British Journal of Pharmacology (2006) 149, 797–801
 0007–1188/06 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Reciprocal regulation of human soluble and particulate guanylate cyclases *in vivo*

M Madhani[1], M Okorie[2], AJ Hobbs[1] and RJ MacAllister[2]

[1]*Wolfson Institute for Biomedical Research, University College London, London, UK and* [2]*Centre for Clinical Pharmacology, University College London, London, UK*

Background & purpose: We demonstrated previously that reciprocal regulation of soluble (sGC) and particulate (pGC) guanylate cyclases by NO and natriuretic peptides coordinates cyclic cGMP-mediated vasodilatation *in vitro*. Herein, we investigated whether such an interaction contributes to vascular homeostasis in mice and humans *in vivo*.
Experimental approach: Mean arterial blood pressure (MABP) changes in anaesthetized mice were monitored in response to i.v. administration of cGMP- and cAMP-dependent vasodilators in wild-type (WT), endothelial NO synthase (eNOS) and natriuretic peptide receptor (NPR)-A knockout mice. Forearm blood flow (FBF) in response to intra-brachial infusion of ANP (25, 50, 100, 200 pmol min^{-1}) in the absence and presence of the NOS inhibitor N^G-methyl-L-arginine (L-NMA; 4 μmol min^{-1}) and the control constrictor noradrenaline (240 pmol min^{-1}) was assessed in healthy volunteers.
Key results: Sodium nitroprusside (SNP; NO-donor) and atrial natriuretic peptide (ANP) produced dose-dependent reductions in MABP in WT animals that were significantly enhanced in eNOS KO mice. In NPR-A K mice, SNP produced a dose-dependent reduction in MABP that was significantly greater than that in WT mice. Responsiveness to the cAMP-dependent vasodilator epoprostenol was similar in WT, eNOS KO and NPR-A KO animals. ANP caused vasodilatation of the forearm resistance vasculature that was significantly greater in individuals lacking endothelium-derived NO (i.e. L-NMA treated).
Conclusions & implications: These data demonstrate that crosstalk occurs between the NO-sGC and ANP-pGC pathways to regulate cGMP-dependent vasodilatation *in vivo* in both mice and humans. These findings have implications for understanding the link between natriuretic peptide activity and cardiovascular risk.
British Journal of Pharmacology (2006) **149,** 797–801. doi:10.1038/sj.bjp.0706920; published online 3 October 2006

Keywords: nitric oxide; atrial natriuretic peptide; cyclic GMP; vasodilatation; particulate guanylate cyclase; soluble guanylate cyclase; *in vivo*

Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine-3′,5′-monophopshate; eNOS, endothelial nitric oxide synthase; FBF, forearm blood flow; KO, knockout; L-NMA, N^G-methyl-L-arginine; MABP, mean arterial blood pressure; NA, noradrenaline; NO, nitric oxide; pGC, particulate guanylate cyclase; sGC, soluble guanylate cyclase; SNP, sodium nitroprusside; WT, wild-type

Introduction

Natriuretic peptides (NP), including atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) play important roles in cardiovascular homeostasis. ANP and BNP are produced predominantly in the cardiac atria and ventricles, respectively, in response to hypervolaemic states and increase generation of cyclic guanosine-3′,5′-monophosphate (cGMP) to exert their biological activities, including vasodilatation and natriuresis/diuresis (Maack, 1996; Melo *et al*., 1998). Aberrant production or activity of these mediators contributes to cardiovascular disease. This is exemplified in knockout (KO) mice, in which the genes encoding ANP and BNP have been deleted, which exhibit hypertension and cardiac hypertrophy (John *et al*., 1995; Tamura *et al*., 2000). Moreover, plasma levels of BNP have been used for many years in the diagnosis of congestive cardiac failure (Richards *et al*., 2004). Recently, BNP (and N-terminal pro-BNP) has been promoted in cardiovascular risk stratification, with increased BNP associated with two- to fourfold increase in the incidence of myocardial infarction and stroke (Schillinger, 2005). However, the mechanism whereby increased plasma BNP might precipitate complications of atherosclerotic vascular disease is unknown.

In the peripheral vasculature, cGMP-dependent regulation also involves locally generated nitric oxide (NO), released from vascular endothelial cells in response to chemical mediators (e.g. bradykinin) and shear stress. NO and NP induce increases in intracellular cGMP levels via distinct

Correspondence: Dr AJ Hobbs, Wolfson Institute for Biomedical Research, University College London, Cruciform Building, Gower Street, London WC1E 6AE, UK.
E-mail: a.hobbs@ucl.ac.uk
Received 30 June 2006; revised 7 August 2006; accepted 25 August 2006; published online 3 October 2006

isoforms of guanylate cyclase (GC). NO activates the cytoplasmic heterodimeric haemoprotein soluble GC (sGC) (Hobbs, 1997), while ANP activates the membrane-bound protein, particulate GC (pGC) (Winquist *et al*., 1984; Drewett *et al*., 1995). Stimulation of either cyclase results in the conversion of GTP to the intracellular second messenger cGMP, which is responsible for mediating the majority of cardiovascular effects of these mediators.

We have demonstrated previously that the NO–sGC–cGMP system is influenced by the ambient concentration of NO, possibly through a cGMP-dependent process (Hussain *et al*., 1999). In addition, we have reported that cGMP derived from pGC also desensitizes blood vessels to the effects of NO (Hussain *et al*., 2001; Madhani *et al*., 2003). NP-induced downregulation of the vascular NO pathway might explain the observed increase in vascular risk associated with high plasma concentrations of BNP in humans, and possibly contribute to the increased adverse cardiovascular events associated with the BNP-mimetic nesiritide (natrecor) (Sackner-Bernstein *et al*., 2005a, b).

Despite convincing *in vitro* data (Hussain *et al*., 2001; Madhani *et al*., 2003; Sabrane *et al*., 2003), it remains unclear whether the NO and NP systems reciprocally interact in this way *in vivo*. Therefore, in the present study we have employed endothelial nitric oxide synthase (eNOS) and natriuretic peptide receptor (NPR)-A KO mice to model chronic deficiencies in NO- and ANP-mediated signalling to investigate the responsiveness to cGMP- and cAMP-dependent vasodilators *in vivo*. Moreover, we have undertaken translational studies in healthy volunteers to demonstrate whether reciprocal regulation of cGMP-mediated vasodilatation occurs in humans *in vivo*.

Methods

Materials

ANP (rat) and sodium nitroprusside (SNP) were purchased from Sigma Chemical Co. (Dorset, UK). Epoprostenol (Flolan) was purchased from GlaxoSmithKline (Middlesex, UK). Human ANP and N^G-methyl-L-arginine (L-NMA) were purchased from CLINALFA (Laufelfingen, Switzerland). NA was purchased from Abbott laboratories Ltd (Queenborough, Kent, UK).

Measurement of mean arterial blood pressure

C57/BL6 (wild-type), eNOS KO (in-house colony) and NPR-A KO mice (kind gift of Oliver Smithies, University of North Carolina (Oliver *et al*., 1997); male, 20–25 g) were anaesthetized with 1.5% isoflourane and placed supine on a thermostatically controlled heating blanket (37°C). To measure systemic mean arterial blood pressure (MABP), the left common carotid artery was isolated and a fluid-filled (heparin; 100 U ml^{-1} diluted in 0.9% saline) 0.28-mm internal diameter cannula (Critchely Electrical Products Pty Ltd, Auburn, Australia) introduced into the artery. The right femoral vein was cannulated for drug administration. After a 10-min period of stabilization, mice were given 50 μl intravenous (i.v.) bolus injections of either ANP (0.1, 1.0, 10.0 and 100.0 μg kg^{-1}) or SNP (0.1, 0.3, 1.0, 3.0 and 10 μg kg^{-1}). To determine if changes in MABP in response to SNP and ANP were cGMP-specific, the cAMP-dependent vasodilator epoprostenol was administered (0.03, 0.1, 0.3, 1.0, 3.0 and 10 μg kg^{-1}; 50 μl i.v. bolus injections). Sequential doses of ANP, SNP or epoprostenol were given 5–10 min apart to allow restoration of baseline blood pressure between doses. MABP was measured using a P23 XL transducer (Viggo-Spectramed, Oxnard, CA, USA) and recorded onto a pre-calibrated PowerLab system (ADInstuments, Castle Hill, New South Wales, Australia).

Forearm blood flow measurements

Studies were approved by the local University College London Hospital (UCLH) research ethics committee. Thirteen healthy male volunteers taking no medication were recruited (age 19–40 years) and asked to refrain from any caffeine-containing substances 12 h before each study. All studies were performed in a temperature-controlled laboratory (24–26°C). A 27-gauge stainless-steel needle (Cooper's Needle Works, Birmingham, UK) was inserted into the brachial artery of the non-dominant arm under local anaesthesia (2% lignocaine). Drugs were dissolved in 0.9% sodium chloride solution and were infused at 0.5 ml min^{-1}. Forearm blood flow (FBF) was recorded in both arms using venous occlusion plethysmography as described previously (Chan *et al*., 2001). During recording periods, the hands were excluded from the circulation by inflation of wrist cuffs to 200 mm Hg. Measurement of basal blood flow was made over a 15-min period, after which blood flow responses to ANP (25, 50, 100, 200 pmol min^{-1}, each dose for 5 min) were recorded. After allowing restoration of baseline blood flow (saline infusion for 15 min), the response to ANP was repeated to test for tachyphylaxis. On a separate day, the effect of ANP was determined in the presence of L-NMA (4 μmol min^{-1} pre-infused for 15 min and co-infused with ANP). To control for the action of L-NMA to increase baseline forearm vascular resistance, in a further series of experiments, responses to ANP were assessed in the presence of noradrenaline (NA; 240 pmol min^{-1} pre-infused for 15 min and co-infused with ANP).

Statistical analyses

Data shown are mean ± s.e.m. Changes in MABP in responses to SNP, ANP and epoprostenol were calculated as the maximum change in blood pressure minus the responses to bolus injections of saline (50 μl). Data were analysed by two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. A P-value of <0.05 was taken as an appropriate level of significance.

In FBF studies, the ratio of flow in the infused/non-infused (control) arm was calculated for each measurement period. Vasodilatation was expressed as the percentage increase in the ratio of FBF (infused/non-infused arm) relative to the immediately preceding baseline flow, as described previously (Chan *et al*., 2001). The area under the dose–response curve (AUC) was calculated to provide a summary measure of drug response for comparison between studies. AUC values (expressed in arbitrary units) were compared by one-way ANOVA followed by Bonferroni's multiple comparison test.

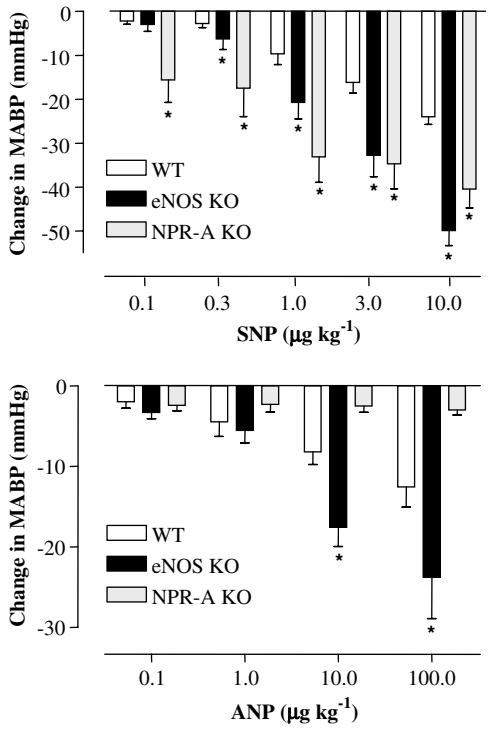

Figure 1 Mean changes in systemic blood pressure following i.v. administration of SNP (upper panel) and ANP (lower panel) in WT, eNOS and NRA-KO mice ($n \geqslant 5$; *$P < 0.05$ significantly greater than WT). Data are expressed as mean ± s.e.m.

Results

Effect of SNP and ANP on haemodynamic responses in mice

SNP decreased MABP in a dose-dependent manner in wild-type (WT), eNOS KO and NPR-A KO animals. However, the fall in MABP in WT mice was smaller than that observed in both KO strains (Figure 1). In eNOS KO mice, ANP caused a larger dose-dependent decrease in MABP than in WT animals (Figure 1). ANP did not cause a significant change in MABP in NPR-A KO mice (Figure 1).

Effect of epoprostenol on MABP in WT and eNOS KO mice

To ascertain whether changes in MABP in response to SNP and ANP were cGMP-specific, the vasodilator activity of epoprostenol (a cAMP-dependent vasodilator) was measured in WT and eNOS KO animals. Epoprostenol produced dose-dependent decreases in MABP in both eNOS KO and WT mice. However, the magnitude of epoprostenol-induced decreases in MABP was not significantly different between the two strains (Figure 2).

Effects of L-NMA and NA on changes in FBF in responses to ANP

Intra-brachial infusions of ANP induced dose-dependent increases in FBF; on repeat infusion, there was a diminution of the response to ANP, consistent with tachyphylaxis (Figure 3). NA (23.5 ± 4.7%) and L-NMA (21.7 ± 6.4%) caused similar reductions in baseline blood flow ($P > 0.05$; $n \geqslant 5$). The effect of baseline vasoconstriction by NA was manifest

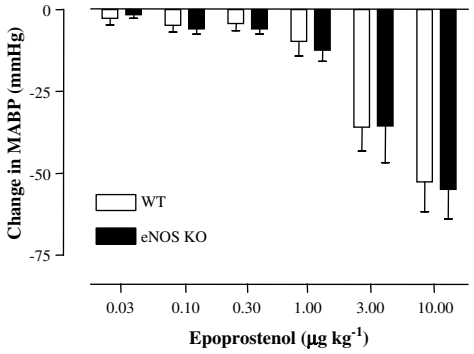

Figure 2 Mean changes in systemic blood pressure following i.v. administration of epoprostenol in eNOS WT and KO mice. Data are expressed as mean ± s.e.m. ($n \geqslant 5$).

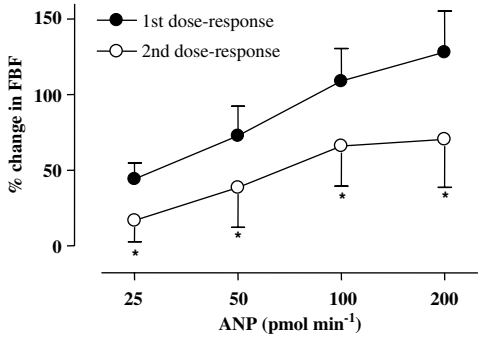

Figure 3 Percentage change in FBF during cumulative administration of ANP in two sequential dose–response curves; initial curve (closed circles) and 15 min later (open circles). *$P < 0.05$ vs control (1st dose–response curve). Data are expressed as mean ± s.e.m. ($n \geqslant 5$).

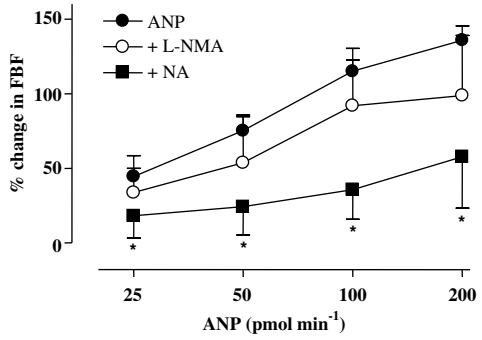

Figure 4 Percentage change in FBF during cumulative administration of ANP in the absence (closed circles) and presence (open circles) of L-NMA or NA (closed squares). *$P < 0.05$ vs ANP alone and ANP plus L-NMA. Data are expressed as mean ± s.e.m. ($n \geqslant 5$).

by a reduction in the response to ANP (AUC = 17697 ± 893.6 for control vs 7351 ± 2020 for L-NMA; $P < 0.05$; $n \geqslant 5$; Figure 4). In contrast, with L-NMA the effect of ANP was not significantly different from control (AUC = 12345 ± 3129; $P > 0.05$; $n \geqslant 5$; Figure 4).

Discussion

This study demonstrates that reciprocal regulation of vascular tone by sGC and pGC is evident in mice and

humans *in vivo*. In eNOS KO mice, a model for chronic NO shortage, the potency of SNP and ANP was significantly increased when compared to WT animals. In addition, during chronic deficiency of ANP-mediated signalling, as occurs in NPR-A KO mice, the potency of SNP (i.e. NO) was significantly increased compared to WT controls. Epoprostenol (a cAMP-dependent vasodilator) caused similar haemodynamic changes in both the WT and eNOS KO animals, suggesting that the reciprocal regulation of vascular tone is specific to cGMP-generating systems. In healthy human volunteers, ANP caused a dose-dependent increase in FBF; when altered baseline tone was taken into account, inhibition of endogenous NO synthesis by L-NMA significantly enhanced the dilator effect of ANP.

We recently demonstrated that NO and ANP cooperatively regulate cGMP levels in conduit and resistance arteries *in vitro* (Madhani *et al*., 2003). To determine whether the studies with isolated arteries reflected *in vivo* effects on blood pressure, we assessed the sensitivity of the sGC and pGC pathways on MABP in anaesthetized mice. In this series of experiments, MABP changes in response to the NO donor, SNP and ANP were compared in control WT, eNOS KO and NPR-A KO mice. SNP decreased the blood pressure in WT animals in a dose-dependent manner. However, in eNOS KO mice, responses to NPR-A activation (i.e. ANP administration) and sGC stimulation (i.e. SNP administration) were both enhanced significantly, intimating that loss of endothelium-derived NO results in upregulation of the sensitivity of both sGC and pGC *in vivo*. These data are in agreement with *in vitro* studies showing a chronic alteration in endothelial NO production in human and murine isolated arteries results in changes in the vasorelaxant responses to ANP (Hussain *et al*., 2001; Madhani *et al*., 2003), and observations made in smooth muscle-specific NPR-A KO mice, which exhibit an increased sensitivity to NO donors (Sabrane *et al*., 2003).

Studies in NPR-A KO animals confirmed that the two cGMP-generating pathways are linked in a reciprocal manner *in vivo*. As expected, ANP did not cause a fall in MABP in NPR-A KO mice. However, decreases in MABP in response to SNP were significantly greater in NPR-A KO animals when compared to WT littermates. To ascertain if the heightened vascular sensitivity in eNOS and NPR-A KO mice was specific for cGMP-dependent pathways, we assessed the hypotensive effects of the cAMP-dependent vasodilator, epoprostenol. MABP was reduced in a dose-dependent manner by epoprostenol with an identical potency in WT and eNOS KO mice, confirming that the sensitivity of the adenylate cyclase-cAMP signalling cascade is not altered by changes in ambient NO. These observations define clearly a reciprocal regulation of cGMP-mediated responses in the mammalian vasculature *in vivo*, with deficiencies in either NO- or NP-mediated signalling being compensated for by the alternate cGMP-generating pathway.

The effect of NO deficiency on the response to ANP in humans *in vivo* was studied using the NOS inhibitor L-NMA. Infusion of constrictors in the human forearm can reduce dilator responses through functional antagonism (Barba *et al*., 1999). To take this into account in the current study, we compared ANP-dependent vasodilatation in the presence of equi-effective constrictor doses of L-NMA and NA. Indeed, when NA was used as a constrictor, there was a substantial reduction in the sensitivity to ANP in the human forearm, as a result of functional antagonism. In contrast, in the presence of L-NMA, the increase in FBF in response to ANP was not significantly different from that obtained under control conditions, and greater than responses in the presence of NA. These observations suggest that following loss of endothelium-derived NO (i.e. in the presence of L-NMA), the sensitivity of NPR-A/pGC is increased. The dose of L-NMA used in this study (4 μmol min^{-1}) is close to the top of the dose–response curve for L-NMA in the forearm ($\sim EC_{80}$) (Vallance *et al*., 1989; Dawes *et al*., 2001); a larger dose of L-NMA (e.g. 16 μmol min^{-1}), causing maximal constriction in the forearm (Dawes *et al*., 2001), is likely to accentuate the hypersensitivity to ANP. These observations are consistent with our previous finding that, in isolated human vessels, the responses to ANP were enhanced by NOS inhibition (Hussain *et al*., 2001). In concert, these data highlight a reciprocal interaction of cGMP-dependent signalling in the human vasculature.

Our results can be contrasted with previous reports demonstrating that responses to ANP are attenuated by L-NMA, suggesting that, at least in part, ANP-mediated relaxations are dependent on endothelial NO release (Brunner and Wolkart, 2001; Sugamori *et al*., 2002). However, the idea that ANP releases NO to produce a vasodilator response remains controversial, given its well-characterized action to directly generate cGMP. Our data suggest alternative explanations for prior observation in animals and humans. Firstly, functional antagonism produced by inhibition of eNOS will complicate the interpretation of studies that do not include a non-NO-dependent vasoconstrictor (Sugamori *et al*., 2002). Secondly, we observed that sequential administration of ANP causes tachyphylaxis that could explain reduced ANP responses when using this experimental design (Brunner and Wolkart, 2001; Sugamori *et al*., 2002). This observation is in line with the well-characterized dephosphorylation and desensitization of NPR-A (most likely by protein phosphatase 2A) (Potter and Garbers, 1992). Repeating ANP response curves on separate days minimizes the confounding caused by tachyphylaxis.

Heterologous regulation of the cyclase pathways provides a physiological mechanism to integrate the regulation of cGMP-dependent vascular tone, linking the paracrine and endocrine functions of NO and natriuretic peptides (i.e. soluble and pGCs). Moreover, it is possible that such integration provides a means to compensate for dysfunction of either cGMP-dependent pathway. For instance, endothelial dysfunction characterized by reduced activity of NO-sGC signalling occurs in patients with cardiovascular risk factors and established atherosclerosis (Cai and Harrison, 2000). Here, reduced sGC activity might be compensated for by increased pGC-derived cGMP (i.e. natriuretic peptide activity). Conversely, increased activity of the pGC-cGMP pathway that occurs in diseases such as congestive heart failure (as a result of increased plasma natriuretic peptide levels) (Nakaoka *et al*., 1988) might downregulate NO-sGC signalling, promoting vasoconstriction associated with heart failure. Whether natriuretic peptide-induced reduction in

NO bioactivity provides a mechanism for the positive association between plasma BNP (which like ANP relies exclusively on NPR-A activation for cardiovascular actions) and cardiovascular risk remains to be determined (Tsutamoto *et al*., 1997; Wang *et al*., 2004). Therapeutically, our data suggest that not only will NPR-A activation be associated with tachyphylaxis of the pGC system, but will also extend to sGC-dependent pathways. Therefore, reciprocal interaction between sGC and pGC might contribute to tachyphylaxis during chronic administration of natriuretic peptides (Tsutamoto *et al*., 1992, 1993; Komarek *et al*., 2004), neutral endopeptidase inhibitors (Corti *et al*., 2001) and organic nitrates (Munzel *et al*., 1996; Parker and Parker, 1998).

Acknowledgements

This work was supported by The Wellcome Trust and the British Heart Foundation. AJH is the recipient of a Wellcome Trust Senior Research Fellowship.

Conflict of interest

The authors state no conflict of interest.

References

Barba G, Mullen MJ, Donald A, MacAllister RJ (1999). Determinants of the response of human blood vessels to nitric oxide donors *in vivo*. *J Pharmacol Exp Ther* **289**: 1662–1668.

Brunner F, Wolkart G (2001). Endothelial NO/cGMP system contributes to natriuretic peptide-mediated coronary and peripheral vasodilation. *Microvasc Res* **61**: 102–110.

Cai H, Harrison DG (2000). Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* **87**: 840–844.

Chan NN, MacAllister RJ, Colhoun HM, Vallance P, Hingorani AD (2001). Changes in endothelium-dependent vasodilatation and alpha-adrenergic responses in resistance vessels during the menstrual cycle in healthy women. *J Clin Endocrinol Metab* **86**: 2499–2504.

Corti R, Burnett Jr JC, Rouleau JL, Ruschitzka F, Luscher TF (2001). Vasopeptidase inhibitors: a new therapeutic concept in cardiovascular disease? *Circulation* **104**: 1856–1862.

Dawes M, Chowienczyk PJ, Ritter JM (2001). Quantitative aspects of the inhibition by *N*(*G*)-monomethyl-L-arginine of responses to endothelium-dependent vasodilators in human forearm vasculature. *Br J Pharmacol* **134**: 939–944.

Drewett JG, Fendly BM, Garbers DL, Lowe DG (1995). Natriuretic peptide receptor-B (guanylyl cyclase-B) mediates C-type natriuretic peptide relaxation of precontracted rat aorta. *J Biol Chem* **270**: 4668–4674.

Hobbs AJ (1997). Soluble guanylate cyclase: the forgotten sibling. *Trends Pharmacol Sci* **18**: 484–491.

Hussain MB, Hobbs AJ, MacAllister RJ (1999). Autoregulation of nitric oxide-soluble guanylate cyclase-cyclic GMP signalling in mouse thoracic aorta. *Br J Pharmacol* **128**: 1082–1088.

Hussain MB, MacAllister RJ, Hobbs AJ (2001). Reciprocal regulation of cGMP-mediated vasorelaxation by soluble and particulate guanylate cyclases. *Am J Physiol Heart Circ Physiol* **280**: H1151–H1159.

John SW, Krege JH, Oliver PM, Hagaman JR, Hodgin JB, Pang SC *et al*. (1995). Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science* **267**: 679–681.

Komarek M, Bernheim A, Schindler R, Steden R, Kiowski W, Brunner-La Rocca HP (2004). Vascular effects of natriuretic peptides in healthy men. *J Cardiovasc Pharmacol Ther* **9**: 263–270.

Maack T (1996). Role of atrial natriuretic factor in volume control. *Kidney Int* **49**: 1732–1737.

Madhani M, Scotland RS, MacAllister RJ, Hobbs AJ (2003). Vascular natriuretic peptide receptor-linked particulate guanylate cyclases are modulated by nitric oxide-cyclic GMP signalling. *Br J Pharmacol* **139**: 1289–1296.

Melo LG, Veress AT, Ackermann U, Sonnenberg H (1998). Chronic regulation of arterial blood pressure by ANP: role of endogenous vasoactive endothelial factors. *Am J Physiol* **275**: H1826–H1833.

Munzel T, Kurz S, Heitzer T, Harrison DG (1996). New insights into mechanisms underlying nitrate tolerance. *Am J Cardiol* **77**: 24C–30C.

Nakaoka H, Imataka K, Kitahara Y, Fujii J, Ishibashi M, Yamaji T (1988). Relationship between plasma levels of atrial natriuretic peptide and cyclic guanosine monophosphate in patients with heart diseases. *Jpn Circ J* **52**: 30–34.

Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL *et al*. (1997). Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci USA* **94**: 14730–14735.

Parker JD, Parker JO (1998). Nitrate therapy for stable angina pectoris. *N Engl J Med* **338**: 520–531.

Potter LR, Garbers DL (1992). Dephosphorylation of the guanylyl cyclase-A receptor causes desensitization. *J Biol Chem* **267**: 14531–14534.

Richards AM, Lainchbury JG, Troughton RW, Espiner EA, Nicholls MG (2004). Clinical applications of B-type natriuretic peptides. *Trends Endocrinol Metab* **15**: 170–174.

Sabrane K, Gambaryan S, Brandes RP, Holtwick R, Voss M, Kuhn M (2003). Increased sensitivity to endothelial nitric oxide (NO) contributes to arterial normotension in mice with vascular smooth muscle-selective deletion of the atrial natriuretic peptide (ANP) receptor. *J Biol Chem* **278**: 17963–17968.

Sackner-Bernstein JD, Kowalski M, Fox M, Aaronson K (2005a). Short-term risk of death after treatment with nesiritide for decompensated heart failure: a pooled analysis of randomized controlled trials. *JAMA* **293**: 1900–1905.

Sackner-Bernstein JD, Skopicki HA, Aaronson KD (2005b). Risk of worsening renal function with nesiritide in patients with acutely decompensated heart failure. *Circulation* **111**: 1487–1491.

Schillinger M (2005). Cardiovascular risk stratification in older patients: role of brain natriuretic peptide, C-reactive protein, and urinary albumin levels. *JAMA* **293**: 1667–1669.

Sugamori T, Ishibashi Y, Shimada T, Sakane T, Takahashi N, Ohata S *et al*. (2002). Nitric oxide-mediated vasodilatory effect of atrial natriuretic peptide in forearm vessels of healthy humans. *Clin Exp Pharmacol Physiol* **29**: 92–97.

Tamura N, Ogawa Y, Chusho H, Nakamura K, Nakao K, Suda M *et al*. (2000). Cardiac fibrosis in mice lacking brain natriuretic peptide. *Proc Natl Acad Sci USA* **97**: 4239–4244.

Tsutamoto T, Kanamori T, Morigami N, Sugimoto Y, Yamaoka O, Kinoshita M (1993). Possibility of downregulation of atrial natriuretic peptide receptor coupled to guanylate cyclase in peripheral vascular beds of patients with chronic severe heart failure. *Circulation* **87**: 70–75.

Tsutamoto T, Kanamori T, Wada A, Miyauchi N, Murata T, Kinoshita M (1992). Limitations of compensation by endogenous atrial natriuretic peptide in heart failure. *Jpn Circ J* **56**: 489–493.

Tsutamoto T, Wada A, Maeda K, Hisanaga T, Maeda Y, Fukai D *et al*. (1997). Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. *Circulation* **96**: 509–516.

Vallance P, Collier J, Moncada S (1989). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* **2**: 997–1000.

Wang TJ, Larson MG, Levy D, Benjamin EJ, Leip EP, Omland T *et al*. (2004). Plasma natriuretic peptide levels and the risk of cardiovascular events and death. *N Engl J Med* **350**: 655–663.

Winquist RJ, Faison EP, Waldman SA, Schwartz K, Murad F, Rapoport RM (1984). Atrial natriuretic factor elicits an endothelium-independent relaxation and activates particulate guanylate cyclase in vascular smooth muscle. *Proc Natl Acad Sci USA* **81**: 7661–7664.