Nebivolol Increases Arterial Distensibility In Vivo

Carmel M. McEniery, Matthias Schmitt, Ahmad Qasem, David J. Webb, Alberto P. Avolio, Ian B. Wilkinson, John R. Cockcroft

Abstract—Arterial stiffness is a key determinant of cardiovascular risk in hypertensive patients. β-Blockers appear to be less effective than other drugs in improving outcome in hypertensive patients, and a potential explanation may be that β-blockers are less effective in reducing arterial stiffness. The aim of this study was to assess the direct effect of β-blockade on pulse wave velocity (PWV), a robust measure of arterial distensibility, using a local, ovine, hind-limb model. In addition, we hypothesized that the vasodilating β-blocker nebivolol, but not atenolol, would increase arterial distensibility in vivo. All studies were conducted in anesthetized sheep. PWV was recorded in vivo using a dual pressure-sensing catheter placed in the common iliac artery. Intraarterial infusion of nebivolol reduced PWV by 6±3% at the higher dose (P<0.001), but did not alter mean arterial pressure (change of −1±3 mm Hg, P=0.1). In contrast, atenolol had no effect on PWV (P=0.11) despite a small drop in mean pressure (change of −5±3 mm Hg, P<0.01). Infusion of glyceryl trinitrate led to a dose-dependent fall in PWV, and 2 nmol/min produced a similar reduction in PWV to the higher dose of nebivolol (500 nmol/min). The effect of nebivolol on PWV was significantly attenuated during coinfusion of L-arginine (P=0.003) and also during coinfusion of butoxamine (P=0.02). These results demonstrate that nebivolol, but not atenolol, increases arterial distensibility. This effect of nebivolol is mediated through the release of NO via a β-adrenoceptor–dependent mechanism. Thus, nebivolol may be of benefit in conditions of increased large artery stiffness, such as isolated systolic hypertension. (Hypertension. 2004;44:305-310.)

Key Words: blood pressure • nitric oxide • arteries • hemodynamics • receptors, adrenergic β

Arterial stiffness is an important independent predictor of mortality in a number of patient populations, including hypertensive patients.1-4 Therefore, a better understanding of the structural and functional factors regulating large artery stiffness may lead to the development of specific therapies to reduce cardiovascular risk. Although the use of β-blockers in the treatment of hypertension is widespread, the precise effect of these agents on arterial stiffness is controversial.5-11 This is partly because concomitant reductions in mean arterial pressure (MAP) also lead to a decrease in stiffness, making interpretation of any direct effects of β-blockers on the large arteries more difficult.

Nebivolol is a relatively new vasodilating highly-selective β, adrenoceptor antagonist. It differs from conventional nonvasodilating β-blockers, such as atenolol, in that it stimulates NO production, which leads to vasodilatation. Indeed, we have shown previously that nebivolol, but not atenolol, causes vasodilatation in the human forearm vascular bed and that this effect can be blocked by inhibitors of NO synthase.12

More recently, we13 and others14 have shown that NO is an important regulator of arterial distensibility. Therefore, we hypothesized that nebivolol would increase local arterial distensibility in vivo through the stimulation of NO production, whereas atenolol would not. The aim of this study was to test this hypothesis in an ovine hind-limb model, with local drug infusions, thereby avoiding the confounding influence of changes in MAP. In addition, we investigated the pharmacological mechanisms by which nebivolol influenced distensibility. Intravascular measurements of pulse wave velocity (PWV) were used as a robust measure of arterial distensibility in vivo.

Methods

All experiments were conducted at the University of New South Wales, Australia, in adult crossbred Suffolk sheep aged between 12 and 18 months. The study was approved by the University’s Animal Care and Ethics Committee. Anesthesia was induced by intravenous injection of 600 to 900 mg of sodium phenobarbitone (Rhone Merieux) and maintained by inhalation of 2% to 3% halothane, as described previously.15 Animals were spontaneously breathing throughout and studied in the supine position.

Hemodynamic Measurements

Simultaneous pressure waves were recorded using a 6F dual pressure-sensing catheter (Gaeltec) as described previously.13 The iliac PWV was calculated using the foot-to-foot methodology as described previously.13 Heart rate (HR) was calculated over the measurement period from a simultaneously recorded ECG.
Drugs
Nebivolol (A. Menarini Ltd) and atenolol (AstraZeneca) were infused at equimolar concentrations (250 and 500 nmol/min) for 5 minutes each. Glyceryl trinitrate (GTN; Schwarz) was infused at 2, 4, and 8 nmol/min for 5 minutes each. \( \text{L}^- \)-monomethyl-\( \text{L}^- \)-arginine (\( \text{L}^- \)-NMMA; Clinalfa) was coinfused with nebivolol at 10 \( \mu \)mol/min. Butoxamine (Sigma) was infused alone for 5 minutes and then coininfused with nebivolol at 3 \( \mu \)mol/min. These doses and duration of infusions were based on pilot studies (data not shown) and published literature.12,13

Protocol
Following 30 minutes of saline infusion, baseline measurements of iliac PWV, MAP, and HR were recorded in triplicate, or until measurements were stable. Recordings were then made during the last 20 seconds of each drug infusion. Infusion of drugs through the catheter exposed the arterial segment under study to the drug, whereas infusion through the sheath did not, because this was located distally to the pressure sensors (Figure 1). Because the common iliac artery is nonbranching, this methodology, which has been described previously,13 enables indirect drug effects, such as those produced by changes in flow or reflex activation, to be taken into account by comparing the effect of infusion via the catheter with infusion via the sheath.

Study 1: Effect of Nebivolol and Atenolol on PWV
Nebivolol was infused at 500 nmol/min via the sheath and then infused via the catheter at 250 and 500 nmol/min in 6 animals. It was not possible to infuse both atenolol and nebivolol within the same animal because of the propensity for systemic effects. Therefore, in another 6 animals, atenolol was infused via the catheter at 250 and 500 nmol/min. Atenolol was not infused via the sheath because pilot studies suggested that atenolol did not alter PWV.

Study 2: Comparison of the Effect of GTN and Nebivolol on PWV
A dose-response curve was constructed using the NO donor GTN, which was infused via the catheter in 6 sheep at 2, 4, and 8 nmol/min. Following a 20-minute washout period with saline, nebivolol was then infused via the catheter at 500 nmol/min.

Study 3: Effect of Inhibiting NO Synthase on the Response to Nebivolol
In 6 animals, nebivolol (500 nmol/min) was coinfused via the catheter with the NO synthase inhibitor, \( \text{L}^- \)-NMMA (10 \( \mu \)mol/min). This dose of \( \text{L}^- \)-NMMA has previously been shown to substantially inhibit the effect of exogenous acetylcholine on iliac distensibility.13

Study 4: Role of \( \beta_2 \) Adrenoceptors in the Response to Nebivolol
In 8 animals, nebivolol was infused via the catheter at 500 nmol/min. Following a 20-minute washout period, the selective \( \beta_2 \) adrenoceptor antagonist, butoxamine (3 \( \mu \)mol/min), was infused alone for 5 minutes and then coinfused with nebivolol for a further 5 minutes via the catheter.

Data Analysis
All results are expressed as means±SD, unless otherwise stated. Data were analyzed using paired or unpaired Student t tests where appropriate and repeated measures ANOVA. Bonferroni test was used for post hoc comparisons. \( P<0.05 \) was considered significant.

Results
Study 1
Infusion of nebivolol (500 nmol/min) via the sheath did not change iliac PWV (3.60±0.42 versus 3.54±0.39 m/s; \( P=0.3 \)) or MAP (99±11 versus 98±10 mm Hg; \( P=0.4 \)). However, infusion of nebivolol via the catheter reduced iliac PWV in a dose-dependent manner, by \(-3±3\%\) and \(-6±3\%\), respectively (\( P<0.001 \), Figure 2). The effect of the higher dose was significant when compared with infusion via the sheath (\( P=0.005 \)). In contrast, atenolol did not alter the iliac PWV (change of \( 2±1\% \) and \( 3±5\% \); \( P=0.11 \)). The effect of nebivolol differed significantly from that of atenolol (\( P<0.001 \), Table).

Although there was no change in MAP following infusion of nebivolol via the catheter, there was a small but significant drop in MAP following infusion of the higher dose of atenolol. However, when the overall effects of the 2 agents

Figure 1. Schema showing infusions via the catheter (proximal) and sheath (distal). P1 indicates pressure sensor 1; P2, pressure sensor 2. (Figure redrawn from McEniery CM, Qasem A, Schmitt M, Avolio AP, Cockcroft JR, Wilkinson IB. Endothelin-1 regulates arterial pulse wave velocity in vivo. J Am Coll Cardiol. 2003;42:1975–1981).

Figure 2. The effect of intraarterial infusion of atenolol (n=6, ■) and nebivolol (n=6, □) via the catheter on iliac PWV. Values represent means±SEM. \( P<0.001 \), ANOVA, nebivolol vs atenolol. \( * P<0.05, ** P<0.01 \), Bonferroni test.
were compared, they were not significantly different. In addition, there was a significant reduction in HR after the higher doses of nebivolol and atenolol, but again, there was no significant difference between drugs (Table).

**Study 2**
Infusion of GTN via the catheter at doses of 2, 4, and 8 nmol/min significantly reduced PWV in a dose-dependent manner (change of $-5 \pm 3\%$, $-11 \pm 4\%$, and $-12 \pm 4\%$, respectively; $P<0.001$; Figure 3). In comparison, infusion of nebivolol via the catheter at 500 nmol/min significantly reduced PWV by $-6 \pm 3\%$ ($P<0.001$).

**Study 3**
There was no change in PWV when nebivolol was coinfused with L-NMMA in 6 animals (change of $0 \pm 3\%$ and $-1 \pm 2\%$, respectively), which differed significantly from the response to nebivolol alone ($P=0.003$; Figure 4). Both MAP and HR were unchanged during these studies.

**Study 4**
Again, infusion of nebivolol via the catheter at 500 nmol/min significantly reduced PWV by $-5 \pm 1\%$ ($P<0.001$). Infusion of butoxamine alone had no effect on PWV (change of $0 \pm 4\%$; $P=0.9$), and there was no significant change in the PWV when butoxamine was coinfused with nebivolol (change of $-1 \pm 5\%$; $P=0.6$). This differed significantly from the response to nebivolol alone ($P=0.02$; Figure 5). Both MAP and HR were unchanged during these studies.

**Discussion**
Arterial stiffness is a key determinant of cardiovascular risk, and drugs that reduce stiffness as well as blood pressure may confer a survival advantage in patients with stiffened arteries. Previous data concerning the effects of $\beta$-blockers on arterial stiffness are conflicting. Nevertheless, in all of these studies $\beta$-blockade was accompanied by a fall in MAP, and so it is difficult to determine whether any reduction in arterial stiffness was because of a direct effect on the arterial wall or an indirect effect via a decrease in distending pressure, a problem made more difficult because of the nonlinear relationship between MAP and arterial stiffness. The aim of this series of experiments was to investigate the direct effect of $\beta$-blockade on arterial distensibility.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>250</th>
<th>500</th>
<th>ANOVA Nebivolol vs Atenolol</th>
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<tbody>
<tr>
<td>Iliac PWV, m/s</td>
<td>3.56±0.42</td>
<td>3.62±0.41</td>
<td>3.65±0.38</td>
<td>3.47±0.35</td>
</tr>
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<td>MAP, mm Hg</td>
<td>106±10</td>
<td>106±7</td>
<td>100±10$^†$</td>
<td>102±4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>130±13</td>
<td>128±12</td>
<td>126±12</td>
<td>126±5</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>89±11</td>
<td>86±12</td>
<td>83±12</td>
<td>87±4</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>42±9</td>
<td>42±10</td>
<td>42±10</td>
<td>40±4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>131±10</td>
<td>132±7</td>
<td>119±7$^†$</td>
<td>130±17</td>
</tr>
</tbody>
</table>

DBP indicates diastolic blood pressure; PP, pulse pressure. Data are means±SD, and doses are quoted as nmol/min. Comparisons with baseline are indicated by $^*P<0.05$ and $^†P<0.01$, Bonferroni test. Overall significance for atenolol vs nebivolol (ANOVA) is indicated in the final column.

**Figure 3.** The effect of intraarterial infusion of nebivolol ■ and GTN ■ (n=6) via the catheter on iliac PWV. Values represent means±SEM.

**Figure 4.** The effect of inhibiting NO synthase with L-NMMA on the response to nebivolol. Values represent means±SEM. $^*P=0.003$, Student t test, L-NMMA+nebivolol ■ vs nebivolol □.
Effect of Inhibiting NO Synthase on the Response to Nebivolol

Confusion of L-NMMA and nebivolol significantly attenuated the effect of nebivolol on iliac PWV, indicating that endothelium-derived NO, to a large extent, mediates the effect of nebivolol on PWV. This is in agreement with our previous findings in the ovine hind-limb vascular bed that NO regulates large artery distensibility in vivo and extends our previous work in the human forearm vascular bed, showing that nebivolol, but not atenolol, causes peripheral vasodilatation via the release of NO. Moreover, at clinical doses, nebivolol increases stimulated and basal production of NO in patients with essential hypertension.

Effect of Inhibiting β2 Adrenoceptors on the Response to Nebivolol

In vitro data suggest that the release of NO from endothelial cells is inhibited by β2 adrenoceptor blockade, although this is not a universal observation. In this study, infusion of the β2 adrenoceptor antagonist, butoxamine, alone, had no effect on PWV. However, it abolished the effect of nebivolol on PWV, suggesting that stimulation of β2 adrenoceptors by nebivolol is involved in the NO-dependent effect of nebivolol on PWV. This is in keeping with previous data in humans.

Taken together, the current data demonstrate that nebivolol, but not atenolol, acts directly on the arterial wall to increase arterial distensibility. The vasodilatory effects of nebivolol are comparable to the effect of low doses of GTN and are mediated by NO, at least in part, via the stimulation of β2 adrenoceptors. These data indicate that β-blockers differ in their acute effects on arterial distensibility in vivo and that vasodilating agents such as nebivolol may be of greater benefit in conditions associated with increased arterial stiffness, such as isolated systolic hypertension.

Study Limitations

The use of general anesthesia may have influenced our results to some degree. Because we did not measure vessel diameter, we are unable to identify whether PWV changed because of a change in wall thickness or wall stiffness in the present investigation. It is unlikely that the dose of atenolol used here was too low because infusion of the higher dose of atenolol caused a slight decrease in HR, suggesting systemic “spillover.” Similarly, it would seem that the doses used were not too high because in pilot studies (data not shown) infusion of atenolol at lower doses had no effect on HR or arterial distensibility. In addition, although we explored the role of β2 adrenoceptors as a potential mechanism underlying the release of NO in response to nebivolol, we did not investigate whether this mechanism was dose-dependent. Other mechanisms such as those involving 5HT1A receptors and β1 adrenoceptors have also been described.

The present study used the ovine iliac artery as a model of large arteries in humans. It was not possible to conduct the experiments in the aorta because this would have led to the use of systemic dosing and the associated changes in MAP that we sought to avoid. However, although the iliac artery is predominantly a muscular rather than elastic artery, there is a progressive increase in smooth muscle content moving dis-

Figure 5. The effect of inhibiting β2 adrenoceptors with butoxamine on the response to nebivolol. Values represent means±SEM. P=0.02, Student t test, Butoxamine+nebivolol ■ vs nebovolol □.

Comparison of the Effect of GTN and Nebivolol

Infusion of the NO donor, GTN, caused a significant and dose-dependent reduction in iliac PWV, as we and others have observed previously. The response to 2 mmol/min GTN was comparable to that produced by infusion of 500 mmol/min nebivolol, suggesting a modest NO-related effect of nebivolol on large arteries and in keeping with our observations that L-NMMA inhibits the effect of nebivolol on arterial distensibility.

Essentially, using local drug infusions coupled with intravascular measurements of PWV.

Effect of β-Blockade on Iliac PWV

Infusion of nebivolol via the sheath (distal to the site of measurement) did not alter iliac PWV or MAP, suggesting that there were no systemic or reflex effects from the infusion. In contrast, there was a significant dose-dependent reduction in PWV when nebivolol was infused via the catheter (proximally), indicating an increase in arterial distensibility. This was not accompanied by any change in MAP, but there was a significant decline in HR with the higher dose of nebivolol. Nevertheless, this was only 3 bpm, which is not likely to modify PWV significantly in light of previous data indicating that HR has no effect or only a modest effect on PWV. This is in agreement with our previous findings in the ovine hind-limb vascular bed that NO regulates large artery distensibility in vivo and extends our previous work in the human forearm vascular bed, showing that nebivolol, but not atenolol, causes peripheral vasodilatation via the release of NO.

Moreover, at clinical doses, nebivolol increases stimulated and basal production of NO in patients with essential hypertension.  

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tally from the ascending aorta along the arterial tree. Indeed, the iliac artery is similar to the abdominal aorta and still
confers an important buffering component during each ventricular ejection. Moreover, noninvasive assessment of “aortic” PWV in humans also encompasses the carotid, iliac, and femoral arteries. Therefore, we feel that assessing changes in iliac artery stiffness provides useful information concerning large artery function, but clearly the applicability of the current results to humans requires confirmation. Nevertheless, the diminished effect of nebivolol during coin
fusion with L-NMMA observed in the current study is in agreement with findings in human resistance vessels. Finally, the current study examined the acute local effects of atenolol and nebivolol on arterial distensibility, to determine the specific actions of these agents on vessel properties, while avoiding the confounding influence of systemic changes in MAP. However, further studies are required to determine the chronic effects of nebivolol on arterial distensibility.

Perspectives
The lack of effect of atenolol on iliac PWV observed in the present study raises the hypothesis that conventional 
  β-blockers may not be as effective as other antihypertensive agents in directly reducing arterial stiffness in humans. This might provide one potential explanation as to why atenolol was less effective than losartan in reducing cardiovascular risk in the recent Losartan Intervention For Endpoint reduc
  tion in hypertension (LIFE) study31 and showed no benefit was less effective than losartan in reducing cardiovascular over placebo in the Medical Research Council (MRC) study.32 However, further investigations clearly would need to compare directly the effects of the 2 agents on large artery stiffness in humans. Nevertheless, recent outcome data in patients with end-stage renal disease highlight the importance of targeting antihypertensive therapy toward reducing arterial stiffness as well as blood pressure.16 Finally, our confirmatory observations that GTN produces a dose-dependent re
  duction in PWV independently of changes in MAP may help explain why NO donors such as isosorbide dinitrate produce sustained reductions in blood pressure in subjects with hypertension.33 Together, our observations indicate that the NO pathway may be an effective therapeutic target in the treatment of conditions associated with increased arterial stiffness, such as isolated systolic hypertension.

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