Natriuretic Peptides

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

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Abstract—The Kv7 family of voltage-gated potassium channels 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. DOI: 10.1161/HYPERTENSIONAHA.114.04373.)

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>BNP>ANP.3 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 vasodilation 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 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.7,10,11 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_{ATP} channel blocker (glibenclamide; 10 μmol/L; P=0.74), a BK_{Ca} channel blocker (paxilline; 1 μmol/L; P=0.74), or a K_{IR} 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 Kv7.4/7.5 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),...
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

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**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.** 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).

Figure 4. Kv7.4 currents are enhanced by application of cGMP. Representative traces from a voltage step protocol of control currents in human embryonic kidney 293 cells stably transfected with Kv7.4 (A) and after the application of 100 μmol/L cGMP (B) and then after application of 10-μmol/L linopirdine (C). The time course of the maximum current at 20 mV is shown in D. Mean data (±SEM) is shown in E, n=7 to 8. 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.

Figure 5. Natriuretic peptide (NP)–mediated vasorelaxations are impaired in spontaneously hypertensive rats (SHRs). A, Representative traces of relaxations to atrial NP (ANP) in the aorta (left) and renal artery (right) in normotensive (black) and SHR (gray) animals after constriction with 3-μmol/L methoxamine (indicated by dot). In SHR, 1-μmol/L pinacidil was added at the end of the experiment. Mean dose response curves to atrial NP (B), C-type NP (CNP; C), and sodium nitroprusside (SNP; D) in aortae and to ANP in renal artery (E) are significantly impaired in SHR (dashed line) compared with NT (solid line) animals. Each point is the mean of 6 to 13 animals±SEM. A Bonferroni post hoc test was performed following a 2-way ANOVA, where *P<0.05, **P<0.01, and ***P<0.001. Results were considered nonsignificant when P>0.05.
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 vasodilatations 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, HM1556, 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 7.5. 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 arteries). These findings add to growing appreciation that 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 7.5. 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 arteries 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 SHRs (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 factor 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. CNP stimulates NPR-B to increase cellular cGMP, which produces membrane hyperpolarization and relaxations that are mainly sensitive to blockers of BKCa with some contribution from glibenclamide-sensitive KATP channels (eg, guinea pig carotid arteries). CNP also relaxes rat mesenteric arteries via NPR-C and the subsequent stimulation of tertiapin Q-sensitive Kir3.1 to 3.4 channels by β3 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 Kir3.1 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, 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 BK<sub>Ca</sub> 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 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.

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Disclosures
None.

References


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### Novelty and Significance

- 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

Natriuretic peptide vasorelaxations are mediated by Kv7 channels, and this process is compromised in hypertension.

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### What Is New?

- Although the mechanism of natriuretic peptide vasorelaxation remains unknown, we reveal new insights.

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