Contribution of Kv7 Channels to Natriuretic Peptide Mediated Vasodilation in Normal and Hypertensive Rats

Jennifer B. Stott, Vincenzo Barrese, Thomas A. Jepps, Emma V. Leighton, Iain A. Greenwood

Abstract—The Kv7 family of voltage-gated potassium channels are expressed within the vasculature where they are key regulators of vascular tone and mediate cAMP-linked endogenous vasodilator responses, a pathway that is compromised in hypertension. However, the role of Kv7 channels in non–cAMP-linked vasodilator pathways has not been investigated. Natriuretic peptides are potent vasodilators, which operate primarily through the activation of a cGMP-dependent signaling pathway. This study investigated the putative role of Kv7 channels in natriuretic peptide–dependent relaxations in the vasculature of normal and hypertensive animals. Relaxant responses of rat aorta to both atrial and C-type natriuretic peptides and the nitric oxide donor sodium nitroprusside were impaired by the Kv7 blocker linopirdine (10 μmol/L) but not by the Kv7.1-specific blocker HMR1556 (10 μmol/L) and other K+ channel blockers. In contrast, only the atrial natriuretic peptide response was sensitive to linopirdine in the renal artery. These Kv7-mediated responses were attenuated in arteries from hypertensive rats. Quantitative polymerase chain reaction showed that A- and B-type natriuretic peptide receptors were expressed at high levels in the aorta and renal artery from normal and spontaneously hypertensive rats. This study provides the first evidence that natriuretic peptide responses are impaired in hypertension and that recruitment of Kv7 channels is a key component of natriuretic peptide–dependent vasodilations. (Hypertension. 2015;65:676-682.

Key Words: cyclic GMP ■ KCNQ potassium channels ■ muscle, smooth, vascular ■ natriuretic peptides

The guanylate cyclase cyclic GMP (cGMP) signaling cascade is a key vasodilator pathway involved in regulating vascular smooth muscle tone.1 In vascular smooth muscle, an increase in cGMP is produced by 2 primary mechanisms: activation of soluble guanylate cyclase by agents including nitric oxide (NO) or activation of guanylate cyclase–linked transmembrane natriuretic peptide receptors (NPR-A and NPR-B), which are stimulated endogenously by atrial, B-type, or C-type natriuretic peptides (ANP, BNP, or CNP, respectively).2 NPR-A is preferentially activated by ANP>>BNP>>CNP, whereas NPR-B has a rank order of potency of CNP>>ANP>>BNP.3,4 ANP and BNP are potent vasodilators synthesized predominantly in cardiac tissues, and raised levels are associated with heart failure and myocardial infarction.4 CNP, like ANP and BNP, is formed as a propeptide but far lower levels circulate compared with the other 2 natriuretic peptides. However, CNP is released by the endothelium, which has led to speculation that CNP may act in a localized manner to contribute to vasodilations on a regional basis.5

Despite their pronounced effects in the vasculature, the effect of natriuretic peptides in hypertension and the mechanisms underlying regional vasodilatation remain unknown. Recently, KCNQ-encoded potassium channels (termed Kv7.1–7.5) have been identified as important regulators of vascular tone.5 In the vasculature Kv7.1, 7.4 and 7.5 isoforms are consistently expressed with little or no contribution from Kv7.2 or Kv7.3,6,7 and the dominant molecular species in cerebral and mesenteric arteries is a Kv7.4/7.5 heterotetramer.8,9 Blockade of Kv7 channels results in the contraction of vessels at rest, whereas Kv7 activators relax precontracted arterial vessels or inhibit vasoconstrictions.2,10,11 A reduction in Kv7.4 protein has been described in hypertensive models, where Kv7-dependent relaxations are attenuated.12,13 Kv7 channels are also a key end point of endogenous vasodilator responses mediated by Gs-linked, cAMP-dependent pathways.8,13,15 However, the role of Kv7 channels in mediating the effects of other endogenous vasodilator signaling pathways has yet to be investigated. The aim of this study was to determine whether cGMP-dependent vasorelaxations produced by ANP and CNP, as well as the NO donor sodium nitroprusside (SNP), were affected by Kv7 channel blockade or impaired in arteries from hypertensive rats, where Kv7.4 abundance is reduced.

Methods

Experimental protocols are available in the online-only Data Supplement. All experiments were performed in accordance with the UK Animal (Scientific Procedures) Act 1986.
Results

Kv7 Channels Contribute to Aortic and Renal cGMP-Dependent Vasodilations

Cumulative application of ANP and CNP to segments of aorta precontracted with 3 μmol/L methoxamine produced concentration-dependent relaxations (Figure 1A and 1B, i and ii). Responses to both natriuretic peptides were attenuated markedly by previous incubation with linopirdine (10 μmol/L), which is a selective Kv7 blocker without discrimination for individual isoforms. SNP also produced concentration-dependent relaxations of rat aortae that were impaired in the presence of linopirdine (Figure 1B, iii). Similar to the aorta, relaxations to ANP in the renal artery were significantly inhibited in the presence of 10-μmol/L linopirdine. However, neither CNP- nor SNP-mediated relaxations were affected by linopirdine (P=0.77 and P=0.88 for CNP and SNP, respectively; Figure 1C). Because 10-μmol/L linopirdine produced contractions at rest in both the aorta and the renal artery (Table S1 in the online-only Data Supplement), the inhibition of responses may be because of functional antagonism of the vessels. Therefore, we performed further experiments using 3-μmol/L linopirdine, which is sufficient to block Kv7 channels but does not produce as robust as constriction of the vessel (1.5±0.6 versus 4.4±0.8 mN in aorta; 0.3±0.2 versus 1.9±0.5 mN in renal artery; 3 versus 10 μmol/L). Vasodilations that were inhibited by 10-μmol/L linopirdine were similarly attenuated by 3-μmol/L linopirdine (ie, ANP and CNP in aorta and ANP in renal artery; Figure S3). In contrast, no impairment in the response to ANP in the aorta was observed in the presence of a Kv7.1-specific blocker (HMR 1556; 10 μmol/L; P=0.95), a nonselective blocker of Kv channels (4 aminopyridine; 1 mmol/L; P=0.46), a K<sub>ATP</sub> channel blocker (glibenclamide; 10 μmol/L; P=0.74), a BK<sub>Ca</sub> channel blocker (paxilline; 1 μmol/L; P=0.74), or a K<sub>IR</sub> channel blocker (tertiapin Q; 1 μmol/L; P=0.98; Figure 2). Similar effects were seen for CNP responses in the aorta (Figure S4) and for ANP in the renal artery (Figure S5). These data suggest that relaxations evoked by natriuretic peptides are mediated by stimulation of Kv7.4/ Kv7.5 channels.

Further experiments investigated the signaling pathways underlying with Kv7-mediated relaxations. A competitive inhibitor of cGMP signaling (rp-8-Br-PET-cGMP; 3 μmol/L) produced a significant inhibition of the relaxant responses in the aorta to ANP, CNP, and SNP (Figure 3A–3C). The relaxant response to ANP in the renal artery was also inhibited significantly in the presence of rp-8-Br-PET-cGMP (Figure 3D), suggesting a role for cGMP signaling in these responses. To confirm the role of this pathway further, we used 10-μmol/L ODQ to inhibit the soluble guanylate cyclase pathway, which completely inhibited the effect of SNP in the aorta (Figure S6). Because cross talk exists between the cGMP and the cAMP signaling pathways, and the latter is already implicated in Kv7 channel activation, natriuretic peptide relaxant responses were further tested in the presence of 1-μmol/L H-89 (a protein kinase A inhibitor) but had no effect on these relaxations (P=0.92 ANP aorta, P=0.99 CNP aorta, and P=0.96 for ANP renal artery; Figure S7), while producing an impairment of 1-μmol/L forskolin responses in aorta and in renal artery (reduced relaxation by 22.6±6.3% and 17.2±5.4%, respectively; n=4). Likewise, endothelium denudation had no effect on these responses (P=0.75 and P=0.19 for ANP and CNP in aorta, respectively, and P=0.74 for ANP in renal artery).

Results

Kv7 Channels Contribute to cGMP Vasodilations
confirming that the natriuretic peptides work directly on the smooth muscle (Figure S8).

cGMP Activation of Kv7.4 Currents

There are no data on the effect of cGMP on Kv7 channels. Because Kv7.4 is dominantly expressed and has been implicated in dilatory responses to various endogenous molecules, we established whether application of cGMP enhanced K+ currents in human embryonic kidney cells stably expressing Kv7.4. Whole cell electrophysiological recordings showed that in these cells K+ currents were significantly increased after stimulation with cGMP, without a change in activation or deactivation kinetics, which was completely abolished by subsequent application of linopirdine (Figure 4) or preincubation with this agent (n=4, data not shown).

Kv7-Dependent cGMP-Mediated Relaxations Are Compromised in Spontaneously Hypertensive Rat

Previous studies have shown that relaxations to Kv7 activators or to Kv7-linked Gs-coupled receptor agonists were compromised in aorta and renal arteries from spontaneously hypertensive rats (SHR), which is associated with a reduction in Kv7.4 protein.12,13 As cGMP signaling is vital to the normal functioning of the vasculature, dysfunction of any stage of this pathway can lead to cardiovascular disease. Therefore, we determined whether Kv7-dependent, cGMP-linked responses were attenuated in arteries

![Figure 2](image1.png)

**Figure 2.** Blockade of other potassium channels did not affect Kv7-dependent responses. Relaxations to ANP in the aorta in the absence (solid line) or presence (dashed line) of HMR1556 (A), glibenclamide (B), paxilline (C), and tertiapin Q (D). Each point is the mean of 4 to 6 animals±SEM. A Bonferroni post hoc test was performed after a 2-way ANOVA. Results were deemed nonsignificant when P>0.05.

![Figure 3](image2.png)

**Figure 3.** Relaxant responses to natriuretic peptides (NPs) and sodium nitroprusside (SNP) are mediated by cGMP. In the presence of 3 μmol/L, RP-8-Br-PET-cGMP (dashed line), concentration effect curves of atrial NP (ANP; A), C-type NP (CNP; B), and SNP (C) in the aorta and to ANP in the renal artery (D) show significant attenuation. Each point is the mean of 6 to 8 animals±SEM. A Bonferroni post hoc test was performed after a 2-way ANOVA, where *P<0.05, **P<0.01, and ***P<0.001. Results were considered nonsignificant when P>0.05.
from SHR. In the aorta from SHRs, the relaxant responses to all 3 agents were compromised significantly (Figure 5B–5D). The ANP response was also significantly attenuated in renal arteries from SHRs (Figure 5E). This was not because of a reduction in NPR expression because quantitative polymerase chain reaction showed a modest increase in NPR-A abundance in arteries from SHRs and no change in NPR-B (Figure 6A). However, a significant reduction of Kv7.4 protein was seen in both the aorta and the renal artery from SHR when compared with NT animals (Figure 6B and 6C).
Discussion and Conclusions

ANP is a potent vasodilator and natriuretic molecule that is raised in patients with hypertension and heart failure. Despite these marked physiological effects and the role in pathophysiological processes, little is known about the mechanisms by which this peptide produces vasodilatation, except for mediating a rise in cGMP levels in the smooth muscle cells after stimulation of NPR-A receptors. Moreover, there is little clear information as to whether ANP-evoked vasodilations are reduced in hypertensive models. The present study shows that ANP-mediated relaxations of aorta and renal arteries were attenuated by an inhibitor of cGMP signaling and the pan-Kv7 blocker linopirdine but not affected by the Kv7.1-specific blocker, HMR1556, or a host of other potassium channel blockers (for BKCa, KATP, or Kir). Previously, recruitment of BKCa channels has been determined to underlie a small (∼20%) component of ANP-mediated relaxations of guinea pig aorta, whereas unspecified Kv channels have been indicated to be involved in these relaxations in rat aorta. Our data suggested that ANP-evoked relaxation of these arteries is driven mainly through the activation of vascular Kv7.4 or Kv7.5. This finding was supported, circumstantially, by the observation that ANP-mediated responses were impaired in arteries from SHRs where Kv7.4 protein is reduced (present study) and that K+ currents generated by the overexpression of Kv7.4 in human embryonic kidney cells were enhanced by cGMP (although the caveat to these latter studies is that Kv7.4 is likely to exist in a heterotetramer with Kv7.5, as shown for cerebral and mesenteric arteries8,9 and may be modulated by different auxiliary subunits). These findings add to growing appreciation that Kv7.4 and 7.5 proteins, probably as 7.4/7.5 heteromers, are involved in these relaxations in rat aorta.17 Our data suggested that ANP-evoked relaxation of these arteries is driven mainly through the activation of vascular Kv7.4 or Kv7.5. This finding was supported, circumstantially, by the observation that ANP-mediated responses were impaired in arteries from SHRs where Kv7.4 protein is reduced (present study) and that K+ currents generated by the overexpression of Kv7.4 in human embryonic kidney cells were enhanced by cGMP (although the caveat to these latter studies is that Kv7.4 is likely to exist in a heterotetramer with Kv7.5, as shown for cerebral and mesenteric arteries8,9 and may be modulated by different auxiliary subunits). These findings add to growing appreciation that Kv7.4 and 7.5 proteins, probably as 7.4/7.5 heteromers, are key functional end points for several endogenous vasodilators, including β-adrenoceptor agonists (renal artery), calcitonin-gene–related peptide (cerebral artery), and adenosine (coronary artery). The downregulation of Kv7.4 in arteries from SHRs4,8,12,14 (present study) provides a mechanism to explain impaired relaxations by natriuretic peptides in aorta and renal arteries from SHRs, where levels of NPR-A and NPR-B actually increase. Indeed, the increase in receptor expression may be a compensatory mechanism to try and overcome the deficiencies in this signaling pathway.

CNP activates guanylate cyclase–linked NPR-B receptors and NPR-C, commonly considered a clearance receptor. It is stored in endothelial cells and produces membrane hyperpolarization of vascular smooth muscle in many beds leading to it being proposed as a candidate endothelial-derived hyperpolarizing factor18 although the role as a global diffusible hyperpolarizing factor has largely been refuted because many of the criteria for an endothelial-derived hyperpolarizing factor are not met by CNP.19,20 CNP stimulates NPR-B to increase cellular cGMP, which produces membrane hyperpolarization and relaxations that are mainly sensitive to blockers of BKCa,21 with some contribution from glibenclamide-sensitive Kir3.1 channels (eg, guinea pig carotid arteries).21 CNP also relaxes rat mesenteric arteries via NPR-C and the subsequent stimulation of tertiapin Q-sensitive Kir3.1 to 3.4 channels by βγ G proteins although we failed to obtain relaxant responses to CNP or ANP in this vessel (Figure S9), even though acetylcholine produced large, rapid responses. The reason for this discrepancy is not obvious. In the present study, blockers of BKCa, KATP and 3.1 to 3.4 had little or no effect on CNP-mediated relaxations of rat aorta, whereas linopirdine produced a marked impairment in response. Similar to CNP relaxation of guinea pig aorta,16 these relaxations were attenuated by a cGMP signaling inhibitor, suggesting that...
these effects were mediated via NPR-B receptor activation. Intriguingly, CNP-induced relaxation of the renal artery was not sensitive to linopirdine unlike those produced by ANP. This may represent the presence of localized intracellular signaling microdomains within vascular smooth muscle, which act to regulate endogenous vasodilator responses finely. The logical corollary to this is that NPR-A, but not NPR-B, is located close to Kv7 channels in the renal artery myocytes, which would account for the differences in Kv7 sensitivity of ANP and CNP responses.

NO donors like SNP have previously been shown to stimulate BKCa channels to produce arterial relaxation. The present study reveals that in the rat aorta, SNP-induced relaxations were prevented by previous incubation with linopirdine supporting a coupling between cGMP and Kv7 channels in this tissue. However, linopirdine failed to affect SNP-dependent relaxations in the renal artery similar to CNP responses. It is noteworthy that SNP responses are less sensitive to the cGMP inhibitor rp-8-Br-PET-cGMP, so it is possible that the large, nonlocalized, rise in cGMP produced by SNP would activate many diverse signaling pathways and overwhelm the Kv7 response. The colocalization of various ion channels to natriuretic receptors and cGMP signaling could be a defining feature of different vascular beds, and further experiments are needed to understand the complexities of cGMP and ion channel coupling throughout the vasculature.

In conclusion, we propose that Kv7 channels display artery-specific contributions to cGMP-dependent vasodilations, and the impaired cGMP-linked vasorelaxations in the SHR may be because of reduced levels of Kv7.4 in the vasculature. These findings emphasize the importance of Kv7 channels in the maintenance of vascular tone and mediating endogenous vasodilator responses.

Perspectives

Hypertension is an epidemic affecting >25% of the world’s population and is a major risk factor in the development of stroke and cardiovascular disease. Natriuretic peptides are potent vasodilators, and aberrations in circulating levels are noted in hypertension and heart failure. However, the molecular mechanisms involved in mediating natriuretic peptide vasorelaxations and whether their vasorelaxant properties are preserved in hypertension was unclear. This study is the first to identify that natriuretic peptide–induced vasodilations are impaired in hypertension, and that these responses functionally couple to Kv7 channels via a cGMP-linked pathway. These findings further enhance our knowledge of key intracellular signaling pathways that regulate Kv7 channels in the vasculature and also provide further insight into the pathogenesis of hypertension.

Acknowledgments

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Disclosures

None.

References


**Novelty and Significance**

**What Is New?**
- Natriuretic peptide vasorelaxations are compromised in hypertension.
- Natriuretic peptide vasorelaxations are mediated by Kv7 channels.
- cGMP activates Kv7.4 channels.

**What Is Relevant?**
- Natriuretic peptides are potent vasodilators and markers for cardiovascular disease.

**Summary**
- Although the mechanism of natriuretic peptide vasorelaxation remains unknown, we reveal new insights.
Contribution of Kv7 Channels to Natriuretic Peptide Mediated Vasodilation in Normal and Hypertensive Rats

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CONTRIBUTION OF Kv7 CHANNELS TO NATRIURETIC PEPTIDE MEDIATED VASODILATION IN NORMAL AND HYPERTENSIVE RATS

Short title: Kv7 channels contribute to cGMP vasodilations

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Methods

Animals

Experiments were performed using either normotensive (NT) male Wistar rats or spontaneously hypertensive (SHR) male Wistar rats, between 12-15 weeks of age (Charles River UK Ltd) culled by cervical dislocation. All experiments were performed in accordance with the UK Animal (Scientific Procedures) Act 1986.

Myography

Aortae and renal arteries were dissected and cleaned of adherent fat in ice cold Krebs solution containing (in mmol/L): 125 NaCl, 4.6 KCl, 2.5 CaCl₂, 25.4 NaHCO₃, 1 NaH₂PO₄, 0.6 MgSO₄ and 10 Glucose. Segments (~2 mm) were mounted in a myograph (Danish Myo Technology, Aarhus, Denmark) for isometric tension recording. The chambers contained Krebs solution, maintained at 37 °C and aerated with 95 % O₂ / 5 % CO₂. Vessels were normalized to 90% of the diameter at 100mg Hg and challenged with 60mmol/L KCl to assess viability. A dose response curve to methoxamine was performed in NT, SHR and in the presence of 10μmol/L linopirdine to assess differences in vascular reactivity in these conditions, and to determine the concentration of agonist to produce an 80% constriction of the vessel for the relaxation experiments. Whilst the reactivity of the vessels to methoxamine in the presence of linopirdine or in the SHR did differ at lower concentrations, we found that 3μmol/L methoxamine produced approximately 80% constriction in all conditions (Fig. S1). This concentration also produced contractions of all vessels that matched the contraction elicited by 60mmol/L KCl (Fig. S1). Therefore, we ensured that vessels were constricted by the same extent in all conditions. Following pre-constriction with 3μmol/L methoxamine, responses to ANP (100pmol/L-30nmol/L), CNP (1-300nmol/L) or SNP (1nmol/L-1μmol/L) were assessed in the presence or absence of linopirdine (3 and 10μmol/L), rp-8-Br-PET-cGMP (3μmol/L), HMR1556 (10μmol/L), 4-aminopyridine (1mmol/L), glibenclamide (10μmol/L), paxilline (1μmol/L), tertiapin Q (1μmol/L ), ODQ (10μmol/L ) H-89 dihydrochloride hydrate (1μmol/L) or vehicle control. The effects of these agents on the basal tone of vessels can be found in Table S1.

Electrophysiology

Whole cell patch clamp experiments were performed using HEK293 cells stably expressing Kv7.4. Cells were maintained in Modified Eagle’s Medium supplemented with 10% foetal bovine serum, 1% non-essential amino acids, 1% L-glutamine, 1% sodium pyruvate and 1% penicillin/streptomycin in a 37°C incubator with 5% CO₂. On the day of experiments, cells were dislodged by brief trypsin treatment and plated onto glass coverslips for at least 30 mins to allow for adherence before experiments were performed. The external media was exchanged for a solution containing (in mmol/L): 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂ and 10 HEPES. Patch pipettes with a resistance of 4-10 MΩ were filled with an internal solution containing (in mmol/L): 110 K⁺ gluconate, 30 KCl, 0.5 MgCl₂, 5 HEPES and 0.1 EGTA. Data was recorded and analysed using pClamp 9.0 software (Axon Instruments, Sunnyvale, CA, USA). Cells were held at a resting potential of -50mV and current amplitude was monitored continuously by applying a test pulse to 20mV every 20s. To generate current-voltage relationships cells were held at a potential of -50mV and tested with a voltage step protocol, stepping the voltage by increments of 10mV from -70 to 20mV at 15s intervals.
**Western Blot**

Western blot experiments were performed as previously described\(^\text{13}\). Briefly, renal artery and aorta from normotensive and spontaneously hypertensive rats were homogenized in ice cold lysis buffer (mmol/L: 20 Tris Base, 137 NaCl, 2 EDTA, 1% nonidet P-40, 10% Glycerol, pH 8, and 10 \(\mu\)L/mL protease inhibitor cocktail; Sigma-Aldrich, U.K.), incubated on ice for 15 min and centrifuged (15 min, 4 °C, 1000 rpm). 20 \(\mu\)g per lane of total protein were loaded onto SDS-PAGE gels (4% to 12% Bis-Tris; Invitrogen, U.K.), subjected to electrophoresis and transferred onto a PVDF-membrane (GE healthcare, UK). Membranes were then blocked in 5% milk powder in PBS-Tween 20 (0.1%; PBS-T) for 1h at room temperature and incubated overnight at 4°C with an anti-K\(_\text{v7.4}\) antibody (sc-5041, Santa Cruz Biotechnology, USA; dilution 1:200). Antibody specificity was tested using protein lysates from HEK293 cells transfected with K\(_\text{v7.4}\) and non-transfected controls, which showed one clear band at 77kD in the transfected cells only (n=5, Fig. S2). Membranes were then incubated with an anti-rabbit horseradish peroxidase-conjugated antibody (Sigma-Aldrich, U.K; dilution 1:25000) for 1h at room temperature, and reactive bands were detected by chemiluminescence (ECL; GE Healthcare, UK). A mouse anti-\(\beta\)-actin antibody (dilution, 1:5000; Sigma-Aldrich) was used to check for equal protein loading.

**Quantitative PCR**

Total RNA was extracted from all arteries using the RNeasy Micro Kit (Qiagen, Manchester, UK) and reverse transcribed as described previously\(^\text{13}\). QPCR was used to measure abundance of specific mRNAs using the SYBR-Green detection technique and specific primers, as previously described\(^\text{13}\). Quantification cycle (Cq) for each gene of interest was calculated and normalized to the reference gene DNA topoisomerase (TOP1) using the \(2^{-\Delta\text{Cq}}\) method; no template controls were run alongside all reactions to assess contamination. Amplification efficiency was calculated after running a qPCR using serial dilutions (from 1 to 1:10000) of a template to obtain a standard curve. Amplification efficiency (E) was calculated from the slope of the standard curve using the \(E = 10^{\frac{-1}{\text{slope}}}\) formula, and expressed as percentage using the following formula: % Efficiency = (E – 1) x 100%. The value obtained for the three different set of primers are: 94.5% for NPR1, 101.9% for NPR2 and 104.1% for TOP1. Primers with efficiencies between 90-110% were deemed suitable for use in the calculation of \(\Delta\text{Cq}\) in accordance with the current guidelines. Primers and mastermix for the qPCR experiments were purchased from Primerdesign (Southampton, UK). Information on primers used can be found in Table S2.

**Drugs**

All chemicals and drugs were purchased from Sigma-Aldrich (Dorset, UK) unless otherwise stated. C-type natriuretic peptide (CNP) and terciapin Q were obtained from Tocris (Bristol, UK) and HMR1556 was supplied by Janssen Pharmaceuticals. All drugs were prepared in either DMSO or distilled water.

**Statistics**

All data are expressed as mean ± SEM, where n equals the number of animals from which arteries were used. Multiple comparisons were performed by two-way ANOVA with Bonferroni post-hoc analysis.
Tables
Table S1 – Effect of drugs on basal tone

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<th>Aorta</th>
<th>Renal Artery</th>
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<td>10μmol/L Linopirdine</td>
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Table S2 – Primer sequences

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Supplementary Figures

Figure S1

Vascular reactivity of aortae and renal arteries. Contraction to methoxamine (100nmol/L-10μmol/L) in NT (black) or SHR (red) animals or the presence of 10μmol/L (green) in aortae
(Ai) or renal arteries (Bi). Force generated in response to 3μmol/L methoxamine in NT (black) or SHR (grey) aortae (Aii) and as a % of 60mM KCl contraction (Aiii) and in the renal arteries (Bii and Biii, respectively).

Figure S2

Representative Western Blot showing the band detected by the anti-K\textsubscript{v}7.4 antibody in non-transfected (ctl) or kcnq4-transfected (K\textsubscript{v}7.4) HEK cells. Membranes were re-probed for anti Gβ-subunit to check for equal protein loading (n=5).
Figure S3

*Natriuretic peptide responses are sensitive to 3μmol/L linopirdine.* Relaxations to ANP (A) or CNP (B) in the aorta (blue), or to ANP in the renal artery (C, green) in the absence (solid line) or presence (dashed line) of 3μmol/L linopirdine. Each point is the mean of 3-4 animals ± S.E.M. A Bonferroni post-hoc test was performed following a two-way ANOVA where p<0.05 is denoted (*), p<0.01 is denoted (**) and p<0.001 is denoted (***) . Results were deemed non-significant when p>0.05.
Blockade of other potassium channels did not affect Kv7 dependent responses. Relaxations to CNP in the aorta in the absence (solid line) or presence (dashed line) of HMR1556 (A), Glibenclamide (B), Paxilline (C) and Tertiapin Q (D). Each point is the mean of 4 to 6 animals ± S.E.M. A Bonferroni post-hoc test was performed following a two-way ANOVA. Results were deemed non-significant when p>0.05.
Blockade of other potassium channels did not affect Kv7 dependent responses in renal artery. Relaxations to ANP in the renal artery in the absence (solid line) or presence (dashed line) of HMR1556 (A), Glibenclamide (B), Paxilline (C) and Tertiapin Q (D). Each point is the mean of 4 to 6 animals ± S.E.M. A Bonferroni post-hoc test was performed following a two-way ANOVA. Results were deemed non-significant when p>0.05.
Figure S6

*Effect of soluble guanylate cyclase inhibition on SNP response in the aorta.* Relaxation to SNP in the aorta in the absence (black) or presence (blue) of 10μmol/L of ODQ. Each point is the mean of 4 animals ± S.E.M. A Bonferroni post-hoc test was performed following a two-way ANOVA. Results were deemed non-significant when p>0.05.
Role of PKA in linopirdine sensitive natriuretic peptide vasorelaxations. Relaxations to ANP (A) or CNP (B) in the aorta (blue), or to ANP in the renal artery (C, green) in the absence
(solid line) or presence (dashed line) of 1μmol/L H-89. Each point is the mean of 3-5 animals ± S.E.M. A Bonferroni post-hoc test was performed following a two-way ANOVA. Results were deemed non-significant when p>0.05.

Figure S8

**Effect of endothelial denudation on linopirdine sensitive natriuretic peptide responses.** A. Maximum relaxation to 10μmol/L carbachol in the aorta (left) and renal artery (right) to test endothelial denudation. Relaxations to ANP in the aorta (B) and renal artery (C) in vessels with endothelium present (control) and in denuded vessels. Relaxations to CNP in the aorta (D) in vessels with endothelium present (control) and in denuded vessels. Each point is the mean of 3 animals ± S.E.M. Results were deemed non-significant when p>0.05.
Figure S9

*Effects of ANP and CNP in the mesenteric artery.* Responses to ANP (A) or CNP (B) in the renal artery. No significant difference between natriuretic peptide and time control was seen (p=0.95 ANP and p=0.34 CNP). Each point is the mean of 5 animals ± S.E.M. Percentage relaxation to mesenteric arteries to 10μM carbachol (CCh), and then subsequently to either 300nM CNP or 30nM ANP. Each bar is the mean of 3 animals ± S.E.M.