Role of natriuretic peptides in regulation of conduit artery distensibility

Matthias Schmitt, Ahmed Qasem, Carmel McEniery, Ian B. Wilkinson, Vicki Tatarinoff, Kate Noble, John Klemes, Nicola Payne, Michael P. Frenneaux, John Cockcroft, and Albert Avolio. Role of natriuretic peptides in regulation of conduit artery distensibility. Am J Physiol Heart Circ Physiol 287: H1167–H1171, 2004; 10.1152/ajpheart.00101.2004.—Arterial distensibility, assessed by the pulse-wave velocity (PWV), is an independent predictor of cardiovascular risk. We investigated whether natriuretic peptides, acting locally, modify conduit artery distensibility in vivo. All studies were conducted in anesthetized sheep (n = 18) by using a validated ovine hindlimb model. In brief, the PWV was calculated, with the use of the foot-to-foot methodology, from two pressure waveforms recorded simultaneously with a high-fidelity dual pressure-sensing catheter placed in the common iliac artery. Drugs were infused either proximally, via the catheter to perfuse the segment of artery under study, or distally, via the sheath to control for any reflex changes in flow or sympathetic activation. First, the effects of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and c-type natriuretic peptide (CNP) were studied. Second, the role of endogenous ANP was investigated by infusing the natriuretic peptide receptor type A (NPRA)-selective receptor antagonist A71915. Third, A71915 was infused. Infusion of CNP or des-[Gln18 Ser 19 Gly 20 Leu 21 Gly 22]-ANF-(4-23)-NH₂ (cANF), an ANP analog that selectively binds to the NPRC receptor (14, 21, 37). The in vivo effects of ANP on large artery vasomotor control, however, have not been investigated. Therefore, we aimed to establish whether NPs, acting locally, modify regional distensibility in large muscular arteries.

To investigate the potency and mechanisms of action, we performed four studies. First, we performed a comparative dose-response study assessing the effects of ANP, brain natriuretic peptide (BNP), and c-type natriuretic peptide (CNP) on PWV in a previously validated ovine hindlimb model (40).

Second, to determine the contribution of endogenous ANP to basal arterial distensibility, we studied the effects of the natriuretic peptide receptor type A (NPRA)-selective receptor-antagonist A71915 (16) on resting PWV. Third, we assessed the effects of A71915 on ANP-induced changes in PWV. Fourth, to establish the role of the NPRC receptor in regulation of regional iliac artery distensibility, we studied the effects of des-[Gln18Ser19Gly20Leu21Gly22]-ANF-(4-23)-NH₂ (cANF), on PWV.

METHODS

Studies were conducted in 18 adult, cross-bred Suffolk sheep aged between 12 and 18 mo and weighing between 37 and 60 kg, at the University of New South Wales, Sydney, Australia. The studies were approved by the University’s Animal Care and Ethics Committee. Anesthesia was induced by an intramuscular injection of Zoletil 100 (10 mg/kg) (Virbac, Peakhurst, New South Wales, Australia) and maintained by inhalation of 2–3% isofluorane, administered via a Boyle’s rebreathing apparatus with an oxygen flow rate of 2 l/min. Animals were studied in the supine position, breathing spontaneously.

Aortic pulse-wave velocity (PWV), a measure of distensibility, is an important independent determinant of cardiovascular risk (6, 7). Structural components within the arterial wall (mainly collagen and elastin), together with transmural pressure and smooth muscle tone, are all important regulators of arterial distensibility (2, 3). Vascular smooth muscle tone is dependent on locally derived signaling molecules, such as nitric oxide (NO) (40) and endothelin-1 (26), but may also be influenced by endocrine- and paracrine-acting natriuretic peptides (NPs). Although atrial natriuretic peptide (ANP) is a potent vasorelaxant on smaller conduit and resistance vessels in vivo and in vitro, there is considerable heterogeneity in responsiveness dependent on size and site of the vessels (18–21, 37). The in vivo effects of ANP on large artery vasomotor control, however, have not been investigated. Therefore, we aimed to establish whether NPs, acting locally, modify regional distensibility in large muscular arteries.

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and calculates the transit time from the foot-to-foot delay, as previously described (40). The iliac PWV is calculated from the transit time and the fixed distance between the recording sites (50 mm), which is inversely related to arterial distensibility by the equation of Bramwell and Hill (9):

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

where \( V \) is artery volume, \( \Delta V \) is change in volume, \( \Delta P \) is change in pressure, and \( \rho \) is blood density (assumed to be constant during the study). Heart rate (HR) is derived over the measurement period from a simultaneously recorded electrocardiogram.

Doses. Human α-ANP, BNP, CNP (Clinalfa; Läufelfingen, Switzerland), A71915, and cANF (Bachem, St. Helens, UK) were all prepared in 0.9% saline in an aseptic manner on the day of the study. The NP doses and infusion periods were based on our previous experience in the human forearm (33) and were titrated to produce local and not systemic effects.

Protocol-surgical preparation. The femoral artery was identified by palpation, and a 30-mm segment of each artery was exposed by limited dissection into which a 7-Fr sheath was inserted. The arterial catheter was then positioned in the common iliac artery with the tip below the level of the aortic trifurcation (sheep have a large mediastinal artery), as previously described (40). Saline was infused through the sheath or catheter at 1 ml/min for a period of 30 min to allow for stabilization of the preparation. At the end of this period, measurements of iliac PWV, MAP, and HR were taken. This was followed by infusion of ANP, BNP, or CNP via the sheath. Measurements of iliac PWV, MAP, and HR were then recorded in duplicate or until measurements were stable (within 10% of each other). All drugs were infused at 1 ml/min for 5 min in doses as outlined below, and repeated pressure waveforms were recorded for 20 s during the last minute of each infusion period. Potentially confounding changes in flow were controlled by drug infusion via the catheter and the sheath. Infusion of drugs through the catheter exposes the arterial segment under study to the drug, whereas infusion via the sheath does not because the drug is delivered distal to the pressure sensors (see Fig. 1).

With the exception of study 3, no two drugs were infused in the same limb. Where animals were studied twice, hemodynamics had returned to baseline and a minimal washout period of 40 min was interposed before the contralateral limb was studied.

Study 1: ANP, BNP, and CNP dose-response studies. ANP (6), BNP (6), or CNP (6) (0.3 nmol/min) was infused via the sheath, and two baseline recordings were taken. This was followed by infusion of equimolar doses of ANP (0.03, 0.15, and 0.3 nmol/min), BNP (0.03, 0.15, and 0.3 nmol/min), or CNP (0.15 and 0.3 nmol/min) via the catheter, in an incremental cumulative fashion.

Study 2: effects of A71915 on resting PWV. After baseline measurement during normal saline via the catheter, the NPR\(_{\alpha}\)-selective receptor antagonist A71915 was infused at an infusion rate of 6.1 nmol/min (6), and measurements of HR, MAP, and PWV were repeated.

Study 3: effects of ANP-A71915 coinfusion. In four sheep, baseline measurements during normal saline infusion via the catheter were followed by coinfusion of ANP (0.15 nmol/min)-A71915 (6.1 nmol/min), a further measurement, infusion of ANP (0.15 nmol/min) alone, and a final measurement.

Study 4: effect of cANF on regional PWV. The role of the NPR\(_c\)-receptor in mediating NP-induced changes in regional PWV were studied by infusing the NPR\(_c\)-selective agent cANF (6). After baseline measurements during normal saline infusion via the catheter, infusion of cANF was commenced at a dose rate of 0.3 nmol/min.

Statistics. Data are expressed as means ± SE. Data were analyzed by two-way ANOVA with post hoc comparison to baseline or by Student’s t-test where appropriate. A value of \( P < 0.05 \) was considered significant.

RESULTS

Changes in HR and MAP are summarized in Table 1. Importantly, in the ANP, BNP, and CNP dose-response studies, NP infusion distal to the common iliac artery (via the sheath) did not have any (reflex) effects on (proximal) PWV.

Study 1: effects of ANP, BNP, and CNP on iliac PWV. ANP, and to a lesser degree BNP, dose dependently decreased regional PWV. The results, using 0.3 nmol/min sheath infusion as baseline, are summarized in Table 1. Additionally, the ANP and BNP dose-response curves (using normal saline infusion as baseline) are shown in Fig. 2, A and B, respectively.

Study 2: effect of A71915 on iliac PWV. The effects of A71915 on iliac PWV are shown in Fig. 3. A71915 increased iliac PWV from 2.97 ± 0.13 to 3.06 ± 0.13 m/s; \( P < 0.01 \).

Study 3: effect of ANP-A71915 coinfusion on iliac PWV. Iliac PWV did not change significantly during ANP-A71915 coinfusion (PWV increased from 3.32 ± 0.2 m/s during normal saline infusion to 3.36 ± 0.2 m/s during coinfusion; \( P = \) not significant). When A71915 was stopped and ANP infusion continued alone, PWV fell to 3.23 ± 0.1 m/s (\( P < 0.01 \)).

Study 4: effects of cANF on iliac PWV. cANF infusion had no significant effect on resting PWV (see Fig. 4).

DISCUSSION

Large artery stiffness, the inverse parameter distensibility, and aortic PWV are powerful, independent predictors of cardiovascular risk (6, 7). Whereas age-related arterial stiffening was thought to be mainly the consequence of structural
changes within the arterial wall, we (26, 40) and others (22) recently demonstrated a significant degree of functional regulation.

In the present study, we investigated the role of NPs in the functional regulation of large artery distensibility.

The principal findings were as follows. First, we demonstrate that ANP, and to a lesser degree BNP, regulate regional large artery distensibility. Second, the effect of ANP was completely antagonized by the NPRA-selective receptor blocker A71915, suggesting that this effect was solely mediated via the NPRA receptor. Third, A71915 infusion increased resting PWV, suggesting that basal ANP plasma levels contribute to resting large artery distensibility. Fourth, neither CNP nor the NPRC-selective receptor agonist cANF altered regional PWV, suggesting that the NPRB and NPRC receptors do not acutely modify large artery distensibility in vivo.

Effect of ANP and BNP on large conduit arteries. ANP and BNP exert well-documented vasorelaxant effects in the resistance vasculature in vivo (19, 37). Furthermore, in organ bath experiments, various NPs have been shown to stimulate cGMP production and to dilate rings of smaller conduit arteries, including human internal mammary (1, 8, 19, 21). However, compelling evidence of a biological effect of ANP on in vivo large artery function, including resting distensibility, is lacking. In the present study, we provide direct evidence that ANP modulates regional large artery distensibility. The study design rules out central sympathetic or general systemic effects. The results therefore reflect local ANP actions.

**CNP, a vasoactive NP?** The natriuretic peptide system consists at least of ANP, BNP, both predominantly myocardial in origin, and CNP, largely of endothelial cell origin (24). ANP and BNP are believed to exert their vasorelaxant effects mainly through binding to NPRA, a membrane-bound guanylate cyclase (GC)-coupled receptor that signals via the second messenger cGMP. CNP in contrast is the natural ligand for the NPRB receptor. All three peptides have high-binding affinity to the NPRC receptor (23), the latter being devoid of a GC domain. The NPRC receptor (previously believed to mainly act as a clearance receptor) has now been recognized to play a crucial role in mediating the antiproliferative action of the NPs. Activation of NPRC inhibits adenylate cyclase, increases phospholipase C activity, and has very recently been shown to mediate CNP-induced endothelium-derived hyperpolarizing factor (EDHF)-dependent vasorelaxation in mesenteric resistance arteries (14).

In contrast to the latter study, neither cANF-NPRC nor CNP-NPRB/C interaction elicited any immediate regional arterial effects in the present study. The latter is not surprising given that the amount of EDHF-dependent vasorelaxation is believed to decrease with increasing vessel diameter (34, 36). Furthermore, it has long been noticed that the effects of NPs are size

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Table 1. Effects of ANP, BNP, and CNP on hemodynamics

<table>
<thead>
<tr>
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<th>ANP, nmol/min</th>
<th>BNP, nmol/min</th>
<th>CNP, nmol/min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sheath (0.3)</td>
<td>0.15</td>
<td>0.3</td>
</tr>
<tr>
<td>Iliac PWV, m/s</td>
<td>3.6±0.2</td>
<td>3.3±0.1†</td>
<td>3.3±0.1‡</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84±5</td>
<td>82±7</td>
<td>82±6</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>141±6</td>
<td>142±10</td>
<td>142±8</td>
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Continuous data are presented as means ± SE; n = 18 sheep, 6 for each group. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, c-type natriuretic peptide; PWV, pulse-wave velocity; MAP, mean arterial pressure; HR, heart rate. *P < 0.05; †P < 0.01; ‡P < 0.001.
and site dependent (18, 21). The potential vasodilatory role of CNP remains controversial. Whereas some in vitro (5, 14) and in vivo studies (17, 29) (the latter following local drug administration) have reported vasodilatory effects, at least at pharmacological concentrations, others (4, 12, 13, 30) have failed to demonstrate important hemodynamic effects at pathophysiological plasma concentrations. Our study suggests that CNP has no vasodilatory potency, at least in large muscular conduit arteries.

Effects of basal ANP plasma levels on large artery distensibility. The effects of NPs on vascular function have traditionally been investigated assessing changes in blood pressure, central hemodynamics, vascular tone, blood flow, and resistance in response to either exposure to exogenous ANP (27, 32, 37) or in response to inhibition of breakdown of endogenous NPs (28). Most studies have employed doses of ANP that result in elevation of plasma peptide concentrations to values far above normal (15, 38). Richards et al. (31) demonstrated that even very low dose infusions caused significant hemodynamic effects. However, acceptance of ANP as a physiologically significant vasoactive hormone depends on the demonstration of changes in vascular tone in response to inhibition of the effects of basal plasma levels. Studies by Brunner and Woelkert (10), using the ANP analog A71915, provided evidence that ANP antagonism contributes to regulation of basal coronary and total peripheral resistance, at least in rodents. Using the same receptor antagonist, we previously demonstrated that basal ANP plasma levels contribute to regulation of regional vascular volume and venous tone in healthy volunteers. Furthermore, we (33) demonstrated that A71915 dose dependently antagonized ANP but not sodium nitroprusside-induced changes in forearm blood flow. Here we extend these findings to large conduit arteries. Indeed the present study, to the best of our knowledge, is the first to show that basal ANP plasma levels contribute to resting large artery distensibility in vivo.

Clinical considerations. Decreased arterial distensibility (increased stiffening) causes increased PWV so that wave reflection affects the systolic rather than the diastolic part of the wave, creating a secondary rise in pressure in late systole thereby increasing afterload without adequately augmenting coronary perfusion pressure (39). The sequelae are isolated systolic hypertension (ISH), left ventricular hypertrophy, and ultimately heart failure. Wave reflection itself arises from a myriad of sites at which there is a change in vascular impedance, including the aorto-iliac bifurcation. Besides the largely age-dependent and at present untreated structural abnormalities (fatigue and fracture of elastin), it is now well recognized that functional changes, most importantly smooth muscle tone, will also affect PWV. Interestingly, patients with ISH exhibit lower baseline ANP plasma levels compared with age-matched patients with essential hypertension but exhibit a higher renal ANP sensitivity (35). If the same were true for their vasculature, then NP-based treatment regimes may prove particularly useful in this subgroup of hypertensive patients. Indeed, NPs have the potential to counteract both pathological remodeling and increased vascular tone. Here we show that ANP-NPRA-interaction acutely modulates regional PWV, thereby providing a potential mechanism by which NP based treatment regimes may exert beneficial effects in ISH.

Limitations. The present study used an ovine hindlimb as a model of large arteries in humans. Therefore, general concerns of transferring data obtained from animal research to humans will apply to this study. However, whereas ovis aries and Homo sapiens for example differ markedly in their digestive system, for most published cardiovascular parameters, the sheep is similar to the human if allowance is made for the smaller weight of the average sheep. Besides these cardiovascular similarities and the surgical suitability the sheep was the animal of choice for the present work because there is possibly no other mammal (apart from Homo sapiens) in which the cardiovascular effects of NPs have been equally well characterized (11–13). In addition, inhibition of basal NO production with N\textsuperscript{G}-monomethyl-L-arginine has a similar effect on arterial distensibility in ovine (40) and human iliac artery (own unpublished data) in vivo.

Furthermore, a nonhomolog NP system was used in the present study. Given the different degree of species variation between the amino acid sequence of human and ovine NP (minor for ANP and CNP but relatively large for BNP), the absolute vascular responses of, in particular, the BNP study regimes may prove particularly useful in this subgroup of hypertensive patients. Indeed, NPs have the potential to counteract both pathological remodeling and increased vascular tone. Here we show that ANP-NPRA-interaction acutely modulates regional PWV, thereby suggesting that NPR\textsubscript{A} and NPR\textsubscript{C} do not acutely influence distensibility in vivo.

Although PWV is a measure of distensibility, factors that influence 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 of these parameters are responsible for the observed changes in PWV in the present study. However, it would seem unlikely that alterations in vascular resistance in the hindlimb is responsible for the changes in PWV because infusion of NPs via the sheath had no effect.

In conclusion, ANP, and to a lesser degree BNP, play a role in regulating regional large artery distensibility via the NPR\textsubscript{A} receptor. Neither CNP nor cANF altered PWV, suggesting that NPR\textsubscript{A} and NPR\textsubscript{C} do not acutely influence distensibility in vivo.
REFERENCES


