Heart Rate Dependence of Aortic Pulse Wave Velocity at Different Arterial Pressures in Rats

Isabella Tan, Mark Butlin, Ying Yi Liu, Keith Ng, Alberto P. Avolio

Abstract—Arterial stiffness, as measured by aortic pulse wave velocity (PWV), is an independent marker of cardiovascular disease and events in both healthy and diseased populations. Although some cardiovascular risk factors, such as age and blood pressure, show a strong association with PWV, the association between heart rate (HR) and PWV is not firmly established. Furthermore, this association has not been investigated at different arterial blood pressures. To study effects of HR on aortic PWV at different mean arterial pressures (MAPs), adult (12 weeks; n = 7), male, anesthetized Sprague-Dawley rats were randomly paced at HRs of between 300 and 450 bpm, at 50-bpm steps. At each pacing step, aortic PWV was measured across a physiological MAP range of 60 to 150 mmHg by infusing sodium nitroprusside and phenylephrine. When compared at the same MAP, increases in HR resulted in significant increases in PWV at all of the MAPs > 80 mmHg (ANOVA, P < 0.05), with the greatest significant change of 6.03 ± 0.93% observed in the range 110 to 130 mmHg. The positive significant association between HR and PWV remained when PWV was adjusted for MAP (ANOVA, P < 0.001). These results indicate that HR dependency of PWV is different at higher pressures than at lower pressures and that HR may be a confounding factor that should be taken into consideration when performing analysis based on PWV measurements. (Hypertension. 2012;60:528-533.)

Key Words: heart rate ■ arterial pressure ■ arterial stiffness ■ pacing ■ rats

Aortic pulse wave velocity (PWV), a surrogate measure of arterial stiffness, has been shown to be an independent predictor of all-cause and cardiovascular mortality,1 as well as a marker for cardiovascular diseases and events in a number of populations.2–4 In 2007, the European Guidelines for the Management of Hypertension5 added increased PWV as one of the factors that influenced prognosis of hypertensive patients as an early index of large artery stiffening, further establishing the parameter as a potentially important clinical measure. As the usage of PWV increases in the clinical setting, the importance of understanding its association with other cardiovascular factors, such as heart rate (HR), also increases. In addition, because elevated HR itself has been identified as a risk factor for cardiovascular morbidity and mortality in both healthy and diseased populations,6–9 there lies the possibility that any HR effect on PWV can compound the risk that increased HR or PWV individually poses. Although the association between HR and arterial stiffness has been investigated previously in both human10–15 and animal models,16–18 a consistent relationship between the two has yet to be established given the limited number of studies and contradictory results. Furthermore, HR-induced effects on arterial stiffness have not been investigated in association with different levels of arterial pressure, components of which themselves have been shown to be strongly correlated with PWV.19,20 This study thereby aims to examine the effect of HR changes on PWV at different mean arterial pressures (MAPs) in the rat aorta.

Methods

Animal Preparation
Adult, male, Sprague-Dawley rats (n = 7) aged 12 weeks and weighing 408 ± 25 g (mean ± SD) were studied. Rats were anesthetized with an IP injection of urethane (1.3 g/kg in 0.1 g/mL of saline solution). Two single-pressure sensor catheters (Sciensite 1.2F pressure catheter and Millar 1.4F Mikro-Tip pressure catheter) were placed in the upper thoracic and abdominal aorta via the left carotid and femoral artery, respectively, for continuous blood pressure measurement. Polyethylene tubes were inserted into the left femoral vein for drug delivery. To achieve atrial pacing, a custom-made 2.7 F bipolar catheter connected to an isolated pulse stimulator (A-M Systems Inc, model 2100) was inserted into the right external jugular vein and introduced into the right atrium. Appropriate placement of the pacing electrode was confirmed by the presence of P waves and unchanged P-Q interval and QRS complex in the paced ECG signal as compared with the sinus rhythm ECG signal. All of the procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and the experimental protocol was approved by the institution’s animal ethics committee.

Measurements
Blood pressure and ECG signals were continuously recorded with a Cambridge Electronic Design Power1401 data acquisition system (Spike2 version 7, CED, Cambridge, United Kingdom) at a sampling rate of 2000 Hz. Pulse sequences of 5.0 to 7.5 Hz (300–450 pulses

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per minute) were triggered using Spike2 via the isolated pulse stimulator. A pulse amplitude range of 0.6 to 1.0 V was used with a square pulse duration of 2 ms. HR was calculated from diastolic troughs of the blood pressure pulse because of the presence of pacing stimulus artifacts in the ECG signal. The distance between the 2 pressure sensors was measured postmortem, and aortic PWV values for each pressure pulse were calculated by dividing the measured distance by the time difference between the foot of the 2 pressure waves. The foot of the pressure wave was determined by the second derivative peak of the pressure signal for each cardiac cycle.

**Experimental Protocol**

Each rat was given multiple IV injections of a bradycardic agent, zatebradine (1 mg/kg in 1 mg/mL of saline solution) to reduce the animal’s HR to <350 bpm (n=7) and, where possible, <300 bpm (n=5). Each bolus was administered 10 minutes apart and ≤4 administrations were given to any 1 rat. After 5 minutes of stabilization at sinus rhythm, rats were paced in a random sequence of 300, 350, 400, and 450 bpm. At each pace rate, the rats were allowed to stabilize for 5 minutes before the MAP was raised with phenylephrine (30 μg/kg per minute IV) and lowered with sodium nitroprusside (30 μg/kg per minute IV). Each drug was infused until the blood pressure plateaued. Subsequent infusion of the opposing drug was given after the blood pressure had returned to baseline. At the end of each pacing step, HR was allowed to return to sinus rhythm and to stabilize for another 5 minutes before the next pacing step began.

**Data and Statistical Analyses**

Baseline PWV, HR, and MAP before and after zatebradine were measured and compared by paired t test to detect any differences at resting MAP at the 2 different HRs achieved. At each paced HR, PWV measurements were averaged for each rat across every 10 mm Hg of MAP between 60 and 150 mm Hg. Mean PWVs measured at the different HRs were compared at the same MAP range by way of 1-way ANOVA for repeated measures. Where a difference was detected, post hoc paired Student t test with simple Bonferroni correction applied was performed to determine which HRs resulted in significant differences in PWV. Effects of HR on PWV independent of MAP interaction were also examined by correcting PWV values for MAP at 100 mm Hg and then comparing corrected PWV at different HRs using repeated-measures ANOVA and post hoc paired Student t tests. Data are presented as mean±SEM. For ANOVA, a P<0.05 was considered significant. Paired Student t tests were considered significant for P<0.0167 (corresponding with the Bonferroni corrected P value for P<0.05, for multiple group comparisons).

**Results**

**Effect of HR on PWV at Different MAPs**

With administration of zatebradine, HR decreased significantly from 393±41 to 270±36 bpm (P<0.001). This was accompanied by a significant decrease in PWV from 4.5±0.6 to 4.2±0.5 m/s (P<0.05) and a nonsignificant decrease in MAP from 95±13 to 93±9 mm Hg (P=0.74).

Across the range of MAP measured, PWV generally increased as HR increased (Figure 1). The effect of HR on PWV was significant at all MAPs >80 mm Hg (Table). Post hoc analysis showed that significant differences in PWV were observed among all of the HRs but not at all of the MAPs. Significant differences in PWV for a difference in HR of 50 bpm were only observed at MAPs between 110 and 130 mm Hg, whereas differences in PWV were detected across a wider range of MAPs for HR differences of 100 and 150 bpm. PWVs measured at 300 and 450 bpm were significantly different at all of the MAPs >80 mm Hg, and PWVs at 350 and 450 bpm were significantly different at all of the MAPs >90 mm Hg. Differences between PWVs measured at 300 and 400 bpm were significant at all of the MAPs >80 mm Hg except at 100 to 110 mm Hg and 130 to 140 mm Hg.

The largest significant change in PWV (6.03±0.93%) was observed between 300 and 450 bpm at 110 to 120 mm Hg. The lowest significant change in PWV (2.05±0.5%) was observed between 300 and 350 bpm at 110 to 120 mm Hg.

**Effect of HR on PWV Independent of MAP**

When PWV measurements were corrected for a reference MAP of 100 mm Hg, HR was still shown to significantly affect PWV (P<0.001). The MAP-normalized PWV at 450 bpm was significantly different compared with all of the normalized PWVs at lower pacing rates (Figure 2).

**Discussion**

To our knowledge, this is the first study to have investigated HR effects on PWV over a range of MAPs. The results of our study indicate that aortic stiffness, as measured by PWV, is affected by acute, sustained changes in HRs across the normal physiological MAP range. The changes in PWV corresponding with changes in HR were different at different MAPs, with minimal nonsignificant changes occurring at the lower MAP range (60–80 mm Hg) and maximal significant changes occurring at the middle to high MAP range (110–130 mm Hg). Furthermore, our results show that the effect of HR on PWV is evident even in the absence of HR and MAP interaction. These findings are consistent with other studies that have also found arterial stiffening effect of increased HR in both animals17,18 and humans.10,11,13,14,21

To date, studies on the effect of HR on arterial stiffness have resulted in conflicting findings. Although some studies have found a significant effect of HR on arterial stiffness, others have found no association.15,16,22 Indeed, there are
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PWV.19,20,24 In a study by Liang et al,12 a decrease in HR effects may have influenced the PWV measurement, where PWV was used as the measure for arterial stiffness, existing studies may be affected by several factors. First, Similarly, a study by Albaladejo et al22 found a nonsignificant both MAP and PWV, and when HR was increased to 100 study by way of pacing resulted in a significant increase in

Figure 2. Pulse wave velocity (PWV) corrected for a reference mean arterial pressure (MAP) of 100 mmHg. Data shown as mean±SEM. The effect of heart rate (HR) remains evident, with corrected PWV at 450 bpm being significantly different from corrected PWV at all of the other pacing rates.

tantly increased. It is difficult to interpret, therefore, whether the increases in PWV were attributed to an increase in MAP rather than in HR. In our current study, PWVs at different HRs were analyzed at the same MAP values to remove the influence of MAP in our analysis. Furthermore, by correcting PWV for a reference MAP of 100 mmHg, our study found that PWV was still significantly associated with HR even in the absence of MAP interaction.

Second, it has been argued that the algorithm or method used to determine PWV can also introduce errors when investigating the relationship between HR and PWV, at least in humans.25 However, a more recent comparison study between 2 timing algorithms (intersecting tangent foot-to-foot and maximum systolic upstroke) in the determination of PWV and the influence of HR on both showed that, whereas PWV is critically dependent on the algorithm used to determine the pulse transit time between 2 measurement sites, HR was still shown to be positively correlated with PWV regardless of which method was used.26 In our study, PWV was determined using a foot-to-foot method, where the foot of the wave was determined by the second derivative peak of the pressure wave. The accuracy of this method has been shown to be similar to that of the intersecting tangent method.27

Third, HR changes were achieved differently in different studies, either by pacing or by pharmacological means. The latter may introduce additional factors that could have directly or indirectly affected the arterial stiffness through systemic effects, whereas pacing, as is the method used in this study, directly and independently controls HR. Although we decreased HR pharmacologically using zatebradine, all of the PWV measurements were taken at paced HRs. Moreover, studies have shown that zatebradine selectively acts on the If current28–30 and does not affect cardiovascular function directly.31 Therefore, it is presumed that the changes in PWV observed in this study were related to changes in HR alone.

<table>
<thead>
<tr>
<th>MAP, mm Hg</th>
<th>300 bpm</th>
<th>350 bpm</th>
<th>400 bpm</th>
<th>450 bpm</th>
<th>P, ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>60–70</td>
<td>3.94±0.22</td>
<td>4.04±0.17</td>
<td>4.06±0.15</td>
<td>4.04±0.15</td>
<td>NS</td>
</tr>
<tr>
<td>70–80</td>
<td>3.99±0.20</td>
<td>4.10±0.15</td>
<td>4.15±0.15</td>
<td>4.16±0.16</td>
<td>NS</td>
</tr>
<tr>
<td>80–90</td>
<td>4.06±0.18</td>
<td>4.20±0.14</td>
<td>4.26±0.15*</td>
<td>4.28±0.14*</td>
<td>0.007</td>
</tr>
<tr>
<td>90–100</td>
<td>4.15±0.17</td>
<td>4.32±0.13</td>
<td>4.38±0.13*</td>
<td>4.42±0.13†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100–110</td>
<td>4.30±0.16</td>
<td>4.50±0.13</td>
<td>4.54±0.12</td>
<td>4.62±0.13†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>110–120</td>
<td>4.45±0.15</td>
<td>4.66±0.13*</td>
<td>4.70±0.13*</td>
<td>4.84±0.12†‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>120–130</td>
<td>4.63±0.11</td>
<td>4.84±0.13</td>
<td>4.95±0.14†</td>
<td>5.07±0.13†‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>130–140</td>
<td>4.97±0.12</td>
<td>5.19±0.12</td>
<td>5.23±0.12</td>
<td>5.34±0.14†</td>
<td>0.001</td>
</tr>
<tr>
<td>140–150</td>
<td>5.30±0.12</td>
<td>5.54±0.15</td>
<td>5.61±0.13</td>
<td>5.77±0.15†</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. Values in the last column represent results for repeated-measures 1-way ANOVA for HR at each MAP bin. PWV indicates pulse wave velocity; HR, heart rate; MAP, mean arterial pressure; NS, not significant.

*Post hoc comparisons between HRs with Bonferroni correction applied are shown as *P<0.05 when compared with 300 bpm
†Post hoc comparisons between HRs with Bonferroni correction applied are shown as P<0.05 when compared with 350 bpm.
‡Post hoc comparisons between HRs with Bonferroni correction applied are shown as P<0.05 when compared with 400 bpm.

studies that have found an opposite effect of HR on arterial stiffness, where an increase in HR resulted in an increase in arterial compliance and distensibility.27 Discrepancies among existing studies may be affected by several factors. First, where PWV was used as the measure for arterial stiffness, MAP effects may have influenced the PWV measurement, because MAP has been shown to be strongly correlated with PWV.19,20,24 In a study by Liang et al,12 a decrease in HR with metoprolol from 70 to 56 bpm did not result in a significant decrease in carotid-femoral PWV in humans. However, an increase in HR from 56 to 80 bpm in the same study by way of pacing resulted in a significant increase in both MAP and PWV, and when HR was increased to 100 bpm, neither MAP and PWV was significantly changed. Similarly, a study by Albaladejo et al22 found a nonsignificant increase in PWV with an increase in HR, but MAP signifi-
The mechanistic relationship between HR and arterial stiffness is still largely unknown. Studies in sympathectomized rats showed that sympathetic influence did not play a part in arterial stiffening in the presence of increased HR in elastic arteries,\textsuperscript{18,32} whereas several investigators have attributed the stiffening effect at higher HRs to the shortened time for vessels to recoil from an expanded state.\textsuperscript{11,17} Another plausible explanation is that, as HR increases, the viscosity component of the arterial wall’s viscoelasticity also changes.\textsuperscript{16,33} In a seminal study by Bergel\textsuperscript{34} on the dynamic elastic properties of the arterial wall, it was observed that stiffness, as defined by the dynamic elastic modulus, increased in both elastic and muscular arteries of the dog between frequencies of 0 to 2 Hz. Contrary to studies by Mangoni et al\textsuperscript{17} and Mircoli et al,\textsuperscript{18} this increase in stiffness was greater in the muscular arteries. The viscous component of the dynamic elastic modulus was relatively small at all frequencies between 2 and 18 Hz, although there was a small increase.

Results from more recent studies on the frequency dependency of arterial wall viscosity have not resulted in a convergence in findings. Although some investigators have found that wall viscosity decreases with HR with no change in arterial stiffness,\textsuperscript{35} others have found that, at higher frequencies, in the range of HRs tested in this study (300–450 oscillations per minute), the rat aorta wall may become more susceptible to stiffening,\textsuperscript{36} especially at higher pressures.\textsuperscript{33} Our finding that the effect of HR on PWV was more evident at higher MAP is consistent with the latter observation. Further investigation with in vitro studies may be useful in understanding the mechanisms involved in arterial stiffening at increasing HRs, but it should be noted that, in humans, the aorta is more distensible in vivo than when in vitro\textsuperscript{37} and that there are limited data on the viscoelastic response of the modulus of elasticity in humans.

As with other pacing studies, observations from this study are limited to acute changes in HR and may not be directly comparable with studies on the long-term effects of HR on PWV. Furthermore, a change in HR by way of pacing uses a different mechanism to pathological tachycardia, which can be influenced by many factors, such as neural mechanisms and endocrine disorders. However, studies in humans on the relationship between elevated resting HR and PWV have also demonstrated a significant, positive association.\textsuperscript{13,38,39} Although the mechanisms involved in long-term HR effects on arterial stiffness are also unknown, the fact that both short- and long-term changes in HR significantly change PWV suggests that HR must in some way affect arterial stiffness, whether by mechanistic changes or functional changes. As mentioned before, pacing directly and independently controls HR and, therefore, is useful in solely examining the frequency dependency of the mechanisms involved in arterial stiffening.

When results obtained from the rat model are applied to humans through appropriate scaling, it is of note that the changes in PWV that we obtained were very similar to the PWV changes obtained in the human study by Lantelme et al in 2002.\textsuperscript{11} A comparison of the changes in PWV for HR changes was made at an MAP of 90 mmHg (the subjects’ mean pressure as calculated from the given systolic and diastolic pressure values in the study by Lantelme et al\textsuperscript{11}), with the lowest pace rate (300 bpm for our study, 60 bpm for the study by Lantelme et al\textsuperscript{11}) used as the reference for PWV comparisons. A 5:1 ratio between rat and human HRs was chosen based on the general resting HRs of rats and humans (300 and 60 bpm, respectively). Given a human with body mass (BM) of 80 kg and HR 60 bpm and a 0.4-kg rat with HR of 300 bpm, an allometric relationship can be obtained where HR (in bpm) is inversely related to BM (kg; HR=227×BM\textsuperscript{0.25}). Given the variability of this scaling across species, the multiplier and exponent are of similar order to published values for a large range of species (241 and −0.25, respectively).\textsuperscript{40} As HR increased, PWV increased by a similar order in both the study by Lantelme et al\textsuperscript{11} and the study presented here (Figure 3). In our study, PWV increased by between 1% and 4% for HR increases of between 17% and 50%, whereas in the study by Lantelme et al,\textsuperscript{11} PWV increased by between 1% and 7%.

The significant PWV changes observed in our study were between 2% and 6%. Although these changes in PWV were moderate, it has been shown in humans that a 14.8% change in aortic PWV is equivalent to ≈10 years of arterial aging.\textsuperscript{24} Although some reservations must be made in extrapolating animal data from rodents to humans, the observed PWV changes in this study were similar to those observed in humans, and these changes would indicate an equivalent arterial aging of ≈1 to 4 years. Moreover, in our current study, these PWV changes were equivalent to a rise in MAP of ≈10 mmHg in both the low and high MAP range at all of the HRs (data not shown). Such increases in MAP can translate to an increased stroke and all-cause mortality risk of 20% and 14%, respectively.\textsuperscript{41}

Our finding that aortic PWV increases more with increased HR at higher MAP than at lower MAP prompts further investigation into the synergistic effects of these 3 factors and whether the combination of elevated HR and increased PWV would affect the prognosis for hypertensive individuals differently than either factor alone. Elevated HR has been shown to be an independent risk factor for cardiovascular disease.
and mortality in both healthy and diseased populations. In the hypertensive population, all-cause mortality significantly increased with increased HR, and increased HR has been shown to be associated with the accelerated progression of PWV more so than in the normotensive population. A review by Bangalore et al comparing β-blockers suggested that, in patients being treated with β-blockers, a lower HR was associated with an increased risk of cardiovascular events and mortality. However, the Conduit Artery Function Evaluation Study demonstrated that this increased risk may be a result of the increased aortic systolic blood pressure (relative to brachial cuff systolic pressure) associated with lower HRs in subjects on blood pressure-lowering medication.

The presented study has several potential limitations. First, the sample size was relatively small, thus there may not have been enough statistical power, especially for comparisons <300 bpm. However, the sample size was still sufficient for comparisons at higher HR as significance was still achieved. Second, the animals were under urethane-induced anesthesia, which in itself could have altered vascular tone by way of relaxation of smooth muscle. Notwithstanding, any potential effect of anesthesia and duration under anesthesia were normalized by randomization of the order in which the HR was paced in each rat. Third, a bradycardic agent, zatebradine, was used to first lower the HR before pacing began. Drugs may induce systemic effects that can directly or indirectly affect arterial stiffness. However, studies have shown that zatebradine selectively acts on the If current and does not affect cardiovascular function directly. In addition, the use of vasoactive agents phenylephrine and sodium nitroprusside may be suspected to have an independent effect on the aorta. However, the doses used have been shown to have no direct effect on the aorta compared with passive means of lowering and increasing MAP (unpublished findings, at 70 mmHg, sodium nitroprusside 4.1 ± 0.3 m/s, passive means 4.2 ± 0.3 m/s, P = 0.32; at 120 mmHg, phenylephrine 5.8 ± 0.5 m/s, passive means 5.8 ± 0.4 m/s, P = 0.58), and the primary site of action is considered to be the peripheral vasculature. In addition, the dose of both phenylephrine and sodium nitroprusside was identical at each HR; therefore, if there was any effect on the aorta, it would be consistent throughout the study.

It is known that PWV increases with age. However, this study addresses the interaction among PWV, MAP, and HR, with the confounding factor of age avoided by investigating rats at a single age. Further studies might investigate whether the relationship among PWV, MAP, and HR changes with age.

Perspectives
Our study found that an increase in HR results in an increase in PWV at the middle to high MAP range (80–150 mmHg). The PWV differences, although statistically significant, were modest, and further study would be required to confirm whether it implicates a physiologically significant compound risk in PWV, in particular for the hypertensive population. However, when these changes (≤6% change in PWV for 50% change in HR) are considered as equivalent sustained changes in human populations, they are associated with substantial relative additional risk of ≥14% for all-cause sustained and 20% for stroke. Hence, HR may be an important confounding factor in PWV measurements and, thus, should be incorporated when analyzing arterial stiffness as measured by PWV.

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Disclosures
None.

References
Summary
This study demonstrates that HR is positively associated with PWV and that the magnitude of this association is greater at higher MAPs.

Novelty and Significance

What Is New?
This study establishes the relationship between HR and PWV over a physiological range of MAPs in rats.

What Is Relevant?
A significant positive association between HR and PWV was found, with the sensitivity most pronounced at higher MAPs.


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