Effect of a Nonhypotensive Long-term Infusion of ANP on the Mechanical and Structural Properties of the Arterial Wall in Wistar-Kyoto and Spontaneously Hypertensive Rats

M.C. Mourlon-Le Grand, P. Poitevin, J. Benessiano, M. Duriez, J.B. Michel, and B.I. Levy

A nonhypotensive dose of atrial natriuretic peptide (ANP) was infused (60 pg/kg body wt per day s.c. by osmotic pump) for 25 days in 16-week-old normotensive Wistar-Kyoto rats (WKYS, n=12) and age-matched spontaneously hypertensive rats (SHRs, n=12). During the infusion period, systolic blood pressure, urinary volume, and cyclic guanosine monophosphate (cGMP) excretion/12 hr were measured once a week in both groups. Then mechanical and morphological properties of the arterial wall and plasma ANP levels were assessed and compared with those from control groups of SHRs (n=8) and WKYS (n=8) receiving a saline vehicle. The compliance (CC) of the in situ localized carotid artery was measured for pressures ranging from 25 to 175 mm Hg under control conditions and after “poisoning” of smooth muscle tone by potassium cyanide. After pressure fixation, the medial thickness, elastin and collagen contents, and the size and number of nuclei were measured in the thoracic descending aorta. In WKYS, ANP did not modify either mechanical or structural properties of the arterial wall or biochemical parameters. Conversely, in ANP-treated SHRs, CC was significantly increased compared with untreated SHRs under basal conditions (p<0.03) and after potassium cyanide poisoning (p<0.02). Structural properties were also modified by ANP in SHRs, i.e., medial thickness (129.3±4.1 versus 113.1 ±3.3 μm, p<0.01) and nuclear size (8.81 ±0.28 versus 5.52±0.20 μm², p<0.0001) in untreated and treated SHRs, respectively. Furthermore, urinary volume and cGMP content were significantly increased during ANP infusion in treated SHRs (p<0.05). The present results indicate concomitant modifications of mechanical and structural properties of the arterial wall in SHRs chronically treated with low doses of ANP. These long-term effects of ANP could be involved in the remodeling of the arterial wall observed during hypertension and could have beneficial effects on cardiovascular diseases in chronic sustained hypertension. (Arteriosclerosis and Thrombosis 1993;13:640–650)

KEY WORDS • atrial natriuretic peptide • histomorphometry • arterial wall mechanical properties • cyclic GMP

Atrial natriuretic peptide (ANP) has multiple effects such as an increase of diuresis, natriuresis, urinary excretion of cyclic guanosine monophosphate (cGMP), and glomerular filtration rate and a selective regional relaxation of vascular smooth muscle cells that is partially mediated by the activation of particulate guanylate cyclase via its second messenger, cGMP. The vasorelaxant effects of ANP have been extensively demonstrated in in vitro preparations but not so much in in vivo models. Recent studies have demonstrated that long-term infusion of ANP at low doses reduces blood pressure in several animal models of hypertension, including spontaneously hypertensive rats (SHRs). In this strain, it has been previously shown that the antihypertensive properties were more marked when the plasma levels of ANP were modestly but chronically elevated than when they were markedly and acutely increased. In SHRs, elevated systemic resistances are associated with an increase in arteriolar and arterial wall thicknesses that are related to an increase in smooth muscle cell mass and collagen content. These morphological modifications are associated with changes in arterial wall mechanical properties (i.e., increase in stiffness). Moreover, it has recently been reported that ANP has in vitro antiproliferative effects. There is no information concerning the in vivo effects of long-term infusion of ANP on the structure and mechanical properties of the arterial wall. In all models of hypertension, alterations of vascular structure have been described. In most cases, drug-induced reduction of hypertension induced a normalization of the structure of the arterial wall. Therefore, it has been very difficult to differentiate between the antihypertensive and the antihypertrophic effects induced by a drug when experiments were performed in vivo. Thus, a nonhypotensive long-term infusion of ANP was performed in SHRs and in the normotensive con-
control Wistar-Kyoto rats (WKYS). The mechanical properties of the carotid artery were assessed using an experimental model that allowed us to measure the static mechanical properties of the in situ localized vessel. Mechanical properties were measured before and after abolition of smooth muscle tone to evaluate the respective participation of smooth muscle cells and extracellular matrix. Furthermore, sections of descending thoracic aorta were subjected to automatic image analysis to measure the effects of ANP on the histological structure of the aorta. Finally, urinary volume and cGMP excretion were measured once a week during treatment. Plasma blood samples were collected on the last day (25th) of treatment to determine plasma ANP levels.

Methods

Experimental Model

Thirty 12-week-old male WKYS weighing 337±7 g were compared with 30 age-matched male SHRs weighing 304±8 g. They were randomly divided into four groups: normotensive control (NC, n=13, 330±5 g); normotensive ANP treated (NT, n=17, 335±2 g); hypertensive control (HC, n=13, 291±10 g); and hypertensive ANP treated (HT, n=17, 310±6 g). Before ANP treatment, body weight was significantly lower in SHRs compared with WKYS (p<0.05).

A continuous administration of ANP (28-7ANP; Sigma Chemical Co., St. Louis, Mo.) was delivered for 25 days by osmotic minipumps (Alzet model 2MLA, Palo Alto, Calif.) with a constant flow of 2.5 μL/hr. Before implantation, the 2,000-μL reservoir of the pumps was filled with either sterile vehicle (0.9% NaCl) or a solution of ANP (10⁻¹⁰ M), adjusted to release 2.5 pg/kg body wt per hour (3.10⁻¹⁴ M/L per hour) of the peptide. Minipumps were then subcutaneously implanted in rats under light ether anesthesia.

Before initiation of the study, systolic blood pressure (SBP) was measured for 3 days by the tail-cuff method in conscious animals that were habituated to this technique. Rats were warmed before measurement for 10 minutes at 38°C. Heart rate was derived from the pulse signal (BP recorder 8006, Ugo Basile, Varese, Italy). SBP was measured three times a week during the 25-day period. Animals were weighed weekly. At the end of this 25-day period, the weights of rats were NC, 310±14 g; NT, 315±7 g; HC, 293±5 g; and HT, 303±6 g. At the end of this period, body weight was significantly lower in SHRs compared with WKYS (p<0.001).

ANP treatment for 25 days did not affect body weight in both strains compared with their respective control groups.

In a first series of experiments (five NC, five NT, five HC, and five HT), urine samples were collected once a week during treatment to measure urine excretion and cGMP content. After 25 days of treatment, blood samples were collected to measure plasma ANP levels. Then mechanical studies were performed under pentobarbital anesthesia, and rats were then killed; the thoracic aorta was then excised for morphological study (eight NC, 12 NT, eight HC, and 12 HT). Animal care complied with the principles of laboratory animal care formulated by the National Society for Medical Research in the guide for the care and use of laboratory animals (NIH publication No. 8223, revised 1985).

Measurements of Static Mechanical Properties of the Carotid Artery

Animals were anesthetized with sodium pentobarbital (50 mg/kg body wt i.p.) and kept at a constant body temperature by a thermostat-controlled blanket. After induction of anesthesia, the trachea was cannulated and then connected to a rodent respirator (frequency, 50 beats/min; tidal volume, 4.5 mL; model 680, Harvard Apparatus, South Natick, Mass.). A midsternal thoracotomy was performed, and the root of the left carotid artery was exposed. The upper portion of the left carotid artery was catheterized with a nylon tube 80 cm long (0.6-mm i.d.) filled with an iso-osmotic/iso-oncotic Tyrode's solution mixed with albumin (4%) and Evans blue dye (0.03%). The presence of protein in the flushing and incubating solutions preserved the endothelium and maintained a physiological osmotic pressure gradient across the vessel wall. Then the root of the left carotid artery was dissected, and a removable clamp was positioned at the junction of the aortic arch and the carotid artery. This preparation allowed us to isolate, in situ, 20–23 mm of unexposed carotid artery (Figure 1). The advantages of such a model are the following: 1) It preserves the integrity of the endothelium in the isolated segment of artery. At the end of the experiment after excising and washing, the absence of fixation of Evans blue dye by the wall indicated that the endothelial surface remained unaltered. 2) It keeps the non-dissected, unexposed, and nonexcised segment of carotid artery at its physiological length and in its normal fluid environment. 3) It avoids collapse of the vessel.

Protocol

At the beginning of the measurement period, the artery was subjected to atmospheric pressure for 5
Displacements of the meniscus of 1 mm corresponded to a carotid volume variation of 0.28 μL. To record the volume-pressure relation, the artery was subjected to increasing pressures from 50 to 175 mm Hg (or 200 mm Hg for SHRs) at increments of 25 mm Hg. The duration of each increment was 5 minutes, during which time the position of the meniscus was recorded every 15 seconds. At each pressure, we noted an initial rapid inflow (30–45 seconds), corresponding to the relaxation of vascular smooth muscle; then the inflow was constant and corresponded to the fluid filtration through the vascular wall. An estimate of the initial increase in volume was obtained by extrapolating the constant portion of the inflow curve to the time when the pressure was established (Figure 2). The volumes of the segment of isolated artery at each pressure were used to construct a volume-pressure relation for each rat (Figure 3a), for which the slope of the curve represented the carotid compliance (CC). Therefore, CC corresponded to the variation of the volume contained in the carotid artery as a function of pressure (dV/dP). Because of the sigmoidal shape of the volume-pressure relation, CC is maximum at intermediate pressure values (Figure 3b).

Finally, the artery was washed and then filled with a saline solution of potassium cyanide (100 mg/L). The cyanide solution was left in the artery for 30 minutes to poison the vascular smooth muscle cells. Another series of measurements of the pressure-volume relation were then performed. The CC was thus measured in each animal under baseline conditions and after total abolition of vascular smooth muscle tone, i.e., under passive conditions (collagen and elastin fibers of extracellular matrix).

Morphological Study
A segment of 2–3 cm from the descending thoracic aorta was removed and fixed at operating pressure (corresponding to the mean arterial pressure of each rat) in a saline solution with 4% formaldehyde. This segment was embedded in paraffin. Three successive sagittal sections of 5-μm thickness were treated by specific staining to obtain a monochromatic color associated with the various structures to be studied in the aortic media. Sirius red was used for collagen staining, orcein for elastin, and hematoxylin after periodic acid oxidation for nuclear staining.

Morphometric analysis was performed with a specialized automated image processor (NS 1500, Nachet-Vision, Paris, France). This processor is based on morphological mathematical principles and is software controlled. Different algorithms were developed to analyze each of the three structures shown by the staining in each of the three successive sections.

For image processing, the image is sent to the processor via a video camera and can then be viewed on a TV monitor. The control of luminosity was automatically adjusted by the software to obtain similar levels of contrast, taking into account the total luminosity transmitted by the video camera. This analog image is then digitized as follows. Each elementary point (pixel) is automatically compared with a threshold; if the gray level of the pixel exceeds this threshold, the pixel is given the numeric value 1; otherwise, it is given the numeric value 0. Threshold determination is a complex operation mainly involving pixel ensembles. Threshold parameters are the size of such pixel groups and their local contrast (top-hat transformation). The threshold
was determined using the top-hat algorithm to minimize variations in nuclear staining and background.

For data processing, this binary image is then processed to 1) eliminate background and artifacts, 2) delineate the zones of interest and the reference zone, and 3) extract and measure the parameters from the various zones of interest.

The first algorithm analyzed the mean medial thickness by measurements of the distance between the internal and external elastic laminae (70 measurements in each section). The medial elastin network was analyzed in terms of the relative area and mean thickness of elastin lamellae and laminae; the measurements and calculations were made for 10 fields in each section. The second algorithm analyzed the collagen matrix by measurement of the relative area density of collagen fibers in 20 contiguous fields in each Sirius red-stained section. Elastin and collagen densities were defined as the ratio of the surface stained by orcein or Sirius red, respectively, to the surface of the studied field. The third algorithm counted the number of nuclei within 20 fields of a 7,442-μm² area of measurement in each section and measured the mean area of each nucleus. A two-step procedure (conditional opening then conditional closing\textsuperscript{21}) leads to the elimination of all particles under a predetermined size. The final result is retention of the images of the nuclei, without any “holes” or deformations due to the structuring element (hexagon). The image processor automatically eliminates “borderline” nuclei before measuring the number of nuclei per unit of surface.

Repetitive measurements were performed, pooled, and averaged for the three algorithms in the corresponding stained sections of the aortic wall media of each animal. Moreover, the morphological analyses were performed twice in a blinded fashion by two independent researchers, and the results were stored on a floppy disk.

**Determination of Plasma ANP Concentration**

After the rats were decapitated, 3 mL of blood was collected in tubes containing peptidase inhibitors (EDTA, phenylmethylsulfonyl fluoride, and aprotinin) for the assay of immunoreactive ANP (ANP-ir) in rat plasma. Tubes were immediately centrifuged for 10 minutes (3,000g, 4°C) and frozen at −40°C. ANP-ir was extracted on Sep-Pak C18 cartridges and measured by radioimmunoassay with anti-ANP antibodies. These antibodies presented a 100% cross-reaction with rat ANP, and the limit of detection was about 3 pg rat ANP per tube.
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**Figure 5.** Panel a: Line plot of carotid compliance measured in anesthetized normotensive Wistar-Kyoto rats receiving chronic subcutaneous administration of a saline solution (NC) compared with anesthetized normotensive Wistar-Kyoto rats receiving chronic subcutaneous administration of atrial natriuretic peptide (ANP) (NT group; mean±SEM). Differences were not significant. Panel b: Line plot of carotid compliance measured in anesthetized spontaneously hypertensive rats receiving chronic subcutaneous administration of a saline solution (HC) compared with anesthetized spontaneously hypertensive rats receiving chronic subcutaneous administration of ANP (HT group; mean±SEM). Carotid compliance was significantly higher in HT than in HC animals (p<0.03). P, pressure.

Determination of Urinary cGMP Concentration

Urine samples were collected once a week for 12 hours during the night (days 7, 14, and 21), centrifuged for 10 minutes (3,000g, 4°C), and frozen at -40°C. The samples were then assayed for cGMP content by radioimmunoassay (Amersham).

Statistical Analysis

Results are expressed as mean±SEM. The experimental design allowed us to use a two-way analysis of variance to provide evidence of differences relating to the experimental models and/or treatment and interaction. To compare the pressure-compliance relation obtained in the different groups, the area under the CC-pressure curve was calculated for each rat, and a two-way analysis of variance was performed. The differences between groups were evaluated using the Newman-Keuls test.

Results

**Hemodynamics**

Figure 4 indicates that noninvasive SBP was not significantly modified in NT rats compared with NC rats. In addition, SBP was not significantly modified from the beginning to the 13th day of treatment between HC and HT rats; nevertheless, SBP was signifi-
cantly reduced from the 13th until the last day of treatment in HT rats compared with HT SHRs ($p<0.05$). Furthermore, when invasive aortic blood pressure was measured after 25 days of treatment in anesthetized, open-chest rats, SBP and mean arterial pressure were significantly lower in ANP-treated SHRs compared with untreated SHRs (186±4 versus 159±8 mm Hg, $p<0.02$, and 167±3 versus 146±7 mm Hg, $p<0.05$, respectively), whereas diastolic blood pressure was not significantly modified in SHRs (148±3 versus 131±7 mm Hg, $p=0.1$). Finally, invasive aortic blood pressure was not decreased in the NT compared with the NC group.

**Mechanical Properties of the In Situ Isolated Carotid Artery**

Minimal CC values were obtained for the lower and higher pressure levels corresponding to "flat" parts of the pressure–volume relation (Figure 3b). Maximal values of CC were obtained in the operating pressure range between 75 and 125 mm Hg for WKYs and 100–150 mm Hg for SHRs.

Under control conditions in the NC group, carotid artery compliance (i.e., CC) ranged from 1.68±0.31 to 10.73±1.53×10⁻³ μL/mm Hg per millimeter of vessel length. In ANP-treated rats (NT), CC ranged from 3.09±0.54 to 10.61±0.90×10⁻³ μL/mm Hg per millimeter of vessel (Figure 5a). In the HC group, CC ranged from 1.47±0.31 to 4.89±0.86×10⁻³ μL/mm Hg per millimeter of vessel, and in the HT group from 2.27±0.73 to 6.97±0.72×10⁻³ μL/mm Hg per millimeter of vessel (Figure 5b). There was no significant difference in measured CC between the NC and NT groups (Figure 5a). In contrast, CC was significantly higher in the HT group compared with the HC rats for the whole range of pressures ($p<0.03$; Figure 5b). Moreover, for the whole range of pressures, CC values in SHRs were significantly lower than in WKYs in both control and treated groups ($p<0.01$).

After poisoning of the smooth muscle cells by potassium cyanide, CC ranged from 1.27±0.26 to 12.69±2.13×10⁻³ μL/mm Hg per millimeter of vessel length in the NC group. In ANP-treated rats (NT), CC ranged from 2.13±0.29 to 12.70±1.16×10⁻³ μL/mm Hg per millimeter of vessel (Figure 6a). In the HC group, CC ranged from 0.91±0.26 to 6.48±1.13×10⁻³ μL/mm Hg per millimeter of vessel, and in the HT group from 1.63±0.36 to 8.39±1.03×10⁻³ μL/mm Hg per millimeter of vessel (Figure 6b). For the whole range of pressures, CC values were significantly lower in SHRs than in WKYs in untreated and treated groups ($p<0.05$). There was no difference in measured CC between NC and NT groups after potassium cyanide poisoning (Figure 6a). In contrast, CC was significantly higher in the HT than the HC group ($p<0.05$). Moreover, the potassium cyanide–induced compliance increase was significantly larger in the HT group than in the respective control group (HC) ($p<0.02$).

**Morphometry**

Typical examples of longitudinal sections of the descending thoracic aorta that were specifically stained are...
FIGURE 8. Photomicrographs of typical examples of longitudinal sections of the descending thoracic aorta after Sirius red staining for collagen content in the wall. Upper left panel, normotensive control (NC); upper right panel, hypertensive control (HC); lower left panel, normotensive treated (NT); lower right panel, hypertensive treated (HT). ×600.

represented in Figures 7–9. Morphometric parameters are summarized in Table 1. Aortic medial thickness was increased by 30% in the HC group versus the NC group (p<0.001). ANP treatment had no effect on medial thickness in WKYs, whereas it induced a significant reduction in arterial wall thickness in the SHRs (p<0.01).

In parallel, the relative density of elastin was significantly lower in the HC versus the NC group (p<0.01). ANP had no significant effect on elastin density. In addition, the mean thickness of elastin lamellae was similar in the four studied groups.

In SHRs, the relative density of collagen was significantly increased compared with normotensive rats (p<0.001); treatment by ANP had no effect on collagen density in both strains.

No difference in the number of nuclei per millimeter of aorta was observed between NC and HC groups. A significant increase in the number of nuclei was observed in the NT group versus the NC group (p<0.05).

In contrast, no difference in the number of nuclei was observed in the HT versus the HC group. The nuclear cross-sectional area was significantly larger in HC compared with NC rats (p<0.001). Treatment induced a significant decrease in the size of the nuclei in both strains (p<0.001).

Plasma ANP Concentration

Plasma ANP levels were significantly higher in the HC group compared with the NC group (574±81 versus 337±15 pg/mL plasma, p<0.05). Plasma ANP levels were not significantly modified in treated groups compared with untreated groups in both strains (Figure 10).

Urine Excretion and Urinary cGMP Content

In both strains, urine excretion was higher in treated than in untreated rats. In the HT group, urine excretion increased significantly from the seventh to the 14th day of treatment (3.3±0.6 versus 6.0±0.5 mL/12 hr, p<0.05) and remained constant until the 21st day of treatment (6.1±1.1 mL/12 hr) (Figure 11a).

Urinary cGMP concentration was higher in treated than in untreated rats. In the HT group, cGMP content increased significantly from the seventh to the 14th day of treatment and then reached a plateau (39.9±6.3 versus 61.6±1.2 nmol/12 hr, p<0.01). This increase remained constant until the 21st day of treatment (56.1±4.3 mL/12 hr; Figure 11b).

Discussion

Sustained essential hypertension is associated with an increase of arteriolar resistances and an increase of the thickness of the arterial wall, partially due to hypertrophy of smooth muscle cells. Several authors have suggested that smooth muscle cell hypertrophy may be a primary abnormality preceding the development of hypertension. Moreover, in SHRs the increase in stiffness of the arterial wall could participate in hemodynamic alterations.

It is now well established that ANP plays a vasodilator role; however, when used at the same dose, ANP
seemed more efficacious when infused chronically as compared with a bolus injection. There is controversy concerning the in vivo vascular actions of ANP because of the apparent disparate mechanisms of vascular regulation after either bolus administration or long-term infusion of ANP. As suggested by Winquist and Hintze in a recent review, this was not based on the method of studying ANP's vascular actions but on the physiological function that one envisioned for ANP.

In the present experiment, ANP did not significantly change noninvasive SBP in both strains during the 25-day infusion period. The only significant decrease was observed from the 13th until the last day of treatment in HT rats compared with HC SHRs. Furthermore, a significant decrease of invasive SBP and mean arterial blood pressure was obtained in anesthetized, treated SHRs. These results indicate that blood pressure was actually lower in the HT than the HC group at the end of treatment. Garcia et al have reported that long-term infusion of ANP (300 ng/kg body wt per hour) for 7 days in conscious SHRs and WKYs induced a drop of the initial SBP in SHRs. Using a lower dose of ANP (100 ng/kg body wt per hour), Cachofeiro et al reported a fall in arterial pressure 5 days after beginning the ANP infusion in SHRs. In both studies, infusion of ANP at higher doses than those used in the present work did not modify the arterial pressure in WKYs. Also, it is well known that high concentrations of ANP result in a reduction in the number of ANP receptors (downregulation). Thus, it is possible that the silent C-receptors (ANP clearance receptors), which represent 90–95% of total binding sites, should not be activated (at least during the first 2 weeks) and saturated in the presence of a low dose of ANP as used in the present study. Thus, they could not play their epuration role as they do when all the B receptors are occupied by ANP. This could explain why low doses of ANP appear to have paradoxically larger vascular effects than do high doses.

Mechanical Properties of Arteries

The measurement of CC provides direct information about the static mechanical behavior of the carotid wall under different experimental conditions. In the present study, CC was measured in situ in nonisolated, unexposed vessels maintained at physiological longitudinal stress. These experimental conditions allowed us to measure more physiologically relevant values than those obtained in vitro from strips or arterial rings. Indeed, in the present experimental model, the artery kept its in vivo length, whereas it was reduced in in vitro studies. Moreover, in our experiments, the vessel was not preconstricted with norepinephrine, angiotensin II, or potassium chloride, as needed in in vitro studies.

Under control conditions and after abolition of vascular smooth muscle tone, a higher stiffness was observed in the carotid arteries of SHRs compared with
smooth muscle appeared to be more effective in controlling the static mechanical properties of the carotid artery in SHRs than in WKYs. The significant difference in CC measured after abolition of vascular smooth muscle tone may be related to differences in protein content of the extracellular matrix of the arterial wall and/or to an increase in smooth muscle mass.7,10,11

Under control conditions with active smooth muscle tone, CC was higher in ANP-treated SHRs than in untreated SHRs (p<0.03). In the same way, after potassium cyanide poisoning of smooth muscle cells, CC was higher in ANP-treated SHRs than in untreated SHRs (p<0.05). Furthermore, the potassium cyanide-induced increase in CC was larger in treated than in untreated SHRs (p<0.02). These results suggest that the increase in CC induced by ANP treatment was mainly related to structural modifications of the carotid wall and not to the relaxation of smooth muscle cells.

Most of the previous studies on mechanical responses of vessels to ANP have been performed on in vitro preparations. Cohen and Schenck49 have shown that arteries from rats were relaxed by ANP, whereas veins were not. Strips from large vessels (aortic or renal arteries) seemed more sensitive to the peptide than those from smaller vessels.44-45 Additionally, Meisheri et al46 suggested that the vasorelaxant effect of ANP could be related to prevention of the release of Ca2+ from intracellular storage sites. This could produce an inhibition of phosphatidylinositol hydrolysis and a rise in cGMP.47 Therefore, the dependence on extracellular versus intracellular origins of Ca2+ for excitation–contraction coupling in vascular beds could be an important determinant in the overall responsiveness to ANP. Other in vitro studies with vascular strips of rat aorta have also shown that ANP induced a profound inhibition of the contraction induced by norepinephrine or angiotensin II, independent of the presence or absence of the endothelium. Nevertheless, it is difficult to compare the short-term effects of ANP in vitro obtained in these studies with our results observed in a long-term in vivo rat model. Therefore, morphological study of the arterial wall was important to consider when interpreting the mode of action of ANP on the large vessels.

**Morphological Analysis**

In untreated SHRs, aortic wall thickness was significantly larger (by 30%) than that of untreated WKYs. These results are in agreement with preceding studies that have demonstrated an increase in medial thickness in different models of hypertension.7,10-12 Similar nuclear densities in untreated WKYs and SHRs were associated with significantly larger nuclei in SHRs than in WKYs, suggesting that the increase in medial thickness in SHRs was related to smooth muscle hypertrophy. This is in agreement with previous studies, which showed hypertrophy of smooth muscle cells in different types of hypertension.9

The increase in nuclear cross-sectional area in SHRs is concordant with results reporting an increase in DNA content and polyploidy in hypertension.27 This increase in the size of nuclei could be related to protein synthesis by smooth muscle cells. It is now well established that collagen content is increased in hypertension.10,11 Using the same morphometric methods as in the present study, we reported a similar increase in collagen content in hypertensive rats.18,41

**TABLE 1. Morphometric Parameters of the Aortic Wall Media in Each Experimental Group**

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<thead>
<tr>
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<th>Normotensive</th>
<th>Hypertensive</th>
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<tr>
<td><strong>Media thickness (µm)</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>NS 99.8±1.3</td>
<td>NS 102.4±2.6</td>
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<tr>
<td>Treated</td>
<td>NS 102.4±2.6</td>
<td>NS 113.1±3.3</td>
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<tr>
<td>Elastin density (%)</td>
<td></td>
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<tr>
<td>Control</td>
<td>NS 25.9±1.4</td>
<td>NS 26.1±1.5</td>
</tr>
<tr>
<td>Treated</td>
<td>NS 26.1±1.5</td>
<td>NS 22.3±1.4</td>
</tr>
<tr>
<td>Thickness of elastin fiber (µm)</td>
<td></td>
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<tr>
<td>Control</td>
<td>NS 3.22±0.09</td>
<td>NS 3.36±0.11</td>
</tr>
<tr>
<td>Treated</td>
<td>NS 3.29±0.12</td>
<td>NS 3.30±0.15</td>
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<tr>
<td>Collagen density (%)</td>
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<tr>
<td>Control</td>
<td>NS 8.4±0.6</td>
<td>NS 10.6±0.9</td>
</tr>
<tr>
<td>Treated</td>
<td>NS 10.6±0.9</td>
<td>NS 15.8±1.6</td>
</tr>
<tr>
<td>Nuclei number (per millimeter aortic section)</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>* 406±16</td>
<td>NS 437±21</td>
</tr>
<tr>
<td>Treated</td>
<td>NS 471±17</td>
<td>NS 408±19</td>
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<tr>
<td>Nuclear cross-sectional area (µm²)</td>
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</tr>
<tr>
<td>Control</td>
<td>* 7.08±0.22</td>
<td>NS 8.81±0.28</td>
</tr>
<tr>
<td>Treated</td>
<td>NS 7.08±0.22</td>
<td>NS 8.81±0.28</td>
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NS, not significant. Values are mean±SEM. *p<0.05, †p<0.01, ‡p<0.001.
In the present study, the relative amount of elastin was lower in SHRs compared with WKYs. The mean thickness of elastic fibers was similar in both strains. Conversely, the relative amount of collagen was higher in untreated SHRs compared with untreated WKYs. These results show that hypertrophy of smooth muscle cells in untreated, 16-week-old SHRs was not associated with an increase in elastin content but with an increase in collagen content.49

Vascular hypertrophy in hypertension is widely considered to be an adaptive process in response to increased arterial wall stress. The medial thickness could have deleterious effects on the damping function of the systemic network and could participate in the atherosclerosis process. Furthermore, increased arterial stiffness participates in the increase in left ventricular afterload in hypertension. Therefore, the reduction or limitation of vascular hypertrophy could be an interesting therapeutic target.

In a recent in vitro study, Itoh et al50 have shown that ANP acts both as an antiproliferative as well as an antihypertrophic factor.14,52 In the SHR, augmented secretion of ANP, which has natriuretic, vasorelaxant, and antihypertrophic properties, might be considered as a compensatory mechanism to limit the progression of hypertension by reducing vascular tone as well as by inhibiting vascular hypertrophy. In the present study, histomorphology of the aortic wall in ANP-treated SHRs provided evidence for a significant reduction of aortic medial thickness. The size of the nuclei of smooth muscle cells was significantly decreased in both ANP-treated WKYs and SHRs compared with their respective control groups. These results might be related to the antiproliferative effect of ANP observed in vitro. Probably because of a high turnover rate, elastin fiber size was not affected by 3 weeks' treatment with ANP. These results suggest that in the present experimental conditions, ANP could have more marked antiproliferative than antihypertensive effects. In addition to those obtained by Dzau's group,14 our results suggest that an infusion of a very low dose of ANP for several weeks could induce results similar to those obtained in vitro after some hours or days (4) of incubation with higher concentrations of ANP.

Plasma ANP was not affected by infusion of the peptide in both strains. In contrast, the infusion of a low dose of ANP induced an increase in the diuresis and urinary cGMP excretion in the hypertensive rats. These results are in agreement with a previous study with a larger ANP dose.10 Because of the short half-life of ANP and the variation in plasma ANP levels induced by stress during blood collection, it appears that urinary cGMP is a better marker of the physiological effects of ANP than is plasma ANP content. The main finding of the present study was to provide evidence for concomitant modifications of the mechanical and structural (histological) properties of the arterial wall and urinary cGMP content in SHRs chronically treated with a low dose of ANP. These long-term effects of ANP could be involved in the remodeling of the arterial wall that is observed during hypertension and could have beneficial effects on the cardiac and vascular diseases in chronic sustained hypertension.

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References

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