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# Structure and reactivity of small arteries in aging

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**Abstract**

**Objective:** Increased pulse pressure has been observed in aging subjects, but the impact on the structure and reactivity of small arteries has been scarcely evaluated. **Methods:** This study presents the modifications of vascular structure and function observed in female rats of 5, 18 and 32 months of age, and their relation to the prevailing hemodynamic status. Geometry and reactivity of perfused and pressurized basilar and mesenteric small arteries were analyzed in vitro using a video dimension analyzer. **Results:** Mean arterial pressure was similar in the three age groups, and only pulse pressure was increased in the oldest group. Media thickness and cross sectional area increased in basilar and mesenteric arteries of the oldest rats and these structural abnormalities were positively related to pulse pressure but not to mean, systolic or diastolic arterial pressure. Only minor changes of vascular reactivity were noted with age: there was a decreased contraction to angiotensin II in mesenteric arteries and an enhanced contraction to endothelin-1 in the basilar arteries. **Conclusion:** In conclusion, aging is associated with increased pulse pressure and hypertrophy of basilar and mesenteric resistance arteries, suggesting that this hemodynamic variable may influence cerebral and peripheral vascular structure in aging. © 1998 Elsevier Science B.V.

*Keywords:* Aging; Basilar artery; Mesenteric artery; Vascular remodeling; Pulse pressure; Rat

## 1. Introduction

With age, the vascular wall of large peripheral vessels undergoes structural changes that may contribute to the age-related increase in systolic arterial pressure [1]. These include increased stiffness, thickening of the media and enlargement of the lumen diameter [2]. Few studies have directed their attention at the structure of small arteries in the context of advanced physiological aging [3]. By different means of investigation, an increased wall thickness has been reported in the hindquarter of normotensive rats [4], small muscular and ear arteries of rabbits [5] and small arteries from several human vascular beds studied post-mortem [6,7]. In contrast, one study reported an atrophy of cerebral arterioles associated with a decreased distensibility of the vessel wall in aged rats [8]. However, in most studies, the structural alterations have not been related to the hemodynamic status prevailing in aging.

Similar to vascular structure, reports on vascular reactivity of small arteries in the context of aging are also scarce and none are available for the female sex. Indeed, most of the studies were performed in large arteries [3] and it is known that resistance vessels differs in several important aspects, most probably due to their different function [9]. Even in conduit arteries, regional differences have been noted. Indeed, in a recent study, the release of nitric oxide (NO) was reduced with age in the rat aorta, but not in pulmonary circulation [10]. Since hemodynamic or vascular structural changes present in aging may alter reactivity of resistance arteries, it will be evaluated in relation to the structural and hemodynamic conditions prevailing.

Since pulsatile stretch has been shown to increase vas-

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cular smooth muscle cell proliferation [11], it is of particular interest to determine if long term increased pulse pressure can have an impact on the structure of smaller resistance vessels. In addition, pulse pressure has been associated with an increased cross-sectional area (CSA, an index of vascular wall hypertrophy) of pial arterioles in the context of hypertension [12], but it is not known if this can happen independently of changes in mean arterial pressure. In this study, we examined the effect of aging (18 months) and advanced aging (32 months), characterized by a selective increase in pulse pressure, on vascular remodeling and reactivity of the rat basilar and small mesenteric arteries, as directly assessed in perfused and pressurized in vitro conditions.

## 2. Materials and methods

Rats of the RORO strain were purchased from Biological Research Laboratories (Füllinsdorf, Switzerland) at 5 (adult), 18 (old) and 32 (very old) months of age ($n = 7$ per age group; female sex). These rats, originally of the Wistar strain, were outbred for twenty years in Hoffmann-La Roche laboratories. Their life expectancy is approximately 36 months. The rats were anesthetized (thiopental, 50 mg/kg, intra peritoneal) and a short polyethylene catheter (internal diameter: 0.58 mm) was inserted in the left femoral artery and connected to a pressure transducer (Letica PRI 256/2, Letica S/A, Hospitalet, Spain) to allow for the determination of systolic, diastolic arterial pressure and heart rate. During the anesthesia, the average of a 15 min recording was used to calculate mean and pulse pressure. All these procedures were approved by the Commission for Animal Research of the canton of Bern, and conform with the Guide for the care and use of laboratory animals of the NIH.

The animals were then decapitated and the basilar artery as well as a segment of a fourth branch of the mesenteric arterial bed (closest segment to the ileum) were isolated under a dissecting microscope in cold Krebs solution of the following composition (in mmol/l; control solution): NaCl 118.6, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.1, edetate calcium disodium 0.026, glucose 10.1. The arteries were then inserted and sutured on two small glass cannula positioned in a vessel chamber (Living Systems Instrumentation, Burlington, VT, USA) and superfused with control solution maintained at 37°C and oxygenated (95% $O_2$, 5% $CO_2$). The vessel perfusion chamber was positioned on the stage of an inverted microscope (Nikon, TSM-F) and the amplified image was transmitted, by a video camera, to a monitor and a video dimension analyzer (V91, Living Systems Instrumentation), allowing for the measurements of lumen diameter and wall thickness. With this technique it is possible to distinguish between the adventitia and the media, and the latter was used for calculations and comparisons. Longitudinal stretch was controlled by adjusting the vessel length to a value slightly superior to the one required to produce a small bending of the vessel [13].

The mesenteric arteries were allowed to equilibrate for 60 min with a perfusion of control solution containing 1% bovine serum albumin at a constant and optimal perfusion pressure of 30 mmHg [14] and their resting lumen diameter and media thickness were recorded. The basilar arteries were equilibrated for 60 min in a calcium free control solution to prevent myogenic tone. The perfusion pressure was then increased from 25 to 55 mmHg in 10 mmHg steps and the efferent pressure was adjusted to maintain a constant flow. In basilar arteries, the vascular structure was determined at each of the four pressure steps in maximally relaxed conditions (confirmed by the inefficacy of papaverine to further relax the artery).

In the functional experiments, all drugs were applied extraluminally and each section of the protocol was preceded by a washout period of 45 min. In the mesenteric artery, the following protocol was performed: (1) a single dose of angiotensin II (Ang II, $10^{-7}$ mol/l), (2) a concentration–response curve to norepinephrine (NE, $10^{-9}$–$3 \times 10^{-5}$ mol/l), (3) a concentration–response curve to acetylcholine (Ach, $10^{-9}$–$10^{-5}$ mol/l) after a 40% precontraction of the vessel with norepinphrine, (4) similar experiment with sodium nitroprusside (SNP, $10^{-10}$–$10^{-6}$ mol/l) and (5) a concentration–response curve to endothelin-1 (ET-1, $10^{-11}$–$10^{-8}$ mol/l). In the basilar artery, only a concentration–response curve to ET-1 ($10^{-11}$–$10^{-8}$ mol/l) was studied since contractions to serotonin were very weak.

All the drugs were obtained from Sigma Chemicals (Buchs, Switzerland), except for ET-1 which was obtained from Calbiochem-Novabiochem (Läufelfingen, Switzerland). The CSA and the growth index were calculated according to the formulas previously described [15,16] (see legend of Table 2). Since CSA does not change with changes in pressure, a mean of the values obtained at four different pressures (see above) was calculated for the basilar artery (Fig. 2). The distensibility of the basilar artery is expressed as μm changes per mmHg of pressure increase and represents the slope of the pressure–lumen diameter curve. Contractions are expressed as the percentage of decrease in lumen diameter from the baseline diameter. Relaxations are expressed as the percentage of increase in lumen diameter from the extent of precontraction. For each individual concentration–response curve, the maximum response and the half maximum effective concentration (expressed as negative logarithm, $pD_2$) were calculated by non-linear regression. Values are expressed as mean ± S.E.M, except for correlation analysis which show the actual data. Statistical evaluation was done by one-way ANOVA with Bonferroni's correction for multiple comparisons [17] or by one sample analysis (growth

Table 1
Characteristics of the adult, old and very old rats studied

| | Adult (5 months) | Old (18 months) | Very old (32 months) |
|---|---|---|---|
| Number of rats | 7 | 7 | 7 |
| Body weight (g) | 222±5 | 316±5* | 294±4* |
| Mean arterial pressure (mmHg) | 86±5 | 103±8 | 88±6 |
| Pulse pressure (mmHg) | 21±1 | 21±1 | 25±1* |
| Heart rate (beat/min) | 311±12 | 330±11 | 303±11 |

Arterial pressure and heart rate were measured in anesthetized conditions.* $P < 0.05$ as compared to 5 months old rats.

Table 2
Morphological characteristics of basilar and small mesenteric arteries in adult, old and very old rats

| | Adult (5 months) | Old (18 months) | Very old (32 months) |
|---|---|---|---|
| *Basilar artery (35 mmHg)* | | | |
| Lumen diameter (μm) | 302±5 | 316±14 | 316±14 |
| Media thickness (μm) | 24.8±1.0 | 29.5±2.5 | 35.6±1.7[a] |
| Media/Lumen ratio (%) | 8.2±0.3 | 9.6±1.1 | 11.4±0.8[a] |
| Growth index (% from control) | - | 24.1±8.7[b] | 54.2±9.7[b] |
| *Mesenteric artery* | | | |
| Lumen diameter (μm) | 233±9 | 271±8 | 279±21 |
| Media thickness (μm) | 16.4±1.0 | 17.1±1.3 | 20.2±0.6[a] |
| Media/Lumen ratio (%) | 7.0±0.3 | 6.4±0.5 | 7.5±0.6 |
| Growth index (% from control) | - | 19.3±10.4 | 46.3±12.3[b] |

For the basilar artery, the data is presented only at 35 mmHg of perfusion pressure. Similar results and statistics were obtained at other pressures. The growth index is calculated as a ratio of the difference between the treatment cross-sectional area (CSAt, Fig 2) and the control CSA ($CSA_c$) over CSAc ($CSA_t$-$CSA_c$ /$CSA_c$). Thus, the control group has a growth index of zero.[a] $P < 0.05$ as compared to 5 months old rats (ANOVA).[b] The 95% Confidence interval does not include 0.

index). Pearson's correlation coefficients were calculated by linear regression. $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Animals

Body weight was higher in old (18 months) and very old (32 months) rats than in adult animals (Table 1). Probably due to their very old age, the 32 months old rats were lighter than 18 months old animals and seemed to have reduced general activity (subjective observation). Mean arterial pressure tended to be higher in rats of 18 months of age (Table 1), as did systolic and diastolic arterial pressures (n.s., data not shown). Pulse pressure was significantly enhanced only in very old rats and was similar in the 5 and 18 months old groups (Table 1). Heart rate was not different among the groups.

### 3.2. Vascular structure

Aging had no influence on the lumen diameter of the basilar or small mesenteric arteries, although there was a tendency for mesenteric arteries to have a larger lumen with age (Table 2). Different perfusion pressures applied to the basilar arteries gave similar increments in the lumen diameter for adult, old and very old rats, respectively (Fig. 1). Indeed, the slope of the relationship between perfusion pressure and lumen diameter was very similar among the groups (Fig. 1, inset). The media thickness and media CSA were significantly increased in both vascular beds of very old rats, but the media/lumen ratio was augmented only in the basilar artery (Fig. 2A, Table 2). Indeed, in the mesen-

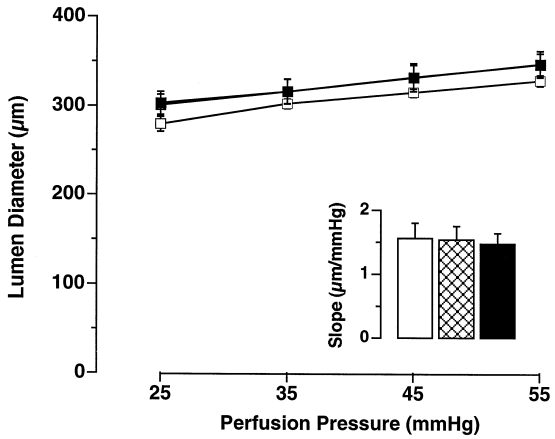


Fig. 1. Change of lumen diameter as a function of in vitro perfusion pressure in basilar arteries from 5 months (□), 18 months (⊠) and 32 months (■) old rats. Please note that the curves from old (18 months) and very old rats (32 months) are superimposed. The inset represents the slope (μm/mmHg) obtained from the linear regression of the curves shown in the graphic. The order of the bars is the same as the symbol description above. There was no statistical difference between the three groups.

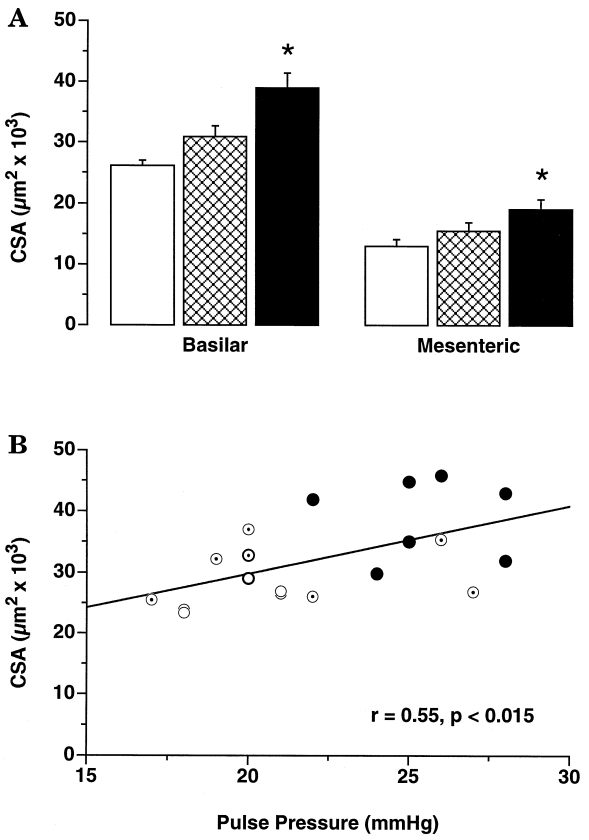


Fig. 2. A) Cross-sectional area (CSA) of the basilar and small mesenteric arteries of adult (5 months, □), old (18 months, ⊠) and very old (32 months, ■) female rats ($n = 7$/group). The CSA was calculated with the media thickness and not with the total wall thickness. $^{*}P < 0.05$ as compared to 5 months old rats. B) Relationship between the mean CSA of the basilar artery and in vivo pulse pressure of 5 (○), 18 (⊙) and 32 (●) months old rats. Similar results were obtained in the mesenteric arteries.

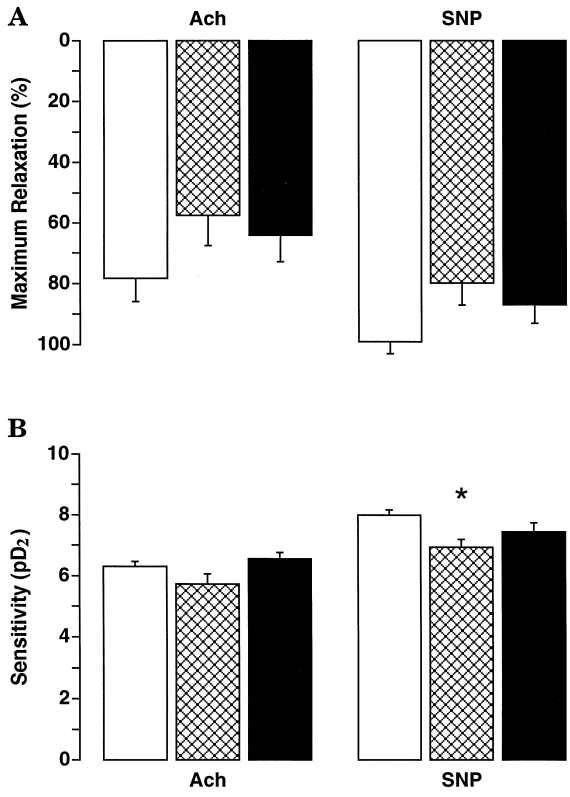


Fig. 3. Maximal relaxation (A) and sensitivity (B) of preconstricted mesenteric arteries stimulated with acetylcholine (Ach) and sodium nitroprusside (SNP) in adult (5 months, □), old (18 months, ⊠) and very old (32 months, ■) female rats ($n = 7$/group).

teric artery, the increase in lumen diameter prevented the media/lumen ratio to be different in very old as compared to adult rats. The growth index was significant in both 18 and 32 months old groups for the mesenteric artery, but only in the very old group for the basilar artery.

There were positive correlations between pulse pressure and CSA ($r = 0.55$, $p < 0.05$, Fig. 2B) or media thickness ($r = 0.53$, $p < 0.05$) in basilar arteries. Similar findings were obtained in mesenteric arteries ($r = 0.47$ and $r = 0.55$ respectively, $p < 0.05$). However, pulse pressure was not correlated with media/lumen ratio (basilar: $r = 0.45$, $p = 0.05$; mesenteric: $r = 0.36$, $p = 0.12$). Structural parameters were not related to any other hemodynamic variable such as mean, systolic or diastolic arterial pressure.

### 3.3. Vascular reactivity

Maximal endothelium-dependent relaxations to Ach and endothelium-independent relaxations to SNP of mesenteric arteries were not significantly different among the groups, but a similar tendency for reduced relaxations with aging were noted with both agents (Fig. 3). In terms of sensitivity, the concentration–response curve to SNP was shifted

Table 3
Reactivity of small mesenteric arteries to vasoconstrictors in adult, old and very old rats

| | | Adult (5 months) | Old (18 months) | Very old (32 months) |
|---|---|---|---|---|
| Angiotensin II | ($10^{-7}$ M) | 67.6 ± 4.5 | 53.8 ± 5.3 | 50.5 ± 5.8 * |
| Norepinephrine | Max | 85.1 ± 2.2 | 80.2 ± 5.8 | 85.9 ± 1.5 |
| | $pD_2$ | 6.02 ± 0.08 | 6.19 ± 0.10 | 6.23 ± 0.14 |
| Endothelin-1 | Max | 84.1 ± 0.9 | 83.0 ± 3.2 | 84.5 ± 2.1 |
| | $pD_2$ | 9.29 ± 0.07 | 9.12 ± 0.09 | 9.13 ± 0.04 |

Max.: Maximum contraction; $pD_2$: negative log of the concentration producing half of the maximal contraction. Both were calculated for each animal using non linear regression and the mean ± s.e.m. is presented for each group. Due to tachyphylaxis, only one dose of angiotensin II was applied to the vessels. $^{*}P < 0.05$ as compared to 5 months old rats (ANOVA).

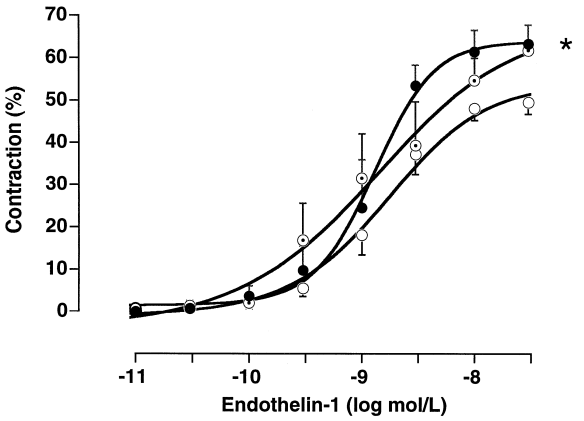


Fig. 4. Concentration-response curves to endothelin-1 (ET-1) of basilar arteries in adult (5 months, ○), old (18 months, ⊙) and very old (32 months, ●) female rats ($n = 7$/group). * The maximum contraction in the very old group is significantly different from the 5 months old rats.

to the right (less sensitive) only in the old rats (Fig. 3) and relaxations to Ach followed a similar pattern (n.s.).

In mesenteric arteries, there was a selective decrease in contractions to Ang II in very old rats, while NE and ET concentration–response curves were not modified (Table 3). In contrast, however, the maximal contraction to ET-1 were enhanced with age in the basilar artery (Fig. 4). There were no relationships between reactivity and either vascular structure (CSA) or hemodynamic changes (pulse pressure).

## 4. Discussion

Advanced age in rats was associated with increased pulse pressure without changes in MBP. There was a tendency for MBP to rise in 18 months old rats, but this subsided in older animals. A similar hemodynamic pattern has been observed with aging in rabbits [5], rats [4] and man [3], although MBP remained significantly elevated in the animal species. Our study, therefore, offers the advantage of having a selective increase in pulse pressure in comparison to previous studies, thus making it possible to isolate the effect of this important hemodynamic parameter on vascular structure and reactivity.

Advanced aging was also associated with an increased media thickness in both vascular beds studied, as well as an increased media/lumen ratio in the basilar artery. These structural changes do not appear to be the result of eutrophic remodeling [16] as there was no reduction of lumen diameters. Furthermore, the media CSA was significantly increased indicating hypertrophic remodeling. Changes in vascular geometry cannot be imputed on changes in distensibility, as this parameter remained unchanged in basilar arteries at the study pressures. Our results, therefore, add precision in terms of nature of vascular remodeling during aging to the previous studies reporting weight increase of arterial segments in old rabbits [5], as well as increased vessel wall thickness of small arteries evaluated at autopsy from a heterogeneous human population [7]. However, they are at variance with a study of 24–27 months old Fisher 344 rats, showing a relative atrophy of the cerebral arterioles [8] (see below). The intermediate results obtained in the 18 months old group confirms modest changes of the geometry of resistance arteries in the hindquarter of 21 months old rats as compared to younger controls [4]. It is noteworthy that, although there exists exceptions such as Fisher 344 rats [8], a study by Burek and Hollander [18], suggested that rats older than 30 months seem to better represent old age ($>$ 70 years of age) in man.

Vascular CSA and media thickness were positively related to pulse pressure and not to any other pressure parameter in this study of advanced aging. A simple correlation does not necessarily imply any causal relationship [19]. However, it is well accepted that hemodynamic changes can induce modification of the vascular structure [16,20]. Accordingly, at least two studies in experimental models of hypertension that have used different approaches to alter the local or systemic hemodynamic conditions have suggested that pulse pressure could be an important factor to induce adaptive changes in the vascular geometry [12,21]. In addition, pulsatile stretching has been shown to promote growth of vascular smooth muscle cells in culture, again lending support for a role of pulse pressure to induce hypertrophy of the vessel wall [11]. Furthermore, Fisher 344 rats showed a relative atrophy of cerebral arterioles and pulse pressure was slightly reduced [8]. It is therefore reasonable to suspect a causal link between pulse pressure and the vascular hypertrophy. It must be noted, however, that correlation coefficients ($r$) around 0.55 suggest that 30% ($r^2$) of the changes of CSA could be explained by the variability of pulse pressure [19]. Thus, it is not possible to exclude the participation of other factors in the hypertrophy of small arteries that we observed. Alternative or additional explanations include a decreased force generated by vascular smooth muscle cells with age requiring a thicker media, or a slight reduction in cardiac output requiring a greater peripheral resistance [3]. On the other hand, body weight does not appear to contribute to the alterations, since the old rats had a greater body weight than the very old rats ($p < 0.05$), without marked changes in their vascular structure. One limitation of our study is the fact that pulse pressure was measured at the level of the femoral artery, but not directly at the level of smaller arteries. However, in the very old rats, it is tempting to assume that the form and velocity of the pressure wave reaching the small arteries is altered, as it has been generally suggested in aging [3,22].

Maximal relaxations generated by endogenous formation of NO or other EDRFs (with Ach) or by exogenous application of NO (with SNP) as well as sensitivity to these agents were slightly altered ($p < 0.05$ for the sensi-

tivity to SNP), but with a consistent pattern. These observations, more apparent in old rather than in very old rats, suggest that the alterations may result from a decreased responsiveness of vascular smooth muscle cells to NO, but not to a decreased production of NO or other EDRFs by endothelial cells. This is in contrast to a report using 14 months old rats and showing reduced sensitivity to a muscarinic agonist but not to SNP [23]. However, consistent with our results, most studies in resistance arteries do not support a marked alteration of endothelial function with age [1,24,25], in contrast to studies looking at conduit arteries [23,26]. It must be noted, however, that resistance vessels seem to depend more on an endothelium-derived hyperpolarizing factor (EDHF, also stimulated by Ach) for tonic vasorelaxation than do conduit arteries [27,28]. This may help to explain the discrepancy between small and large vessels in the context of aging [23].

There was regional heterogeneity in the responsiveness of small arteries to exogenous ET-1. Indeed, contractions were enhanced in the basilar, but not in the mesenteric arteries. Contractions to ET-1 have also been reported to be increased in the coronary circulation with age [24]. In contrast, previous experiments in mesenteric arteries from aging Fischer 344 rats showed a reduced sensitivity to ET-1 [29]. In most studies on small arteries, including the present, stimulation of the $\alpha$1-adrenoceptors have not demonstrated any difference in contraction [23,25,30]. In contrast to previous studies [24,30], however, contractions to Ang II were reduced in very old rats. This discrepancy may relate to the sex of the animals as contractions to the peptide are markedly greater in adult female (68%) than in male rats (30%) [31].

We have previously shown in $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME)-induced hypertension that systolic arterial pressure per se was responsible for the increased media/lumen ratio of the basilar artery through eutrophic remodeling [15]. Our present results suggest that a selective pulse pressure increase, in the context of physiological advanced aging, is associated with vascular hypertrophy of small arteries of the peripheral and cerebral circulations with very limited alterations of vascular reactivity. The process (eutrophic or hypertrophy remodeling) involved in the adaptation of the vessel wall may therefore depend on the nature of the hemodynamic changes to which the vessels are exposed. However, there is a common goal; that is to minimize the impact of hemodynamic alterations, and associated changes in wall tension, on small artery function.

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