Effect of Hypertension on Fibronectin Expression in the Rat Aorta*

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Interactions between extracellular fibronectin and vascular cells are thought to influence the phenotype of those cells. To determine if changes in fibronectin expression accompany the phenotypic changes of vascular tissue characteristic of experimental hypertension, steady state mRNA levels for fibronectin were determined in aortas of normotensive and hypertensive rats. A 3-6-fold increase in fibronectin mRNA was observed in aortic tissue of hypertensive rats following 3 weeks of treatment with deoxycorticosterone and salt, whereas if rats were treated only with deoxycorticosterone or salt alone, no changes occurred. The changes were reversed by normalization of blood pressure. The increases observed were localized to aorta and not to the periaortic tissue. Angiotensin II infusion using osmotic minipumps also caused an increase in fibronectin expression. Age-dependent increases in aortic fibronectin mRNA occurred in several rat strains, and the combined effects of hypertension and aging were greater than either variable alone. A clear distinction between the expression of fibronectin mRNA and that for collagen or tropoelastin were found in hypertensive and aging models. Aortic fibronectin was also increased in the hypertensive rats as determined by Western blot analysis. The findings indicate that elevation in blood pressure increases fibronectin expression in rat aorta and suggest that such changes may influence the aortic cellular responses to hypertension.

Fibronectin is a widely distributed glycoprotein localized in the extracellular matrix of various cell types and has been implicated functionally in the regulation of several cellular processes, including adhesion, differentiation, motility and transformation (reviewed in Refs. 1 and 2). In vascular tissue, fibronectin has been demonstrated immunohistochemically in aorta and other large vessels (3-5), but the insoluble nature of the extracellular matrix has made quantitation difficult (6-8). Interactions between fibronectin and cultured vascular smooth muscle cells induced a phenotypic change from a contractile to a synthetic state, and it has been suggested that the cellular changes that occur during atherosclerosis may be due to fibronectin-cell interactions (9). A recent study has shown that alternatively spliced variants of fibronectin are present in aortic tissue, and that following vascular injury, proliferating smooth muscle cells switch from one variant to another (10). This phenomenon was observed using both tissue culture and in vivo animal models (10).

Transforming growth factor-β (TGF-β) has been shown to influence fibronectin expression in fibroblasts and endothelial cells, and it was suggested that several effects of TGF-β on cellular growth were mediated by changes in fibronectin production (11-15). We reported recently on the increased steady state mRNA level of TGF-β in rat aorta associated with experimental hypertension induced by treatment with deoxycorticosterone and excess salt (16). The present study was designed to determine if changes in fibronectin expression occurred following experimental hypertension in the rat.

EXPERIMENTAL PROCEDURES

Experimental Animal Models (i) Deoxycorticosterone/salt hypertension and related control groups. Male Wistar rats weighing 250-300 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were uninephrectomized at 10 weeks of age and a subcutaneous pellet (Innovative Research of America, Toledo, OH) containing 100 mg of deoxycorticosterone acetate (DOC) was implanted 1 week following uninephrectomy. Unless otherwise indicated, animals were given 0.9% NaCl as drinking water. Control treatments included uninephrectomy alone, uninephrectomy plus DOC implant but with tap drinking water and low sodium diet (0.4% NaCl; Teklad, Madison, WI), DOC implant alone, and uninephrectomy without DOC implant but with 0.9% NaCl as drinking water. (ii) Angiotensin II infusion. Male Wistar rats weighing 250-300 g (Charles River Breeding Laboratories, Inc., Wilmington, MA) were made hypertensive by subcutaneous infusion of angiotensin II (125-153 ng/min) with an Alzet model 2001 or 2002 osmotic minipump (Alza, Palo Alto, CA). Control animals had a minipump implanted that contained only the vehicle. (iii) Spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) were purchased from Taconic Farms, Germantown, NY. (iv) 10-week-old and 40-week-old male Wistar rats were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA. (v) 5-week-old and 100-week-old Fischer rats were obtained from the National Institutes of Health, Bethesda, MD.

Systolic blood pressure was measured by tail cuff plethysmography and a photoelectric cell detector as described previously (17). Blood pressure measurements were made at frequent intervals, and the reported values were averages of values obtained 1 day prior to killing the rat. Animals were killed by an overdose of pentobarbital, and the aortae were dissected, rapidly cleaned of periaortic tissue, and immediately frozen in liquid nitrogen.

RNA Isolation and Analysis—Total RNA from aortic tissue was extracted using the guanidinium thiocyanate/cesium chloride centrifugation method (18). RNA concentration was determined by UV spectrophotometry, and 20 μg of total RNA was separated through 0.8% agarose, 1.4 M formaldehyde gel electrophoresis. Northern blotting to nylon membranes (GeneScreen Plus, Du Pont-New England Nuclear) was performed as described previously (16), and hybridiza-

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‡ The abbreviations used are: DOC, deoxycorticosterone acetate; SDS, sodium dodecyl sulfate; TGF-β, transforming growth factor-β; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.
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RESULTS

The DOC/salt model of experimental hypertension in the rat involves uninephrectomy followed by administration of DOC, a steroid with mineralocorticoid activity, and an excess of dietary salt, given by substituting saline for water. Fig. 1A shows a Northern blot analysis for fibronectin using aortic RNA taken from rats treated with or without DOC and salt. Each lane contains 20 μg of total aortic RNA obtained from animals treated as designated for 3 weeks. A, the effect of different treatment regimens on the steady state mRNA level of fibronectin in aorta. UN, uninephrectomy alone; UN/salt, uninephrectomy and administration of 0.9% NaCl (saline) in drinking water; UN/DOC, uninephrectomy, implantation of a DOC pellet, and low sodium diet; DOC/salt, implantation of a DOC pellet alone; UN/DOC/salt, uninephrectomy, implantation of a DOC pellet, and saline-drinking; Regression, 6-week administration of a diuretic (chlorothiazide) and a low salt diet after a 3-week UN/DOC/salt treatment. Mean and S.D. of the systolic blood pressure (SBP) are given in mm Hg. The absence of a strong signal for fibronectin mRNA was seen (UN/DOC/salt). This increase was associated with higher systolic blood pressure (191 mm Hg) than in the other groups, although animals given salt but no DOC (UN/salt) had mild increases in systolic blood pressure (154 mm Hg) but no obvious change in fibronectin expression. The last lane contains RNA from uninephrectomized rats treated with DOC and salt for 3 weeks followed by a period of reversal of hypertension by cessation of DOC/salt treatment and institution of a low salt diet and diuretic therapy (500 mg/liter chlorothiazide included in the drinking water) for 6 additional weeks. The absence of a strong signal for fibronectin mRNA indicates that reversal of DOC/salt treatment was associated with a reversal in the increased levels of fibronectin mRNA.

Fig. 1B contrasts the intensity of the signal for fibronectin mRNA from aortic tissue with that of periaortic tissue and brown adipose tissue, using samples from control and hypertensive animals. Equivalent amounts of total RNA (20 μg) were applied to each lane prior to Northern blot analysis. The strongest signals were obtained using aortic tissue, and the 6-fold increase in aortic fibronectin mRNAs seen following DOC/salt hypertension was clearly greater than the 1.4-fold increase seen in periaortic tissue. Thus the increased steady state mRNA levels can not be accounted for by the relatively small amount of contaminating periaortic tissue that is difficult to completely remove from aortae prior to isolation of RNA.

Steady state mRNA levels for fibronectin, tropoelastin, collagen, and β-actin in aortae of animals made hypertensive by two different models of experimental hypertension, angiotensin II infusion and DOC/salt treatment, are shown in Fig. 2. The data shown are representative of other RNA samples taken from additional rats treated in a similar manner. Lanes 2 and 3 contain RNA from rats treated with subcutaneous...
injection of angiotensin II (133 ng/min) using an osmotic minipump for 3 and 10 days, respectively. Fibronectin mRNA levels were elevated in both cases when compared to a representative control sample taken from rats infused only with vehicle (lane 1). When the RNA was rehybridized using cDNA probes for either tropoelastin or collagen type I, major components of the extracellular matrix, there were no consistent changes in steady state mRNA levels for those substances.

However, fibronectin mRNA was consistently increased in all RNA samples tested, showing a 2.0 ± 0.3-fold increase (mean ± S.E., n = 6) when comparing six separate RNA samples from 17 rats treated with angiotensin II versus four separate RNA samples obtained from 9 control rats. Thus, the increase in gene expression of all components of the extracellular matrix was relatively specific and not associated with a general increase in gene expression of all components of the extracellular matrix.

Fig. 2 (lanes 5 and 6) contains aortic RNA from animals subjected to DOG/salt treatment for 2 and 3 weeks, respectively. Fig. 2 (lane 4) contains RNA from uninephrectomized control rats that were untreated for 2 weeks following uninephrectomy. When hypertension was produced by DOG/salt treatment, there was an increase in steady state mRNA levels for aortic fibronectin. Densitometric analysis comparing five separate RNA samples from 15 DOG/salt-treated rats versus four separate RNA samples from 14 uninephrectomized control rats showed a 5.1 ± 1.5-fold increase (mean ± S.E., n = 5) following 2 and 3 weeks of treatment. When the same RNA samples were rehybridized with cDNA probes for tropoelastin or collagen type I, increased steady state mRNA levels were not seen; rather, there was a decrease in tropoelastin mRNA levels and no obvious change in collagen type I mRNA. In one study using aortic RNA from rats treated with DOG/salt, we did observe a moderate increase in collagen mRNA, but that finding was not consistent whereas we invariably observed increased fibronectin expression.

The increase in fibronectin mRNA levels was also characteristic of a genetic model of hypertension. Fig. 3A shows measurements of fibronectin mRNA in aortic samples from SHR and age-matched WKY. Densitometric comparisons indicated an approximate 2-fold increase in the SHR at all ages past 5 weeks, whereas the youngest group showed a 3.3-fold greater signal in the WKY than the SHR. In both strains, increasing age was associated with an increased signal. Again, when the same RNA samples were hybridized with cDNA probes for collagen or tropoelastin, a clear dissociation between the regulation of steady state mRNA levels between fibronectin and either collagen or tropoelastin was apparent. The steady state mRNA level of β-actin was slightly and

### Table 1

| Age (weeks) | WKY | SHR |
|------------|-----|-----|
| 5          | 115 | 116 |
| 10         | 112 | 113 |
| 20         | 124 | 127 |
| 30         | 144 | 137 |
| 40         | 216 | 187 |
| 50         | 216 | 174 |

**Mean SBP (SD mmHg)**

- **WKY** Mean SBP: 115 ± 1 SD mmHg
- **SHR** Mean SBP: 187 ± 1 SD mmHg

### Fig. 3

**The effect of age and hypertension on the steady state mRNA level for fibronectin in rat aorta.** Each lane contains 20 μg of total RNA. Mean and S.D. of the systolic blood pressure (SBP), the number of rats pooled for each RNA sample, and the statistical significance of the blood pressure difference among age-matched groups are shown at the bottom of each lane. *p < 0.05; **p < 0.01. A, total aortic RNA from different ages (from 5- to 40-week-old) of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) were used for Northern blot analysis for fibronectin, collagen α2(I), tropoelastin, and β-actin. The same nylon membrane was rehybridized for the four different probes. B, total aortic RNA from 5- and 40-week-old Fischer rats (left panel), age-matched (40-week-old) Wistar, WKY, and SHR (middle panel), and 5- and 100-week-old Fischer rats (right panel) were used for Northern blot analysis for fibronectin. kb, kilobases.
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An increase in steady state mRNA and protein for fibronectin in the rat aorta was found following induction of two forms of experimental hypertension. In addition, aortic fibronectin mRNA was shown to increase with age in several rat strains and these age-related changes appeared to be enhanced in the presence of hypertension. It has been suggested that the changes that occur normally in the vessel wall with age are accelerated by experimental hypertension (27, 28). Increased fibronectin expression appeared to be associated with aging in four rat strains studied, and hypertension appeared to accelerate age-related changes further, based on the comparative data between 40-week-old Wistar, WKY, and SHR rats shown in Fig. 3B. The contrast between fibronectin and other major components of the aortic extracellular matrix was most evident when compared in the SHR where the marked increases in fibronectin mRNA were clearly distinguished from the decreased levels of mRNA for either collagen or tropoelastin.

In a recent study, changes in fibronectin expression were documented in cultured vascular smooth muscle cells that were converted from a contractile to synthetic phenotype (10). In addition, the appearance of an alternatively spliced variant of fibronectin was found in vivo in the aortic intima following the induction of intimal lesions by balloon injury in the rat or atherosclerotic lesions in man (10). The in vivo hypertensive models that were used in the present study are known to produce medial changes such as hypertrophy and polyploidy (27, 29, 30). Our observations suggest that the increase in aortic fibronectin mRNA was localized to aorta, and not to the periaortic tissue, which includes adipocytes and several other cell types (31). Other studies on gene expression in vascular tissue showed that pulmonary hypertension induced increased elastin expression in adventitial fibroblasts, perhaps stimulated by a paracrine mechanism originating from vascular smooth muscle (32). Studies on expression of the various components of the renin-angiotensin system in vascular tissue have implicated the periaortic adipocyte as the major producer of angiotensinogen (31).

The mechanism responsible for increased aortic fibronectin expression in hypertensive animals is difficult to assess. TGF-β has been shown to regulate the expression of fibronectin and other components of the extracellular matrix in fibroblasts and aortic endothelial cells (11-15). However, studies with cultured vascular smooth muscle cells have shown that TGF-β does not induce fibronectin (33). In addition to TGF-β, substances shown to influence fibronectin expression in cultured cells include glucocorticoids (34), CAMP (34), interleukin-6 (35), epidermal growth factor (36), platelet-derived growth factor (36,37), glucose (38), heparin (39,39), and tumor necrosis factor (40). Responses to these substances differ markedly depending on the cell type and culture conditions. There is little evidence for any of these agents influencing fibronectin expression in cultured vascular smooth muscle cells and no evidence to date for any hormone or growth factor influencing aortic fibronectin biosynthesis in vivo.

The functional significance of altered aortic fibronectin expression in the hypertensive rat also is difficult to assess at present. Hypertrophy is a common response of vascular smooth muscle to experimental hypertension or to vasoactive agents such as angiotensin II or norepinephrine. An early event in the induction of hypertrophy is thought to be a shift in the cell cycle from G0 to G1, and fibronectin has been implicated as one of the early response genes that may be rapidly expressed when cells are exposed to certain growth

**Fig. 4. Western blot analysis for fibronectin in rat aorta from three different hypertensive models.** Each lane contains 15 μg of protein from the SDS extract of an individual rat aorta (see “Experimental Procedures”). Purified rat plasma fibronectin (100 μg) was used as a reference (FN). Immunodetection was performed using a goat anti-human polyclonal antibody to fibronectin as a first antibody and chemiluminescence emitted from oxidized luminal as a detection method as described under “Experimental Procedures.” Systolic blood pressure (SBP) for the individual animals used is shown at the bottom of each lane. Top panel, Control, uninephrectomized rats. DOC/salt, rats treated with DOC/salt for 21 days. Middle panel, Control, rats with subcutaneous saline infusion for 6 days. AII, rats treated with subcutaneous angiotensin II infusion (125 ng/min) for 6 days. Bottom panel, Wistar, 20-week-old Wistar rats; SHR, 20-week-old spontaneously hypertensive rats.
it is not possible at present to establish with certainty what fibronectin in both physiological and pathological processes, the multiplicity of functions that have been attributed to factors that initiate cell cycle changes (36). However, despite role fibronectin has in vascular tissue of either normotensive or hypertensive animals. The consistent and long-lived intensive rats suggest a possible role for fibronectin in mediating increase in aortic fibronectin mRNA levels in aging or hypertensive conditions, but additional studies concerning the localization of fibronectin in aorta and relative contribution of specific isoforms of fibronectin will be necessary to determine if such a role exists.

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