Pressure dependency of aortic pulse wave velocity in vivo is not affected by vasoactive substances that alter aortic wall tension ex vivo

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Butlin M, Lindesay G, Viegas KD, Avolio AP. Pressure dependency of aortic pulse wave velocity in vivo is not affected by vasoactive substances that alter aortic wall tension ex vivo. Am J Physiol Heart Circ Physiol 308: H1221–H1228, 2015. First published March 13, 2015; doi:10.1152/ajpheart.00536.2014.—Aortic stiffness, a predictive parameter in cardiovascular medicine, is blood pressure dependent and experimentally requires isobaric measurement for meaningful comparison. Vasoactive drug administration to change peripheral resistance and blood pressure allows such isobaric comparison but may alter large conduit artery wall tension, directly changing aortic stiffness. This study quantifies effects of sodium nitroprusside (SNP, vasodilator) and phenylephrine (PE, vasoconstrictor) on aortic stiffness assessed by aortic pulse wave velocity (aPWV) in vivo and direct measurement of aortic stiffness in ex vivo aortic rings in animal models. Methodology and results: Vasoactive drugs PE and SNP induce pressure-dependent aPWV changes and aortic stiffness changes, respectively. With age beyond 50 yr, systolic blood pressure increases and diastolic pressure decreases, a condition described as isolated systolic hypertension. A major contributing factor to isolated systolic hypertension is the stiffening of large arteries, which increases the magnitude and decreases the time of the reflected pressure wave. This augments the systolic peak of pressure. McEniery et al. (18) have demonstrated a positive correlation between the development of isolated systolic hypertension and increasing aortic stiffness. Aortic stiffness is a pressure-dependent parameter attributable to the fundamental properties of arterial design (24). Generally, any decrease in blood pressure will cause a decrease in aortic stiffness. However, the magnitude of this change in aortic stiffness is dependent on the structural and functional elements of the aortic wall. Therefore, blood pressure-lowering therapy may induce a different magnitude of decrease in aortic stiffness in different subjects and subpopulations depending on the structural components of the aortic wall. As a result, it is critical to consider, not only the isobaric condition of arterial stiffness, but also the dependency of stiffness on blood pressure changes. Although increased aortic stiffness and isolated systolic hypertension in the elderly are correlated, the mechanisms underlying the increased vascular stiffness are not fully established. Further study is required in both humans and in animal models of disease and vascular changes to obtain a greater understanding of the development of aortic stiffness.

The study presented here investigates a methodology for establishing the pressure dependency of aortic stiffness in animals. The approach utilizes phenylephrine (PE, vasoconstrictor) and sodium nitroprusside (SNP, vasodilator) to alter systemic blood pressure through modulation of peripheral resistance while continuously measuring aPWV. However, PE and SNP are vasoactive drugs and may have a direct effect on the smooth muscle and stiffness of the aorta, thus directly altering aPWV. The in vivo concentrations of these drugs that alter arterial pressure are of the same order as concentrations that cause substantial contraction and relaxation of smooth muscle in aortic rings in ex vivo myography preparations. The logical conclusion of this observation is that the vasoactive drugs may also act on the aorta in vivo and directly alter aPWV through alteration of wall tension. This study addresses this question by quantifying the effect of PE and SNP on aortic contractile properties and stiffness both ex vivo and in vivo, respectively. In doing so, it assesses the suitability of vasoactive means of altering systemic blood pressure while measuring aPWV. Within the approach, it also demonstrates a robust method of measuring and assessing isobaric aPWV and the pressure dependency of aPWV.

MATERIALS AND METHODS

Male, 12-wk-old Sprague-Dawley rats (n = 7, Animal Resource Centre, Perth, Australia) were shipped to Macquarie University at 11 wk of age and housed for 1 wk before commencement of experiments.

AORTIC STIFFNESS IS AN INDEPENDENT predictor of cardiovascular risk, both in specific disease subtypes (1, 12) and in the general population (17, 29). It has also been shown to have an additive predictive value above and beyond traditional cardiovascular risk factors (19). The importance of aortic stiffness was highlighted when carotid femoral pulse wave velocity, as a surrogate for aortic pulse wave velocity (aPWV) and aortic stiffness assessment stiffness in humans, was adopted by the European Society of Hypertension as a recommended test in the diagnosis and management of hypertension (13, 16).

With age beyond ~50 yr, systolic blood pressure increases and diastolic pressure decreases (18), a condition described as isolated systolic hypertension. A major contributing factor to

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All animals were housed in a temperature-controlled environment (21°C) with a 12-h:12-h light/dark cycle before experiments. Rats were fed standard chow and water ad libitum. The Macquarie University Animal Ethics Committee approved all animal procedures.

Establishing Pressure Dependency of Aortic Stiffness

Rats were anaesthetized with an intraperitoneal injection of urethane (1.3 g/kg) with anesthesia maintained throughout the procedure with intravenous boluses of urethane as required. Heart rate was measured from an electrocardiogram recorded in a type II three-lead electrode configuration. Polyethylene tubing (polyethylene-10, 0.6 mm outer diameter, 0.3 mm inner diameter) was introduced into the femoral vein for systemic infusion of vasoactive drugs. A perivascular ultrasound flow probe (Transonic TS420 system, 2PSB2326 flow probe) was fitted to the abdominal aorta in a suprarenal position using a ventral approach. A suture was placed around the inferior vena cava, also using a ventral approach, and externalized and fixed to a micromanipulator for modification of venous return through partial occlusion of the vena cava.

Two high-fidelity pressure-sensor 1.2-Fr catheters (Transonic Scienseqe) were introduced into the descending aorta via the left or right femoral and left carotid arteries. The pressure sensors were positioned, using investigation of the waveform characteristics, in the upper thoracic and lower abdominal aorta for the measurement of aPWV (Fig. 1). The position of the pressure sensors was confirmed by visual inspection at the conclusion of the experiment, at which point the distance between the two sensors was measured using a wet suture placed on the aorta between the two points, retrieved, and measured using Vernier calipers. All physiological waveforms were sampled at 2 kHz using a Cambridge Electronic Design (CED) 1401 data acquisition system with associated Spike2 software (Version 7.09, CED).

Measurement of aPWV. The transit time of the pressure pulse from the proximal to the distal sensor was calculated using the foot as a time-point marker within the pressure wave (Fig. 1) (6, 19). A custom software algorithm written in CED Spike2 software interpolated by spline fit each pressure waveform to a sampling rate of 10 kHz, before calculating the second derivative of that waveform and locating the peak of the second derivative within each pulse. Interpolation at an increased sampling rate was used to prevent aliasing of PWV results at higher values. Previous analysis (not shown) indicated that analysis of the 10-kHz data provided the same MAP-PWV relationship as the 2-kHz data but without aliasing at higher PWV values. The peak of the second time derivative of pressure was taken to define the time of the diastolic foot of the pressure waveform. The known distance between the pressure sensors was divided by the transit time for each pulse to calculate aPWV. The aPWV and corresponding MAP was calculated for each individual cardiac pulse. All waveform characteristics were extracted using custom script files written in house for the Spike2 software.

Alteration of systemic blood pressure with vasoactive drugs. Active alteration of blood pressure was achieved through the systemic effects of intravascular infusion of an exogenous nitric oxide donor, SNP (30 μg/kg per min, David Bull Laboratories), to lower blood pressure and an α1-adrenergic agonist, PE (30 μg/kg per min, Sovereign Medical), to raise blood pressure. Solutions were made in saline at a concentration of 30 μg/ml such that the venous infusion rate did not exceed 0.5 ml/min, all rats weighing under 500 g. Infusion was continued until a plateau of pressure was achieved. Analysis was conducted on the period following infusion, as arterial blood pressure returned to baseline conditions.

Alteration of systemic blood pressure by changing venous return. To obtain a vasoactive independent reduction in blood pressure, the inferior vena cava was partially occluded to reduce venous return. This was done without drug infusion for comparison in the blood pressure range achieved with SNP infusion (~50–95 mmHg). Venous occlusion was also conducted during continuous PE infusion to reduce blood pressure in the range of ~150–95 mmHg in a high-concentration PE setting for comparison to a low-concentration PE setting (Fig. 2). This method of passively decreasing blood pressure across the PE (high) range of pressure was used because trialed methods of passively increasing blood pressure (increased venous return, body tilt) did not achieve a MAP increase >5–10 mmHg.

Estimation of In Vivo Concentration of Vasoactive Drugs

The in vivo concentration of the infused vasoactive drugs, PE and SNP, is the difference between the amount infused and the amount bound and used by vascular smooth muscle or cleared through other means.

The infusion rate was defined, per the experiment, at 30 μg/kg per min. The rate at which the vasoactive drug exited the system was assumed to be exponential in nature and of the same decay constant (τ) with respect to time (t) as the decay of MAP from peak values following PE infusion or trough values following SNP infusion, back to baseline (of asymptote MAPbaseline) (Fig. 2). A three-parameter exponential decay model (constants c, MAPbaseline, τ; Eq. 1) was fitted to the MAP decay by minimization of the minus log likelihood function using the statistical software R (version 3.1.0) and a dose-response curve software library (10).

\[
\text{MAP}(t) = \text{MAP}_{\text{baseline}} + (c - \text{MAP}_{\text{baseline}}) e^{-t/\tau}
\]  
(1)

Once the exponential decay constant (τ) was calculated, the in vivo concentration of the vasoactive drug (\(X(t)\)) was calculated by summing the decay in each time period (t) to the end duration of the infusion in seconds (\(t_{\text{end}}\)) with the infusion rate (\(R = 0.5 \mu g/kg \text{ per s} \)) multiplied by the mass of the rat (m) for each second of the infusion (Eq. 2, \(t \leq t_{\text{end}}\)). The in vivo concentration following the infusion (Eq. 2, \(t > t_{\text{end}}\)) was calculated assuming exponential decay from the peak concentration of vasoactive drug (\(X(t)_{\text{peak}}\)) at the conclusion of the infusion (\(t = t_{\text{end}}\)). Calculations assumed immediate and uniform mixing of the drug throughout the blood volume. Examples of the resulting in vivo drug concentration curves are provided in Fig. 2.
A separate group of 12-wk-old Sprague-Dawley rats \((n = 8)\) were anaesthetized with an intraperitoneal injection of urethane \((1.3 \text{ g/kg})\), followed by a high dose of intravenous urethane. The heart was exposed and the aorta perfused with cold Krebs solution by injection through the left ventricle. The aorta was then excised and cleaned of surrounding tissue, and two nonbranching aortic rings of between 2 and 3 mm in length were cut immediately distal to the aortic arch (thoracic aorta) and immediately proximal to the aortic bifurcation (abdominal aorta).

The aortic rings were placed in an organ bath \((\text{Danish Myo Technology Multi-Wire Myograph System, 620M})\). The ring was mounted on metal rods of 200-\(\mu\)m diameter in warmed \((37^\circ \text{C})\) Krebs solution, with one rod attached to a force gauge and the other to a micrometer screw gauge. The aortic rings were incrementally stretched up to an equivalent pressure of 13.3 kPa over the course of 1 h. The extension was then set to 90% of the extension at 13.3 kPa and the preparation allowed to stabilize for 30 min or until a steady, baseline force was registered. Potassium chloride \((60 \text{ nM})\) was added to the bath and contractile force allowed to plateau before we washed the vessel in Krebs solution several times, until the baseline force was again registered. PE was then added in incremental doses from \(5 \times 10^{-10}\) to \(1 \times 10^{-5} \text{ M}\) concentration, and the force was allowed to plateau and was recorded at each concentration. The preparation was washed out, and a single dose of PE was added at a concentration that would cause 70–80% of the maximum contraction achieved with a PE concentration of \(1 \times 10^{-5} \text{ M}\). SNP was then added at increasing concentrations from \(5 \times 10^{-12}\) to \(5 \times 10^{-7} \text{ M}\) to induce vasorelaxation.

The contractile force \((F)\) in Newtons was converted to equivalent pressure \((P)\) using the measured longitudinal length of the sample \((l)\) and the internal circumference \((C)\) of the sample at the set point of 90% of the extension at 13.3 kPa \((\text{Eq. 3, derived from the Laplace Law})\) and expressed in mmHg. The response to PE was calculated as the pressure change from baseline conditions. The response to SNP was calculated as the percentage of relaxation compared with baseline (theoretical 100% relaxation) and contraction in response to the initiating PE concentration (0% relaxation).

\[
[X]_t = \begin{cases} 
\sum_{i=1}^{t} [X]_{i-1} \times e^{-r} + R \cdot m & t \leq t_{end} \\
[X]_{max} \times e^{-r(t-t_{end})} & t > t_{end}
\end{cases}
\]

\((2)\)

\(\text{Ex Vivo Response of Aortic Smooth Muscle to Vasoactive Agents}\)

A separate group of 12-wk-old Sprague-Dawley rats \((n = 8)\) were anaesthetized with an intraperitoneal injection of urethane \((1.3 \text{ g/kg})\), followed by a high dose of intravenous urethane. The heart was exposed and the aorta perfused with cold Krebs solution by injection through the left ventricle. The aorta was then excised and cleaned of surrounding tissue, and two nonbranching aortic rings of between 2 and 3 mm in length were cut immediately distal to the aortic arch (thoracic aorta) and immediately proximal to the aortic bifurcation (abdominal aorta).

The aortic rings were placed in an organ bath \((\text{Danish Myo Technology Multi-Wire Myograph System, 620M})\). The ring was mounted on metal rods of 200-\(\mu\)m diameter in warmed \((37^\circ \text{C})\) Krebs solution, with one rod attached to a force gauge and the other to a micrometer screw gauge. The aortic rings were incrementally stretched up to an equivalent pressure of 13.3 kPa over the course of 1 h. The extension was then set to 90% of the extension at 13.3 kPa and the preparation allowed to stabilize for 30 min or until a steady, baseline force was registered. Potassium chloride \((60 \text{ nM})\) was added to the bath and contractile force allowed to plateau before we washed the vessel in Krebs solution several times, until the baseline force was again registered. PE was then added in incremental doses from \(5 \times 10^{-10}\) to \(1 \times 10^{-5} \text{ M}\) concentration, and the force was allowed to plateau and was recorded at each concentration. The preparation was washed out, and a single dose of PE was added at a concentration that would cause 70–80% of the maximum contraction achieved with a PE concentration of \(1 \times 10^{-5} \text{ M}\). SNP was then added at increasing concentrations from \(5 \times 10^{-12}\) to \(5 \times 10^{-7} \text{ M}\) to induce vasorelaxation.

The contractile force \((F)\) in Newtons was converted to equivalent pressure \((P)\) using the measured longitudinal length of the sample \((l)\) and the internal circumference \((C)\) of the sample at the set point of 90% of the extension at 13.3 kPa \((\text{Eq. 3, derived from the Laplace Law})\) and expressed in mmHg. The response to PE was calculated as the pressure change from baseline conditions. The response to SNP was calculated as the percentage of relaxation compared with baseline (theoretical 100% relaxation) and contraction in response to the initiating PE concentration (0% relaxation).

\[
P = \frac{F/l}{C/2\pi}
\]

\((3)\)

\(\text{Statistical Analysis}\)

Results throughout are expressed as means ± SE unless otherwise stated. Analysis was conducted using the statistical package, R (version 3.1.0).

In vivo data were averaged in 1-mmHg incremental bins of MAP for statistical analysis. Comparison was made between aPWV, pulse pressure (PP), heart rate (HR), and mean abdominal aortic flow (Q). The shaded region indicates the data used in analysis, which commences at the peak (PE, PE + VO) or trough (SNP, VO) of MAP. The top row gives the calculated in vivo concentration of vasoactive drug. A: end of PE infusion. B: venous occlusion while continuing to infuse PE. C: end of infusion of SNP. D: start of venous occlusion. E: start of gradual decrease in venous occlusion.

![Fig. 2. An example of infusion of the vasoactive drugs phenylephrine (PE) and sodium nitroprusside (SNP) and the use of venous occlusion (VO) to reduce venous return for a passive reduction in arterial blood pressure. Each dot represents a single pressure pulse with results calculated for mean arterial pressure (MAP), aPWV, pulse pressure (PP), heart rate (HR), and mean abdominal aortic flow (Q). The shaded region indicates the data used in analysis, which commences at the peak (PE, PE + VO) or trough (SNP, VO) of MAP. The top row gives the calculated in vivo concentration of vasoactive drug. A: end of PE infusion. B: venous occlusion while continuing to infuse PE. C: end of infusion of SNP. D: start of venous occlusion. E: start of gradual decrease in venous occlusion.](http://ajpheart.physiology.org/)

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binned individual rat data with a second-order polynomial by linear regression and comparing the coefficients of the fit by paired t-tests. Myography dose-response curves \([f(x)]\) were fitted using a four-parameter \((a, b, c, d)\) log-logistic function (10) \((\text{Eq. } 4)\). Differences between abdominal site (thoracic, abdominal) dose-response curves were assessed by ANOVA. The 50% and 90% effective concentration \((\text{EC}_{50}, \text{EC}_{90})\) and maximum contractile or relaxation response were compared by paired t-tests.

\[
f(x) = b + \frac{c - b}{1 + \exp(a \cdot (\ln(x) - \ln(d)))} \quad (4)
\]

RESULTS

MAP range was reduced to \(<60\) mmHg with intravenous infusion of SNP and to \(>150\) mmHg with PE infusion. Venous occlusion achieved a MAP reduction to \(<60\) mmHg, and venous occlusion during PE infusion achieved a pressure drop from the peak pressure during vasoconstriction down to \(~90\) mmHg of mean pressure.

Aortic PWV showed a curvilinear relationship with MAP, with increasing blood pressure giving rise to increased aPWV (Fig. 3). There was a greater aPWV sensitivity to blood pressure at higher, compared with lower, blood pressures, as indicated by the increasing slope with increasing pressure in Fig. 3. This relationship was accurately described by a second-order polynomial fitted by least-squares regression (Table 1).

Aortic PWV was greater during PE infusion with reduced venous return at mean pressures of 100 (\(P < 0.01\)) and 105 mmHg (\(P < 0.05\)). No statistical difference was detected in aPWV between the SNP infusion and reduced venous return methods of lowering blood pressure (Fig. 3).

Pressure sensitivity of aPWV differed between the vasoactive drug methods of changing blood pressure and reduced venous return methods of changing blood pressure (Table 1).

Venous occlusion caused a reduction in abdominal aortic flow that was significantly different from drug-induced changes for the pressure range of 100–140 mmHg (Fig. 3). Heart rate increased because of venous occlusion through probable baroreceptor effects at pressures between 85 and 110 mmHg (Fig. 3).

At the calculated maximum in vivo concentration of PE \((7.0 \pm 1.8 \times 10^{-7} \text{ M})\), ex vivo wire-mounted vessels had an equivalent change in wall tension per unit area of 52 mmHg (thoracic) and 112 mmHg (abdominal). At the calculated maximum in vivo concentration of SNP \((4.2 \pm 0.6 \times 10^{-7} \text{ M})\), ex vivo wire-mounted aortic sections demonstrated 96% smooth muscle relaxation. The abdominal aorta exerted a greater contractile force in response to PE than thoracic sections (Figs. 4 and 5). There was no difference in the dose response of thoracic and abdominal sections to SNP (\(P = 0.17\), Figs. 4 and 5).

DISCUSSION

As arterial stiffness is a blood pressure-dependent variable, assessment of arterial stiffness through PWV measurement needs ideally to be compared at the same distending pressure. Additionally, the pressure sensitivity of the change in PWV provides additional information characterizing vasculature hemodynamics. These kinds of assessments require driving a blood pressure change most commonly through infusion of vasoactive drugs such as PE and SNP to modulate peripheral resistance. This study shows that, despite estimated in vivo concentrations of PE and SNP having a contractile and relaxation effect in an ex vivo preparation, the drugs have a negligible effect on transient aPWV compared with passive (altering venous return) mechanisms of changing blood pressure.

This is a novel observation, and as such the study was not designed to elucidate the underlying mechanisms for the differing performance of the aorta in in vivo and ex vivo condi-
Act any difference in the in vivo and ex vivo setting. Myography is an idealized condition to maximize exposure to the administered drug. The aorta in vivo is exposed only on the luminal surface to a flowing source of the circulating drug. These conditions would provide a much slower rate of absorption into the aortic wall for smooth muscle activation.

PWV assessment in rodents is well established. Noninvasive PWV techniques include optical methods in the peripheral vasculature (8) and carotid femoral or aortic PWV assessment by either tonometry (14) or noninvasive Doppler techniques (7). The invasive approach used in this study is also an established technique (4), including correcting for blood pressure differences between strain and treatment groups with acute administration of vasoactive drugs (6). The present study quantifies the effect of such vasoactive agents on the site of interest. Such analysis provides greater volumes of data to observe changes in arterial blood pressure was used as a best approximation in the absence of developing a method to instantly measure the blood concentration of drugs once infused may be different again depending on uptake throughout the vasculature. A modeling approach based on the observed changes in arterial blood pressure was used as a best approximation in the absence of developing a method to instantly measure the blood concentration of drugs within the aorta.

Instantaneous changes in blood pressure drive an opposing change in sympathetic activity through the baroreceptor pathway. An arterial pressure increase driven by PE infusion will cause greater stretch of baroreceptors in the aorta and carotid arteries with a feedback of decreased sympathetic nerve activity, heart rate, and cardiac output. A reduction in arterial pressure through venous occlusion will have the opposite effect. The presence of sympathetic and parasympathetic input in the in vivo environment may contribute to the differences observed to those found ex vivo. Sympathetic and parasympathetic activity was not measured in the present study. However, Fig. 3 shows a baroreceptor heart rate rise with blood pressure lowering during venous occlusion with PE infusion (95–150 mmHg) and a heart rate fall with blood pressure rise following venous occlusion (MAP 50–95 mmHg). These same effects were not present following infusion of PE and SNP and may be related to the rate of blood pressure change.

### Table 1. Average coefficients for polynomial regression

<table>
<thead>
<tr>
<th></th>
<th>SNP</th>
<th>VO</th>
<th>P</th>
<th>PE</th>
<th>PE + VO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPWV, m/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>4.2 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>0.05</td>
<td>3.5 ± 0.8</td>
<td>2.6 ± 0.9</td>
<td>0.34</td>
</tr>
<tr>
<td>b (×10⁻²)</td>
<td>-1.6 ± 0.6</td>
<td>0.5 ± 0.5</td>
<td>0.02</td>
<td>-2.2 ± 1.4</td>
<td>0.3 ± 1.4</td>
<td>0.09</td>
</tr>
<tr>
<td>c (×10⁻⁴)</td>
<td>2.2 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.01</td>
<td>3.5 ± 0.7</td>
<td>2.0 ± 0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>344 ± 89</td>
<td>437 ± 27</td>
<td>0.33</td>
<td>202 ± 102</td>
<td>577 ± 110</td>
<td>0.01</td>
</tr>
<tr>
<td>b (×10⁻³)</td>
<td>2.3 ± 2.4</td>
<td>-0.1 ± 0.7</td>
<td>0.33</td>
<td>3.8 ± 1.5</td>
<td>-1.4 ± 2.0</td>
<td>0.02</td>
</tr>
<tr>
<td>c (×10⁻⁴)</td>
<td>-15.57 ± 15.29</td>
<td>0.04 ± 4.14</td>
<td>0.36</td>
<td>-14.20 ± 5.46</td>
<td>3.30 ± 7.85</td>
<td>0.03</td>
</tr>
<tr>
<td>Aortic blood flow, ml/min</td>
<td>12.4 ± 9.4</td>
<td>-0.9 ± 6.9</td>
<td>0.38</td>
<td>-25.2 ± 12.2</td>
<td>21.4 ± 60.8</td>
<td>0.52</td>
</tr>
<tr>
<td>a (×10⁻¹)</td>
<td>4.8 ± 2.9</td>
<td>5.2 ± 2.2</td>
<td>0.92</td>
<td>10.9 ± 3.8</td>
<td>-1.4 ± 8.5</td>
<td>0.30</td>
</tr>
<tr>
<td>b (×10⁻²)</td>
<td>-2.5 ± 1.7</td>
<td>-1.7 ± 1.4</td>
<td>0.77</td>
<td>-4.7 ± 1.7</td>
<td>1.2 ± 3.0</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE and are average coefficients for the polynomial regression: $y = a + b \cdot MAP + c \cdot MAP^2$ for mean arterial pressure (MAP) ranges achieved by infusion of sodium nitroprusside (SNP) and phenylephrine (PE), and through venous occlusion (VO), where $y$ is aortic pulse wave velocity (aPWV), heart rate, or aortic blood flow.
Previous studies have investigated the isolated contribution of sympathetic nerve activity (15) and heart rate (26) on rat aPWV. Complete withdrawal of sympathetic nerve activity in the normotensive rat causes a 0.3–3.9% reduction in aPWV (15). Sympathetic activity would be near silenced only during periods of rapid pressure increase, such as during the initial phase of PE infusion. These periods of rapid pressure changes were not used in the analysis (Fig. 2). Future studies could account for the relatively small contribution of the sympathetic nervous system to aPWV by use of an alternate vasoconstrictor, infusing at different rates to alter the rate of pressure change, or by sympatho-inhibition through administration of hexamethonium.

Heart rate also has a positive correlation with aPWV, although again with a small effect, with at most a 6% change in aPWV with a heart rate increase of 150 beats/min (26). Heart rate changes achieved in the present study with venous infusion

Table 2. Aortic hemodynamics after intravenous infusion of saline

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Saline Infusion</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>414 ± 6</td>
<td>414 ± 5</td>
<td>0.995</td>
</tr>
<tr>
<td>Mean pressure, mmHg</td>
<td>97 ± 3</td>
<td>97 ± 2</td>
<td>0.997</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>30 ± 1</td>
<td>30 ± 1</td>
<td>0.916</td>
</tr>
<tr>
<td>aPWV, m/s</td>
<td>3.92 ± 0.06</td>
<td>3.91 ± 0.05</td>
<td>0.973</td>
</tr>
</tbody>
</table>

Values are means ± SE. Intravenous infusion of saline at 1 ml/min via the femoral vein did not alter aortic hemodynamics compared with baseline conditions (3).
of drugs and with venous occlusion did not exceed 40 beats/min. However, this maximum difference did occur where aPWV differences were detected between PE and venous occlusion, with higher heart rate occurring with higher aPWV (Fig. 3). Therefore, the higher heart rate may, at least in part, be a contributor to the small but higher aPWV observed with greater in vivo concentration of PE.

Reduction of venous return causes a reduction in aortic flow (Figs. 2 and 3). Reduced flow will reduce flow-dependent endothelial release of nitric oxide, reducing the magnitude of vessel relaxation. This could be a further contributing factor to the small but higher aPWV observed during venous occlusion with PE infusion. Endothelial function was not assessed in this study but is commonly assessed, ex vivo, in the rat aorta. Replication of in vivo conditions in an ex vivo setup has shown that endothelial function does contribute to abdominal aortic wall viscosity (2). Therefore, the reduced aortic flow during venous occlusion may have reduced endothelial function and thus may partly contribute to the observed increased aortic stiffness.

The relationship between vessel stiffness and diameter is complex. An example of this complex relationship was highlighted by Dobrin and Rovick (5), who demonstrated that smooth muscle contraction resulted in a decreased elastic modulus with increased pressure but decreased vessel radius. The ex vivo aortic diameter used in this study for all testing was 90% of the extension required for an equivalent 100-mmHg distension. This diameter would be approximately in the middle of the pressure range obtained in vivo. However, in vivo aortic diameter was not measured. Matching a measured in vivo diameter and drug concentration at each distending pressure and matching these parameters in an ex vivo scenario might provide further information on whether the vessel diameter is a contributing factor to the difference.

The peak of the second time derivative of pressure was used to locate the pressure-waveform foot for pulse transit-time measurement. A previous algorithm-comparison study in rats found that the foot-to-foot approach of transit-time measurement was most accurate (19); however, the peak of the second-derivative approach was not addressed in that particular study. As the foot of the rat aortic pressure wave is quite acute, with no flattening of the late diastolic portion of the waveform, the peak of the second derivative provides a consistent clear marker of the pressure-waveform foot that is rapidly assessed. The accuracy of the foot-finding approach might be improved by comparing multiple points surrounding the pressure-waveform foot (27).

In this particular study, two single-sensor catheters were placed in the descending aorta, and the distance between the sensors was measured postmortem. The data in this study were analyzed in a pair-wise fashion, with each rat as its own control. Thus the accuracy of distance measurement does not impact on the findings, provided the pressure sensors are not moved during the experiment. The pressure sensors were fixed tightly in place, and the animal position was not moved throughout the duration of measurement. For unpaired, strain comparison or treatment-comparison studies, where the accuracy of distance measurement is more pertinent, a highly accurate distance can be achieved by using a single catheter with two pressure sensors a fixed distance apart. The use of SNP and PE to vary arterial blood pressure during aPWV measurement provides valuable additional information for pressure-matched aPWV measurement and to quantify the pressure sensitivity of aPWV. The use of these vasoactive drugs has a negligible direct effect on functional large artery stiffness in rats. However, the calculated in vivo concentration of these drugs does alter contractile properties of an ex vivo preparation of aortic sections. Future studies might investigate the source of this difference, commencing with research into the kinetics of absorption of PE and SNP in vivo and ex vivo.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.B. and A.P.A. conception and design of research; M.B., G.L., and K.D.V. performed experiments; M.B. analyzed data; M.B., G.L., and K.D.V. interpreted results of experiments; M.B. prepared figures; M.B. drafted manuscript; M.B. and A.P.A. edited and revised manuscript; M.B., G.L., K.D.V., and A.P.A. approved final version of manuscript.

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