Erectile dysfunction: an early marker for hypertension? A longitudinal study in spontaneously hypertensive rats

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Erectile dysfunction: an early marker for hypertension? A longitudinal study in spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol 288: R276–R283, 2005. First published August 5, 2004; doi:10.1152/ajpregu.00040.2004.—Erectile dysfunction (ED) is another manifestation of vascular disease. We evaluated the natural history of ED in the spontaneously hypertensive rat (SHR) and the respective participation of associated pathophysiological modifications, i.e., endothelial dysfunction and tissue remodeling. SHR and their normotensive counterparts [Wistar-Kyoto rats (WKY)] of 6, 12, and 24 wk of age (n = 12) were used to evaluate erectile function, erectile and aortic tissue reactivity, and remodeling. Erectile responses in SHR are reduced at all ages (P < 0.001). In both aortic and erectile tissues of SHR and WKY, relaxations to ACh are altered progressively with age, although more markedly in SHR. They are decreased at 12 wk of age in erectile tissue of SHR compared with WKY (maximal relaxation: −19.2 ± 2.8% vs. −28.3 ± 3.9%, P < 0.001) but only at 24 wk of age in aortas (−47.9 ± 6.4% vs. −90.5 ± 2.9%, P < 0.001). Relaxations to sodium nitroprusside are unaltered in aortic rings of both strains but enhanced in erectile tissue of SHR at 12 wk of age. Major modifications in the distribution of collagen I, III, and V in SHR occur in both types of tissue and are detectable sooner in erectile tissue compared with aortic tissue. The onset of ED is detectable before the onset of hypertension in the SHR. Structural and functional alterations, while similar, occur earlier in erectile compared with vascular tissue. If confirmed in humans, ED could be an early warning sign for hypertension, and common therapeutic strategies targeting both ED and hypertension could be investigated.

Hypertension; endothelial dysfunction; remodeling

IT IS WELL KNOWN that men with hypertension (HTN) have a significantly higher prevalence of erectile dysfunction (ED) than the general population (11, 16). Furthermore, the incidence of ED is associated with the duration and severity of HTN (11). Interestingly, 8–10% of untreated hypertensive patients suffer from ED at the time of diagnosis of HTN (19).

The basal tone of corpus cavernosum smooth muscle is controlled by complex events coordinated at the level of the central and peripheral nervous system. The sympathetic nervous system ensures flaccidity by producing an α-adrenergic-dependent tone of the corporal smooth muscle maintaining the penis in a flaccid state, thus minimizing intracavernosal blood flow and pressure (ICP) (13). On sexual stimulation, penile erection, occurring in response to the activation of proerectile autonomic pathways, is greatly dependent on adequate inflow of blood to the erectile tissue and requires coordinated arterial endothelium-dependent vasodilatation and sinusoidal endothelium-dependent corporal smooth muscle relaxation (3). Nitric oxide (NO) is the principal peripheral proerectile neurotransmitter that is released by both nonadrenergic, noncholinergic neurons and the sinusoidal endothelium to relax corporal smooth muscle through the cGMP pathway (5, 15), resulting ultimately in increased ICP (3). This increase in ICP activates pressure-dependent veno-occlusive mechanisms to limit the outflow of blood, thus further promoting elevated ICP and erectile response. The increased blood flow is thus ultimately driven by the force of the arterial pressure (13). Any factors modifying the basal corporal tone, the arterial inflow of blood to the corpora, the synthesis/release of neurogenic or endothelial NO within the corpora and/or the veno-occlusive mechanism are prime suspects for being involved in the pathophysiology of ED.

We have previously shown that erectile tissue from spontaneously hypertensive rats (SHR) with established hypertension presented functional alterations (6) mirroring those present in arteries from both conductance and resistance vasculature, i.e., an elevated vasconstrictor tone (29) and decreased endothelium-dependent vasodilatations (18). To go a step beyond, modifications of extracellular matrix composition (remodeling) at the vascular level are present or may even precede onset of HTN (1, 12), thus modifying the overall dilatory capacity of the vessel (12). Interestingly, remodeling associated with hypertension may also occur at the corporal level (9, 14, 23, 26), although the timeline of these modifications is unknown at this time.

Therefore, the primary goal of this study was to study the natural history of ED associated with HTN in vivo in a well-established model of genetic HTN, i.e., SHR and its normotensive control rat, the Wistar-Kyoto rats (WKY). Our second objective was to investigate the participation of the pathophysiological mechanisms implicated in ED in the SHR by studying in parallel the modifications occurring with time at the vascular level and at the level of the erectile tissue in terms of I tissue reactivity by in vitro isometric tension studies and...
2) modifications in cellular and extracellular composition (remodeling).

METHODS

Male SHR/Kyο®Rj male rats and WKY/Kyο®Rj rats at 6, 12, and 24 wk of age were obtained from Elevage Janvier (Le Genest-St-Isle, France). All procedures were performed in accordance with the "Guiding Principles for Research Involving Animals and Human Beings" established by the American Physiological Society (2) and the legislation on the use of laboratory animals (NIH publication no. 85–23, revised 1996, and Animal Care Regulations in force in France as of 1988).

In vivo evaluation of erectile function. As previously described (6, 13), SHR (n = 12/age) and WKY (n = 12/age) were anesthetized with an intraperitoneal injection of xylazine (10 mg/kg) and ketamine (90 mg/kg), tracheotomized and maintained at 37°C. The carotid artery was catheterized to record arterial pressure (AP), and a 21-gauge needle was inserted into one of the corpus cavernosum of the penis to record ICP simultaneously via pressure transducers (Elcomatic 750). The cavernous nerve (CN) was exposed at the lateral aspect of the prostate and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100, Phymep, France). For each animal, 24 wk of age were compared with WKY (113 ± 4 vs. 96 ± 4 mmHg, P < 0.001, respectively) but not at 6 wk of age [94 ± 4 vs. 83 ± 2 mmHg, not significant (NS)].

Evaluation of erectile function in vivo. Erectile responses to CN stimulation from WKY were identical at 6, 12, and 24 wk of age compared with WKY (113 ± 4 vs. 96 ± 4 mmHg, P < 0.001, respectively) but not at 6 wk of age [94 ± 4 vs. 83 ± 2 mmHg, not significant (NS)].

Extent of extracellular matrix solubilization determined by differential 4°C/30°C pepsin treatment. Before collagen phenotyping, pepsin digestion (pepsin:collagen ratio of 1:5 in 0.5 M acetic acid) was performed at 4°C for 48 h. After centrifugation, a second total digestion (same pepsin:collagen ratio at 30°C for 72 h) was performed on the pellet. Quantity of collagen was determined on each pepsin extract by colorimetric assay of hydroxyproline.

Typing of collagen. Type I, III, and V collagens in the pooled pepsin extracts were separated on 7.5% SDS-PAGE in reducing conditions (17). Collagen α-chains were stained by Coomassie blue, identified using standard collagen I, III, and V samples, and quantified in triplicate by densitometric analysis (Syngene GeneTools), and collagen proportions were calculated as previously described (17): % of collagen I equals 100 × [α2(I)] × 3/total α-chains; % of collagen III = 100 × [α1(III)] – [α2(II)] × 2/total α-chains; and % collagen V = 100 × [α1(V)] × 1.5/total α-chains.

Smooth muscle cell content (α-actin determination). Total protein concentration was measured with a Bradford assay, and equal amounts of protein (0.5 μg) were separated on 10% denaturing SDS-PAGE, electroblotted on nitrocellulose, probed with a monoclonal anti-α-actin antibody (ABCAM), and detected by enhanced chemiluminescence (Roche, Germany) on autoradiographic film (Kodak Bio-Max Light film). Densitometric results (Quantity one software, Bio-Rad) were expressed in relative optical density of the α-actin protein normalized by an internal standard. This internal standard was a fixed amount of an aortic sample run on the same gel as all other samples and following the same detection process as the samples with which it was run. Thus normalization with this internal standard ensured comparable results from one gel to another.

Values were expressed as means ± SE. Comparisons were performed using one-way or two-way ANOVA with repeated measures followed by Bonferroni’s complementary analysis where relevant. A P value <0.05 was considered to be significant.

RESULTS

MAP was significantly higher in anesthetized SHR of 12 and 24 wk of age compared with WKY (113 ± 4 vs. 96 ± 4 and 121 ± 4 vs. 96 ± 4 mmHg, P < 0.001, respectively) but not at 6 wk of age [94 ± 4 vs. 83 ± 2 mmHg, not significant (NS)].

Evaluation of erectile function in vivo. Erectile responses to CN stimulation from WKY were identical at 6, 12, and 24 wk of age. Likewise, in SHR, there was no further worsening of erectile responses to CN stimulation with time, indicating that the ratio of ICP/MAP remained equivalent for a given frequency, and thus that the rise in ICP varied proportionally to the rise in MAP in these SHR (Fig. 1). Strikingly, however, the magnitude of the erectile responses was drastically reduced in SHR compared with WKY rats at all time points and all frequencies except 1 Hz (P < 0.001, Fig. 2).

Isometric tension studies on aortic rings and corporal strips. In both aortas and corporal strips, the initial tension generated in response to phenylephrine (Phe; 10−6 or 10−5 M, respectively) was not modified with time in WKY, while it was significantly decreased in 12- and 24-wk-old SHR compared with younger 6-wk-old SHR (in corporal strips, 206 ± 18 and 177 ± 18 vs. 334 ± 23 g/g wet wt; in aortic rings, 405 ± 60 and 473 ± 76 vs. 741 ± 82 g/g wet wt, for 12- and 24-wk-old SHR compared with younger 6-wk-old SHR, respectively). These developed tensions were significantly decreased in aortic rings of 24-wk-old SHR compared with age-matched WKY (405 ± 60 vs. 756 ± 98 g/g wet wt) and as soon as 12 wk of age in corporal strips (206 ± 18 vs. 265 ± 18 g/g wet wt). In subsequent experiments, care was taken to obtain comparable...
precontraction levels with Phe before inducing concentration-
dependent relaxant responses to ACh or SNP in both WKY
and SHR.

In aortic rings, maximal relaxation responses to ACh were
reduced progressively with increasing age in SHR starting at
12 wk of age, whereas they were unchanged in WKY. Inter-
estingly, the pD2 values in 6-wk-old WKY indicate a particular
sensitivity and responsiveness to ACh at this age (8.44 ± 0.27
vs. 7.43 ± 0.20 and 7.60 ± 0.08 at 12 and 24 wk of age, P <
0.05 and P < 0.01, respectively). Furthermore, at 24 wk of age,
the concentration-response curve was significantly different
between WKY and SHR, indicating a clear alteration of the
ACh-induced endothelium-dependent relaxations in aortic
rings from SHR (Fig. 3). Maximal relaxation to SNP and pD2
values did not differ significantly among the different age
groups in WKY or in SHR, except at 24 wk of age (pD2 values
of 8.72 ± 0.12 in SHR vs. 9.11 ± 0.09 in WKY, P < 0.05,
Fig. 4).

In corporal strips, in both WKY and SHR, maximal relax-
ation responses to ACh were clearly diminished at 12 and 24
wk of age compared with 6 wk of age, indicating a particular
sensitivity and responsiveness to ACh at that age. Furthermore,
the concentration-response curves to ACh were significantly
altered in SHR compared with WKY as soon as 12 wk of age,
while pD2 values were significantly augmented (7.02 ± 0.19
vs. 6.46 ± 0.12, P < 0.05, Fig. 3). Maximal relaxation
responses to SNP were slightly reduced with increasing age in
WKY but not in SHR. The degree of relaxation elicited by SNP
was even greater in SHR starting at 12 wk of age, with greater
pD2 values compared with WKY (7.11 ± 0.08 vs. 6.46 ± 0.12,
P < 0.001, Fig. 4).

Vascular and penile remodeling. In both aortic and erectile
tissues, there was a significant increase in total protein content
with age in both strains, with WKY tissue containing more
protein per gram of wet weight than age-matched SHR (P <
0.05, Table 1). In both strains, while total collagen remained

Fig. 2. Effect of cavernous nerve stimulation at
increasing stimulation frequencies on the intracav-
ernous pressure (ICP) of SHR vs. WKY rats of
various ages. Results are expressed as ratio ΔICP/
MAP (%), where ΔICP is the difference between
ICP in the flaccid state and ICP during the plateau
phase of the erectile response, and MAP is the mean
arterial pressure during the tumescence phase (n =
12/age/strain). ***P < 0.001, 2-way ANOVA, SHR
vs. age-matched WKY.
constant with age in aortic rings, it increased significantly with age in the erectile tissue, with a more pronounced accumulation in SHR compared with WKY, especially at 24 wk of age ($P < 0.05$, Table 1).

A 4°C pepsin treatment solubilized >95% of total collagen in aortic tissue from both strains and >90% in erectile tissue from WKY whatever the age (Table 1). In contrast, the proportion of 4°C pepsin-soluble collagen decreased significantly with age in erectile tissue from SHR and was significantly lower in SHR compared with WKY starting as early as 6 wk of age (Table 1). An additional extraction step with pepsin at 30°C was thus performed to allow the solubilization of up to 90% of total collagen present in SHR erectile tissue, and the two pepsin extracts were mixed for further analysis.

Both aortic and erectile tissue from WKY and SHR aorta contained type I, III, and V collagens, with type I as the major collagen present. Most interestingly, the relative distribution of collagen subtypes was similar in erectile and aortic tissues although great differences remain between WKY and SHR in both types of tissues. Indeed, collagen I/III ratio increased with time in WKY aortas ($P < 0.05$), whereas it strongly decreased at 12 and 24 wk of age in SHR aortas compared with WKY (Fig. 5, right). In corpus cavernosum, the collagen I/III ratio is already significantly reduced in SHR erectile tissue at 6 wk of age compared with age-matched WKY erectile tissue and continues to decrease thereafter. Collagen V accumulation was increased in SHR aortas compared with WKY at 24 wk of age, whereas it was detectable as soon as 12 wk of age in the erectile tissue (Fig. 5, left).

While there was no difference in the $\alpha$-actin content of aortic and erectile tissue in SHR with time, there was a significant increase in $\alpha$-actin content in 12-wk-old WKY compared with 6- and 24-wk-old WKY in both erectile and aortic tissues and in 12-wk-old WKY compared with age-matched SHR in aortic tissues (Table 1, Fig. 6). On the other hand, when comparing WKY and SHR at 6 and 24 wk of age, there was no difference in aortic $\alpha$-actin content while there was a significant increase in $\alpha$-actin content in erectile tissue of SHR compared with WKY (Table 1, Fig. 6).

**DISCUSSION**

Because we and others had already evidenced that SHR with established HTN (6, 14) or other hypertensive rats (DOCA salt and stroke-prone SHR) (9) had ED compared with normotensive rats, the present study aimed to evaluate the natural history.
of corporal and vascular structural and functional abnormalities due to the progression and development of HTN and its consequences on erectile function in the SHR. We report that the magnitude of erectile responses is considerably reduced in the SHR compared with age-matched WKY, whatever the age, indicating that the onset of ED is detectable before the onset of HTN, without further deterioration with time. Interestingly, similar structural and functional alterations occur in the endothelium of vascular and corporal origin, but with a different time course. Indeed, our data demonstrate altered corporal endothelium-dependent relaxations occurring earlier than aortic alterations, as soon as HTN is established. Moreover, consistent parallel changes in the cellular and acellular tissue composition from SHR are also detectable at an earlier time point in the erectile compared with the aortic tissue and characteristic of a fibrotic remodeling.

A finding of clinical relevance in these experiments is that, despite the fact that the magnitude of an erectile response is directly driven by the magnitude of the arterial blood pressure (13), erectile responses elicited by CN stimulation are already decreased in the prehypertensive SHR and do not evolve with time thereafter. For obvious reasons, the measurements could not be obtained in freely moving conscious rats, and we have previously looked at the possibility of interference of anesthetics on these erectile responses, thus selecting ketamine/xylazine anesthesia as the agent of choice (6). Nonetheless, the development of hypertension in the SHR has already been well described and the age of 6 wk is regarded to be largely prehypertensive (24). If confirmed in humans, this finding could be of utmost interest because it could confer the valuable property for ED to be an early warning sign/sentinel for HTN as it has been postulated for cardiovascular conditions in general. Supporting this suggestion is the fact that 8–10% of untreated hypertensive patients suffer from ED at the time of diagnosis of HTN (19).

We have also investigated the evolution with time of corporal endothelium-dependent and -independent relaxations implicated in the local physiology of penile erection and compared it with modifications occurring at the aortic level. Although the important control site for pressure is at the level of the small arteries/arterioles, the parallel observations between corporal and aortic tissue are of value because endothelium-dependent corporal relaxation mechanisms, as in the aorta, rely mainly on the release of biologically active NO, while smaller vascular beds (i.e., resistance vessels) include the concomitant release of other endothelium-derived substances, i.e., endothelium-derived hyperpolarizing factor (4). Indeed, previous studies have suggested that the penile vasculature and the erectile

Fig. 4. Relaxations induced by increasing concentrations of SNP (10^{-10} \text{ mol/l} to 10^{-5} or 10^{-3} \text{ mol/l}, depending on the tissue) in aortic rings (right) and corporal strips (left) from WKY and SHR at 6 (A), 12 (B), and 24 wk of age (C) (n = 12 per age per strain). §§§P < 0.001, SHR vs. WKY, 2-way ANOVA; *P < 0.05, **P < 0.01, ***P < 0.001, Bonferroni’s complementary analysis.
tissue could undergo modifications similar to those occurring in the systemic vasculature (6, 14, 23, 29). In the present study, we show that both corporal and aortic endothelium-dependent relaxation responses to ACh are reduced progressively with increasing age in SHR compared with WKY, although the time course of that alteration is different between the two types of tissue. Indeed, corporal endothelium-dependent alterations occur at an earlier age than aortic alterations, as soon as HTN is established. This suggests that the erectile tissue of SHR is not protected from the functional changes induced by chronic exposure to high blood pressure; it could even be at the front line of the development of endothelial dysfunction and thus be an early target end organ.

Interestingly, we found that erectile tissue from both WKY and SHR possesses a particular sensitivity and responsiveness to ACh and SNP at 6 wk of age, and this particular reactivity is lost with time in both WKY and SHR, except for reactivity to SNP in the SHR. A maturation of the calcium-sensitizing pathway involving RhoA-Rho kinase, the common downstream modulator of smooth muscle tone, could be involved, as shown in Fig. 5. Evolution of collagen I/III ratio (top) and collagen V (in mg/g protein; bottom) in aortas (right) and erectile tissue (left) of WKY and SHR with time. Values are expressed as means ± SE (n = 12 per age per strain). §§§P < 0.001, SHR vs. WKY, 2-way ANOVA; ***P < 0.001, Bonferroni’s complementary analysis.

### Table 1. Vascular and penile remodeling in WKY and SHR with time

<table>
<thead>
<tr>
<th>Tissue/Group</th>
<th>Protein, mg/g protein</th>
<th>Collagen, mg/g protein</th>
<th>Collagen Extraction, %</th>
<th>α-Actin, OD</th>
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<tbody>
<tr>
<td><strong>Aorta</strong></td>
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<tr>
<td>WKY</td>
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<td></td>
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<tr>
<td>6 wk</td>
<td>232.5 ± 8.3</td>
<td>437.5 ± 4.9</td>
<td>&gt;95</td>
<td>1.56 ± 0.26a</td>
</tr>
<tr>
<td>12 wk</td>
<td>250.9 ± 5.3</td>
<td>443.7 ± 5.3</td>
<td>&gt;95</td>
<td>2.87 ± 0.27</td>
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<tr>
<td>24 wk</td>
<td>265.0 ± 8.6</td>
<td>450.1 ± 5.2</td>
<td>&gt;95</td>
<td>1.01 ± 0.32a</td>
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<tr>
<td>SHR</td>
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<tr>
<td>6 wk</td>
<td>214.1 ± 1.3a,b,e</td>
<td>426.2 ± 6.3</td>
<td>&gt;95</td>
<td>1.84 ± 0.12</td>
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<tr>
<td>12 wk</td>
<td>244.3 ± 0.4a</td>
<td>422.9 ± 6.0</td>
<td>&gt;95</td>
<td>1.81 ± 0.20</td>
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<tr>
<td>24 wk</td>
<td>250.2 ± 4.0a</td>
<td>439.9 ± 9.2</td>
<td>&gt;95</td>
<td>1.29 ± 0.14</td>
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<tr>
<td><strong>Corpus cavernosum</strong></td>
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<tr>
<td>WKY</td>
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<tr>
<td>6 wk</td>
<td>178.0 ± 1.9p,e</td>
<td>261.1 ± 7.8</td>
<td>93.4 ± 1.3</td>
<td>0.69 ± 0.14a</td>
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<tr>
<td>12 wk</td>
<td>156.0 ± 6.7</td>
<td>242.0 ± 9.0</td>
<td>90.1 ± 1.4</td>
<td>2.11 ± 0.14</td>
</tr>
<tr>
<td>24 wk</td>
<td>189.0 ± 7.9</td>
<td>315.1 ± 5.9</td>
<td>19.9 ± 1.2</td>
<td>1.13 ± 0.34a</td>
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<td>SHR</td>
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<tr>
<td>6 wk</td>
<td>160.9 ± 0.4p,e,g</td>
<td>237.0 ± 5.1e</td>
<td>70.0 ± 1.4e</td>
<td>2.26 ± 0.4b</td>
</tr>
<tr>
<td>12 wk</td>
<td>159.8 ± 2.0p</td>
<td>260.0 ± 6.7e</td>
<td>64.9 ± 1.5e</td>
<td>2.08 ± 0.21</td>
</tr>
<tr>
<td>24 wk</td>
<td>175.8 ± 2.1</td>
<td>355.0 ± 14.8e</td>
<td>58.9 ± 1.6e</td>
<td>2.73 ± 0.33b</td>
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| **Values are expressed as means ± SE (n = 12 per age per strain). a P < 0.05, b P < 0.01, c P < 0.001 vs. age-matched Wistar-Kyoto rats (WKY). Student’s t-test. d p < 0.05 vs. WKY 12 wk, e p < 0.05 vs. WKY 24 wk. f P < 0.05 vs. spontaneously hypertensive rats (SHR) 12 wk, g P < 0.05 vs. SHR 24 wk, 1-way ANOVA. OD, optical density.**
already evidenced in vessels (21, 29). On the other hand, the increased sensitivity or effectiveness of SNP in corporal tissue from SHR at 12 and 24 wk of age is in agreement with previous results obtained in our laboratory (6) and could be a tissue-specific compensatory upregulation mechanism of the soluble guanylate cyclase pathway to defective endothelium-dependent relaxations (25). It is, however, not powerful enough to overcome other alterations present in the prehypertensive and hypertensive SHR and responsible for the altered erectile responses to sexual stimulation, suggesting that other mechanisms are involved. It is noteworthy, indeed, that impairment of the erectile responses to CN stimulation is already detectable in SHR as soon as 6 wk of age while corporal and aortic endothelium-dependent alterations occur only once HTN is established. Thus other mechanisms need to be addressed to understand ED in the prehypertensive SHR.

We have also evaluated the remodeling of the vascular and erectile tissues during the developmental stages of HTN in the SHR to evaluate its potential participation to ED. Indeed, it is well recognized that vascular remodeling precedes the onset of HTN and participates in the long-term resistance changes associated with HTN (1, 27) and that structural changes in the penile vasculature also occur and may participate in the impairment of corporal smooth muscle relaxation leading to HTN-associated ED (14, 23, 26). Our data evidence the fact that striking and consistent changes in the distribution of collagen phenotypes in both erectile and aortic tissues from SHR compared with WKY occur. We clearly show greater percentage distribution of type V collagen and lower percentage distribution of collagen I/III in SHR, both in aortic and erectile tissues. These changes were detectable at an earlier time point in the erectile tissue compared with the aortic tissue (6 vs. 12 and 12 vs. 24 wk of age, respectively). These modifications in collagen distribution may be related to both the increase in smooth muscle mass we observed even in prehypertensive animals, at least at the level of the corpus cavernosum, and the predominance of a synthetic phenotype predisposing the vessels to the increase of extracellular matrix deposition. Indeed, the fact that these modifications occur both at the vascular and the corporal level points to a common biosynthesis defect of extracellular matrix by the smooth muscle cells.

Interestingly, such collagen III overexpression has often been reported as the hallmark of the fibrosis-related alterations in several tissues during hypertension (8, 20). The relative augmentation of collagen III and V could contribute to the formation of less structured and less functional heterotypic collagen fibrils, as previously described (7). Thus a general fibrotic process occurs during the development and evolution of HTN, as previously suggested (8, 20). Such alterations of collagen I/III and collagen V proportions in heterotypic fibrils of collagen could lead to functional disturbances of the collagenous network within the erectile and aortic tissue from SHR and thus be implicated in the pathogenesis of ED in these animals. Furthermore, we found that the proportion of 4°C pepsin-soluble collagen decreased significantly with age in erectile tissue from SHR and was significantly lower in SHR compared with WKY starting as early as 6 wk of age. Although this preliminary observation needs further investigation, it could be an early indication of a progressive cross-linkage of collagen in erectile tissue of SHR, contributing to its resistance to digestion by pepsin treatment and resulting in the formation of a fibrotic and less functional tissue.

In summary, and to the best of our knowledge, this is the first and most exhaustive report performed both in vivo and in vitro to investigate the progression of corporal and vascular structural and functional abnormalities associated with development of HTN and its consequences on erectile function in SHR. Important features of fibrotic alterations of the corporal tissue were detected in the prehypertensive SHR and concomitant with ED. These structural changes do not have an impact on the endothelium-dependent and -independent relaxations of aortic and erectile tissue in the prehypertensive SHR. Although this result may be surprising, inappropriate extracellular matrix composition of the corpora cavernosa may nonetheless be responsible for altered mechanical properties of the erectile tissue, leading to corporal veno-occlusive dysfunction. Indeed, although relaxation of the erectile tissue is preserved, it may have lost the characteristic compliance of the fibroelastic frame of the penis (22), resulting in an inability to expand the trabeculae against the tunica albuginea and compress the subtunical venules. The clinical consequences for such hemodynamic alterations are an excessive outflow of lacunar blood through the subtunical venules, which prevents adequate penile rigidity, leading to corporal veno-occlusive dysfunction and thus ED.

This study provides exhaustive experimental support to investigate common therapeutic strategies targeting both ED and HTN, i.e., inhibitors of the renin-angiotensin system (ACE inhibitors or AT1 receptor antagonists), all the more since recent studies have suggested that erectile function of the SHR could be recovered after such antihypertensive therapy (10, 14). In particular, this modelization could be particularly useful to investigate innovative pharmacological strategies acting upon remodeling. Indeed, this pioneering target could both address the modifications occurring at the level of the general vasculature but also specifically at the level of an original target end organ, i.e., the penis.

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