Angiotensin II Promotes Thoracic Aortic Dissections and Ruptures in Col3a1 Haploinsufficient Mice

Julie Fangeroux, Hany Nematalla, Weiwei Li, Marc Clement, Estelle Robidel, Michael Frank, Emmanuel Curis, Hafid Ait-Oufella, Giuseppina Caligiuri, Antonino Nicoletti, Albert Hagege, Emmanuel Messas, Patrick Bruneval, Xavier Jeunemaitre, Sonia Bergaya

Abstract—Vascular Ehlers-Danlos syndrome is a dramatic inherited disease caused by mutations of type III collagen (COL3A1) gene, associated with early-onset occurrence of arterial ruptures. Col3a1+/− heterozygous mice, the only vascular Ehlers–Danlos syndrome model available to date, have no spontaneous early vascular phenotype. Our objective was to determine the susceptibility of Col3a1+/− mice to develop arterial ruptures under high blood pressure (BP) conditions induced by a 4-week infusion of angiotensin II (AngII). AngII (1 μg/kg per minute) significantly and comparably increased systolic BP in Col3a1+/− and Col3a1+/+ mice but led to a higher premature mortality rate in Col3a1+/− mice compared with Col3a1+/+ mice (73% versus 36%; P=0.03), particularly during the first-week infusion (55% versus 0%). Echocardiography and histological analysis evidenced that early deaths were caused by thoracic aortic ruptures preceded by dissections and associated with low aortic collagen fibrils content. Remarkably, lowering the dose of AngII (0.5 μg/kg per minute) rescued the first-week premature deaths of Col3a1+/− mice while decreasing the rises in systolic BP (P=0.05 compared with the high-dose AngII), resulting in similar mortality rates in both groups of mice at the end of the 4-week period (30% versus 50% in Col3a1+/− and Col3a1+/+ mice; P=0.30). Finally, norepinephrine infusion (3.9 μg/kg per minute) did not induce significant mortality in both groups, whereas it significantly increased systolic BP, comparably with the high and with the low dose of AngII in Col3a1+/− mice (P=0.53 and P=1.00, respectively). Our findings demonstrated the extreme sensitivity of Col3a1 insufficient mice to prematurely develop thoracic aortic ruptures in response to AngII and its associated high levels in BP. (Hypertension. 2013;62:00–00.)

Key Words: angiotensin II ■ aortic dissection and rupture ■ collagens type III and type I ■ mouse model ■ vascular Ehlers-Danlos syndrome

Vascular Ehlers–Danlos syndrome (vEDS) is a rare inherited autosomal dominant disorder caused by mutations of COL3A1 gene resulting in quantitative and qualitative abnormalities of type III collagen in human.1–4 Type III collagen belongs to the fibrillar collagen family and represents, with type I collagen, the second most abundant collagen in blood vessels.3 Collagen is the main determinant of the arterial sensitivity to rupture.6 Consequently, vEDS is mainly characterized by arterial dissections and ruptures in young adults, leading to important morbidity and premature death. Medium and large caliber arteries are predominantly affected by these ruptures, including thoracic (TA) and abdominal (AA) aorta.3,7 These arterial ruptures may be preceded by dissections or aneurysms but spontaneous arterial ruptures are the most common cause of sudden death. Currently, there is no systematic vascular surgery or even therapeutic or preventive treatment available for vEDS patients.8,9 Recently, the first prospective study conducted in vEDS patients evidenced a preventive effect of the celiprolol, a cardioselective β1 receptor antagonist with partial β2 agonist properties, which decreased the incidence of vascular complications in vEDS patients, but without any change in their hemodynamic parameters.10 A mouse model of the disease has been generated on a BALB/c genetic background, by a complete and ubiquitous inactivation of the Col3a1 gene.11 Col3a1−/− homozygous mice exhibit a severe perinatal mortality, and Col3a1+/− heterozygous mice are viable and do not have a reduced life expectancy.12 Col3a1+/− mice present a very mild phenotype as suggested by a low incidence in vascular events, such as the delayed onset in internal elastic laminae fragmentations, becoming apparent in aging mice only and which could be prevented by a long-term treatment of doxycycline, a broad spectrum matrix metalloproteinase inhibitor.13,14 Because the vascular features of these mice do

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not recapitulate the human disease, we, therefore, decided to induce experimentally vascular dissections or ruptures in young adults Col3a1+/− and Col3a1+/− BALB/c mice, to investigate whether Col3a1+/− mice are more susceptible to vascular ruptures than wild-type mice as observed in vEDS patients. Among the different experimental methods developed to study the formation and progression of vascular disease, the chronic infusion of angiotensin II (AngII) has consistently been used as a model of aortic dissections and arterial ruptures notably in hypercholesterolemic mice.5,16 Chronic infusion of AngII through the use of osmotic mini-pumps induced a rapid and severe premature lethality in Col3a1+/− mice caused by thoracic aortic dissections and ruptures. Aortic ruptures were correlated with significantly less collagen fibrils production in early deceased Col3a1+/− mice compared with Col3a1+/− mice which survived longer. We further showed that this may have conferred to Col3a1+/− TAs, an important fragility in response to AngII toxicity and its associated rises in blood pressure (BP) as the incidence of early complications was significantly reduced when lowering BP or when infusing another agonist with comparable pressor effect.

Methods
A detailed description of all methods is presented in the online-only Data Supplement.

Results

Basal Characterization of Col3a1+/− Mice
We first evaluated in young adult Col3a1+/− and Col3a1+/− BALB/c mice different basal systemic and vascular parameters reported in Table S1 in the online-only Data Supplement. Basal systolic BP (SBP) was slightly decreased in Col3a1+/− mice compared with Col3a1+/− mice, with no modification in their heart rate values. Aortic diameters of TA and AA, as well as their respective media thicknesses, were comparable between both groups of mice, although adventitial collagen content was lower in Col3a1+/− aortas compared with Col3a1+/− aortas. Consistently, histological colorations of aortic sections showed normal vascular wall rearrangement in both groups of mice as previously reported (Figure S1). As expected, type III collagen mRNA expression was significantly reduced by ≈50% in TA and AA of Col3a1+/− mice compared with Col3a1+/− mice (Table S2). However, mRNA expression of type I collagen and metalloproteinase types 2 and 9 (MMP-2 and MMP-9) remained unchanged in Col3a1+/− mice compared with Col3a1+/− mice.

Chronic Infusion of AngII Leads to Sudden and Premature Deaths in Col3a1+/− Mice
AngII mini-pumps (1 μg/kg per minute during 28 days) were implanted in Col3a1+/− and Col3a1+/− mice (n=11 mice in each group). AngII infusion induced lethality in both groups of mice (Figure 1A). However, mortality was more rapid and dramatic in Col3a1+/− mice when compared with Col3a1+/− mice. During the first week of infusion, 55% of Col3a1+/− mice died versus 0% in Col3a1+/− group. At the end of the 28-day period of infusion, our results showed a 73% versus a 36% mortality rate in Col3a1+/− and Col3a1+/− mice, respectively (logrank test, P=0.03). For both groups, AngII infusion induced a rapid and significant increase in SBP during the 28-day period (P=10−10 compared with basal SBP; Figure 1B). The magnitude of the hypertension and the percentage in survival rates observed in the wild-type mice are in accordance with what was previously reported in BALB/c mice in response to chronic AngII infusion.17 Importantly, SBP was comparable between the 2 groups of mice over the 28-day period (P=0.64). However, over the same period of time, heart rate was significantly reduced in Col3a1+/− when compared with Col3a1+/− mice (P=0.001). Remarkably, during the first week of infusion, early deaths occurred in Col3a1+/− mice with the highest rises in SBP (Figure S2).

Premature Deaths in Col3a1+/− Mice Are Caused by Thoracic Aortic Ruptures and Can Be Preceded by Aortic Dilatations
Using echocardiography, we were able to measure in vivo aortic diameters of the 11 Col3a1+/− and the 11 Col3a1+/− mice, before and during the AngII infusion experiment described above (Figures S3 and S4). Interestingly, days during which we noticed the appearance of an aortic dilatation just preceded death of the corresponding mouse. In Col3a1+/− mice, 5 aortic dilatations were observed, both in the ascending and in the suprarenal regions, and among them 3 corresponded to early deceased mice (at days 2, 4, and 11; Figure S3). Thus, ≈37% of early dead mice in this group (3/8 mice) did exhibit aortic dilatations before their death.

Location of these aortic dilatations matched the autopsy and the histological observations that followed. At autopsy of Col3a1+/− mice, only hemothorax were observed, suggesting that all death cases probably ended in ruptures of the TA. Detailed analysis showed that large hematomas were predominantly observed surrounding the heart at the aortic root and arch and some were also observed in the descending TA (close to the thoraco-abdominal junction; Table). Even if we were unable to determine the precise site of aortic rupture for each death case, histological analysis evidenced that all premature deaths could be associated with major thoracic aortic dissections. 75% of them were more prominent in the ascending aorta (aortic arch) rather than in the descending aorta of Col3a1+/− mice (Table). Hematoxylin/eosin staining showed that major dissections were often represented by massive intramural hematomas particularly located beneath the external elastic lamina (Figure 2). Orcein

Table. Penetrance of the Aortic Dissection Phenotype in Col3a1+/− and Col3a1+/− Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dead Mice</th>
<th>Autopsy Observation</th>
<th>Histological Analysis</th>
<th>Penetration of Aortic Arch Dissection</th>
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<td></td>
<td></td>
<td>Suspicion of Aortic Rupture</td>
<td>Major Aortic Dissection</td>
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<td>Ascending Aorta</td>
<td>Descending Aorta</td>
<td>Abdominal Aorta</td>
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<tr>
<td>Col3a1+/−</td>
<td>4/11</td>
<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Col3a1+/−</td>
<td>8/11</td>
<td>6/8</td>
<td>4/8</td>
<td>0/8</td>
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coloration showed, in these regions, fragmentations of some elastic laminae within the media. Remarkably, when dissections occurred in Col3a1<sup>+/−</sup> mice in response to AngII infusion, they developed in the same regions as the ones observed for Col3a1<sup>−/−</sup> mice (Figure S5). Finally, histological analysis of infrarenal AA from early deceased mice did not reveal evident signs of media pathology or dissections in both groups of mice (Figure S6).

**Decreased Vascular Collagen Content of Ruptured Aorta From Col3a1<sup>+/−</sup> Mice**

Vascular effect of AngII infusion overtime was revealed by the significant increase in media thickness evidenced in Col3a1<sup>−/−</sup> and Col3a1<sup>+/−</sup> mice (Figure S7) and to the same extent as previously reported. To examine the concomitant fibrotic effect of AngII which could, if altered, explain the aortic fragility of Col3a1<sup>−/−</sup> TA, we quantified vascular collagen content in AngII-infused Col3a1<sup>+/−</sup> and Col3a1<sup>−/−</sup> mice using their thoracic aortic sections stained with Picro-Sirius red (Figure 3A). Early ruptured Col3a1<sup>−/−</sup> aortas (before day 5) had a lower collagen content compared with those examined after 2 to 4 weeks of AngII exposure, as illustrated by the scatter-plot graphs (Figure 3B). Indeed, early ruptured aortas failed to increase their collagen content in the adventitia compared with basal condition (P=0.48) and in contrary to surviving mice which succeeded in increasing significantly their level of adventitial collagen in response to AngII (P=0.04 when compared with early dead mice; Figure S8). Compared with basal condition, early dead mice were, however, able to significantly increase their collagen content in response to AngII in the media layer (P=0.02), but at a much lower degree (100x less) than the surviving mice (P=0.05 when compared with early dead mice). Importantly, we further showed that Col3a1<sup>−/−</sup> TA were indeed unable to significantly increase their levels of collagens mRNA after 5 days of AngII infusion, in contrary to Col3a1<sup>+/−</sup> TA (Table S3). Interestingly, for each Col3a1<sup>−/−</sup> mouse, collagen content of the media layer correlated with the corresponding media thickness (Figure S9). In contrast to what was observed in TA, AngII significantly increased collagen fibrils formation in the adventitial layer of AA from early dead Col3a1<sup>−/−</sup> mice (P=0.01) and this time to a comparable level with surviving Col3a1<sup>+/−</sup> mice (P=0.07; Figure S10).

**BP Contribution to AngII-Induced Aortic Ruptures**

To precise the respective contribution of BP and AngII toxicity per se on premature deaths, we tested a chronic infusion of either a lower dose of AngII (0.5 μg/kg per minute; n=10 mice per group; Figure 4A) or norepinephrine (NE; 3.9 μg/kg per minute; n=8 mice per group; Figure 4B), expecting similar rises in BP with 2 different mechanisms. We used linear mixed-effects models approach (see Methods for details), to compare SBP among the 3 different pharmacological treatments (high-dose of AngII, low-dose of AngII, and NE). Our data showed that for both groups of mice, low-dose of AngII and NE infusions induced a significant increase in SBP over the 28-day period of infusion compared with their respective basal SBP (P=2×10<sup>−5</sup> and P=0.0009, respectively; Figure S11). Rises in SBP were similar between these 2 treatments within each group of mice (P=1.00 in each group of mice) and were also comparable between the 2 groups of mice in each treatment (P=0.53 and P=1.00, respectively, for the low-dose of AngII and the NE experiments). Despite these similar increases in SBP, NE and low-dose AngII did not induce the same lethality (Figure 4A and 4B, red lines). NE failed to induce premature lethality in contrast to the low-dose of AngII which induced a significant and comparable mortality rate in both groups of mice (30% and 50% in Col3a1<sup>−/−</sup> and Col3a1<sup>+/−</sup> mice, respectively; logrank test, P=0.30).

In Col3a1<sup>−/−</sup> mice, the lethality was significantly reduced under the low-dose of AngII compared with the high-dose of AngII (logrank test, P=0.01), particularly during the first week of infusion. SBP was also significantly reduced during the first week of infusion under the low-dose infusion compared with the high-dose infusion (P=0.05). Therefore, lowering the dose of AngII and its associated rises in SBP rescued the premature mortality observed in Col3a1<sup>−/−</sup> mice during the first week of infusion. Importantly, in Col3a1<sup>+/−</sup> mice, SBP was...
comparable between the high-dose infusion of AngII and NE infusion ($P=0.53$), particularly during the first week of infusion ($P=0.96$). Thus, rapid and important increases in SBP induced by an agonist other than AngII were not sufficient to induce premature deaths in this group (Figure 4B, red and black lines).

**Discussion**

This study evidences for the first time the arterial fragility of Col3a1 insufficient mice and their susceptibility to develop thoracic aortic dissections and ruptures in response to AngII. As observed in vEDS patients, in this AngII infusion model, Col3a1$^{+/−}$ mice died prematurely and significantly more when compared with Col3a1$^{+/+}$ mice. The haploinsufficiency in type III collagen confers an important fragility to the thoracic aortic wall in response to rapid and important rises in SBP induced by a high-dose of AngII. In this model, BP seems to be a major trigger of this fragility since (1) early deceased Col3a1$^{+/−}$ mice had also the highest rises in SBP, and (2) lowering by half the dose of AngII and its associated increases in SBP (26 to 13 mm Hg) rescued the first-week premature deaths observed in Col3a1$^{+/−}$ mice. This is consistent with the contribution of collagen to the mechanical properties of the arterial wall and the preponderance of type III collagen in the media. However, NE infusion in Col3a1$^{+/−}$ mice also increased SBP during the first-week infusion, and comparably with the low- and with the high-dose of AngII, but did not induce premature mortality. This favors the hypothesis of AngII toxicity per se, independently of BP, to be critical in the occurrence of arterial ruptures in this model and possibly also in vEDS patients.

Concomitantly to arterial hypertension, AngII induces vascular remodeling through smooth muscle cell hypertrophy and secretion of proteins of the extracellular matrix, which if altered could contribute to aortic fragility. Indeed, we showed that TA from Col3a1$^{+/−}$ mice failed to increase significantly their levels of collagens expression in response to 5 days of AngII infusion, in contrast to wild-type mice. In addition, Col3a1$^{+/−}$ mice not only possessed less aortic collagen fibrils in basal condition compared with wild-type mice but their early ruptured TAs had a lower production in aortic collagen in response to AngII compared with mice that survived longer. Aortic collagen content corresponds to mature cross-linked type I collagen fibrils, formed of type I and type III collagens. As type III collagen is crucial for type I collagen fibrillogenesis, the haploinsufficiency in type III collagen could thus also brake the time-course in collagen fibrils formation, thereby increasing the intrinsic arterial fragility of Col3a1$^{+/−}$ mice.
fragility of Col3a1−/− mice. Another important observation of our study was that premature mortality of Col3a1−/− mice was due to dissections and ruptures that occur predominantly in TA rather than in AA. Strikingly, reduction in (proα1-) type I collagen in mice lead to similar aortic pathology as the one observed in Col3a1−/− mice. In these mice, early deaths were correlated with aortic dissections (also associated with less adventitial collagen), whereas the histology of other large vessels remained normal. Even if Rahkonen et al were unable to determine the precise site of aortic ruptures for each individual death case, they observed in some cases that the origin of the dissections was close to the aortic root. Thus, it seems that both collagens are critical in the pathogenesis of aortic dissections and ruptures, specifically in the ascending aorta, a lesion-prone segment likely associated with highly variable shear and wall stress patterns. More recently, mice bearing deletion of a region of chromosome 1 have unexpectedly been correlated with a predicted null allele of the Col3a1 gene. Interestingly, these mutant mice exhibited a phenotype which closely resembles to that of our AngII-induced phenotype in Col3a1−/− mice, such as premature deaths associated with dissections and ruptures of the ascending or descending TA. Taken together, this indicated that type III collagen plays a major role in TA integrity rather than in AA and reveals important possible pathogenic differences between TA and AA as it has been already evoked.

In conclusion, we demonstrated for the first time that chronic AngII infusion in Col3a1−/− mice induced thoracic aortic dissections and ruptures which may be preceded by aneurysms, thus leading to premature and sudden death. These thoracic aortic ruptures were associated with a low production in collagen fibrils which might confer an important fragility to this
region in response to rapid and important rises in SBP and to AngII toxicity per se.

**Perspectives**

A majority of human vEDS cases are caused by missense mutations (glycine substitutions) in COL3A1 gene, but cases of COL3A1 haploinsufficiency caused by specific mutations have also been reported. Primary complications of these patients are only limited to vascular complications (ruptures, dissections, or aneurysms), with a majority of dissecting affecting notably AA and TA. These ruptures might be favored by rapid changes in BP. Therefore, despite the inherent limitations of the Col3a1 haploinsufficiency mouse model, we established a suitable and interesting inducible model to further determine novel therapeutic approaches for this disease. The extreme sensitivity of Col3a1 insufficient mice to AngII toxicity per se in addition to its associated high levels in BP could justify testing AngII antagonists in humans. This model also offers the opportunity to test numerous other therapeutics, including blockade of the fibrinolytic activity. Future investigations to specify the molecular signaling pathways involved by AngII during collagen and matrix synthesis and degradation in Col3a1+− mice may also help to gain further insights in the pathogenesis of the overall progression of arterial disease in vEDS.

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**Disclosures**

None.

**References**


**Novelty and Significance**

**What Is New?**

- The extreme sensitivity of type III collagen–deficient arteries to angiotensin II.
- A mouse model of angiotensin II–induced aortic ruptures.

**What Is Relevant?**

- Relevance to a severe arterial inherited disease, vascular Ehlers–Danlos syndrome, with deficiency in type III collagen and yet no satisfactory prevention of arterial ruptures.

**Incentive to test angiotensin II blockers in this condition.**

**Summary**

This study evidences the high susceptibility of Col3a1 insufficient mice to prematurely develop thoracic aortic dissections and ruptures in response to angiotensin II toxicity and its associated high levels in blood pressure.
Angiotensin II Promotes Thoracic Aortic Dissections and Ruptures in Col3a1 Haploinsufficient Mice

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Data Supplement

Angiotensin II promotes thoracic aortic dissections and ruptures in *Col3a1* haploinsufficient mice

**Running title:** *Col3a1* insufficiency leads to aortic rupture

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Supplemental Methods

Animals
Col3a1+/− mice [strain C.129S4(B6)-Col3a1tm1Jae/J SN290] were originally purchased on a BALB/c genetic background from Charles River Laboratories (L’arbresles, France). For all animal studies, we solely used young adult males littermates from wild-type (Col3a1+/+) and Col3a1-haploinsufficient (Col3a1+/−) groups of mice.

All animal studies have been approved and conducted in accordance with the relevant guidelines of the French Ministry of Agriculture (Authorisation Executive Order No. A 75-05-12) for scientific experimentation on animals, European Communities Council Directives and international ethical standards. Col3a1+/− mice were maintained on a BALB/c genetic background in our mouse facility.

Drug Infusion and Treatments
Angiotensin II (AngII) (dissolved at the dose of 1µg/kg/min or 0.5µg/kg/min in saline solution) and norepinephrine (NE) (dissolved at the dose of 3.9µg/kg/min in saline solution with 0.2% wt/vol L-ascorbic acid) were used to fill osmotic mini-pump (model ALZET 2004) as previously described1,2.

For each individual series of drug infusion experiments, young males littermates from Col3a1+/− (n=8 to 11) and Col3a1+/+ (n=8 to 11) mice had comparable ages (from 9 to 12 weeks) and weights (from 23g to 28g) (P>0.05 between groups for weeks and weights comparison within each single series). Filled mini-pumps were, before implantation, equilibrated 24 hours to 48 hours in saline solution, at 37°C. They were then implanted subcutaneously in the back of the mouse previously anesthetized with isoflurane (2%). Surviving mice were all sacrificed at day 28 of AngII infusion.

Systolic Blood Pressure Measurement
Systolic Blood Pressure (SBP) and Heart Rate (HR) were measured on conscious Col3a1+/− and Col3a1+/+ mice using a tail cuff system (BP-2000 Visitech Systems) as described previously3.

Measurements were always performed in the morning. For each animal, the system automatically performed 4 first measurements which were not recorded; then, 10 consecutive measurements of SBP and HR were recorded for each mouse (leading to 10 or less SBP and HR values for each mouse). For our analysis, we only kept SBP and HR measurements for which we had at least 4 (out of 10) values. To avoid procedure-induced anxiety, and for each series of experiments, mice were initially accustomed to the tail cuff system during 3 consecutive days. Then, basal SBP and HR were recorded during 2 to 3 days (which were averaged) just prior mini-pump implantations. At the end of our study, we obtained a total number of n=38 to 40 mice in each group for which we had basal SBP recordings. Next, for each single series of experiments, SBP and HR were systematically measured at days 1, 2, 3, 4, 7, 13 or 14, 20 or 21 and 28, post-implantation.

Echocardiography
Echocardiography was performed using a Vevo 2100 imaging system and a MS400 (18-38 MHz) transducer (VisualSonics Inc, Toronto Ontario, Canada).

The resolution for this device is 30 microns. Mice were sedated in a chamber using isoflurane, 2.0% to 2.5% for induction and maintenance. Mice were shaved from the thorax to the lower abdominal region. Ultrasound transmission gel (Aquasonic 100; Parker Laboratories, Inc, Fairfield, NJ) was applied onto the shaved region of interest. The thoracic aortic arch was imaged along its long-axis using a modified right parasternal transthoracic view, the thoracic
aorta behind the cardiac structures using a modified long-axis parasternal transthoracic view and the suprarenal aorta using an abdominal longitudinal view. Two-dimensional guided colour Doppler helped to target vessel confirmation. Measurements of the systolic diameters of the aorta (using “leading edge to leading edge” technique) were performed offline on digitally stored recorded cine loops at three different levels: one measurement was performed at the level of the ascending aorta, another at the level of the descending aorta and a third one at the level of the suprarenal abdominal aorta, by a single observer blinded to the genotype of the mice. Measurements were performed before (day 0) and throughout AngII infusion at days 1, 2, 4, 7, 14 and 28. Basal aortic diameters at day 0 reported in Table 1 are the mean of one measurement per mice in n=11 mice in each group. During AngII infusion, when aortic dilatation was observed in one of these regions, its maximal diameter was then reported (in the graphs from Supplemental Figures S3 and S4) to illustrate the occurrence and the progression of a dilatation and/or dissection. It has been shown that the variability of in vivo measurements derived from this technique applied to aneurysmal and nonaneurysmal aortas is in the order of 0.1 to 0.01 mm, with excellent agreement between measurements regardless of the size or region of the aorta evaluated 4.

Tissue collection and Morphometric analyses
Col3a1^−/− and Col3a1^+/+ mice from drug infusion experiments were either dissected within the 24 hours after death observation in case of premature death or sacrificed at day 28 of drug infusion if they survived. An additional group of mice representing our control group for basal condition was used for basal histological and quantification analysis only. All mice were sacrificed with a lethal dose of 20% sodium pentobarbital (i.p.) and a midsternal thoracotomy was performed. Heart, thoracic and abdominal aortas were carefully exposed. Heart and thoracic aorta connected to each other and abdominal aortas (from renal to iliac junctions) were isolated and fixed in 4% paraformaldehyde. Before paraffin inclusion, heart was dissociated from the thoracic aorta at the junction between ascending and descending aorta. Then thoracic (TA) and abdominal (AA) aortas were, each of them, cut into 2 to 3 pieces. Each fragment was embedded in paraffin and aortic cross-sections of 6µm were obtained using a microtome. Aortic cross-sections were stained with hematoxylin/eosin, orcein or Picro-Sirius red solutions for imaging and morphometric measurements. Media thicknesses of TA and AA were calculated from orcein stained cross-sections using Archimedes software (Microvision Instruments, France). We used 3 to 6 serial cross-sections for each mouse to perform our measurements. Aortic serial cross-sections were chosen in non symptomatic regions, i.e. non affected by important dissections or ruptures, in order to have the most rigorous estimation of the media thickness. For each cross-section, we performed 10 measurements of the media thickness between the internal and the external elastic lamina. Thus, each mouse has one mean value calculated from 30 to 60 measurements. Measurements were performed by a single individual blinded to the genotype of the animals. Concerning the additional group of mice representing our control group for basal condition, these Col3a1^−/− (n=5) and Col3a1^+/+ (n=5) mice had also comparable ages (10.3 and 11.4 weeks respectively; P>0.05), and did not receive any drug or treatment infusion.

Collagen content quantification
Collagen content of TA and AA aortic wall were measured from aortic cross-sections stained with Picro-Sirius red observed through polarized light using AxioVision® and a Zeiss microscope at 2.5X magnification as described previously 5. Under these conditions, total collagen appears in red and represents mature type I collagen fibrils (formed of type III and type I collagens). We used the Leica Qwin® software to quantify the collagen content both in the adventitia and the media layers. We performed measurements of the surface of the media
and the adventitia layers as well as measurements of the quantity of total collagen, both expressed in arbitrary units. Then collagen content can be expressed in arbitrary units as well as in % of the surface of interest (media or adventitia). We used 1 cross-section for each mouse to perform our quantifications. Measurements were performed by a single investigator blinded to the genotype of the animals. With our quantification technique, we were unable to detect collagen in the media layer of AA from both groups of mice.

**Real time Quantitative-Polymerase Chain Reaction (q-PCR)**

Q-PCR were performed using a qPCR MasterMix Plus for SYBR Green I with Fluorescein (Eurogentec) on thoracic and abdominal aorta isolated from Col3a1+/− and Col3a1+/+ littersmates males mice. Mice were aged of 6.6 and 6.2 months respectively (P>0.05). After anesthesia, using 20% sodium pentobarbital (i.p.), a midsternal thoracotomy was performed. Thoracic and abdominal aortas from Col3a1+/− (n=7 and n=8 respectively) and Col3a1+/+ (n=7) mice were carefully exposed, rapidly dissected and cleaned (by removal of vascular fat and connective tissue surrounding the vessel) in cold PBS. Arteries were crushed in liquid nitrogen and RNA was extracted using subsequent steps of Trizol (TRI Reagent, MRC, Molecular Research Center, Inc), chloroform and ethanol 70%. We then followed the RNeasy Micro Kit (Qiagen) until the RNA elution. Then, total RNA of TA and AA (400ng and 200ng respectively, for each sample) were reverse transcribed (RT+) using random primers (Invitrogen). 0.75ng of cDNA (RT+) from each sample was loaded in duplicate on each q-PCR plate. 0.75ng of cDNA (RT-) of each TA sample was also used as negative control. Q-PCR was then performed using a q-PCR MasterMix Plus for SYBR Green I with Fluorescein (Eurogentec). *Ubiquitin C* was used as the reference gene for all q-PCR to express the level of RNA expression as a ratio gene of interest / *Ubiquitin*. The lecture of CT was performed using the C1000 Touch – Thermal Cycler (Bio-Rad). The list of primers used to amplify Type III Collagen, Type I Collagens, MMP-2 and MMP-9 isoforms are available in Supplemental Table S4. Collagens mRNA expressions reported in Supplemental Table 2 were representative of n=2 different experiments.

In a different experiment, we chronically infused AngII at the dose of 1µg/kg/min during 5 days, in Col3a1+/− and Col3a1+/+ mice (littermates males aged of 16.8±0.7 weeks and 16.7±0.7 weeks, respectively (P>0.05)) in order to investigate if the level of collagens mRNA expression were affected by AngII chronic stimulation. A second group was used as the control group for basal condition (n=5 mice in each Col3a1+/− and Col3a1+/+ group). These mice did not receive any treatment but were sacrificed at the same time as the other group of mice. The increase in SBP and the survival rates that we obtained during the 5 days of AngII infusion were in accordance with what we showed in Figure 1. At day 5 of infusion, AngII-infused mice which did not die earlier, were all sacrificed (n=4 Col3a1+/− and n=5 Col3a1+/+ mice). Thoracic and abdominal aortas were isolated from every mouse, rapidly dissected and cleaned (by removal of vascular fat and connective tissue surrounding the vessel) in cold PBS. They were then snap-freeze in liquid nitrogen in order to be used for RNA extraction and quantitative-PCR as described above.

**Drugs and Reagents**

Sodium pentobarbital (CEVA Santé animale, France); Osmotic mini pumps Model ALZET 2004 (Charles River Laboratories, France); L-ascorbic acid (cat number: W5408M, Fisher Scientific, France); Angiotensin II (cat number: A9525) and L-norepinephrine bitartrate salt (cat number: A9512) were purchased from SIGMA-ALDRICH (France).
Statistical analysis
All data are expressed as mean ± SEM. To compare SBP between the three different pharmacological treatments (high-dose AngII, low-dose AngII and NE) over the 28-days infusion period, we used linear mixed-effects models for variance analysis (type II sums of square). We also used a general linear model adapted to ANOVA two-way repeated measures to compare SBP and HR between Col3a1+/− and Col3a1+/+ mice, over the 28-days period of AngII or NE experiments. Normality and homoscedasticity were assessed before analysis. For all statistical tests performed, \(P \leq 0.05\) was considered to be statistically significant. NS means that \(P > 0.05\), * means that \(P \leq 0.05\) and ** means that \(P \leq 0.01\) between two groups or two conditions.

A student t-test for unpaired series was used to compare between Col3a1+/− and Col3a1+/+ mice: in vivo aortic diameters, basal media thickness, basal collagen content, q-PCR results, ages and weights of the mice, basal SBP and basal HR. A student t-test for paired series was used to compare within each group of mice, SBP measured daily during AngII or NE infusion to their respective SBP measured under basal condition (Figures 1 and S11). Media thicknesses and collagen contents before and during AngII infusion were also compared using a student t-test for paired series within each group of mice (Figures S7, S8 and S10). We used a general linear model adapted to ANOVA two-way repeated measures to compare SBP and HR between Col3a1+/− and Col3a1+/+ mice over the 28-days period of AngII or NE experiments (Figures 1 and S11). To compare SBP between the three different pharmacological treatments (high-dose AngII, low-dose AngII and NE infusions) over the 28-days period (or over the first-week infusion only), we used linear mixed-effects models for variance analysis (type II sums of square). We used similar models to compare SBP over the 28-days period of treatment to its corresponding basal SBP (Figures 1 and S11). Mouse was used as a random effects factor; whereas day, drug infusion (treatment) and group were taken as fixed effects factor; second order interaction between fixed effects factors were considered in the models. This analysis was performed using the R software (v. 2.13.0) with additional packages nlme, car and multcomp. Model assumptions, both on residuals and on random effects structure (including normality and homoscedasticity) were assessed before further analysis. Suited contrasts (ad hoc) were used to compare, over the 28-days period, the three different treatments on SBP and HR. Finally, we performed Kaplan-Meier survival analyses to draw and compare survival rates between the two groups and for each drug infusion condition. A Logrank (Mantel-Cox) test was used to calculate the statistical significance between two survival curves.
References


Supplemental Table S1, Characterization of the $Col3a1^{+/+}$ and $Col3a1^{+/-}$ mice under basal condition.

<table>
<thead>
<tr>
<th>Echographic / Histological measurements</th>
<th>$Col3a1^{+/+}$</th>
<th>$Col3a1^{+/-}$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages (months)</td>
<td>2.59 ± 0.08 (n=45)</td>
<td>2.62 ± 0.07 (n=45)</td>
<td>0.75</td>
</tr>
<tr>
<td>Weights (g)</td>
<td>24.85 ± 0.53 (n=40)</td>
<td>25.41 ± 0.38 (n=40)</td>
<td>0.39</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>121.46 ± 1.97 (n=40)</td>
<td>115.87 ± 1.81 * (n=38)</td>
<td>0.04</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>479.50 ± 7.36 (n=40)</td>
<td>476.93 ± 8.48 (n=38)</td>
<td>0.82</td>
</tr>
<tr>
<td>In vivo Ascending Aortic Diameter (mm)</td>
<td>1.23 ± 0.03 (n=11)</td>
<td>1.28 ± 0.04 (n=11)</td>
<td>0.44</td>
</tr>
<tr>
<td>In vivo Descending Aortic Diameter (mm)</td>
<td>1.08 ± 0.04 (n=11)</td>
<td>1.07 ± 0.04 (n=11)</td>
<td>0.94</td>
</tr>
<tr>
<td>In vivo Suprarenal Aortic Diameter (mm)</td>
<td>1.08 ± 0.05 (n=11)</td>
<td>1.10 ± 0.03 (n=11)</td>
<td>0.72</td>
</tr>
<tr>
<td>Thickness of TA Media (µm)</td>
<td>45.43 ± 1.18 (n=5)</td>
<td>45.09 ± 0.80 (n=5)</td>
<td>0.82</td>
</tr>
<tr>
<td>Thickness of AA Media (µm)</td>
<td>43.93 ± 0.85 (n=5)</td>
<td>43.18 ± 0.93 (n=5)</td>
<td>0.57</td>
</tr>
<tr>
<td>Collagen content in TA (% of surface adventitia)</td>
<td>39.11 ± 7.48 (n=5)</td>
<td>27.99 ± 2.50 (n=5)</td>
<td>0.20</td>
</tr>
<tr>
<td>Collagen content in AA (% of surface adventitia)</td>
<td>16.40 ± 1.93 (n=5)</td>
<td>7.41 ± 2.19 * (n=5)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Aortic diameters were measured in vivo by echocardiography. Media thicknesses were measured from orcein stained cross-sections and collagen content was quantified using aortic cross-sections stained with Picro-Sirius red and observed under polarized light. * means $P \leq 0.05$. 
Supplemental Table S2, Relative mRNA expression of Collagen Type III and I and MMP-2 and 9 in TA and AA of Col3a1+/+ and Col3a1+/− mice calculated by quantitative-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Col3a1+/+ TA</th>
<th>Col3a1+/+ AA</th>
<th>Col3a1+/− TA</th>
<th>Col3a1+/− AA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Type III</td>
<td>1.00 ± 0.04</td>
<td>1.00 ± 0.09</td>
<td>0.41 ± 0.06**</td>
<td>0.52 ± 0.06**</td>
<td>0.000002 0.0008</td>
</tr>
<tr>
<td>Collagen Type I</td>
<td>1.00 ± 0.27</td>
<td>1.00 ± 0.26</td>
<td>0.79 ± 0.07</td>
<td>0.75 ± 0.16</td>
<td>0.48 0.41</td>
</tr>
<tr>
<td>MMP-2</td>
<td>1.00 ± 0.19</td>
<td>1.00 ± 0.15</td>
<td>0.96 ± 0.14</td>
<td>1.31 ± 0.23</td>
<td>0.87 0.28</td>
</tr>
<tr>
<td>MMP-9</td>
<td>1.00 ± 0.07</td>
<td>1.00 ± 0.19</td>
<td>0.81 ± 0.08</td>
<td>0.72 ± 0.10</td>
<td>0.10 0.19</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. ** means $P \leq 0.01$ between Col3a1+/+ and Col3a1+/− mice.
Supplemental Table S3, Effect of a 5-days AngII chronic infusion on Collagens mRNA expression, in TA and AA of *Col3a1*+/+ and *Col3a1*+/− mice, calculated by quantitative-PCR.

<table>
<thead>
<tr>
<th></th>
<th>Gene</th>
<th><em>Col3a1</em>+/+</th>
<th><em>Col3a1</em>+/-</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>AngII</td>
<td>Basal</td>
<td>AngII</td>
</tr>
<tr>
<td><strong>TA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collagen Type III</td>
<td>1.00 ± 0.17</td>
<td>7.32 ± 1.61*</td>
<td>1.00 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Collagen Type I, alpha 1</td>
<td>1.00 ± 0.22</td>
<td>19.18 ± 6.73*</td>
<td>1.00 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Collagen Type I, alpha 2</td>
<td>1.00 ± 0.13</td>
<td>5.17 ± 0.94**</td>
<td>1.00 ± 0.12</td>
</tr>
<tr>
<td><strong>AA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collagen Type III</td>
<td>1.00 ± 0.11</td>
<td>3.65 ± 0.79*</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Collagen Type I, alpha 1</td>
<td>1.00 ± 0.40</td>
<td>6.45 ± 2.55</td>
<td>1.00 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Collagen Type I, alpha 2</td>
<td>1.00 ± 0.27</td>
<td>2.41 ± 0.65</td>
<td>1.00 ± 0.29</td>
</tr>
</tbody>
</table>

Values are mean ± SEM and represent within each group of mice, the relative mRNA expression of each collagen under AngII condition compared to basal condition. Within each group of mice, * means $P \leq 0.05$ and ** means $P \leq 0.01$ between basal condition (n=5 mice per group) and AngII condition (5 days of AngII chronic infusion; n=5 and n=4 *Col3a1*+/+ and *Col3a1*+/- mice respectively).
Supplemental Table S4, List of primers sequence used for quantitative-PCR performed on aortas of Col3al\(^{+/+}\) and Col3a1\(^{+-}\) mice.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ubiquitin</strong></td>
<td>Forward (exon 2) : 5'-'AGCCCAGTGTTACCACCAAG-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse (exon 2) : 5'-'ACCCAAGAACAAGCACAAAGG-3'</td>
</tr>
<tr>
<td><strong>Type III, alpha 1, collagen</strong></td>
<td>Forward (exon 2) : 5'-'ATGCCCACACGCTTCTACAC-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse (exon 4) : 5'-'ACCAGTTGGACATGATTCACAG-3'</td>
</tr>
<tr>
<td><strong>Type I, alpha 1, collagen</strong></td>
<td>Forward (exon 48) : 5'-'GTTCGTGACCGGTACCTTGA-3'</td>
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<tr>
<td></td>
<td>Reverse (exon 50) : 5'-'GGTGAAGCGACTGTTGCCT-3'</td>
</tr>
<tr>
<td><strong>Type I, alpha 2, collagen</strong></td>
<td>Forward (exon 51) : 5'-'GCTGAGGGCAACAGCAGTTACCTA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse (exon 52) : 5'-'GGAACGGCGAGCGAGATGGCTTATT-3'</td>
</tr>
<tr>
<td><strong>MMP-2</strong></td>
<td>Forward (exon 12) : 5'-'GAATGCCATCCCTGATAACCT-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse (exon 13) : 5'-'TCACGCTTTAGACTTTTGT-3'</td>
</tr>
<tr>
<td><strong>MMP-9</strong></td>
<td>Forward (exon 8) : 5'-'CGACATAGACGCCATCCA-3'</td>
</tr>
<tr>
<td></td>
<td>Reverse (exon 9) : 5'-'CATAGTGGGAGGTGCTGTCG-3'</td>
</tr>
</tbody>
</table>
Supplemental Figure S1. Histological characterization of thoracic and abdominal aorta of Col3a1^{++} and Col3a1^{+-} mice under basal condition. Representative images of descending aortic cross-sections (left panels) and abdominal aortas (right panels) of Col3a1^{++} and Col3a1^{+-} mice stained with Haematoxylin/eosin (H/E), Orcein and Sirius red. Images showed no sign of macroscopic vascular lesions with normal rearrangement in both groups of mice. Bars scale represents 400µm for the H/E staining and 200µm for the Orcein and Sirius red staining.
Supplemental Figure S2. Evolution of the SBP during AngII infusion. Graphs report the evolution of the SBP for each individual *Col3a1*+/− (A) and *Col3a1*+/+ mice (B) (n=11 mice in each group) during the AngII (1 µg/kg/min) infusion experiment reported in Figure1. Circles indicate death of the mouse and we additionally reported the exact day of observed death. Filled circles indicate premature death (within the first week of AngII infusion) and open circles indicate death occurring after the first week of AngII infusion. Dotted lines represent days for which we did not obtained SBP recordings.
Supplemental Figure S3. Aortic echocardiography analysis of Col3a1+/− mice. Diameters of the ascending, descending and suprarenal aortas were measured in vivo by echocardiography before (Day 0) and throughout the 28-days of AngII infusion (1μg/kg/min) for each of the Col3a1+/− mice (n=11) used in the AngII experiment reported in Figure 1. A- Each graph reports the evolution of aortic diameters for each individual Col3a1+/− mice. Dilatations in ascending and suprarenal aortas were observed in 3 out of 8 early deceased mice. B- Representative echocardiography images, taken at days 1 and 7 of one Col3a1+/− mice (dead at day 11), evidenced the formation of a dilatation in the suprarenal aortic region. Autopsy and histological observations confirmed the presence of a large dissection within this region (illustrated in the lower panel of Figure 2).
Supplemental Figure S4. Aortic echocardiography analysis of Col3a1+/+ mice. Diameters of the ascending, descending and suprarenal aortas were measured in vivo by echocardiography before (Day 0) and throughout the 28-days of AngII infusion (1µg/kg/min) for each of the Col3a1+/+ mice (n=11) used in the AngII experiment reported in Figure1. A- Each graph reports the evolution of aortic diameters for each individual Col3a1+/+ mice. Only two Col3a1+/+ mice exhibited aortic dilatations in the suprrenal territory and among them, only one died prematurely at day 19 post-AngII infusion. B- Representative echocardiography images, taken at day 1 and at day 28 of one Col3a1+/+ mice (sacrificed at day 28), evidenced the formation of an in vivo dilatation associated with a net dissection in this mouse. Autopsy and histological observations confirmed the presence of a dissection within this region.
Supplemental Figure S5. Histological characterization of thoracic aorta of Col3a1+/+ mice under AngII infusion. Representative images of ascending and descending aortic cross-sections stained with haematoxylin/eosin, orcein and Picro-Sirius red of two Col3a1+/+ mice. The upper lane represents dissections associated with blood infiltration between the different media layers along the ascending aorta of one Col3a1+/+ mice which died at day 9 after AngII infusion. The lower lane shows images of descending aortic cross-sections stained with Haematoxylin/eosin, Orcein and Picro-Sirius red of one Col3a1+/+ mouse sacrificed after 28 days of AngII infusion. Images showed no sign of dissection or elastic laminae fragmentation in this surviving mouse.
Supplemental Figure S6. Histological characterization of abdominal aorta of Col3a1+/+ and Col3a1+/− mice under AngII infusion. Representative images of abdominal aortic cross-sections stained with Haematoxylin/eosin, Orcein and Sirius red of one Col3a1+/+ mouse (deceased at day 14) and one Col3a1+/− mouse (deceased at day 3). We were not able to observe any sign of dissection in AA in both groups of mice. Bars scales represent 200µm.
Supplemental Figure S7. Effect of AngII infusion on media thickness of the thoracic and abdominal aorta in Col3a1^{+/+} and Col3a1^{+/−} mice. A- Scatter-plots representation of media thickness values (in microns) measured for each Col3a1^{+/−} (n=11) and Col3a1^{+/+} (n=11) mouse used for the AngII (1µg/kg/min) infusion experiment, and expressed as a function of their respective days of death, for thoracic and abdominal aortas. Media thickness values obtained in the control group for basal condition are also represented in the graphs. B- Comparison of the media thicknesses (in microns) between basal and AngII conditions. Within each group of mice, we pooled the media thicknesses values measured in the eleven mice which received AngII infusion (regardless of their day of death) and we compared them to the values obtained in a distinct but comparable group of Col3a1^{+/−} (n=5) and Col3a1^{+/+} (n=5) mice representing our control group for basal condition (which did not receive AngII infusion). In TA, media thicknesses were significantly increased after AngII infusion when compared to basal condition, in Col3a1^{+/−} and Col3a1^{+/+} mice (P=0.0005 and P=0.0004 respectively). In AA, media thicknesses were significantly increased after AngII infusion when compared to basal condition in Col3a1^{+/+} mice (P=0.00004) but not in Col3a1^{+/−} mice (P=0.07). Bars represent mean ± SEM. n.s. means P>0.05 and ** means P≤0.01.
Supplemental Figure S8. Effect of AngII infusion on collagen content of the thoracic aortic wall of Col3a1\textsuperscript{+/-} mice, in the adventitia (A) and the media (B) layers. Graphs represent the comparison of the means of collagen values obtained in basal conditions, in early dead mice (pooled values from mice deceased between days 1 to 5) and in surviving mice (pooled values from mice deceased between days 10 to 28). Measurements for basal conditions were performed on a different group of Col3a1\textsuperscript{+/-} (n=5) and Col3a1\textsuperscript{+/-} (n=5) mice which did not received AngII infusion. Bars represent mean ± SEM. n.s. means $P>0.05$ and * means $P\leq 0.05$. 
Supplemental Figure S9. Correlation between media thickness and media collagen content in TA of Col3a1−/− mice. Graph represents the correlation between values of media thicknesses and values of media collagen contents both measured in TA of the eleven Col3a1−/− mice used for the AngII (1µg/kg/min) infusion experiment. The correlation coefficient was calculated from the regression line.
Supplemental Figure S10. Effect of AngII infusion on collagen content of the abdominal aortic wall of Col3a1^{+/+} and Col3a1^{+-} mice. A- Representative images of AA cross-sections stained with Sirius red and pictured with a polarized light microscope and obtained from one Col3a1^{+/+} and one Col3a1^{+-} mouse (both sacrificed at day 28). The red birefringence represents mature collagen fibrils used for collagen quantification. B- Left graph: scatter plot representation of adventitial collagen values (in arbitrary units) measured in each individual mice used for the AngII (1µg/kg/min) infusion experiment, and expressed as a function of their respective days of death. Right graph: the comparison of the means of collagen values obtained in basal conditions, in early dead mice (pooled values from deceased mice between days 1 to 5) and in surviving mice (pooled values from deceased mice between days 10 to 28) showed that AngII infusion significantly increased collagen content in early dead Col3a1^{+-} mice compared to basal condition (P=0.01) and to a level comparable to surviving mice (P=0.07). Measurements for basal conditions were performed on Col3a1^{+/+} (n=5) and Col3a1^{+-} (n=5) mice which did not receive AngII infusion. Measurements for AngII conditions were performed on Col3a1^{+/+} (n=10) and Col3a1^{+-} (n=7) mice used for the AngII infusion experiment. Bars represent mean ± SEM. n.s. means P>0.05 and ** means P≤0.01.
Supplemental Figure S11. Comparison of SBP between different pharmacological conditions of drug infusion in Col3a1+/+ and Col3a1+-/- mice. A- Low-dose of AngII (0.5µg/kg/min). This experiment is representative of 2 independent experiments which both gave comparable results. The graph represents SBP (lower bars – left scale) and HR (upper lines – right scale) measured before (basal) and during AngII (0.5µg/kg/min) infusion. AngII infusion induced a significant increase in SBP over time for both groups ($P=2x10^{-9}$). SBP and HR were comparable between both groups of mice ($P=0.53$ respectively) throughout the duration of the treatment. Data are mean ± SEM. * means $P\leq 0.05$ and ** means $P\leq 0.01$ when daily SBP were compared to basal SBP within each group of mice. B- Norepinephrine (NE) infusion. Lower graph represents SBP (lower bars – left scale) and HR (upper lines – right scale) as a function of days of NE infusion. NE infusion led to a significant increase in SBP over time for both groups ($P=0.0009$). SBP and HR were comparable between both groups of mice ($P=1.00$ respectively) throughout the duration of the treatment. Data are mean ± SEM. * means $P\leq 0.05$ and ** means $P\leq 0.01$ when daily SBP were compared to basal SBP within each group of mice.