Changes in aortic stiffness related to elastic fiber network anomalies in the Brown Norway rat during maturation and aging

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The aim of the present study was to investigate the impact of these particular characteristics of the BN AA on its mechanical properties. For this purpose, we undertook a study of the aortic wall composition, its mechanical phenotype, and the incidence of IEL ruptures in the BN rat compared with a control rat at large-artery stiffness has predictive value for cardiovascular events, independent of classical cardiovascular risk factors (32). Moreover, aortic stiffness is of independent predictive value for total and cardiovascular mortality, coronary morbidity-mortality, and fatal stroke in patients with essential hypertension, diabetes mellitus, or end-stage renal failure, and in the general population in the elderly. Extensive information on these clinical aspects, as well as the pathophysiology and structural bases of arterial stiffness, can be found in different reviews (23, 26, 27, 43).

Any modification in arterial wall structure may induce changes in its mechanical properties. The elastic network is one of the major determinants of arterial stiffness, and experimental and clinical studies have shown that changes in both content and organization of elastic fibers influence arterial wall elasticity (2, 6, 15, 22, 40). For this reason, the study of the aortic mechanical properties of the Brown Norway (BN) rat is of interest, as this rat strain presents certain particularities concerning its aortic elastic fiber network, notably the presence of numerous ruptures in the internal elastic lamina (IEL) in the abdominal segment (9) and a generalized deficit in elastin content (3, 20, 34, 35), resulting in a low elastin-to-collagen ratio (E/C).

The BN is a normotensive strain in which the above-mentioned IEL ruptures develop spontaneously, during growth and aging, in the abdominal aorta (AA) (9). This striking phenotype is not observed in other normotensive rat strains commonly in laboratory use, including the Long Evans (LE) and the LOU, 3 (20, 34) or in hypertensive strains (Ref. 16 and M. Osborne-Pellegrin, unpublished observations). The etiology of IEL rupture is at present unknown. It appears to be primarily a genetically determined character, for which quantitative trait loci have been recently described on chromosomes 5 and 10, but the gene(s) involved has not yet been identified (13, 20).

However, IEL rupture formation in the BN AA can also be influenced by administration of various compounds, such as β-aminopropionitrile and semicarbazide, which both increase IEL rupture (9, 29), and inhibitors of the renin-angiotensin system, which decrease it (14). β-Aminopropionitrile, and probably semicarbazide, induce arterial wall fragility by decreasing cross-linking of collagen and elastic fibers via inhibition of the enzyme lysyl oxidase (29, 38). Inhibitors of the renin-angiotensin system may act hemodynamically by reducing blood pressure and thus decreasing wall stress, but the effect on IEL rupture is, in part, independent of the hypertensive effect (14).

It is known that the E/C plays an important role in arterial mechanical properties. Compared with other strains, in addition to IEL ruptures, the BN rat presents a lower insoluble elastin content and a proportionally higher total collagen content in the aorta, both in the thoracic segment (3), unaffected by the above-mentioned IEL ruptures, and in the abdominal segment (34) where IEL rupture occurs. The insoluble elastin deficit in the BN rat is, at least in part, due to a deficiency in tropoelastin synthesis during the period of rapid growth (34, 35), but some degree of reduced elastin cross-linking cannot be excluded, as the BN rat was shown to have a lower aortic lysyl oxidase activity than the LE (31).
different ages, ranging from before occurrence of the first IEL ruptures (6 wk), through puberty and young adulthood up to 16 mo of age. Pharmacological modulation of IEL rupture formation, as previously described (14), was undertaken at one time point, to gain support for our hypothesis. For the part of the study dealing with growing rats up to 15 wk of age, we used the inbred LOU as a control, as in our more recent genetic studies (11, 20, 35). However, to study the impact of these various parameters on age-dependent arterial stiffening, for technical reasons we were obliged to use the previously characterized LE rat (9, 34) as a control.

Our results showed that, in BN rats, IEL ruptures first appear between 6 and 10 wk of age and continue to increase thereafter. At all ages studied, E/C was lower in BN compared with LOU and LE. Before IEL rupture formation in the BN rat, its aortic stiffness was significantly greater than in LOU, suggesting that a low E/C may contribute to this rigidity. The onset of IEL rupture then appears to contribute to reduce stiffness to reach values comparable to control strains. However, the BN rat presented a lesser increase in stiffness with aging compared with LE, which may be explained in part by the increase in the number of IEL ruptures throughout adulthood.

MATERIALS AND METHODS

Animals

This study was divided into two experiments. Experiment I was performed during the maturation phase (from 6 to 15 wk of age) on 50 male inbred BN and 30 male inbred LOU rats. Experiment II was performed during the aging phase (from 15 to 64 wk of age) on 27 male BN and 21 male outbred LE rats. BN and LE rats were supplied by Elevage Janvier (France), and LOU rats were from our own breeding stock. All rats were maintained in standard conditions on a low E/C may contribute to this rigidity. The onset of IEL rupture then appears to contribute to reduce stiffness to reach values comparable to control strains. However, the BN rat presented a lesser increase in stiffness with aging compared with LE, which may be explained in part by the increase in the number of IEL ruptures throughout adulthood.

Additional BN rats of different ages, spanning the age range used above for the main part of the study, were used for histological studies to illustrate the increase in size of the IEL ruptures with age and to examine the evolution of the different arterial components (elastic fibers, collagen, and smooth muscle cells) at the site of IEL rupture. The study was performed in accordance with the guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996). All procedures were carried out under Authorization no. 75–825 of the Direction Départementale des Services Vétérinaires de Paris, France.

In Vivo Arterial Mechanical Parameters

We simultaneously recorded intra-arterial diameter and blood pressure in the AA, midway between the renal artery and the iliac bifurcation, in pentobarbital-anesthetized rats. Internal arterial diameter was measured with an ultrasonic echo-tracking device (NIUS-02, Asulab SA, Neuchâtel, Switzerland) (24). The relationship between the pressure and the lumen cross-sectional area was fitted with the model of Lengewouters et al. (25), using an arctangent function. Distensibility, a derivative of this function, was used to assess the elastic behavior of the artery. Circumferential wall stress and incremental elastic modulus (Einc), which characterizes the intrinsic mechanical properties of the wall material, were calculated with the above-mentioned parameters and MCSA of the AA determined by histomorphometry. To compare Einc-stress curves, we calculated the mean wall stress within the 800–1,500 kPa range of Einc (MWS800–1500).

Histological Studies

At the end of the in vivo measurement of arterial mechanical parameters, the thoracic aorta (from the aortic arch down to the diaphragm) was rapidly excised, cleaned of blood and periadventitial tissue in ice-cold saline, and stored at −80°C until use for determination of elastin and collagen contents. A catheter was then inserted into the aorta at the level of the diaphragm for perfusion in situ of the AA with buffered formalin at controlled pressure. After 20–30 min of perfusion, and a further 15 min of contact with formalin in situ, the AA was dissected out and stored in formalin.

Measurement of MCSA. A small ring (1 mm in height) was cut from each AA, at approximately the level of the catheter tip in the mechanical studies, and embedded in paraffin. Eight-micrometer transverse sections were stained with orcein to demonstrate elastic fibers, and MCSA was determined by computer directed analysis (Quant'Image software, Talence, France) (24).

Quantification of IEL ruptures. En face preparations were made of the formalin-fixed AAs of each rat (minus the small ring taken for MCSA measurement). Under a dissecting microscope, aortic segments were cleaned of periadventitial tissue, opened longitudinally.

Table 1. General and mechanical parameters during the maturation phase in BN and LOU rats

<table>
<thead>
<tr>
<th></th>
<th>Strain</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Age, wk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>120 ± 2*</td>
<td>226 ± 5</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>89 ± 3*</td>
<td>125 ± 3</td>
</tr>
<tr>
<td>Diameter at 100 mmHg, mm</td>
<td>0.92 ± 0.07*</td>
<td>1.31 ± 0.05</td>
</tr>
<tr>
<td>Distensibility at 100 mmHg, × 10⁻³ mmHg⁻¹</td>
<td>2.4 ± 0.2*</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>MCSA, mm²</td>
<td>0.122 ± 0.007*</td>
<td>0.185 ± 0.006</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. BN, Brown Norway; MAP, mean arterial pressure; MCSA, media cross-sectional area. *P < 0.05 vs. 10 wk in the same strain. †P < 0.05 vs. BN at the same age.
and pinned out luminal side up. The luminal surface was stained with orcein and hematoxylin, to stain the IEL and the endothelial nuclei, respectively, and reveal ruptures in the IEL. After staining, arteries were dehydrated, unpinned, cleared, and mounted on slides for microscopy. Several segments of each aorta were embedded in paraffin to obtain transverse and longitudinal sections. Serial 8-μm sections were stained with Masson’s trichrome, orcein, and picrosirius red to reveal, respectively, the general architecture, elastic lamellae, and collagen fibers.

Determination of Aortic Elastin and Collagen Contents

Elastin and collagen contents were measured on thoracic aortas, as previously described (35). Briefly, the aortic arch was discarded, and, after the length of the aortic segments were recorded using a grid in the eyepiece of a dissecting microscope, whole descending thoracic aortas, without homogenization, were defatted and dried to constant weight, and their weight was recorded. Cell proteins were extracted by 0.3% SDS and subsequently assayed, and insoluble elastin was purified by the hot alkali method (3 × 15 min in 0.1 N NaOH, in a boiling water bath) and quantified by weighing. Proteins in the NaOH extract were then hydrolyzed, and total collagen was determined by assaying the hydroxyproline present in the hydrolysate. Components were expressed either as percentage of aortic dry weight or milligrams per centimeter of aorta.

Statistical Analysis

All values are expressed as means ± SE. Two-factor analysis of variance was performed to compare groups of nontreated rats, followed by a Fisher’s multiple-comparison test. One-way analysis of variance was used to assess the effect of treatment in BN rats. Differences were considered significant at values of $P < 0.05$.

RESULTS

During the Maturation Phase

The BN rat is compared with the LOU rat during the phase of rapid growth and maturation i.e., from 6 to 15 wk of age. Mean values of general, hemodynamic, and arterial parameters of rapid growth and maturation i.e., from 6 to 15 wk of age. Mean values of general, hemodynamic, and arterial parameters of rapid growth and maturation i.e., from 6 to 15 wk of age.

| Table 2. Aortic composition and IEL ruptures during the maturation phase in BN and LOU rats |
|---------------------------------|---|---|---|---|---|---|---|---|---|
| Age, wk                        | 6 | 10 | 15 | 6 | 10 | 15 | 6 | 10 | 15 |
| $n$                            | 6 | 10 | 8  | 15 | 9  | 5  | 6 | 10 | 5  |
| Dry weight of TA, mg/cm        | 1.81 ± 0.04* | 2.34 ± 0.04 | 2.46 ± 0.03* | 1.74 ± 0.03* | 2.42 ± 0.05 | 2.68 ± 0.02** | 0.04 | $<10^{-6}$ | 0.007 |
| Elastin content, mg/cm         | 0.72 ± 0.02* | 0.82 ± 0.02 | 0.80 ± 0.01  | 0.75 ± 0.02* | 1.03 ± 0.03† | 1.09 ± 0.01† | $<10^{-6}$ | $<10^{-6}$ | 0.000001 |
| Collagen content, mg/cm        | 0.41 ± 0.01* | 0.65 ± 0.02 | 0.76 ± 0.01* | 0.33 ± 0.01† | 0.52 ± 0.01† | 0.58 ± 0.01† | $<10^{-6}$ | $<10^{-6}$ | 0.0008 |
| Elastin content, % dry weight  | 39.9 ± 0.5*  | 35.8 ± 0.4  | 32.4 ± 0.3*  | 43.5 ± 0.2†  | 42.4 ± 0.4† | 40.8 ± 0.1†† | $<10^{-6}$ | $<10^{-6}$ | 0.000001 |
| Collagen content, % dry weight | 22.7 ± 0.2*  | 27.8 ± 0.5  | 30.7 ± 0.3*  | 18.9 ± 0.2†  | 21.6 ± 0.3† | 21.6 ± 0.2†† | $<10^{-6}$ | $<10^{-6}$ | $<10^{-6}$ |
| Elastin/collagen ratio         | 1.76 ± 0.04* | 1.29 ± 0.02 | 1.06 ± 0.02* | 2.31 ± 0.03† | 1.97 ± 0.04† | 1.89 ± 0.02†† | $<10^{-6}$ | $<10^{-6}$ | 0.0009 |
| IEL ruptures, total no./AA     | 0.3 ± 0.3†   | 20.2 ± 1.1  | 22.9 ± 1.1   | 0  | 0†  | 0†  | $<10^{-6}$ | $<10^{-6}$ | $<10^{-6}$ |

Values are means ± SE; $n$, no. of rats. TA, thoracic aorta; AA, abdominal aorta; IEL, internal elastic lamina. *$P < 0.05$ vs. 10 wk in the same strain. †$P < 0.05$ vs. BN at the same age.
and LOU was made by calculating the MWS\textsubscript{800-1500} (Fig. 1B). In both strains, MWS\textsubscript{800-1500} increased between 6 and 10 wk of age, indicating a reduction in stiffness, and then decreased between 10 and 15 wk. However, at 6 and 10 wk, the BN AA was significantly stiffer than that of the LOU, a difference that had disappeared by 15 wk.

Aortic composition in BN and LOU during the maturation phase is summarized in Table 2. As expected, dry weight per centimeter and elastin and collagen contents expressed in absolute values (mg/cm) increased from 6 to 15 wk, with the sharpest increase taking place between 6 and 10 wk, in parallel with the growth curve. Collagen increased proportionately more than elastin. When expressed as a percentage, elastin and collagen evolved in opposite directions in both strains, with %elastin decreasing, while %collagen increased. Thus E/C decreased between 6 and 15 wk in both strains. However, the BN presented a significantly lower E/C at all three ages.

As for ruptures or defects in the IEL, these were virtually absent in the BN at 6 wk, but increased markedly between 6 and 10 wk, and increased more slowly thereafter. In contrast, as previously reported (20), no such ruptures were observed in the LOU strain.

Tables 3 and 4 summarize the effect of treatment by two antihypertensive drugs of different classes in the BN at 10 wk on general and arterial parameters. Treatment of the BN with mibebradil or enalapril significantly decreased MAP at 10 wk. Only enalapril significantly decreased distensibility at 100 mmHg and MCSA. Figure 2 shows that only enalapril significantly altered MWS\textsubscript{800-1500} in BN rats, by partially inhibiting the increase between 6 and 10 wk, resulting in increased stiffness compared with the age-matched BN control.

Both treatments caused aortic hypotrophy, with enalapril having a greater effect as it decreased both elastin and collagen, whereas mibebradil only decreased collagen. This resulted in a slightly increased E/C in the mibebradil group compared with control BN rats of the same age, but which was, nevertheless, far from reaching LOU values. As previously reported (14), only enalapril significantly inhibited the formation of IEL ruptures compared with control.

### During the Aging Phase

For technical reasons (see below), the BN rat is compared with the LE rat during the aging phase, i.e., from 15 to 64 wk of age. Mean values of general, hemodynamic, and arterial parameters at 15, 28, and 64 wk are provided in Table 5. MAP evolved differently in the two strains, decreasing slightly with age in the LE, while increasing in the BN. Arterial diameter continued to increase with age, as did body weight. The LE gained far more weight than the BN, which is a “lean” strain, but its arterial diameter increased less with age than in the BN. Distensibility at 100 mmHg significantly decreased with age in the LE, whereas this evolution was less marked in the BN. MCSA similarly (interaction NS) increased with age in both strains and was higher in LE rats than in BN rats.

The arterial wall-stress/elastic modulus curves at 15, 28, and 64 wk in BN and LE are shown in Fig. 3A, and, as for the maturation phase, the comparison between BN and LE was made by calculating MWS\textsubscript{800-1500} (Fig. 3B). It can be seen that, whereas MWS\textsubscript{800-1500} decreased between 15 and 64 wk of age in the LE rat, this evolution was not observed in BN. Thus the BN aorta at 64 wk was significantly less stiff than the LE.

Aortic composition in BN and LE during the aging phase is summarized in Table 6. As for the younger rats, dry weight per centimeter and elastin and collagen contents expressed in absolute values (mg/cm) increased with age, with collagen increasing proportionately more than elastin. When expressed as a percentage, elastin and collagen evolved in opposite directions in both strains with %elastin decreasing, and %collagen increasing.
lagen increasing, causing a net decrease in the E/C between 15 and 64 wk in both strains. The significantly lower E/C, already observed in the younger BN rats compared with LOU, was also observed here in aging BN rats compared with LE.

Total numbers of IEL ruptures in the AA continued to increase throughout the aging period in the BN rat and were absent from the LE rat, with the exception of an occasional small rupture at 64 wk (Table 6). This continuous increase in rupture number in the BN occurs because the ruptures in the IEL, once formed, remain visible throughout life, as the IEL is never properly reconstituted.

Not only do numbers of IEL ruptures increase with age in BN rats, but their size also increases, especially in older rats (see Fig. 4, top). The morphology of the aortic wall at the site of IEL ruptures in male BN rats at different ages using specific staining to demonstrate elastic fibers, cell nuclei, and collagen is also shown in Fig. 4. In young rats (10 wk old), the inner media where the IEL is absent is highly cellular, probably reflecting the repair process, with little elastin and collagen (Fig. 4, left). In older rats (19 wk old), these areas are less cellular, with the accumulation of some elastic and collagen fibers among the smooth muscle cells (Fig. 4, middle). Examination of sites of IEL rupture from much older BN rats (112 wk old, Fig. 4, right) showed that no local fibrosis occurred with aging (Fig. 4, bottom right), as the intensity of picrosirius

**Fig. 2.** Effects in 10-wk-old BN rats of antihypertensive therapy (Co, control; Mib, mibefradil; En, enalapril) during maturation. A: effects of treatment on Einc-wall stress curves. B: MWS800-1500 in the same rats (ANOVA: P = 0.02). *P < 0.05 vs. control.

| Table 5. General and mechanical parameters during the aging phase in BN and LE rats |

<table>
<thead>
<tr>
<th>Strain</th>
<th>Age, wk</th>
<th>n</th>
<th>Body weight, g</th>
<th>MAP, mmHg</th>
<th>Diameter at 100 mmHg, mm</th>
<th>Distensibility at 100 mmHg, ( %/\text{mmHg} )</th>
<th>MCSA, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN</td>
<td>15</td>
<td>11</td>
<td>291</td>
<td>94</td>
<td>1.35 ± 0.05</td>
<td>3.4 ± 0.5</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>LE</td>
<td>15</td>
<td>6</td>
<td>343</td>
<td>48</td>
<td>1.32 ± 0.05</td>
<td>3.6 ± 0.5</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td>BN</td>
<td>28</td>
<td>6</td>
<td>403</td>
<td>114</td>
<td>1.34 ± 0.04</td>
<td>3.4 ± 0.5</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>LE</td>
<td>28</td>
<td>4</td>
<td>481</td>
<td>109</td>
<td>1.33 ± 0.04</td>
<td>3.4 ± 0.5</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td>BN</td>
<td>64</td>
<td>5</td>
<td>529</td>
<td>110</td>
<td>1.33 ± 0.04</td>
<td>3.4 ± 0.5</td>
<td>0.29 ± 0.08</td>
</tr>
<tr>
<td>LE</td>
<td>64</td>
<td>10</td>
<td>630</td>
<td>105</td>
<td>1.33 ± 0.04</td>
<td>3.4 ± 0.5</td>
<td>0.29 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. LE, Long Evans. *P < 0.05 vs. control.
red staining at the site of IEL rupture was similar to that in the
rest of the aortic media, despite the continuing absence of the
IEL.

DISCUSSION

The aim of this study was to determine the repercussions of
the structure of the BN AA, i.e., an elastin deficit causing a low
E/C and the presence of numerous IEL ruptures, on its me-
chanical properties during growth and adulthood. We show
that, during the early maturation phase, aortic stiffness is
higher in BN than in control rats, but in young adulthood
this difference disappears. However, in contrast with other
strains studied, BN aortic stiffness does not increase with
aging; this effect may be explained by the presence of nu-
merous IEL ruptures.

In performing this study on the BN rat, we were confronted
with the difficult choice of a control strain. Our earlier studies
employed the LE rat as a control (9, 31, 34), but this is an
outbred strain, presenting greater genetic variability, and also
has the disadvantage of being of larger body size than the BN
rat, necessitating correction for some parameters, i.e., arterial
caliber, linked to body size. In our laboratory’s more recent
preparations studies (11, 20, 35), we have adopted the LOU inbred
strain as a control, as it does not greatly differ from the BN in
growth characteristics and body weight gain. This strain pre-

ts the same arterial phenotype as the LE, i.e., a higher aortic
E/C compared with BN and an absence of IEL ruptures in the
AA, but older animals from our LOU colony do not survive
general anesthesia for lengths of time compatible with our
present experimental protocol. For this reason, we opted for the
following compromise: to use the LOU for the maturation
study (6–10 wk), and the LE for the aging part (28 and 64 wk),
and studying all three strains at the midpoint of 15 wk.

It is known that aging alters arterial mechanical properties in
different rat models (1, 5, 19, 36). Previous studies have
demonstrated that large-artery stiffness increases with time, in
parallel with the decrease in E/C in rats (7, 21, 42). The role of
the quantitatively minor extracellular matrix proteins has been
little studied. Both glycoproteins and proteoglycans could
contribute to mechanical properties (4, 10). In our study, the aortic
wall is still growing in the older animals, and all of the constitu-
ents increase to accompany this growth. This is the case for
elastin, and collagen, as shown in Table 6, but also for cell
proteins increase to accompany this growth. This is the case for
elastin, and collagen, as shown in Table 6, but also for cell
proteins, which we measured by subtraction (results
not shown). But with the exception of relative elastin and
collagen contents, which appear to evolve in opposite direc-
tions with age, the proportion of the other proteins did not

Table 6. Aortic composition and IEL ruptures during the aging phase in BN and LE rats

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Strain</th>
<th>Strain</th>
<th>Strain</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN</td>
<td>BN</td>
<td>BN</td>
<td>BN</td>
<td>BN</td>
</tr>
<tr>
<td>15</td>
<td>28</td>
<td>64</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Dry weight of TA, mg/cm</td>
<td>2.46 ± 0.33</td>
<td>3.20 ± 0.09</td>
<td>3.44 ± 0.08</td>
<td>2.98 ± 0.05</td>
</tr>
<tr>
<td>Elastin content, mg/cm</td>
<td>0.80 ± 0.01</td>
<td>0.89 ± 0.02</td>
<td>1.09 ± 0.22</td>
<td>1.16 ± 0.02</td>
</tr>
<tr>
<td>Collagen content, mg/cm</td>
<td>0.76 ± 0.01</td>
<td>0.82 ± 0.03</td>
<td>1.24 ± 0.02</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>Elastin content, %dry weight</td>
<td>32.4 ± 0.03</td>
<td>28.0 ± 0.4</td>
<td>28.3 ± 0.3</td>
<td>38.9 ± 0.4</td>
</tr>
<tr>
<td>Collagen content, %dry weight</td>
<td>30.7 ± 0.3</td>
<td>25.5 ± 0.5</td>
<td>33.3 ± 0.5</td>
<td>23.1 ± 0.6</td>
</tr>
<tr>
<td>Elastin/collagen ratio</td>
<td>1.06 ± 0.02</td>
<td>1.09 ± 0.03</td>
<td>0.85 ± 0.01</td>
<td>1.69 ± 0.03</td>
</tr>
<tr>
<td>IEL ruptures, total no./AA</td>
<td>22.9 ± 1.1</td>
<td>48 ± 6.5</td>
<td>74.8 ± 4.1</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Values from BN rats aged 22 wk as values for 28 wk were not available. †P < 0.05 vs. 15 wk in the same strain. ‡P < 0.05 vs. 28 wk in the same strain. §P < 0.05 vs. BN at the same age.
change significantly with age. In view of their constant proportion during aging, it appears improbable that they can explain the age-related variations in stiffness.

In our study, the intrinsic mechanical properties of the wall material were evaluated for the first time by using the Einc-wall stress curves in intact animals during the maturation phase and aging process. It appears that evolution of arterial stiffness with age differs in young and old rats, as illustrated by an increase in elasticity during growth and by a reduction in arterial stiffness during the normal aging process.

In the LOU strain, the maturation phase is characterized by a marked increase in aortic elasticity, as evaluated by MWS800–1500 (an intrinsic parameter that is independent of vessel geometry and intraluminal pressure) between 6 and 10 wk of age, followed by a decrease between 10 and 15 wk, whereas E/C decreased regularly over this period. During this phase, the aorta is increasing rapidly in caliber to accommodate body growth, which is especially rapid between 6 and 10 wk. This process includes increases in both elastin and collagen contents, and so modifications in E/C cannot alone determine the evolution of aortic elasticity during this phase of profound remodeling of the artery wall.

At 6 and 10 wk of age, the BN aorta was more rigid than the age-matched LOU, although both strains presented the same evolution over this period. This difference is compatible with the lower E/C in the BN compared with LOU, while the size of the aorta was not different between strains at either age.

In contrast, in the BN rat, there was no decrease in elasticity between 10 and 15 wk of age. Thus at 15 wk, there was no longer any difference in elasticity between the two strains,
even though there was a marked difference in E/C (−44% in the BN vs. LOU). This suggests that the presence of IEL ruptures in the BN rat may limit the increase in rigidity.

This hypothesis is supported by two other results of our experiments. During aging, elasticity of the aorta decreased in the LE rat, as in many other strains. Our study confirmed that, not only did distensibility, a functional parameter, decrease, but the intrinsic characteristics of the LE aortic wall (Einc-wall stress curves) also evolved during aging, indicating a progressive increase in stiffness. This evolution was accompanied by a progressive, regular decrease in E/C with age. In contrast, in the BN rat, this evolution toward a stiffer aorta was not observed. Although E/C progressively decreased with age, as in the LE, and also remained lower than in the LE at all ages, the BN aortic wall elasticity became significantly greater than that of the LE at 64 wk. During this period, the number of IEL ruptures increased considerably in the BN, whereas negligible numbers were observed in the LE.

The second element in favor of the implication of the IEL ruptures in the increase in aortic elasticity in the BN is provided by the results of the treatment of BN rats with two antihypertensive drugs of different classes. The effects of enalapril, an angiotensin-converting enzyme inhibitor, were a decrease in blood pressure, a certain degree of hypotrophy of the aortic wall, in a homogenous manner, since E/C was not altered, and a significant decrease in elasticity. These effects were accompanied by a decrease of ~50% in the number of IEL ruptures present at 10 wk of age compared with control BN rats. In contrast, treatment with the calcium antagonist mibebradil had largely similar effects on blood pressure and the aortic wall as enalapril, but did not modify the number of IEL ruptures, as previously reported (14), and was without effect on elasticity compared with nontreated rats.

From a purely mechanical point of view, one would expect the presence of such IEL ruptures to increase elasticity, and Campbell and Roach (8) suggested that the presence of large fenestrations in the human cerebral arteries does increase their distensibility. Since the lower E/C in the BN would be expected to contribute to some degree to increased wall stiffness, it is probable that this counteracts, to some extent, the effect of the presence of ruptures at earlier ages. The relatively decreased stiffness observed at 64 wk is probably due to a predominance of the effect of IEL ruptures, which are numerous and much increased in size at this age, overriding any effect of the low E/C. The hypothesis that an increase in IEL rupture number and size corresponds to an increase in elasticity is also coherent with the fact that the material that fills in the gaps left by the elastic recoil of the ruptured IEL does not appear to be more rigid than the rest of the aortic wall. Indeed, histological study showed an absence of fibrosis in the IEL gaps, even in older rats.

Another possible contributing cause for the BN-LE difference in age-induced stiffness may be the difference in elastin content itself. Elastin is a protein that is mainly laid down during development and growth and undergoes very little turnover in adult life, although, in rats, some new elastin continues to be deposited on the already existing elastic lamellae during adulthood. The bulk of the elastin is thus highly susceptible to age-related changes, which involve an increase in associated polar amino acids, the binding and accumulation of calcium and lipids (33), and fragmentation, perhaps due to enzymatic degradation or fatigue processes (for review, see Ref. 12), all of which alter its elastic properties and render it less distensible. In view of the lesser elastin content in the BN rat aorta, such age-related changes should have fewer repercussions on global mechanical properties than in LE aorta.

In conclusion, our study showed that the evolution of abdominal aortic stiffness with age in the BN differed from the control strains, being greater in young growing rats before IEL rupture occurred, but showing a lesser increase with aging, probably due to the presence of numerous IEL ruptures. The BN rat appears to be well adapted to the structural anomalies present in its AA, which appear to have no adverse effects on arterial mechanics or hemodynamics with aging, explaining its apparently normal life span in the absence of hypertension (9). It is noteworthy that, in contrast to one previous report (18), no aneurysms develop in the AA with age in the BN colonies that we have studied, suggesting that IEL ruptures render the vascular wall more susceptible to increased elasticity than to the focal development of excessive lumen dilatation.

Similar ruptures in the IEL exist in human arteries, but have received little attention. Meyer et al. (30), in their extensive study of arteries during fetal and postnatal development, reported that large transverse ruptures in the IEL, visible on gross inspection, appear during postnatal growth in most large- and medium-sized muscular arteries, but also in the iliac arteries, where they begin to develop in the second half of gestation. The authors suggested that these IEL gaps may be the result of longitudinal stretch or may represent structural adaptation to increased hemodynamic load. However, no such ruptures were described in the human AA, a vessel renowned for its susceptibility to atheromatous and aneurysmal pathology, nor was any data available on variations in this phenomenon between individuals. Other groups have shown that various defects in the IEL, detected histologically and including fragmentations and interruptions, occur with age in human arteries, to a greater extent in males than in females, and may be related to subsequent development of intimal thickening, arteriosclerosis, and atherosclerosis (37, 39, 41). Furthermore, recent studies in the apolipoprotein E knockout mouse provide evidence that functional alteration or disruption of the IEL may be a prominent early, if not initial, feature in atherosclerotic lesion development (17, 28). Thus the implication of IEL ruptures in the BN rat in the evolution of arterial mechanical properties with age are, in part, relevant to the human situation and may shed some light on processes occurring during development and growth, which could influence the onset of arterial disease later in life.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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