Morphological and biochemical characterization of remodeling in aorta and vena cava of DOCA-salt hypertensive rats

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Watts SW, Rondelli C, Thakali K, Li X, Uhal B, Pervaiz MH, Watson RE, Fink GD. Morphological and biochemical characterization of remodeling in aorta and vena cava of DOCA-salt hypertensive rats. Am J Physiol Heart Circ Physiol 292: H2438–H2448, 2007. First published January 19, 2007; doi:10.1152/ajpheart.00900.2006.—Arterial remodeling occurs in response to mechanical and neurohumoral stimuli. We hypothesized that veins, which are not exposed to higher pressures in hypertension, would demonstrate less active remodeling than arteries. We assessed remodeling with two standard measures of arterial remodeling: vessel morphometry and the expression/function of matrix metalloproteinases (MMPs). Thoracic aorta and vena cava from sham normotensive and DOCA-salt hypertensive rats (110 ± 4 and 188 ± 8 mmHg systolic blood pressure, respectively) were used. Wall thickness was increased in DOCA-salt vs. sham aorta (301 ± 23 vs. 218 ± 14 μm, P < 0.05), as was medial area, but neither measure was altered in the vena cava. The aorta and vena cava expressed the gelatinases MMP-2, MMP-9, transmembrane proteaseinase MT1-MMP, and tissue inhibitor of metalloproteinase-2 (TIMP-2). Immunohistochemically, MMP-2 localized to smooth muscle in the aorta and densely in endothelium/smooth muscle of the vena cava. Western and zymographic analyses verified that MMP-2 was active in all vessels and less active in the vena cava than aorta. In hypertension, MMP-2 expression and activity in the aorta were increased (59.1 ± 3.7 and 74.5 ± 6.1 units in sham and DOCA, respectively, P < 0.05); similar elevations were not observed in the vena cava. MMP-9 was weakly expressed in all vessels. MT1-MMP was expressed by the aorta and vena cava and elevated in the vena cava from DOCA-salt rats. TIMP-2 expression was significantly increased in the aorta of DOCA rats compared with sham but was barely detectable in the vena cava of sham or DOCA-salt hypertensive rats. These findings suggest that large veins may not undergo vascular remodeling in DOCA-salt hypertension.

matrix metalloproteinase; artery; vein

HYPERTENSION IS A DISEASE accompanied and influenced by significant changes in arterial function. Although changes in endothelial and smooth muscle cell function are important in acute control of total peripheral resistance and blood pressure, changes in blood vessel structure also contribute to long-term alterations in arterial function. In general, arteries undergo remodeling as a consequence of increased wall stress (8, 33, 34, 39, 40). Remodeling includes a reorganization of the scaffolding/structural support provided by the extracellular matrix and hypertrophy and/or hyperplasia of the vascular smooth muscle cells. The result is a reorganized vessel wall of altered mechanical strength and contractile capability. Although remodeling can be considered a valuable protective adaptation to a higher wall stress, it also contributes to the etiology of hypertension by creating an artery with a thicker, less compliant wall (increased vascular stiffness, and/or a reduced luminal area (eutrophic or hypertrophic inward remodeling). Aortic stiffness, inasmuch as it contributes to isolated systolic hypertension in the human, is an excellent example of the complications presented by remodeling (52).

One mechanism important to arterial remodeling is the function of matrix metalloproteinases (MMPs). MMPs, of which there is a large family, serve as proteases that allow the dissolution and reorganization of extracellular matrix, particularly collagen and elastin (5, 13, 39, 46). These zinc-dependent and redox-sensitive proteases serve an important role in arterial remodeling (5, 16, 25, 30, 31, 94), on the basis of studies from a number of groups investigating large conduit and small resistance arteries (6, 50). MMP-2 (gelatinase A, 72 kDa) and MMP-9 (gelatinase B, 92 kDa) have a fairly wide range of different matrix substrates, including the collagens, and these MMPs are expressed ubiquitously in vascular smooth muscle. MMP-9 has also been shown to be activated by MMP-2 (24), whereas MMP-2 activation from a latent to an active form can be attributed to a number of different enzymes, including the transmembrane proteaseinase MT1-MMP (MMP-14) (3). The actions of the MMPs are offset and balanced by tissue inhibitors of matrix metalloproteinase (TIMPs). Thus remodeling of the arterial vessel wall in hypertension can be demonstrated by an imbalance between the function of MMPs and TIMPs.

Another method to investigate remodeling is direct examination of changes in vascular architecture. Although such changes are well documented for arteries (large and small) in multiple forms of hypertension, little is known about whether veins remodel in response to hypertension. Twenty years ago, Mark (41) demonstrated that forearm venous distensibility in vivo was decreased in humans with borderline hypertension, supporting the idea that veins had undergone structural changes in borderline hypertension. This was later supported by Sudhir et al. (54), who demonstrated a decrease in venous distensibility in vitro in human hypertension. Schmid-Schonbein and others suggested that venous tissue can remodel in response to hypertension or elevated pressure (27, 46, 48, 49). The DOCA-salt hypertensive rat demonstrates significant arterial remodeling (33). In this study, we have investigated remodeling through histological examination of sections of an arterial segment known to remodel (thoracic aorta) and a comparable...
venin (vena cava) and also immunohistochemical expression and activity of two important MMPs, MMP-2 and MMP-9, an activator of MMP-2 (MT1-MMP), and an endogenous inhibitor of MMP (TIMP-2).

METHODS

Animal Model

This study was approved by the Michigan State University Institutional Animal Care and Use Committee and was conducted in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals.” Male Sprague-Dawley rats (0.225–0.250 kg body wt; Charles River, Portage, MI) were uninephrectomized, and DOCA (200 mg/kg in silicone rubber) was implanted subcutaneously. Postoperatively, the rats were given a drinking solution of 1% NaCl-0.2% KCl. Sham normotensive rats were uninephrectomized, received no DOCA, and drank normal tap water. All rats were given free access to standard pelleted rat chow (8640 rodent diet, Harlan/Teklad). Animals remained on this regimen for 4 wk before use.

Blood Pressure Protocol

Systolic blood pressures of rats were determined in the conscious state by the tail-cuff method.

Immunohistochemistry and Gross Morphometry Protocols

Thoracic aorta and vena cava were removed from animals anesthetized with pentobarbital sodium (60 mg/kg ip) and cleaned before use. Tissues were cleaned and fixed in formaldehyde. Paraffin-embedded thoracic aorta and vena cava (8 μm) were cut, dewaxed, and subjected to a standard protocol using a kit for 3.3′-diaminobenzadine staining (Vector Laboratories, Burlingame, CA) for immunohistochemical studies. Aortic sections were blocked with 1.5% blocking serum in phosphate-buffered serum (to reduce nonspecific binding of the primary and secondary antibodies) and then incubated for 24 h with MMP-2 antibody (5 μg/ml; Chemicon, Temecula CA), smooth muscle cell α-actin antibody (1 μg/ml; Oncogene Research Products, Boston, MA), or no antibody. Sections were washed three times with PBS, incubated for 30 min with secondary antibody, washed again, and incubated for 30 min with ABC Elite reagent (Vector Laboratories). For detection of antibody binding, sections were incubated for 1 min with a 3,3′-diaminobenzadine developing solution (Vector Laboratories). Binding is represented by a dark brown/black precipitate.

Histological studies with stains including standard Masson’s trichrome and hematoxylin and eosin were performed in the Investigative Histopathology Laboratories of Michigan State University.

All sections (immunohistochemical and histological) were photographed using an inverted microscope (model T2000, Nikon) connected to a SPOT Insight color camera and MetaMorph software. The thickness of the vessel wall relative to the lumen diameter was determined after visualization of the inner and outer walls of the medial layer with use of an electronic pen stylus on a Cintiq touchscreen monitor and MetaMorph software. A microscope-based micrometer was used to calculate wall thickness. Medial area was calculated by subtraction of the inner outlined area from the outer outlined area and is reported in arbitrary units.

Western Protocol

Tissues were isolated directly from the animal, cleaned, and placed directly into liquid nitrogen. In liquid nitrogen, tissues were ground to a powder, and ice-cold homogenization buffer [125 mmol/l Tris (pH 6.8), 4% SDS, 20% glycerol, 0.5 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l orthovanadate, 10 μg/ml aprotinin, and 10 μg/ml leupeptin] was added as follows: 1 ml for one-half an aorta and 0.2 ml for one vena cava. Homogenates were vortexed, sonicated briefly, transferred to a plastic centrifuge tube, and spun at 4°C to pellet debris. Supernatant was separated from the pellet and analyzed for protein concentration (bicinchoninic acid protein kit, Sigma Chemical, St. Louis, MO). Fifty micrograms of total protein were boiled for 5 min with standard 4:1 sample buffer. Proteins were separated on 1-mm-thick 10% SDS-polyacrylamide gels using a Mini Bio-Rad III or Criterion apparatus. A positive control of active MMP-2/MMP-9, TIMP-2, and MT1-MMP (Chemicon) was run in parallel. After transfer, membranes were blocked overnight in 5% milk (4°C, PBS + 0.025% NaN₃). Primary antibodies [Mab 3308 for MMP-2, Mab 3309 for MMP-9, and Mab 770 for TIMP-2 (Chemicon) and MMP-14/MT1-MMP (Calbiochem/EMD IM57, San Diego, CA)] were incubated with blots for 24 h. Blots were then rinsed three times in Tris-buffered saline + Tween (0.1%) and then once in Tris-buffered saline and incubated with the appropriate horseradish peroxidase-linked secondary antibody (1:2,000 dilution; Cell Signaling Technology, Beverly, MA) for 1 h at 4°C with rocking. Enhanced chemiluminescence reagents (ECL, Amersham Life Sciences, Arlington Heights, IL) were used to visualize bands. Gels were stained with Gel Code Blue (Pierce, Rockford, IL) to verify protein loading. and blots were reprobed with smooth muscle α-actin primary antibody (1:1,000 dilution; Oncogene Research Products, Boston, MA) to ensure equal protein loading and to normalize reported data.

Zymographic Protocol

Gelatin-based zymography was performed using 10% gels (Bio-Rad Laboratories, Hercules, CA) and running, renaturing, and developing buffers (Invitrogen, Carlsbad, CA). Fifty micrograms of total protein were loaded in each lane; a positive control of an active MMP-2/MMP-9 mixture was loaded on every gel (Chemicon). Standard electrophoresis was run using zymography running buffer. The gel was renatured for 30 min with rocking and developed with an initial step of 30 min in developing buffer at room temperature with rocking and then at 37°C in a water bath with gentle rocking for 18 h. Gels were stained for 5 min with 0.5% Coomassie blue and then destained using 10% methanol-10% acetic acid-water for ≥4 h with multiple solution changes. For final destaining, the gel was exposed to 50% methanol-1% acetic acid-water for ≥4 h with multiple solution changes and rehydrated with water for ≥6 h. Gels were scanned on a Bio-Rad Fluor-S Imager.

Measurement of 4-Hydroxyproline

Tissues from sham and DOCA-salt hypertensive rats were analyzed for hydroxyproline content, an estimate of collagen content. Samples were dried and hydrolyzed in 1 ml of 12 N HCl for 24 h at 110°C, and hydroxyproline colorimetric analysis was performed as described previously (51, 57). Data are expressed as micrograms of hydroxyproline per milligram of dry weight, and measurements were done in triplicate.

Data Analysis

Values are means ± SE, and n is number of animals. Band density (Western) was quantified using the public domain program NIH Image (version 1.62). For comparison of two groups, the appropriate Student’s t-test or ANOVA with repeated measures was used. ANOVA followed by Student-Newman-Keuls post hoc test was used to compare three or more groups. In all cases, P ≤ 0.05 was considered statistically significant.

RESULTS

Histology and Morphometry

Basic histological differences in the composition of a thoracic aorta and superior vena cava from a normal male Sprague-Dawley rat as highlighted by Masson’s trichrome.
staining are illustrated in Fig. 1A. Significantly more and better organized smooth muscle cell layers (light pink) in the aorta are organized between bundles of elastin (dark pink). Staining of smooth muscle cell α-actin was intense in smooth muscle layers of aorta (dark brown), and was in/near the intima of the vena cava (Fig. 1B). Hypertrophy of the aortic wall was clear in DOCA-salt hypertension, whereas no clear change in the layer of smooth muscle in the vena cava was apparent.

The inner and outer walls of the media of the aorta and vena cava were traced using a stylus mouse, and the total area of the wall, as well as wall thickness, was measured (Fig. 2). Total medial area was increased in DOCA-salt vs. sham aorta (0.632 ± 0.010 vs. 0.400 ± 0.015 mm², *P* < 0.05) but was not altered in the vena cava (0.516 ± 0.009 and 0.596 ± 0.012 mm², respectively). These measurements included the vessel area between the intima and adventitia and excluded fatty tissue. Wall thickness was estimated in the aorta (301 ± 23 and 218 ± 14 μm for DOCA-salt and sham, respectively, *P* < 0.05), but such estimates were difficult in the vena cava because of the uneven, irregular wall thickness throughout the vessel.

MMP-2 was visualized in paraffin-embedded sections of thoracic aorta (Fig. 3) and vena cava (Fig. 4) from sham normotensive and DOCA-salt hypertensive (110 ± 4 and 189 ± 8 mmHg systolic blood pressure, respectively, *n* = 8, *P* < 0.05) rats by immunohistochemical staining. The artery of the DOCA-salt rat demonstrates a significant increase in wall thickness. The aorta from sham and DOCA-salt rats showed prominent staining for MMP-2 in smooth muscle and endothelial cell layers (left vs. right in Fig. 3). Staining of MMP-2 in the aorta from the DOCA-salt rat was observed through the smooth muscle cell layers. Similarly, the vena cava from sham and DOCA-salt rats also stained for MMP-2 in the intima/smooth muscle layer (Fig. 4).

**Western and Zymographic Analyses**

**MMP-2.** Expression and gelatinase activity of homogenates of the aorta and vena cava from sham normotensive and DOCA-salt hypertensive rats were also measured. Representative results of zymographic and Western analyses in the aorta and vena cava are shown in Fig. 5. Active MMP-2 was detected in all aortic samples, but activity was significantly greater in the aorta from DOCA-salt hypertensive than sham rats, as evidenced by a greater loss of Coomassie blue staining in the gel and appearance of a clear MMP-2 band. This is supported by an increased expression of MMP-2 in the aorta from DOCA-salt compared with sham rats in Western analyses (Fig. 5A, bottom). Thus these two biochemical results are consistent with one another. Similarly, analyses of the same

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**Fig. 1.** A: Masson’s trichrome-stained aorta and vena cava from a normal Sprague-Dawley rat. Blue, collagenous material; thick pink lines, collagen; light pink, smooth muscle; brown, nuclei. B: immunohistochemical smooth muscle cell α-actin staining (brown precipitate) in aorta and vena cava of sham and DOCA-salt rats. Images are representative of 4 separate animals.
amount of total protein from the vena cava as was used for the aorta demonstrated a significantly weaker expression of MMP-2 than in the aorta, with a virtually absent MMP-2 activity as detected by zymography. Moreover, MMP-2 expression and/or activity in the vena cava was not increased in DOCA-salt hypertension. Quantitation of Western analyses is presented in Fig. 6.

MMP-9, MT1-MMP, and TIMP-2. In a similar manner, we measured the expression and activity of MMP-9 in these same samples. MMP-9 expression was ~10% of MMP-2 expression in arterial and venous samples, and it was difficult to visualize active zymographic bands in samples from the aorta or vena cava. Western results, which are not shown because of the significant faintness of the bands for MMP-9, are quantified in Fig. 6. By contrast, the positive control of active MMP-9 provided a signal in zymographic analyses (Fig. 5B). Thus, in the aorta and vena cava, MMP-9 was not highly active, nor was it activated in DOCA-salt hypertension.

One of the proteins able to convert latent MMP-2 to active MMP-2 is MT1-MMP, which is also known as MMP-14. Expression of this MMP was measured in the aorta and vena cava from sham and DOCA-salt hypertensive rats, and the results are shown in Fig. 7A. Expression of MT1-MMP was roughly similar in arterial and venous homogenates, and a modest increase in expression was observed in the vena cava, but not aorta, from DOCA-salt compared with sham normotensive rats. Expression of TIMP-2 was also measured. TIMP-2 was visualized in all samples and was significantly upregulated in the aorta from DOCA-salt compared with sham rats (Fig. 7B). By contrast, TIMP-2 was expressed at a significantly lower level in the vena cava than aorta and was not increased in density in DOCA-salt hypertension.

4-Hydroxyproline Measurements

4-Hydroxyproline content was measured as an index of collagen content. Although collagen content was globally higher in the veins than arteries (P < 0.05, ANOVA), values in the DOCA-salt groups were not higher than sham values for the aorta or vena cava (Fig. 8).

DISCUSSION

The circulation is a closed circuit consisting of a high-pressure (arterial) and a low-pressure (venous) side connected through capillary beds. A goal of this circuit is to maintain physiological homeostasis, such that appropriate perfusion/nutrition of organs can occur and wastes can be removed. The venous and arterial circulations work in balance, controlled by neuronal and humoral factors, to enable circulatory homeostasis. In arterial hypertension, physiological control mechanisms dysfunction and adapt to higher-than-normal pressures. The arterial side of the circulation does this, in part, through structural remodeling of the vascular wall, which reduces wall stress and also produces a stronger artery that is able to resist rupture at higher wall tensions. We tested whether the large vessels on the venous side of the circulation demonstrate similar remodeling, as exemplified by changes in structure and MMP activity.
Arterial Remodeling

All the measures that we examined in the aorta isolated from DOCA-salt hypertensive rats confirm and support active remodeling. Specifically, active remodeling was observed as an increase in wall thickness and medial area, increased expression and activity of MMP-2, and increased expression of TIMP-2. The aorta was used as a model, inasmuch as it is the vessel exposed to the highest pressures in the circulation and it demonstrates alterations in contractility in hypertension. Increased expression and activation of arterial MMP-2 or MMP-9 have been observed in a number of experimental models of hypertension, including pulmonary hypertension (40), the nitric oxide synthase-inhibited hypertensive rat (6), the spontaneously hypertensive rat (7), the air-jet stress hypertensive rat (17), and the hypertensive human (58). Arterial remodeling and MMP activation occur, at least in part, in response to increases in wall tension and mechanical stretch (2, 10, 26, 39, 47). The increase in TIMP-2 expression observed in the aorta from DOCA-salt rats is presumably an adaptive increase to the higher-than-normal levels of MMP-2, confirming previous findings. MMP-9 was detected in Western analyses but could not be detected as active in zymographic analyses, suggesting a lower expression and function of MMP-9 than MMP-2 in the aorta. MT1-MMP was investigated as a potential mechanism by which latent MMP-2 could be made active, but this was not observed in these experiments. Thus MMP-2 activation must depend on other factors.

The mechanism of arterial MMP-2 activation within this model is somewhat understood. Endothelin (ET-1) plays an important role in mineralocorticoid (DOCA and aldosterone)-
induced hypertension, and ETA receptor dependence on MMP-2 activation has been observed in the heart (1) and artery (17). More recently, aldosterone-induced hypertension was associated with an increase in NADPH oxidase activity, MMP-2 activity, and NF-κB activation, resulting in cardiac fibrosis (35). The sequence and/or interaction of these players, ET-1, NADPH oxidase, aldosterone, and NF-κB activation, is not yet clear.

**Venous Remodeling?**

We performed, in parallel, similar measures of vessel remodeling in the vena cava. This vein was the model of choice, inasmuch as it is a vessel for which a significant amount of functional contractile data are available, and the diameter of the vena cava is close to that of the aorta (56). Importantly, this vessel is contractile, and thus the smooth muscle in its wall can respond to increases in tension. One of our more interesting findings is the narrowness of expression of smooth muscle in the vena cava compared with the aorta (Fig. 1). In multiple sections of the vena cava stained with Masson’s trichrome, organized and distinct bundles of smooth muscle were not apparent, except for a layer that appeared to be subendothelial.

Expression of MMP-2 in the vena cava is different from that in the aorta. As shown by Western and zymographic analyses, the vena cava does express active MMP-2. However, it is clear that overall expression of this gelatinase was lower in the...
normal vena cava than expression of the same amount of total protein in the aorta, and, in contrast to the aorta, the vena cava did not demonstrate increased expression of MMP-2 (total or functional) in DOCA-salt hypertension. Similarly, TIMP-2 expression was low and was not elevated in DOCA-salt hypertension, nor could MMP-9 be readily observed as active in the vena cava. This may reflect the relative amount of smooth muscle in the artery vs. the vein, although our gel analyses

Fig. 5. A: zymographic (top) and Western (bottom) analyses of MMP-2 activity in aorta from sham and DOCA-salt hypertensive rats. B: zymographic (top) and Western (bottom) analyses of MMP-2 activity in vena cava from sham and DOCA-salt hypertensive rats. Far left lane is a mixture of latent/active MMP-9 and MMP-2 run as a positive control. Blots are representative of 8 pairs of sham/DOCA-salt animals.

Fig. 6. Quantitation of Western analyses for MMP-2 and MMP-9 in aorta and vena cava from sham and DOCA-salt rats. Values are means ± SE (n = 8). *Statistically significantly different from sham aorta. †Statistically significantly different from respective artery.
cannot discriminate between the cell types in which TIMP-2 is present and those in which MMP-9 is present. These measures suggest that the vena cava does not remodel in the same manner as arteries in this model of experimental hypertension.

It could be argued that the timing of our experiment, i.e., 4 wk after initiation of DOCA-salt hypertension and, thus, during established hypertension, may miss an early remodeling event in the veins. Because we did not perform similar experiments within the 1st wk of DOCA-salt exposure, we cannot exclude this idea. However, it is reasonable to suggest that the process of remodeling in arteries and veins must be intrinsically different, because we can measure a change in arterial expression and function of MMP-2 at the 4-wk time point. The veins could revert to a normal expression over the course of these 4 wk. This issue has yet to be investigated.

One finding that is difficult to explain is the increase in MT1-MMP expression in the vena cava of DOCA-salt compared with sham animals. This protein was investigated, because changes in MMP-2 expression and activity can be driven by an increase in MT1-MMP, which can convert latent MMP-2 to active MMP-2. The levels of MMP-2 expression and function were not elevated in the vena cava of the DOCA-salt rat,
but MT1-MMP expression was increased. We did not measure the activity of MT1-MMP, but it appears to have enzymatic activities that are different from those of MMP-2, meaning that they may not serve exactly the same function (22). Thus the physiological outcome of an elevated MT1-MMP expression is unknown, but it may be that we have not measured the end points to which MT1-MMP could contribute.

Veins do have the ability to change structure in response to stress. Deep venous thrombosis injures the venous wall, causing a reaction that has been described as similar to wound healing, associated with increased procollagen, total collagen, MMP-9, and MMP-2 expression (15). The laboratory of Schmid-Schonbein and others has been dedicated to understanding venous hypertension and the remodeling of venous valves (48, 49, 53, 55). In general, an inflammation-based reaction in venous valves that includes an increase in MMP-2 expression has been observed. Neutrophil infiltration has also been observed in postthrombotic veins (32), as has a medial thickening of coronary venules and large veins exposed to arterial levels of pressure (11, 12). More recently, veins have been described as remodeling in venous disease with the loss of venous valvular function (4), an idea that is difficult for us to investigate in the vena cava, which lacks valves. Certainly, the saphenous vein, which is used in vein grafts for coronary bypass procedures, remodels with hypertrophy and eutrophy when placed in an arterial circuit or with elevations in shear stress and pressure ex vivo (27). However, the saphenous vein has histological characteristics similar to those of an artery; therefore, use of this vein as the sole model for determining whether veins remodel its potentially inappropriate.

Hayashi et al. (29) and Monos et al. (44, 45) are among the few groups that have directly studied the effect of chronic blood pressure elevation on the mechanical properties of the venous wall. Hayashi et al. used a rabbit model in which left femoral venous pressure was elevated for 1, 2, or 4 wk by constriction of the external iliac vein (29). Compared with the contralateral normal vein, the femoral vein, which experienced the higher intravascular pressure (16 vs. 7 mmHg), displayed increased wall thickness and elevated vascular tone, whereas wall elasticity and compliance remained the same. MMPs were not investigated in this study. Monos et al. used a different model, in which the rat was exposed to head-up tilt for 2 wk, which increased blood pooling and stress on lower extremity veins. In the tilted animals, a general decrease in distensibility of the saphenous vein was observed when it was studied in vitro (44, 45) and in borderline-hypertensive humans (6, 50). Changes in axial stress have been cited as a stimulus for venous remodeling (11). These studies support the idea that remodeling can occur in venous tissue.

To our knowledge, MMP/TIMP activity in large veins in hypertension has not been studied. Among the reasons for studying MMP function, rather than mechanical measures of remodeling, is the high sensitivity of techniques to measure expression and function of MMP and TIMP proteins (Western and gelatin zymography analyses). MMP activation is also a logical choice for comparison of venous with arterial remodeling, inasmuch as the activation of MMPs in arterial remodeling has been established. In addition to their role in matrix reorganization, MMPs can cleave adenomedullin into vasoactive constrictors, can act as a sheddase that promotes heparin-binding epidermal growth factor release and vasoconstriction, and can process big ET-1 [i.e., ET-(1-38)] into the novel vasoactive ET-1-(1-32) (19–21, 28, 42). Thus it is useful to elucidate and compare the activity of MMPs in arteries and veins.

Perspectives, Limitations, and Questions

Neurohumoral factors, such as norepinephrine, angiotensin II, and ET-1, may contribute to remodeling of blood vessels in a manner independent of changes in wall stress. If this is so, then all blood vessels of the circulation have the potential to remodel. This includes the venous side of the circulation, which functions at a significantly lower pressure (0–7 mmHg) than the arterial circulation (40–200 mmHg). Thus, if neurohumoral factors play a role in remodeling, this should be evident in venous tissue. However, unilaxial strain has been cited as the primary stimulus that upregulates matrix-degrading enzymes in human vascular arterial smooth muscle cells (3) and mechanical stretch, which is cited as a stimulus for MMP-2 expression and release (23, 39, 47). The present studies address whether remodeling of large veins is similar to remodeling of large arteries in hypertension, specifically related to MMP-2 activity. Because we investigated only the aorta and vena cava, we cannot exclude the possibility that our findings are limited to vessels of their particular size and function. An additional limitation to this study is that the veins and arteries were postfixed, rather than fixed by perfusion of the animal. For this reason, our morphometric measurements may have been more difficult in the vena cava.

It is not clear whether remodeling in a vein is necessarily the same process as remodeling in an artery. Remodeling is defined in the artery as a change in vessel wall composition and architecture that allows for an inward or outward boundary movement. Theoretical and experimental evidence suggests that this occurs in response to elevated pressure and wall tension. Central venous pressure in the model of hypertension used here, the DOCA-salt rat, was not significantly elevated.
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