Abdominal Coarctation Increases Insulin-Like Growth Factor I mRNA Levels in Rat Aorta

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We have previously demonstrated specific insulin-like growth factor I (IGF I) mRNA transcripts in cultured endothelial and vascular smooth muscle cells and postulated an important role for IGF I in blood vessel growth responses. The purpose of this study was to characterize IGF I gene expression in a model of aortic coarctation hypertension in the rat. This high-renin model of hypertension is associated with hyperplastic vascular responses. Northern analysis of rat aorta demonstrated four specific IGF I mRNA transcripts sized 7.6, 4.6, 1.8, and 0.9-1.2 kb. Quantitation of aortic IGF I mRNA levels by solution hybridization/RNase protection assay demonstrated induction of IGF I transcripts in the hypertensive aorta; levels more than doubled at 7 days and were still significantly elevated 21 days after coarctation. In situ hybridization analysis indicated that IGF I transcripts were localized primarily to adventitial surfaces in normotensive aorta, with minimal signal detected over vascular cells. In hypertensive aortas, there was an increase in IGF I transcripts primarily over vascular smooth muscle cells. Thus, vascular IGF I gene expression is induced in this model of high-renin hypertension. IGF I may play an important role in autocrine/paracrine-mediated vessel wall remodeling in hypertension. (Circulation Research 1993;72:271-277)

Key Words • hypertension • insulin-like growth factor I • somatomedins • growth substances
• gene expression regulation

Insulin-like growth factor I (IGF I) is a circulating and locally produced protein that is involved in normal growth and development. Synthesis of IGF I is growth-hormone dependent, and the peptide exerts its effects through both endocrine and autocrine/paracrine mechanisms. We have demonstrated IGF I gene expression in cultured endothelial and vascular smooth muscle cells and have hypothesized that this mitogen may play a key role in the intrinsic growth program of the blood vessel wall. Indeed, prior data have indicated that IGF I is an important mitogen for vascular smooth muscle cells and endothelial cells in vitro. The peptide functions essentially as a progression factor as defined by Stiles et al., who demonstrated that quiescent BALB/c-3T3 cells exposed to platelet-derived growth factor (PDGF) became competent to enter the cell cycle but that progression through G1 into S phase required the presence of IGF I.

Our data have indicated that IGF I mRNA levels are increased in quiescent aortic smooth muscle cells exposed to serum or to mitogens contained in serum such as PDGF, consistent with a role for IGF I as an autocrine growth factor for vascular cells. A prior report has indicated an increase in immunoreactive IGF I in rat femoral artery exposed to an increase in vascular load. To better characterize the role of IGF I in the vasculature in vivo, and in particular to study a possible relation between IGF I gene expression and an increase in blood pressure, we have used a model of rat abdominal aortic coarctation hypertension. This model has been shown to be associated with hyperplastic growth responses of vascular smooth muscle and endothelial cells. Our findings indicate a low level of IGF I gene expression in adult rat aorta basally and an induction of IGF I mRNA transcripts in the hypertensive aorta. This induction is localized primarily to the vascular smooth muscle cell layer as detected by in situ hybridization. These findings provide evidence for an important role for IGF I in autocrine/paracrine-mediated vessel wall growth responses in this model of high-renin hypertension.

Materials and Methods

Aortic Banding

Abdominal aortic coarctation was carried out as described by Stanek et al with modifications. Male Sprague-Dawley rats (200–250 g) were anesthetized using a ketamine (87 mg/kg) and xylazine (13 mg/kg) mixture. The abdominal aorta was exposed via a left lateral abdominal incision, and constriction of the aorta was created immediately proximal to the left renal artery using 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 the animals received penicillin/gentamicin prophylaxis. For
sham-operated animals, the aorta was exposed in an identical manner, and the aorta was lifted free from the surrounding tissue proximal to the left renal artery but not ligated. The rats were allowed to recover from anesthesia and were returned to single cages for 7–21 days. Baseline blood pressures were obtained by introducing catheters into the right carotid and right femoral arteries of a control (nonoperated) group of rats of the same age, sex, and weight. After 7, 14, and 21 days, the banded and sham-operated rats were evaluated with femoral and carotid catheters. The animals were then killed and were immediately perfused via the left ventricle with phosphate-buffered saline followed by ice-cold 4% paraformaldehyde. Thoracic aortas from the arch to 1 cm above the coarctation was removed and immediately processed for in situ hybridization. Aortas to be used for Northern analysis and solution hybridization/RNase protection assays were removed rapidly after death and frozen in liquid nitrogen. All aortas processed included the adventitial layer.

**Northern Hybridization**

RNA was prepared from flash-frozen pooled aortas using the guanidium isothiocyanate/cesium chloride method and enriched in polyadenylated (A+) RNA by passage on a oligothymidilic acid cellulose column. Total RNA (10 μg) and A+ RNA (30 μg) were denatured with dimethyl sulfoxide/glyoxal and separated by electrophoresis on a 1% agarose gel before transfer to nitrocellulose or nylon (Genescreen Plus) filters. Filters were prehybridized at 65°C for 5 hours and then hybridized for 24 hours in a solution containing 2.5× Denhardt’s solution, 1 M NaCl, 2 mM EDTA, 0.1% sodium pyrophosphate, 1% sodium dodecyl sulfate, 100 μg/ml denatured herring DNA, and 106 cpm/ml heat-denatured IGF I cDNA probe. The IGF I cDNA was kindly provided by Dr. G.I. Bell, Howard Hughes Medical Institute, University of Chicago, and is random-primed using [32P]dCTP. After hybridization, the filters were washed with 2× standard saline citrate at room temperature, with 2× standard saline citrate+1% sodium dodecyl sulfate at 65°C, and finally for 1 hour in 0.1× standard saline citrate at 65°C before autoradiography.

**Solution Hybridization/RNase Protection Assay**

Quantitation of aortic IGF I mRNA transcripts in the normotensive and hypertensive rats was carried out using solution hybridization/RNase protection assays. Thoracic aortic sections from the arch to 1 cm above the constriction were removed from sham-operated and coarcted animals. Five groups of sham-operated or coarcted rats (three rats per group) were used at each time point. Total RNA was prepared from pooled aortas by the guanidium isothiocyanate/cesium chloride method. Total RNA (40 μg) was hybridized with a 32P-labeled rat exon 3 IGF I riboprobe overnight in a formamide hybridization buffer at 42°C. The plasmid was kindly provided by Dr. P. Rotwein, Washington University School of Medicine. The hybridized RNA was then exposed to RNase A and RNase T1 for 1 hour and run on a 6% sequencing gel. The 965-bp IGF I sequence gives a 182-bp protected band after RNase digestion. As a control for RNA loading and for determination of the specificity of observed changes in IGF I mRNA levels, hybridizations included a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobe. This probe gave a 248-bp protected band after RNase digestion. After autoradiography, protected bands were quantitated by two-dimensional laser densitometry. All changes in IGF I mRNA levels were normalized for the GAPDH signal.

**In Situ Hybridization**

Localization of IGF I mRNA transcripts in vascular tissue was carried out using in situ hybridization. Aortas from four sham-operated and four banded animals were removed after perfusing the aorta with chilled 4% paraformaldehyde at mean arterial pressure before removal. After removal, the aortas were fixed for 1 hour in paraformaldehyde, dehydrated in serial alcohols, and embedded in paraffin. Sections (5 μm) were cut and placed on lysine-coated glass microscope slides. Sections were hybridized with a 35S-labeled exon 3 IGF I riboprobe overnight at 55°C. Slides were then exposed to RNase, followed by a highly stringent 0.1× standard saline citrate wash at 55°C. Slides were then dipped in liquid emulsion and developed after 21 days. This technique allowed localization of IGF I mRNA in the normotensive and hypertensive rat aortas.

**Results**

Abdominal aortic coarctation resulted in a rapid hypertensive response. Mean arterial pressures determined by carotid pressure recordings from anesthetized sham-operated and banded rats are summarized in Figure 1. Sham-operated paired littermates had no significant change in blood pressure as measured up to 21 days postoperatively. Mean arterial pressure in coarcted rats increased by 33% at 7 days and continued to increase gradually over the 21-day period. The weight gain in banded animals did not differ significantly from sham-operated or control (nonoperated) animals (not shown).

Northern analysis of rat aortic RNA demonstrated the presence of four specific IGF I mRNA transcripts as seen in Figure 2, left panel. IGF I mRNA was only detectable when using A+ RNA with autoradiography.
performed for 1–3 days. To quantitate IGF I mRNA levels, we used a highly sensitive solution hybridization/RNase protection assay previously described in our laboratory. As shown in Figure 2, right panel, hybridization of rat aortic total RNA to a ³²P-labeled IGF I exon 3 riboprobe yielded the predicted 182-bp protected band after RNase digestion. Laser densitometry of the autoradiographic signals was used to quantitate IGF I mRNA levels. A riboprobe complementary to the GAPDH sequence was used as an internal control. Levels of IGF I mRNA were normalized for the GAPDH signal.

As illustrated in Figure 3, there was more than doubling of IGF I mRNA levels in hypertensive aortas at 7 days when compared with aortas from sham-operated animals (p<0.001). IGF I mRNA levels in hypertensive aortas then gradually decreased but were still significantly elevated 21 days after induction of hypertension.

Because aortic tissue represents a heterogenous cell population, in situ hybridization analysis was performed to localize IGF I mRNA and to confirm the result demonstrated by solution hybridization/RNase protection analysis. Emulsion autoradiography of sections hybridized to a ³²P-labeled rat IGF I antisense exon 3 riboprobe demonstrated grains localized primarily over adventitial surfaces in aortas from normotensive sham-operated animals (Figure 4A). In hypertensive aortas, there were multiple transcripts detected over the smooth muscle cell layer. Grains were again visible over the adventitia, particularly over adventitial vessels (Figure 4B). Only rare grains were visible over sections of aorta that were hybridized to a sense exon 3 riboprobe, indicating minimal nonspecific binding (Figure 4C).

**Figure 2.** Left panel: Northern analysis of insulin-like growth factor I (IGF I) mRNA. Ten micrograms total RNA (T) and 30 μg polyadenylated enriched RNA (A+) from pooled normotensive rat aortas were hybridized to a ³²P-labeled rat IGF I cDNA probe. Autoradiographic analysis revealed four distinct transcripts, and their sizes are indicated in kilobases (measured from the positions of 28S and 18S RNA). Right panel: Solution hybridization/RNase protection assay. Forty micrograms total RNA extracted 7 days postoperatively from pooled aortas of sham-operated (C) and banded (B) animals was hybridized to [³²P]UTP-labeled IGF I and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antisense riboprobes. After RNase digestion, samples were analyzed by denaturing gel electrophoresis and autoradiography. Protected bands were quantitated by laser densitometry.

**Discussion**

Hypertension results in arterial hypertrophy and medial thickening. Mechanisms involved in the initiation and progression of vascular growth and remodeling in hypertension are poorly understood. We have hypothesized that local induction of IGF I in the blood vessel wall may be important in the pathogenesis of hypertensive vascular disease. Our data indicate an induction of IGF I expression in the hypertensive aorta after abdom-
Figure 4. Photomicrographs showing representative in situ hybridization analysis of insulin-like growth factor I mRNA in aortic coarctation hypertension. Normotensive aortas (panel A) and hypertensive aortas (panel B) were fixed and paraffin-embedded, and sections were hybridized using a $^{35}$S-labeled antisense insulin-like growth factor I exon 3 riboprobe. After RNase digestion, sections underwent emulsion autoradiography and were counterstained with hematoxylin and eosin and photographed using a combination of epiluminescence and transmitted light. White grains indicating a positive signal are visible in vascular smooth muscle cells in the hypertensive aorta as well as over the adventitial surface. There is scant hybridization to vascular cells in the normotensive aorta, but grains are visible over the adventitia. Hybridization of hypertensive aorta using a sense riboprobe (panel C) indicates minimal nonspecific signal. Magnification, ×187.5.
inal aortic coarctation, a high-renin model of hypertension. This induction appears to be specific, since IGF I mRNA levels were normalized for GAPDH expression.

Observations characterizing growth factor expression in hypertension are limited. Sarzani et al.,14 using a deoxycorticosterone acetate–salt (DOCA-salt) model of hypertension, found no induction of either PDGF, IGF I, IGF II, or basic fibroblast growth factor but did demonstrate a significant increase in transforming growth factor-β mRNA levels in hypertensive aortas. Their model differs from the one used in our study in that renin levels are suppressed. Furthermore, the absence of changes in IGF I expression in the DOCA-salt hypertensive model could be related to the mineralocorticoid, since corticosteroids have been shown to decrease IGF I gene expression in vivo.15 It is of note that recent data from the same group have indicated a threefold increase in aortic steady-state PDGF β-receptor mRNA levels in the DOCA-salt model of hypertension.16 Majesky et al17 have recently shown that acute α-adrenergic stimulation in the rat induces aortic PDGF A-chain expression. Preliminary data from Donohue et al18 have shown induction of left ventricular IGF I mRNA levels in low-, moderate-, and high-renin models of hypertension. However, localization of cardiac IGF I transcripts to either cardiomyocytes or vascular cells was not described. Additionally, aortic IGF I mRNA levels were not characterized. Our group has recently demonstrated, in a model of rat ascending aortic banding, an increase in IGF I mRNA levels in the pressure-overloaded left ventricle.19 These findings are compatible with a role for hemodynamic factors in the induction of local IGF I expression in the cardiovascular system. Indeed, preliminary work from our group has shown an increase in IGF I release from cultured endothelial cells exposed to an increase in hydrostatic pressure.20 The relation between hemodynamic parameters and IGF I homeostasis is further substantiated by data from Hansson et al21 indicating increases in IGF I immunoreactivity in the rat femoral artery subjected to an increase in vascular load. It is important to note that our model does not permit definition of exact hemodynamic parameters that may be important in induction of IGF I expression with hypertension. Indeed, a contribution of changes in flow in the thoracic aorta to observed changes in IGF I expression is possible, in view of the 16–22% increase in cardiac index at 4 weeks described by Stanek et al20 in this model.

IGF I circulates at high levels in plasma, where it serves primarily an endocrine function. However, expression of IGF I at the mRNA and protein level has been demonstrated in multiple tissues, consistent with an autocrine/paracrine function for this growth factor (reviewed in Reference 21). The purpose of this study was to determine the possible role of IGF I in autocrine/paracrine–induced vascular remodeling with hypertension. Aortic IGF I protein levels were not determined, but these have previously been shown to be extremely low in quiescent adult rat femoral artery.7 Because tissue injury has been associated with major increases in IGF I immunoreactivity in endothelium and vascular cells,22 our model used thoracic aorta sectioned at least 1 cm above the constriction, well above any detectable granulation tissue. These aortas were compared with identical aortic sections obtained from sham-operated normotensive rats. Aortic sections from below the constriction site were not sufficiently removed from granulation tissue to allow meaningful comparison to thoracic aortic sections. Our findings of a specific increase in aortic IGF I expression with hypertension raises the possibility that this growth factor plays a role in autocrine/paracrine–mediated vascular growth. Because the biological effects of IGF I are greatly modulated by circulating and cell-attached binding proteins (reviewed in Reference 23), it will be important to determine changes in vascular IGF binding protein expression with hypertension.

IGF I has been found to be an important comitogen for vascular smooth muscle cells, acting synergistically with competence growth factors such as PDGF.24 Our laboratory has recently demonstrated induction of IGF I gene expression in quiescent smooth muscle cells exposed to serum or to PDGF.6 We have hypothesized that IGF I plays a central role in autocrine/paracrine–mediated vascular growth, particularly in response to growth factors such as PDGF that may be synthesized locally in the blood vessel wall or released from aggregating platelets. Thus, it is possible that one of the mechanisms implicated in the induction of IGF I expression in the hypertensive aorta may involve increases in vascular PDGF mRNA levels. Another important potential modulator of vessel wall growth, angiotensin II,25 has also recently been shown to induce aortic PDGF receptor expression.16 Because the aortic coarctation hypertension model is associated with high renin levels, it is possible that angiotensin II contributes to the regulation of aortic IGF I mRNA levels. Indeed, preliminary work from our laboratory has shown that angiotensin II increases IGF I mRNA levels and release from quiescent cultured vascular smooth cells.26

In situ hybridization analysis of the hypertensive aortas indicates preferential induction of IGF I gene expression in vascular smooth muscle cells. It is of note that, in the same model of hypertension, Owens and Reidy6 have demonstrated marked increases in endothelial and vascular smooth muscle cell turnover rates. This hyperplastic response was accompanied by morphological signs of endothelial injury. One may thus speculate that induction of IGF I gene expression in this hypertensive model is mechanistically related to the increases in IGF I mRNA levels and IGF I protein levels present in various models of mechanical arterial injury in the rat.22,27,28

In summary, we have shown that IGF I mRNA transcripts in adult rat aorta are localized primarily to adventitial surfaces and are scarce in quiescent vascular cells. Induction of hypertension by abdominal aortic coarctation increases vascular cell IGF I mRNA levels, providing evidence for an important role for IGF I in autocrine/paracrine–mediated vessel wall growth responses. Mechanisms implicated in IGF I gene induction in this model may be multiple and include direct hemodynamic effects as well as neurohumoral regulation of IGF I expression, in particular through the renin-angiotensin system. Our laboratory is currently focused on studying the role of IGF I in various models of hypertension and in various vascular beds to better define the role of this important mitogen in developmental and abnormal growth in the cardiovascular system.
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References

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