Hypertension-Induced Changes of Platelet-Derived Growth Factor Receptor Expression in Rat Aorta and Heart

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Hypertension-associated growth of vascular smooth muscle cells might be mediated in vivo by platelet-derived growth factor (PDGF). Our previous investigations in hypertensive rats failed to demonstrate changes in aortic steady-state mRNA levels of PDGF A or B chains. The current studies were performed to determine whether hypertension might affect the expression of PDGF receptors. We studied PDGF α- and β-receptor gene expression by Northern analysis using human and rat cDNA probes. Studies of tissue distribution revealed that PDGF β-receptor mRNA was most abundant in total aorta and aortic media, whereas the PDGF α-receptor mRNA was most abundant in the lung and was expressed at low levels in aortic tissue. Deoxycorticosterone acetate (DOCA)–salt hypertension induced a threefold increase in aortic steady-state PDGF β-receptor mRNA levels. Aortic PDGF β-receptor expression also was higher in spontaneously hypertensive rats (SHRs) when compared with age-matched normotensive Wistar-Kyoto (WKY) controls. Aortic PDGF α-receptor steady-state mRNA levels were unchanged in DOCA-salt hypertension and were expressed at similar levels in WKY rats and SHRs. Unlike the findings with aorta, cardiac PDGF β- and α-receptor and PDGF B-chain expressions were unchanged in the DOCA-salt model and were decreased in SHRs. These findings indicate that hypertension can increase aortic steady-state mRNA levels for PDGF β-receptor. They also indicate that tissue-specific expression of the genes of the PDGF ligand/receptor system are differentially regulated in hypertension. (Hypertension 1991;17:888-895)

Hypertension has been shown to affect growth of vascular cells. Depending on the rat model of hypertension studied, aortic smooth muscle cell (SMC) hypertrophy, polyplody, or hyperplasia may be induced.1-3 Increased medial SMC mass in smaller arteries and in resistance vessels has been documented as well.4 Aortic SMC hypertrophy or hyperplasia are thought to be secondary adaptations to increased blood pressure, but humoral factors and neurotransmitters also have been implicated. Cell culture studies have shown that SMC hypertrophy, in some cases associated with polyplody, can be induced by several contractile agonists, including catecholamines,5 angiotensin II,6 and arginine vasopressin,7 although it is unclear how these agents influence SMC growth.

Recently, a relation between vasoactive agents and growth factors has been observed in cultured SMC. Angiotensin II–treated SMCs express higher levels of platelet-derived growth factor (PDGF) A-chain mRNA,8 and an acute increase in aortic PDGF A-chain mRNA levels has been reported after infusion into rats of high doses of the α1-adrenergic agonists phenylephrine and methoxamine or of angiotensin II.9 Moreover, a specific increase in binding sites for PDGF BB-chain dimers has been found in cultured SMCs stimulated with norepinephrine and angiotensin II.10 PDGF dimers, which also can be produced by endothelial cells,11 induce SMC proliferation as well as SMC chemotaxis and contraction.12 PDGF dimers formed by various combinations of PDGF A and B chains act by binding to PDGF α- and β-receptors that are activated when dimerized in the αα, αβ, or ββ combination.13,14 The α-receptor binds both PDGF chains, whereas the β-receptor binds only the B chain with high affinity.13,14 Recently, it has been suggested that at least some of the different effects of PDGF AA and BB dimers are due to differences in absolute and relative number of PDGF α- and β-receptors.13,15 It has been reported

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that the low and variable mitogenic and chemotactic activity of the AA homodimer in cultured human skin fibroblasts and rat aortic SMCs may be secondary to the lower expression of the α-receptor in these cells. In vivo, immunoreactive PDGF β-receptor has been found to be increased in human carotid plaques, and in situ hybridization studies have shown relatively high localization of the PDGF B chain and PDGF β-receptor in areas of human plaques. Increased PDGF β-receptor steady-state mRNA levels also have been demonstrated recently in monkey thoracic aorta after cholesterol feeding.

Chronic PDGF BB treatment of quiescent post-confluent SMCs for 72 hours in a serum-free medium can induce SMC hypertrophy without hyperplasia. Under these conditions, PDGF may not be mitogenic but may induce cell hypertrophy associated with partial cell-cycle progression.

We have hypothesized that increased vascular expression of growth factors in response to increased blood pressure or neurohumoral mediators may stimulate SMC growth by autocrine or paracrine mechanisms. However, we found that deoxycorticosterone acetate (DOCA)-salt hypertension did not appear to influence the expression of PDGF A and B chains, as well as of several other genes encoding for SMC growth-inducing polypeptides. In the current study, we have extended this work to determine the effects of hypertension on both the receptors and the A and B chains of PDGF.

Methods

Experimental Animal Models and Tissue Preparation

Male Wistar rats weighing 250–300 g were obtained from Charles River Breeding Laboratories Inc., Wilmington, Mass. Ten-week-old spontaneously hypertensive rats (SHRs) and Wistar-Kyoto (WKY) rats were obtained from Taconic Farms, Germantown, N.Y. The rats were uninephrectomized at 10 weeks of age. A pellet containing 100 mg DOCA (Innovative Research of America, Toledo, Ohio) was implanted subcutaneously 1 week after uninephrectomy. Unless otherwise indicated, animals were given 0.9% NaCl as drinking water. Control treatments included uninephrectomy alone, uninephrectomy plus the DOCA implant but with tap drinking water and a low sodium diet (0.4% NaCl; Teklad Premier Laboratory Diets, Madison, Wis.), and uninephrectomy without the DOCA implant but with 0.9% NaCl as drinking water.

To study the effect of the reversal of DOCA-salt hypertension, the treatment was discontinued after 3 weeks of administration of DOCA and saline. The rats were placed on a low salt diet as above and given chlorothiazide (500 mg/l in drinking water) for 6 additional weeks.

Systolic blood pressure was measured as previously described by tail-cuff plethysmography and a photoelectric cell detector. The reported values represent the averages of blood pressure measurements obtained 1 day before the rats were killed. Animals were killed by an overdose of pentobarbital, and the aortas were quickly dissected, cleaned of periadventitial tissue, and frozen in liquid nitrogen. Three to five aortas were pooled for analyses. Some aortas, after removal of periadventitial tissue, were opened longitudinally, and both the intimal and adventitial sides were scraped with a scalpel blade. Microscopic evaluation of these specimens showed complete removal of intima with few adventitial cells adherent to the media. This preparation is referred to as aortic media. Other tissues also were rapidly obtained from the same animals. Cardiac tissue was processed individually without pooling of samples from different animals. Cultured confluent rat aortic SMCs were obtained as previously described.

RNA Extraction and Analysis

Total RNA was extracted as previously described using minor modifications of the guanidinium thiocyanate/cesium chloride centrifugation method. In brief, tissues were homogenized while still frozen. The homogenate was transferred to “Quick Seal” tubes (Beckman Instruments, Inc., Palo Alto, Calif.) of approximately 13 ml containing 6 ml of 5.7 M CsCl, and the tubes were spun at 36,000 rpm in a 70.1 Ti rotor (Beckman Instruments, Inc.) for approximately 18 hours. Ten or 20 μg total RNA as quantified by ultraviolet spectrophotometry was separated by electrophoresis through 0.9%/4.4 M formaldehyde gel containing 0.5 μg/ml ethidium bromide. The gels were photographed routinely to verify the relative quality and quantity of RNA samples. Negatives of gel pictures were scanned at the level of 28S and 18S ribosomal RNA; only samples with a 28S/18S ratio of greater than or equal to 2 were used in the experiments. Northern blotting and hybridization were performed as described except that the hybridization buffer was 6x SSPE (1xSSPE is 0.15 M NaCl, 0.01 M sodium phosphate, 0.001 M Na2EDTA, pH 7.4), 4% sodium dodecyl sulfate, 10% dextran sulfate, 500 μg/ml heparin, and 100 μg/ml sonicated and denatured salmon sperm DNA. After moderate- or high-stringency washings, blots were exposed to preflashed x-ray film (X-Omat, Eastman Kodak Co., Rochester, N.Y.) between two intensifying screens (Cronex lightning plus, Du Pont Co., Wilmington, Del.) for 6 hours to 15 days at −80°C. Developed film was scanned by laser densitometry with an Ultrascan XL (Pharmacia LKB Biotechnology, Piscataway, N.J.) to quantitate the relative signal intensity of the bands. Because of the technical limitation of blot hybridization analysis, we chose to consider as significant those densitometric differences greater than 30% of signal intensity. The kilobase size of the detected mRNAs was calculated on the basis of the migration of the 18S and 28S ribosomal RNA from the gel wells and by comparison with the RNA “ladder” purchased from Bethesda Research Laboratories, Bethesda, Md.
Complementary DNA Probes

The following probes were used in the study: 1) a human PDGF β-receptor cDNA probe of approximately 2.7 kb encoding from AA 43 to AA 925 (American Type Culture Collection (ATCC), Rockville, Md., catalog no. 59735, deposited by R.G.K. Gronwald and D. Bowen-Pope); 2) a rat PDGF β-receptor mRNA probe corresponding to the interkinase domain and portions of the split tyrosine kinase domain (AA 547 to AA 874 of the human PDGF β-receptor cDNA), which we obtained by polymerase chain reaction amplification of rat aortic cDNA using primers complementary to highly conserved regions of the tyrosine kinase domain; 3) two human PDGF α-receptor cDNA probes obtained from S. Aaronson corresponding respectively to the 3' untranslated region (bases 4,704 to 6,413) and to the coding region from the transmembrane domain to the beginning of the 3' untranslated region (bases 1,730 to 3,454); 4) a Psi I-BamHI fragment of 2.0 kb of the pSM-1 clone of human PDGF B-chain (c-sis proto-oncogene) cDNA (ATCC catalog no. 57051, deposited by R. Gallo) containing the entire coding region; 5) a PDGF A-chain human cDNA probe of approximately 1.3 kb and a rat β-actin cDNA probe of approximately 1.5 kb, obtained as described previously; and 6) rat β-actin cDNA or human glyceraldehyde-3-phosphate dehydrogenase cDNA of approximately 1.2 kb (ATCC catalog no. 57091, deposited by R. Wu), used to detect the expression of “housekeeping” genes to validate the differences in hybridization signal intensities.

Similar results were obtained with the use of either the human or the rat PDGF β-receptor cDNA probes. However, the rat probe required blot washings at higher stringency and shorter autoradiographic exposure times than the human probe.

Results

The relative abundance of the steady-state mRNA of PDGF β- and α-receptors and their ligands in various tissues is shown in Figure 1A. Different levels of expression were seen in these tissues, although it should be remembered that the data obtained are based on the relative abundance of a given mRNA per 20 μg of total RNA used in the Northern blots rather than the total amount in the tissue. Unexpectedly, the most abundant PDGF β-receptor expression was in total aortic tissue and in the aortic media preparation. Total RNA extracted from adherent periadventitial tissue contained only negligible concentrations of PDGF β-receptor mRNA (approximately 10-fold less than in aortic tissue). The lung had about one half the aortic PDGF β-receptor mRNA levels, and the heart had about one fourth. Cultured confluent aortic rat SMC expressed even higher levels than those found in intact aortic tissue, but other tissues rich in “visceral” SMC, such as from the uterus, stomach, and bladder, expressed much lower levels of PDGF β-receptor than aorta (Figure 1B). Both human and rat PDGF β-receptor cDNA probes detected mRNA of approximately 3.6 kb in accordance with previously reported findings in humans, monkeys, and mice.

PDGF α-receptor expression was analyzed using two human cDNA probes that weakly hybridized with a 6.7 kb mRNA that generally was expressed at low levels, sometimes requiring exposures for up to 15 days to detect the specific signal. PDGF α-receptor was most abundant in lung (Figure 1A) and uterus (Figure 1B), whereas it was expressed at the lowest levels in aortic tissue and liver.

The expression of the genes encoding for the PDGF ligands also was studied (Figures 1A and 1B). PDGF B-chain mRNA of approximately 3.3 kb was most abundant in the lung and present in aortic tissue as well as in periadventitia. PDGF A-chain transcripts of 2.9, 2.3, and 1.75 kb were most abundant in lung, aortic tissue, and media and not detectable in periadventitia. In brain tissue, a large amount of a 2.2 kb PDGF A-chain mRNA was detected. PDGF B chain was expressed at low levels in the heart, whereas PDGF A-chain transcripts were almost undetectable there.

Aortic PDGF β-receptor steady-state mRNA levels increased somewhat after 2 weeks of DOCA-salt administration and by 3 weeks of treatment were approximately threefold greater than the levels observed in age-matched uninephrectomized controls (Figure 2A). The levels in periadventitia were low and were not affected appreciably by DOCA-salt administration (Figure 2B). Administration of DOCA to uninephrectomized rats on a low salt diet caused a slight increase in the receptor expression, although not to the extent observed in the DOCA-salt–treated rats (Figure 2C). Moreover, the increased expression returned toward normal levels by treatment of the hypertension with thiazide diuretics and a low salt diet (Figure 2C), suggesting that the changes in PDGF β-receptor gene expression were related to blood pressure elevation. The aortic levels of PDGF α-receptor mRNA showed no consistent changes after DOCA-salt administration (Figure 2A).

The studies were extended to SHRs and WKY rats. At 10 weeks of age, when SHRs had significantly higher blood pressures, PDGF β-receptor steady-state mRNA levels were approximately twofold higher in SHR than in WKY aorta (Figure 3). The mRNA levels for PDGF α-receptors were low in both WKY and SHR aortas (Figure 3).

The proteins encoded by the genes for the PDGF chains and receptors interact both at the level of PDGF dimer formation and at the level of PDGF binding to αα, αβ, or ββ receptor dimers. To determine whether the regulation of expression of these genes in vivo occurs in a reciprocally coordinated fashion, we also studied PDGF A- and B-chain gene expression using the same as well as different blots.

PDGF B-chain mRNA levels showed no obvious changes in either WKY or SHR aortas (Figure 3).
Although PDGF A-chain expression was difficult to study because of the very low mRNA levels and the limited amounts of RNA that can be extracted from rat aortas, we found similar mRNA levels in WKY and SHR aortas (Figure 3).

To investigate the tissue specificity of the observed changes in aorta, we also studied gene expression of PDGF receptors and their ligands in the hearts of the animals. No significant changes were detected in the cardiac mRNA levels of either the α- and β-receptors or PDGF B chain in the hypertensive rats given DOCA-salt in comparison to age-matched uninephrectomized controls (Figure 4). The cardiac PDGF A-chain steady-state mRNA levels were almost undetectable in both treated and control rats.

The steady-state mRNA levels of cardiac PDGF α- and β-receptors and PDGF B chain were all reduced in SHRs in comparison with WKY rats (Figure 5). The very low expression levels of cardiac PDGF A chain precluded determination of changes in hypertensive hearts.
Discussion

The current study was carried out to determine whether PDGF receptors are expressed in rat aorta and whether hypertension influences PDGF receptor gene expression in the rat. We found that both PDGF receptor genes are expressed in aortic tissue and, surprisingly, that aortic tissue and particularly aortic media contained the highest relative amounts of PDGF β-receptor transcripts as compared with a wide number of other tissues studied. Even nonvascular tissues containing a large proportion of SMCs, such as uterus, had several-fold lower transcript levels, whereas cultured confluent rat aortic SMCs highly expressed PDGF β-receptor mRNA.

Aortic PDGF α-receptor expression was relatively low compared with PDGF β-receptor expression, consistent with the recent observation that cultured rat aortic SMCs have approximately 5,000 high-affinity binding sites per cell for $^{125}$I-PDGF AA and approximately 31,000 for $^{125}$I-PDGF BB. In addition, almost 20-fold more β- than α-receptor subunits have been reported to be present in human skin fibroblasts. Recently, a rat PDGF α-receptor cDNA has been cloned, and a message similar in size and tissue distribution to what we found using two human probes has been reported.

Consistent with prior findings from our own and other laboratories, aortic tissue expressed the
genes for PDGF A and B chains. In comparison with other tissues, aortic tissue and the aortic media preparation contained relatively high expression of PDGF A chain, although to a somewhat lesser extent than that observed in lung and brain.

Increased aortic steady-state mRNA levels of PDGF β-receptors were induced in DOCA-salt hypertension, whereas the PDGF α-receptor expression appeared unaffected. We previously reported that the aortic expression of both PDGF chains was not appreciably changed by DOCA-salt treatment, and a similar lack of change was observed in the current study for SHRs when compared with WKY rats. The increase in PDGF β-receptor steady-state mRNA, which could result from increased transcription or decreased degradation of the transcripts, paralleled the increase in blood pressure. Moreover, the increased PDGF β-receptor expression was partially reversed when normotension was reestablished by a low salt diet and treatment with chlorothiazide. We recently have documented the reversal of the changes in gene expression with control of hypertension for aortic fibronectin gene expression as well.

Increased aortic PDGF β-receptor expression also was found in 10-week-old SHRs, in comparison with normotensive age-matched WKY rats. This finding, in a spontaneous model of chronic hypertension, further suggests the association between blood pressure increases and changes in aortic PDGF β-receptor gene expression, although further studies are needed to rule out the possibility that the increased expression may precede the development of hypertension in the SHR.

The increase in PDGF β-receptor gene expression, if followed by a similar increase of the encoded receptor, might be responsible for some of the growth-related changes occurring in arterial SMCs in response to hypertension. It is interesting that previous findings from our laboratory indicated that aortic SMCs cultured from DOCA-salt hypertensive animals had increased rates of migration and proliferation as compared with normotensive controls. Moreover, aortic SMCs from SHRs proliferate more rapidly in culture and synthesize more DNA in
response to PDGF in comparison with normotensive WKY rats.44

In contrast to the findings in aorta, PDGF β-receptor transcripts in cardiac tissue did not increase in either DOCA-salt–treated animals or the SHR. In fact the steady-state mRNA levels for both PDGF receptors and PDGF B-chain actually were lower in SHR than in WKY hearts. The reason for these differences is unknown, although the failure of cardiac myocytes to divide after infancy may reflect a diminished dependence of these cells on PDGF-induced effects that seem to be important for heart development.35

In conclusion, our data indicate that the genes encoding for both PDGF chains and receptors undergo differential regulation in vivo. The gene–tissue–specific changes induced by hypertension may be a component of an adaptive response of the arterial wall to increased blood pressure, which may serve to protect the vessel against increased hemodynamic stresses.

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