Arterial stiffness is a key independent determinant of cardiovascular risk (1,2). Structural components within the arterial wall, mainly collagen and elastin, together with transmural pressure are important determinants of large vessel stiffness (3,4). However, smooth muscle tone can also influence the stiffness of the elastic and muscular arteries (5), suggesting there is also functional regulation of arterial stiffness in vivo.

Nitric oxide (NO) and endothelin-1 (ET-1) are two important mediators released by the vascular endothelium, which exert major, but opposing influences on blood pressure (6,7) and basal vascular tone (8,9). We have recently shown that NO regulates large artery distensibility in vivo (10), and this may explain why conditions that are characterized by reduced NO bioavailability are also associated with increased arterial stiffness. However, the role of ET-1 in the regulation of arterial stiffness is, at present, unclear.

Endothelin-1 exerts its actions on vascular smooth muscle by binding to at least two specific receptor subtypes. The endothelin-A (ETA) receptor is highly expressed on vascular smooth muscle cells and appears to be the major receptor subtype causing vasoconstriction in human (11) and ovine (12) arteries. In contrast, the endothelin-B (ETB) receptor is expressed on vascular smooth muscle cells mediating vasoconstriction, and also on endothelial cells producing vasodilation via the release of NO and prostacyclin (13). Local ETA receptor blockade causes vasodilatation of epicardial vessels (14,15), demonstrating basal tone of ET-1 in larger arteries. Moreover, arterial plasma ET-1 levels are positively correlated with large artery stiffness in patients with coronary artery disease (16). However, ET-1 is largely secreted abluminally by endothelial cells toward the adjacent vascular smooth muscle (17); thus, plasma ET-1 levels are a poor marker of its vascular activity in vivo. Therefore, there are no direct data concerning the role of ET-1 in regulating large artery stiffness.

We hypothesized that basal release of ET-1 contributes to the regulation of large artery stiffness. The aim of this study was to investigate whether endothelin-1, acting locally, regulates arterial distensibility, assessed by measuring pulse-wave velocity in vivo.

METHODS

All studies were conducted in anesthetized sheep. Pulse wave velocity (PWV) was calculated using the foot-to-foot methodology from two pressure waveforms simultaneously recorded with a high-fidelity, dual pressure-sensing catheter placed in the common iliac artery.

RESULTS

Intra-arterial infusion of ET-1 significantly increased iliac PWV by 12 ± 5% (mean ± STD; p < 0.001), whereas infusion of the endothelin-A (ETA) receptor antagonist BQ-123 significantly reduced PWV by 12 ± 4% (p < 0.001). After BQ-123 infusion, exogenously infused ET-1 did not significantly change PWV compared with infusion of saline (change of −0.08 ± 0.11% vs. −0.01 ± 0.07%; p = 0.53). Importantly, infusion of BQ-123 or ET-1 distal to the common iliac artery did not affect PWV.

CONCLUSIONS

These results demonstrate, for the first time, that endogenous ET-1 production directly regulates large artery PWV in vivo. In addition, exogenous ET-1 increases PWV, and this can be blunted by ETA receptor blockade. These observations explain, in part, why conditions that exhibit up-regulation of ET-1 are also associated with arterial stiffening. Therefore, drugs that block ETA receptors may be effective in reducing large artery stiffness in humans, and thus cardiovascular risk. (J Am Coll Cardiol 2003;42:1975–81) © 2003 by the American College of Cardiology Foundation.
New South Wales, Australia. The study was approved by the University’s Animal Care and Ethics Committee. Anesthesia was induced by intravenous injection of 600 to 900 mg sodium phenobarbitone (Rhone Merieux, Queensland, Australia), and maintained by inhalation of 2% to 3% halothane, administered through a Boyle’s rebreathing apparatus with an oxygen flow rate of 2 l/min. Animals were spontaneously breathing throughout and studied in the supine position.

Hemodynamic measurements. All pressure measurements were made using a Gaeltec 6F end-hole catheter (Gaeltec, Skye, United Kingdom) with a 0.46-mm internal lumen, and dual high-fidelity pressure sensors located 10 and 60 mm from the distal end. Calibration of both sensors was performed simultaneously at the start of each experiment using a mercury sphygmomanometer. The analogue signal from the pressure control unit was fed directly into a portable microcomputer using a PowerLab analogue-to-digital converter (AD Instruments, Hastings, United Kingdom). This identification of the distal pressure waveform using the supplied CHART software (Version 4). Data were then exported digitally for analysis with a custom-written MATLAB analysis program (Math Works, Cambridge, United Kingdom). This identifies the foot of each of the simultaneously recorded pressure waveforms and calculates the transit time (TT) from the foot-to-foot delay, as previously described (10). The minimum resolution of the system was a TT difference of 0.1 ms. The iliac PWV was calculated as the fixed distance between the recording sites (50 mm) divided by the TT, and it is inversely related to arterial distensibility by the 1922 equation of Bramwell and Hill (18):

\[
PWV = \sqrt{\frac{V \cdot \Delta P \cdot \rho \cdot \Delta V}{\rho}}
\]

where \( V \) = artery volume, \( \Delta V \) = change in volume, \( \Delta P \) = change in pressure, and \( \rho \) = blood density (assumed to be constant in the present studies). For a distance of 50 mm, the 0.1-ms resolution in TT provides a PWV resolution of 0.025 m/s (assuming a mean TT of 14 m/s). That is, PWV can be estimated to within 0.7%. The repeatability of measurements has been previously reported (10). Heart rate (HR) was calculated over the measurement period from a simultaneously recorded electrocardiogram.

Drugs. All drugs were freshly prepared in an aseptic manner before the start of each experiment, using 0.9% saline as a diluent. The ETA receptor antagonist, BQ-123 (Bachem, Bubendorf, Switzerland) was infused for 15 min at 40 nmol/min, followed by saline. Endothelin-1 (Calbiochem, Nottingham, United Kingdom) was infused continuously for 60 min at 10 pmol/min. These doses and duration of infusions were based on published data (15,19) and on our previous findings that doses active in the ovine iliac artery are approximately equivalent to those used in human forearm blood flow studies in vivo (10). In particular, the regimen of BQ-123 infusion followed by saline was based on previous data in the human forearm vascular bed, demonstrating slow-onset dilation in response to BQ-123, which persisted for 30 to 60 min following the end of the infusion (20). Nevertheless, we performed pilot studies (data not shown) with BQ-123 (10 to 100 nmol/min) and ET-1 (10 pmol/min) to confirm the choice of selected doses and duration of infusions. The NO synthase inhibitor, N\(^{0}\)-monomethyl-\(l\)-arginine (LNMMA) (Clinalfa, Laufelfingen, Switzerland) was infused at 10 \( \mu \)mol/min and noradrenaline (NA) (Abbott, Maidenhead, United Kingdom) was infused at 600 pmol/min. The dose of LNMMA was based on our previous data (10) and the dose of NA was selected from pilot experiments (data not shown), to produce a similar baseline change in PWV as LNMMA (~6%).

Protocol. The distal femoral artery was identified by palpation and a 20-mm segment of artery exposed by limited dissection into which a 6F sheath was inserted. The arterial catheter was then positioned in the common iliac artery, as described previously (10). Saline was infused through the sheath and catheter at 2 ml/min for a period of 30 min to allow stabilization of the preparation. Baseline measurements of iliac PWV, MAP, and HR were then recorded in triplicate, or until measurements were stable (within 3% of each other). All drugs were infused at 2 ml/min, and pressure waveforms were recorded for 20 s, at 15-min intervals during each infusion period. Infusion of drugs through the catheter exposed the arterial segment under study to the drug, whereas infusion through the sheath did not, as this was located distally to the pressure sensors (Fig. 1). Because the common iliac artery is nonbranching, this methodology, which has been described previously (10), allows 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.

Effect of exogenous ET-1. Because of the slow onset and prolonged action of ET-1, it was not possible to administer ET-1 via both the sheath and catheter in the same animal. Therefore, after baseline recordings had been obtained, ET-1 (10 pmol/min) was infused through the
sheath in six sheep. In a further six sheep, ET-1 was infused at the same dose through the catheter, followed by saline for 2 h to determine the offset of effect of ET-1 (Fig. 2A).

**EFFECT OF ET<sub>A</sub> RECEPTOR BLOCKADE WITH BQ-123.** Eight sheep were studied. After baseline recordings had been obtained, BQ-123 (40 nmol/min) was infused through the sheath for 15 min followed by saline for 15 min. The BQ-123 was then infused through the catheter for 15 min followed by saline for 30 min. In four of the sheep, saline was then infused for a further 45 min, and in the remaining four animals, ET-1 was infused for 45 min, in place of saline (both infusions were via the catheter; Fig. 2B).

In an additional control experiment, four sheep received BQ-123 (40 nmol/min), via the sheath only, for 30 min followed by saline for 30 min. This dose and the infusion times of BQ-123 were equal to that infused in the previous experiment (i.e., 15 min via the sheath plus 15 min via the catheter, at 40 nmol/min). This control experiment was conducted to exclude the possibility that any effects observed in the previous experiment could be explained by "cumulative dosing" or delayed onset of BQ-123, rather than a local effect on the arterial wall.

Finally, to examine the role of NO on the observed changes in PWV during ET<sub>A</sub> receptor blockade, BQ-123 (40 nmol/min for 15 min, followed by saline for 30 min) was coinfused with either the NO synthase inhibitor, LNMMA (10 µmol/min; n = 4), or the control constrictor, NA (600 pmol/min; n = 4).

**Data analysis.** All results are expressed as means ± SD, unless otherwise stated, and data corresponding to the greatest change from baseline values are reported in the text. Data were analyzed using repeated-measures analysis of variance (ANOVA), and the Bonferroni test for post hoc comparisons, where appropriate. A p value of < 0.05 was considered significant.

**RESULTS**

**Effect of exogenous ET-1 on PWV.** Infusion of ET-1 via the sheath did not change iliac PWV (3.95 ± 0.46 vs. 3.92 ± 0.28 m/s; p = 0.6) or MAP (106 ± 12 vs. 109 ± 10 mm Hg; p = 0.2), but there was a significant decline in HR (−7 ± 4 beats/min; Table 1). However, there was a gradual and significant increase in iliac PWV of 12 ± 5%, when ET-1 was infused through the catheter (3.54 ± 0.54 vs. 3.98 ± 0.64 m/s after 60 min; p < 0.001; Fig. 3), which had returned to baseline (3.65 ± 0.3 m/s; p = 0.97) 60 min after stopping the ET-1 infusion.

**Effect of BQ-123 on PWV.** Eight sheep received intraarterial BQ-123. There was no change in iliac PWV when BQ-123 was infused via the femoral artery sheath (3.59 ± 0.32 vs. 3.52 ± 0.27 m/s; p = 0.2). However, there was a significant and gradual decrease in the PWV of 12 ± 5% following infusion through the catheter (3.52 ± 0.27 vs. 3.16 ± 0.25 m/s after 45 min; p < 0.001; Fig. 4). Mean arterial pressure was significantly reduced following infusion of BQ-123 both through the sheath (change of −4 ± 4 mm Hg; p = 0.02) and through the catheter (change of −6 ± 4 mm Hg; p < 0.001). However, the magnitude of this change did not differ significantly between the two routes (p = 0.87; Table 2). No change occurred in HR.

Administration of ET-1 or saline through the catheter,
after infusion of BQ-123, did not significantly alter PWV (3.16 ± 0.25 vs. 3.08 ± 0.22 m/s; p = 0.53; and 3.16 ± 0.25 vs. 3.12 ± 0.26 m/s; p = 0.06, respectively; Fig. 4).

Doubling the duration of BQ-123 infusion via the sheath in four sheep (40 nmol/min for 30 min), to provide the same cumulative dose as given in the first series of experiments, did not alter PWV (change of 2 ± 3%; p = 0.58), despite producing exactly the same average change in MAP (−6 ± 2 mm Hg; p = 0.01; p = 0.41 for comparison). Once again, no change occurred in HR.

Co-infusion of BQ-123 and LNMMA produced a significant reduction in PWV (change of −9 ± 3%; p = 0.04), which was similar to that observed when BQ-123 was co-infused with NA, as a control constrictor for LNMMA (change of −8 ± 6%; p = 0.04; p = 0.32 for comparison).

DISCUSSION

Large artery stiffness is a powerful and independent predictor of cardiovascular risk (1,2). Smooth muscle tone influences the stiffness of the elastic and muscular arteries (5), and removal of the vascular endothelium modifies large artery mechanics in vivo (21,22), suggesting a degree of functional regulation of large artery stiffness by endothelium-derived vasoactive mediators. Indeed, we and others have recently shown that NO regulates large artery distensibility (10,23). Such observations may explain why a number of conditions associated with increased large artery stiffness, such as hypertension and hypercholesterolemia, are also associated with endothelial dysfunction, through either reduced bioavailability of NO (24,25) or enhanced vascular activity of endothelium-derived vasoconstrictors such as ET-1 (26,27).

In the current study, we extend our previous findings by demonstrating, for the first time, that selective blockade of ET_A receptors with BQ-123 substantially reduces PWV in the ovine iliac artery. We have also shown that infusion of exogenous ET-1 increases PWV in the ovine iliac artery, and that infusion of an ETA receptor antagonist significantly attenuates this effect. Together, these data suggest that endogenous ET-1, acting via the ET_A receptor, regulates arterial distensibility in vivo.

**Table 1. Effect of ET-1 on Hemodynamics**

<table>
<thead>
<tr>
<th>Sheath</th>
<th>Baseline</th>
<th>ET-1</th>
<th>Catheter</th>
<th>Baseline</th>
<th>ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iliac PWV, m/s</td>
<td>3.95 ± 0.46</td>
<td>3.92 ± 0.28</td>
<td>3.54 ± 0.54</td>
<td>3.98 ± 0.64†‡</td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>106 ± 12</td>
<td>109 ± 10</td>
<td>110 ± 11</td>
<td>110 ± 10</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>148 ± 17</td>
<td>141 ± 16*</td>
<td>126 ± 18</td>
<td>125 ± 15</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. *p < 0.05. †p < 0.01, compared with baseline values. ‡p < 0.001, for change compared with saline infusion via sheath.

ET-1 = endothelin-1; HR = heart rate; MAP = mean arterial pressure; PWV = pulse wave velocity.

**Figure 3.** The effect of intra-arterial infusion of endothelin-1 (ET-1) via the catheter on iliac pulse wave velocity (PWV) (n = 6). Values represent means ± SD; p < 0.001 (ANOVA), compared with saline. *p < 0.01. **p < 0.001, Bonferroni test.

**Figure 4.** The effect of intra-arterial infusion of BQ-123 through the sheath and catheter (triangles, n = 8), followed by endothelin-1 (ET-1) (open squares, n = 4), or saline (closed squares, n = 4) on iliac pulse wave velocity (PWV). Values represent means ± SD; p < 0.001 (ANOVA), compared with saline. *p < 0.001, Bonferroni test.
hemodynamic changes, indicating that the response to exogenous ET-1 was due to a direct action on the local arterial wall, and not the result of potentially confounding changes in systemic MAP. These data thus suggest that infusion of exogenous ET-1 decreases local arterial distensibility in vivo.

**Effect of BQ-123 on PWV.** Infusion of the selective ETA receptor antagonist BQ-123 via the catheter resulted in a significant decrease in PWV, suggesting an increase in arterial distensibility. The time-course of this effect of BQ-123 (slow-onset and persistent for up to 30 min after stopping the infusion) was similar to responses in the human forearm vascular bed in vivo (20). Although the decrease in PWV was accompanied by a small but significant reduction in MAP (~6 mm Hg), previous data relating MAP to PWV suggest that such a change will alter PWV by <3% (29). Moreover, a blood pressure–independent effect of BQ-123 on PWV in the present study is supported by the observation that when BQ-123 was infused via the sheath at either the same, or twice, the dose as given through the catheter, there was no change in the PWV despite reductions in MAP of 4 mm Hg and 6 mm Hg, respectively (NS for both comparisons vs. change via the catheter). Taken together, these data indicate that the majority of the effect of BQ-123 on PWV in the present study is due to a direct action on the arterial wall rather than a drop in MAP, suggesting, for the first time, that blockade of endogenous ET-1–mediated vasoconstriction via the ET<sub>A</sub> receptor increases large artery distensibility in vivo.

Infusion of BQ-123 abolished the effect of exogenous ET-1 on PWV, indicating that the effect of endogenous ET-1 on PWV is, to a large extent, mediated via the ET<sub>A</sub> receptor. Interestingly, when BQ-123 was co-infused with the NO synthase inhibitor LNMMA, the PWV fell by ~9%, which, although less than that observed in the previous experiment, when BQ-123 was infused alone (~12%), was similar to that observed when BQ-123 was co-infused with the control constrictor NA (~8%). Therefore, it is unlikely that increased NO production, via unopposed ET<sub>B</sub> receptor stimulation (for example), is responsible for the observed effect of BQ-123 in the current series of experiments. This is in contrast with data from human studies in vivo, which suggest that NO does contribute to the vascular responses during ET<sub>A</sub> receptor blockade (30,31), most probably via increased stimulation of ET<sub>B</sub> receptors by ET-1. However, a role for ET<sub>B</sub> receptors in the functional regulation of arterial distensibility in the current investigation cannot be fully excluded, because ET<sub>B</sub> receptors also act to release other vasoactive mediators such as prostacyclin (13). Therefore, further work is now required to examine the role of ET<sub>B</sub> receptors in regulating arterial distensibility.

**Physiological importance of ET-1.** The physiological importance of endogenous ET-1 to basal vascular tone in resistance vessels has been demonstrated in vivo by vasodilation in response to both local (9,20) and systemic (7) ET<sub>A</sub> receptor blockade. Moreover, local ET<sub>A</sub> receptor blockade causes vasodilation of epicardial vessels (14,15), demonstrating basal tone of ET-1 in larger arteries. The current findings add to these data by demonstrating a pronounced effect of ETA receptor blockade in a large muscular artery. Furthermore, it appears that ET-1 may be more important to the regulation of large artery stiffness than NO. The ETA receptor blockade with BQ-123 reduced PWV by ~12%. This effect appears greater than the changes we observed during inhibition of basal NO production in the same experimental setting, although in different animals, when PWV increased by only ~3% (10). However, these findings are consistent with data from human in vivo studies, where infusion of BQ-123 increases forearm blood flow by ~60% (9,20) whereas LNMMA reduces forearm blood flow by only ~40% (8,32). In humans, femoral PWV increases by ~5.5% for each decade of life (3). Therefore, if BQ-123 has an effect on PWV in humans similar to that in the ovine iliac artery, inhibition of the ET<sub>A</sub> receptor-mediated actions of endogenous ET-1 would effectively reduce large artery stiffness by ~15 years. Thus, an increased vasoconstrictor activity of ET-1 may, in part, explain the association between premature arterial stiffening and established cardiovascular risk factors such as hypercholesterolemia (33,34) and cigarette smoking (35). Hence, it may be possible to reduce arterial stiffness pharmacologically through drug therapies targeted at reducing vasoconstriction to ET-1. Such therapies, aimed at the large arteries, may reduce the burden of morbidity and mortality from cardiovascular disease.

**Potential considerations.** The present study used the ovine iliac artery as a model of large arteries in humans. Therefore, the applicability of the results to humans requires confirmation. However, as in humans, ET-1 exerts potent
cardiovascular actions in sheep (36), which can be antagonized with BQ-123 (37). In particular, the ovine and human responses to systemic infusion of ET-1 (36,38) and ET_A receptor blockade (7,37) are similar. In addition, inhibition of basal NO production with LNMMA has a similar effect on arterial distensibility in the ovine iliac artery (10), and on human brachial artery (23) in vivo.

The use of general anesthesia may have influenced our results to some degree. However, owing to the need to make very high fidelity recordings, it was not possible to use conscious animals. Nevertheless, we believe that the ovine hind-limb preparation is a useful surrogate model for the effects of drugs and vasoactive mediators in large muscular artery mechanics.

Although the dose regimen of BQ-123 used in the present study has been described previously (15), we may have underestimated the maximal effect of BQ-123. Moreover, the precise mechanisms underlying the observed changes in PWV in response to drug infusions remain unclear, because we did not measure artery diameter. Although PWV is a measure of distensibility, factors influencing distensibility include vessel diameter, wall thickness, and wall stiffness, possibly due to altered relative loading of elastin and collagen fibers within the arterial wall, accompanying changes in smooth muscle tone. Therefore, we are unable to identify which parameters are responsible for the observed changes in PWV in the present investigation. However, it would seem unlikely that alterations in vascular resistance in the hind-limb is responsible for the changes in PWV because infusion of ET-1 and BQ-123 via the sheath had no effect. Similarly, a delayed effect of BQ-123 on resistance vessels is unlikely because when BQ-123 was infused via the sheath for twice as long (i.e., 30 min) and then followed by saline for 30 min, there was no change in the PWV despite a similar reduction in MAP to that observed during other infusions.

A potential limitation in our study design is that although we demonstrated that the effect of exogenous ET-1 on PWV was abolished following BQ-123 infusion, we did not infuse a comparator vasoconstrictor to ensure that a change in baseline did not account for the lack of effect of ET-1 post–BQ-123. However, such an approach was not feasible owing to concerns about the duration of the experiments (both ethical and relating to preparation stability).

Finally, the role of the ET_B receptor was not examined in the current study. Although our findings suggest that NO does not appear to play a major role in the regulation of arterial distensibility during ET_A receptor blockade, endothelial ET_B receptors release other vasoactive mediators such as prostacyclin (13), which may also be important in the functional regulation of large artery distensibility. Therefore, further studies using ET_B antagonists are required to examine the role of ET_B receptors, and to more fully characterize the effects of endogenous ET-1 in regulating large artery distensibility.

Summary. We have demonstrated, for the first time, that endogenous ET-1, acting via the ET_A receptor, regulates large artery distensibility, assessed by measuring PWV in vivo. Such findings confirm and extend our previous observations that there is functional regulation of arterial distensibility, mediated, in part, by locally generated vasoactive factors. Therefore, an increased vascular activity of ET-1 may help to explain the association between established cardiovascular risk factors and premature arterial stiffening. Drugs that block ET_A receptors may be effective in reducing large artery stiffness in humans, and thus cardiovascular risk.

Acknowledgments
We thank Vicki Tatarinoff, Kate Noble, and John Klemes for their technical help with the studies.

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