cells after vascular injury is inhibited by an antibody against basic fibroblast growth factor. Proc Natl Acad Sci USA. 1991;88:3739-3743.


Hypertension increases insulin-like growth factor binding protein-4 mRNA levels in rat aorta.
A Anwar and P Delafontaine

Hypertension, 1994;24:679-685
doi: 10.1161/01.HYP.24.6.679

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/24/6/679

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/