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Natriuretic peptides relax human intrarenal arteries through natriuretic peptide receptor type-A recapitulated by soluble guanylyl cyclase agonists

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Short title: Natriuretic peptides and human kidney

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Abstract

Aim: Natriuretic peptides, BNP and ANP increase renal blood flow in experimental animals. The signaling pathway in human kidney vasculature is unknown. It was hypothesized that BNP and ANP cause endothelium-independent relaxation of human intrarenal arteries by vascular natriuretic peptide receptor-A, but not -B and -C, which is mimicked by agonists of soluble guanylyl cyclase sGC. **Methods**: Human (n=54, diameter: 665 ± 29 µm 95% CI) and control murine intrarenal arteries (n=83, diameter 300 ± 6 µm 95% CI) were dissected and used for force recording by 4-channel wire myography. Arterial segments were pre-contracted, then subjected to increasing concentrations of BNP, ANP, phosphodiesterase 5-inhibitor sildenafil, sGC-activator BAY 60-2770 and -stimulator BAY 41-2272. Endothelial nitric oxide synthase (eNOS) dependence was examined by use of L-NAME and eNOS knockout, respectively. Molecular targets (NPR A-C, sGC, phosphodiesterase-5 and neprilysin) were mapped by PCR, immunohistochemistry and RNA-scope. **Results:** BNP, ANP, sildenafil, sGC-activation and -stimulation caused concentration-dependent relaxation of human and murine intrarenal arteries. BNP responses were independent of eNOS and were not potentiated by low concentration of phosphodiesterase-5-inhibitor, sGC-stimulator or NPR-C blocker. PCR showed NPR-A and C, phosphodiesterase-5, neprilysin and sGC mRNAs in renal arteries. NPR-A mRNA and protein was observed in vascular smooth muscle and endothelial cells in arteries, podocytes, Bowmans capsule and vasa recta. NPR-C was observed in tubules, glomeruli and vasculature. **Conclusion**: Activation of transmembrane NPR-A and soluble guanylyl cyclase relax human preglomerular arteries similarly to phosphodiestase-5 inhibition. The human renal arterial bed relaxes in response to cGMP pathway.

Key Words: Hypertension, eNOS, endothelium, kidney, cGMP

Introduction

The natriuretic peptides (NPs) atrial-, brain and c-type natriuretic peptide (ANP, BNP and CNP) are synthesized and released predominantly by myocardial cells upon extracellular fluid volume stimuli or atrial/ventricle wall stress. In humans, NPs exert effects through natriuretic peptide receptors.

They are membrane-bound with 2 types coupling to guanylyl cyclase (GC) activity (GC-A or NPR-A and NPR-B) and the C-type, which is truncated and without GC activity and mediates internalization and degradation of NPs. In kidney, ANP exihibited differential vascular effects such that rat afferent arterioles relaxed while efferent arterioles constricted which explained stimulatory effects on GFR.[1,2] In nanomolar range, ANP dilated arcuate and afferent but not efferent vessels in rat juxtamedullary nephron preparation[3]. Similar effects were observed in hydronephrotic rat kidney with efferent constriction that translated into increased GFR and filtration fraction in intact kidney[4,5] and this effect was caused by NPR-A[5]. Papillary blood flow was increased by ANP infusion in rat[6]. Such hemodynamic effects are less well recognized for BNP and not characterized in human intrarenal vessels. In patients with acute kidney failure, ANP infusion increased renal blood flow, filtration fraction, and GFR[7,8], but there are no available data whether this effect was by direct interaction with vascular NP receptors. Expression and localization of NPR-C and the neutral endopeptidase neprilysin in human kidney are unknown. The phosphodiesterase that degrades cGMP in human renal vessels has not been demonstrated functionally, but phosphodiesterase (PDE) type-5 immunoreactive protein was present in human kidney vasculature[9] Both NPs[7,8,10-13] and drugs that protect against NP degradation (neprilysin inhibitors) and drugs acting to promote the cGMP pathway by other targets (soluble GC activators/stimulators, PDE inhibitors) have renoprotective effects, found mostly in preclinical studies, but also in humans[14]. Based on these observations, the questions arise whether beneficial renal effects of cGMP pathway stimulation are fully or partially mediated through a vascular action and, in that case, what is the role for endothelial versus smooth muscle cells. In mice, vascular but not endothelial natriuretic peptide receptors yielded relaxation[15,16] The present study was designed to test the hypotheses that the human intrarenal arterial vasculature relaxes in response to NPs, sGC stimulators/activators, and PDE5 inhibitors. It was hypothesized that relaxation to NPs is mediated through receptors on smooth muscle and that PDE-5 in smooth muscle is a predominant pathway for cGMP degradation. The hypotheses were tested first by recordings of isometric tension in a 4 channel myograph setting with artery rings dissected and mounted *ex vivo* from human cancer nephrectomy tissue specimens and compared with murine counterparts. Subsequently, studies of mRNA and protein localization were performed by PCR, RNAscope, immunofluorescence and -histochemistry analyses on human kidney sections, isolated intrarenal artery segments and glomeruli.

Results

In total, 183 artery rings from 54 patients and 162 artery rings from 83 mice were mounted (Supplement table I). Baseline characteristics of human and murine vascular rings, results from viability and functional test are presented in supplement expanded results section.

Prestimulation of artery rings and force responses

In human intrarenal segments from n=6 patients, increasing concentrations of K^+ yielded EC_{50} = 24.3 ± 2.2 mmol/L (Fig. 1A). Subsequent experiments were conducted with 40 mmol/L K^+ (~EC_{70}) which yielded the most consistent responses. In artery segments from n=12 patients, cumulative concentrations of norepinephrine (NE) produced log EC_{50} = -5.8 ±0.2 log mol/L (Fig. 6A). Human artery rings exposed to NE immediately started oscillating and did not maintain stable tension (Fig. 6B). This precluded the use of NE to obtain stable tone to study relaxation. In murine renal segmental artery rings from n=14, cumulatively increasing concentrations of phenylephrine (PE) yielded log EC_{50} = -6.7 ± 0.1 log mol/L (Fig. 1B). Subsequent experiments were conducted with 10^{-6} mol/L PE (~EC_{70}) in murine artery rings.

Response of pre-stimulated artery rings to natriuretic peptides

In both human and murine pre-stimulated arterial rings, BNP and ANP induced concentration dependent relaxation (Fig. 2). In human segments from n=13 patients, a full concentration response curve to BNP could not be generated. The potency was estimated at log IC_{50} = -6.3 ± 1.1 log mol/L assuming a maximal relaxation corresponding to 0 % of pre-stimulation (Fig. 2A). The response to cumulative concentrations of NE in human arteries was not impacted by incubation with a concentration of BNP below IC_{50} (10^{-7} mol/L, Fig. 6A, P>0.05). For ANP, half maximal effect (log IC_{50}) was -7.3 ± 0.2 log mol/L (n=6) and E_{max}= 107 ± 16 % (Fig. 2C). In murine segments, the concentration response curve for BNP yielded a log IC_{50} = -7.2 ± 0.2 log mol/L and E_{max} = 55 ± 8 % relaxation (Fig. 2B, n=15). For ANP, murine segments displayed a log IC_{50} = -8.3 ± 0.2 log mol/L (n=6) and E_{max} = 105 ± 11 % relaxation (Fig. 2D). Time-control experiments documented that K^+-induced tone in human artery rings and PE-induced tone in murine rings was stable over time (Fig. 2F and G, only 1-2 control rings incubated with agonists beyond the time used to obtain concentration-responses). Involvement of eNOS in the relaxation to BNP was tested in human segments by incubating rings with 10^{-4} mol/L L-NAME and by using segments from eNOS-/- mice. In human rings from n=7, L-NAME had no effect on E_{max} or IC_{50} for BNP (Fig. 3A, P=n. s.), but attenuated acetylcholine- induced relaxation (Fig. 3C (E_{min}) and E, P<0.05). In murine segments

from eNOS -/-, BNP elicited a response that was not different from the one observed in rings from wildtype animals for E_{max} or IC_{50} (n=6, Fig. 3B, P=n.s.). The tension observed after phenylephrine precontraction was attenuated in eNOS-/- (Fig. 3D (E_{min}), P<0.05) as were the responses to 10^{-5} mol/L acetylcholine (Fig. 3F, P<0.05).

Effect of a PDE-5-inhibitor on vascular reactivity and impact on BNP response

The PDE-5-inhibitor sildenafil induced concentration-dependent relaxation both in human and murine precontracted arterial rings. In human segments, sildenafil yielded an effect at log IC_{50} = -5.1 ± 0.3 log mol/L and E_{max} = 126 ± 59 % relaxation (Fig. 4E, n=6) and in murine segments at log IC_{50} = -6.2 ± 0.2 log mol/L and E_{max} = 100 ± 11 % relaxation (Fig. 4F, n=11). Incubation with sildenafil for 10 minutes at a concentration which had no independent effect on relaxation (10^{-7} mol/L) did augment the subsequent response to BNP in human rings (n=5, P>0.05) or murine rings (n=8, P>0.05) by comparison of E_{max} and IC_{50} by F-test and by Bonferroni's multiple comparisons test (Fig. 4A and B show relative effect and Fig. 4C-D show effect on absolute tension, all P>0.05).

Vascular responses to soluble Guanylyl Cyclase (sGC)-activator and -stimulator and impact on BNP response

sGC-activator BAY 60-2770 induced concentration dependent relaxation in both human and murine arterial segments. In human segments, log IC_{50} = -5.3 ± 0.4 log mol/L and E_{max} = 105 ± 42 % relaxation (Fig. 5A, n=6). In murine segments log IC_{50} = -7.5 ± 0.3 log mol/L and E_{max} = 74 ± 13 % relaxation (Fig. 5B, n=6). sGC-stimulator BAY 41-2272 induced concentration-dependent relaxation in both human and murine arterial segments. In human segments, a full concentration response curve to BAY 41-2272 could not be generated (Fig. 5C). The potency estimated by assuming a maximal corresponding to 0% of pre-stimulation yielded log IC_{50} = -5.1 ± 12.6 log mol/L (Fig. 5C from n=4 patients). In murine segments, log IC_{50} = -6.5 ± 0.2 log mol/L and E_{max} = 93 ± 13 % relaxation (Fig. 5D, n=5). In murine rings, the application of 10^{-8} mol/L sGC-stimulator BAY 41-2272 did not significantly impact the response to increasing concentrations of BNP (Fig. 5E, IC_{50} = -7.2 +/- 0.3 log mol/L for BNP and -7.4 +/- 0.4 log mol/L for BAY with BNP, n=8, n.s.).

Impact of NPR-C inhibitor on BNP response

In murine arterial segments, the NPR-C inhibitor AZ12107657 (= M372049, 10^{-6} mol/L)[17,18] had no effect on force development in response to phenylephrine. The response to increasing concentrations of BNP was similar with or without NPR-C blocker (Fig. 2E, n=6, P=n.s.).

Localization of NPRs, PDE5 and sGC in human kidney tissue

NPR-A was detected by PCR in kidney cortex and outer medulla and in isolated human glomeruli, but less consistently in microdissected small caliber intrarenal arteries (Fig. 7A and B, Supplement Fig. I). In kidney sections, NPR-A mRNA and protein was associated with glomeruli by RNAscope and immunohistochemistry, which also showed NPR-A in vasa recta and the parietal layer of Bowman´s capsule (Fig. 7C and D, Supplement Fig. II). RNAscope and immunohistochemistry both demonstrated NPR-A in endothelial cells in arteries and arterioles and in scattered smooth muscle cells in intrarenal artery media (Fig. 7C and D and Supplement Fig. II). NPR-A immunoreactivity in glomeruli, arteries and vasa recta was a consistent finding in n=10 human kidney sections across sex, diagnoses and age (Supplement Fig. II). In glomeruli, NPR-A co-localized with synaptopodin by fluorescence-double labeling (Fig. 7E and F), but not with integrin α-8 (Supplement Fig. IV). NPR-A immunoreactivity was found both in descending and ascending vasa recta in the outer medulla (Supplement Fig. VI). NPR-B was not observed in human kidney tissue by PCR or RNAscope (Supplement Fig. V). NPR-C mRNA was detected in cortex and outer medulla and was associated with arteries and glomeruli by PCR (Fig. 7A and B, Supplement Fig. I). RNAscope showed NPR-C mRNA localization in glomeruli, proximal convoluted tubules and tubules in the outer medulla and in arterial smooth muscle cells (Supplement Fig. III). PDE5 mRNA was detected by PCR in renal artery and intrarenal arteries and enriched compared to cortex/outer medulla tissue (Supplement Fig. I). Soluble guanylyl cyclase (sGC) was detected in renal and intrarenal arteries and was enriched compared to cortex/medulla tissue (Supplement Fig. I). Neprilysin was not detected consistently in the intrarenal vasculature but amplified consistently in cortex and medulla tissue (Supplement Fig. I).

Discussion

The present study finds that BNP and ANP concentration-dependently relaxed intrarenal arteries from humans and mice, in agreement with vascular smooth expression of natriuretic peptide receptor type A, NPR-A. This may account for stimulatory effects on renal blood flow in animals and patients[4-8]. Relaxation was induced by the sGC-stimulator and -activators BAY 41-2272 and BAY 60-2770, in accordance with vascular expression of sGC. Significant contribution to metabolism of cGMP by phosphodiesterase -5 (PDE-5) was revealed by relaxation to the PDE-5

blocker sildenafil. The NPR-C receptor was detected in tubules and vasculature, but an NPR-C blocker did not alter vascular responsiveness to NPs. NPR-A responses were independent of endothelial NO synthesis in mice and human intrarenal vessels in agreement with observations in mice of dispensable endothelial NPRA.[15,19] In human cutaneous microvasculature, BNP responses were partially dependent on eNOS activity[20], so at least in humans, endothelial dependence of BNP responses differ between vascular beds. The relaxing effect of NPs on intrarenal arteries was in agreement with NPR-A immunolabelling in smooth muscle in arterioles and in media of larger arteries. In medulla of human kidney, both ascending and descending vasa recta exhibited immunoreactive NPR-A consistently across age, gender and diagnoses (n=10). In rat kidney, isotope labeled ANP bound to structures of the outer medulla compatible with vasa rectae[21,22]. Efferent arterioles in rodents contract in response to ANP[1] but, to our knowledge, there are no data from isolated vasa recta. Medullary blood flow increased by ANP in pig and rat[6,10]. Based on the present immunolabeling and preclinical observations, it is an intriguing possibility that natriuretic peptides relax human vasa recta and increase medullary blood flow.

In healthy humans, plasma concentration of natriuretic peptides[23] is lower than the effective *in vitro* concentrations in the present study and previous studies[23,24]. This difference could be due to the reduced *ex vivo* preparation and it could also relate to the size of the vascular segment. The present study used segments of ~600 µm and the much smaller glomerular arterioles are sensitive to picomolar levels of ANP in rat[1] and in smaller intrarenal rings, ANP displayed an IC_{50} of 9 nmol/L.[24] Other explanatory factors could be prolonged incubation for wash-out of anesthetics and different NPR-A receptor density (increasing with decreasing artery caliber)[1]. The potency of BNP and ANP in murine preglomerular arteries exceeded that of human arteries by one order of magnitude and ANP was more potent than BNP. This difference between ANP and BNP was observed for NPR-A expressed *in vitro*[25]. The species difference was likely caused by the different agonist used to induce baseline tension. The human artery rings showed regular oscillation of vascular tension in response to norepinephrine (Supplement Fig. V-C), a well-known response *ex vivo*[26]. This phenomenon urged the use of potassium to generate stable tension. Vasomotion was not observed with potassium-induced tension as with norepinephrine which confirms dependence on membrane potential fluctuations also in human renal vascular bed[26]. Relaxation in response to NPs depends on potassium channel activation and hyperpolarization in animal vasculature[27]. This dependence on membrane potential changes may account for the reduced potency of NPs in K^+-pre-

stimulated human rings compared to murine rings. The consequent use of human natriuretic peptides, and not murine, could have contributed to different sensitivity.

The present study supports that both ANP and BNP exert effects through the NPR-A since NPR-B expression was not detected in human kidney. NPR-C expression was observed in vascular smooth muscle cells, tubular epithelium and intraglomerular cells in agreement with previous studies[21,22]. The responsiveness of isolated rings to BNP was not altered by high concentrations of an inhibitor of NPR-C. This confirms previous data where ANP and BNP did not depend on NPR-C in renal vessels[17]. The absence of NPR-A in smooth muscle in mice abolished relaxation of renal vessels to ANP[15,19]. It can be concluded that NPR-C does not contribute to renal vascular relaxation to ANP/BNP. The present results support NPR-A as common receptor for ANP and BNP in the renal vasculature. No labeling of tubular compartment for NPR-A was detected in human kidney while there was abundant NPR-C in proximal tubules and distal nephron parts. Because NPR-C clears and commits ANP/BNP for degradation, the data indicate that NPR-A signaling is not likely in human kidney epithelium. A new observation was abundant NPR-A in podocytes and in parietal layer of Bowman´s capsule in accord with ANP binding data from rat[21,22]. NPR-A in podocytes exerted significant protection against hypertensive injury in a DOCA-salt murine kidney injury modul[28]. The wide vascular and epithelial expression of NPR-C in human kidney may account for the significant ~50% renal extraction of ANP[29] that is larger than for BNP. Neprilysin was detected in human kidney tissue but not consistently in vasculature. BNP depends to a larger degree than ANP on renal clearance and impaired renal function is associated with elevated plasma BNP also in the absence of left ventricular dysfunction.[30 31] The present data showed function and expression of phosphodiesterase type 5 (PDE-5) and sGC in human intrarenal arteries in accord with previous data [9,32,33]. Thus, both transmembrane and soluble guanylyl cyclase are functional in human renal vascular smooth muscle. The present study has several limitations; it was *ex vivo* and under isometric condition and thus the multiple inputs that converge on the vessels *in vivo* (flow-mediated NO, sympathetic nerve input and transmural pressure) are absent which may have affected sensitivity and reactivity. Vessels were predominantly from kidney cancer patients and although harvested from normal tissue, the presence of tumor tissue in the kidney could have affected results. Finally, vessels were from aging patients and therefore may exhibit variable degrees of endothelial dysfunction, exaggerated stiffness and altered perivascular adipose tissue. With these limitations in mind it is safe to conclude on the presence of NPRA in preglomerular artery smooth muscle in human kidney. In pathophysiological settings, drugs are available that increase ANP/BNP half-life

and bioavailability (neprilysin inhibitors), stimulate sGC and inhibit cGMP degradation (PDE-5 inhibitors). The present study shows that such drugs may all exert direct vascular effects at the level of intrarenal arteries in human kidneys. Such effects may contribute to observed, beneficial, renal effects of ANP in acute renal failure[7,8] and cardio-pulmonary surgery[10] patients and of sildenafil in preclinical studies of transplantation[34] and warm ischemia[35].

Materials and methods

Detailed description of the methods and materials used is given in the supplement section. Patients that underwent unilateral total nephrectomy for renal cancer were recruited. The use of tissue was approved by the ethics committee of the Region of Southern Denmark (#S-2014-0159) and used only after informed written and oral consent from each patient. 30-45 min after extirpation, tissue was transferred to physiological buffer for dissection (see protocol in supplement), while samples of renal cortex, outer and inner medulla were snap frozen in liquid nitrogen and stored at -80 ^{o}C. Wedge-shaped tissue blocks were cut along the cortical-papillary axis and subsequently paraformaldehyde-immersion fixed for 48h hours and then embedded and stored in paraffin. Subsequently 5 μm sections were cut and mounted on slides for histochemical labeling.

Experiments

All series I-XIV are described in detail in the supplement. Human and murine (wildtype and eNOS -/-) intrarenal arterial segments were suspended in physiological saline solution aerated with 5% CO_2 in air in a Multi Wire Myograph (model 610M, Danish Myo Technology, Hinnerup, Denmark) for real-time measurement of changes in tension during pharmacological manipulation. Active tension in human and murine arterial segments was achieved by elevation of potassium through isosmotic exchange with sodium to induce depolarization (EC_{70} ~ 40 mmol/L) and by phenylephrine (EC_{70} ~ 10^{-6} mol/L), respectively (Supplement Fig. I). Cumulative concentration response curves were generated to BNP (including series with sildenafil, sGC activator, NPR-C inhibitor[17,18], and L-NAME), ANP, sildenafil, a sGC stimulator, and activator (BAY 60-2770 and 41-2272, respectively). PCR, RNAscope and immunohistochemistry were performed in cortical and medullary human kidney tissue and intrarenal arteries as described in supplementary methods.

Data analysis and statistics

Isometric tension recordings were expressed relative to the tension level induced by precontraction or as absolute values (N/m). Concentration-response curves were compared by two-way analysis of variance or for potency (log EC_{50}/IC_{50}) and efficacy (E_{max}) by F-test in Graph Pad Prism 6.07 (GraphPad Software Inc., San Diego, CA, USA). Bonferroni's multiple comparisons test was applied where appropriate. For supplementary fig. 4 (Supplement Fig. IV), comparisons were made by paired two-tailed Student's t-test. P-values <0.05 were considered significant.

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Conflict(s) of Interest

Authors report no conflicts of interest

List of supplemental materials:

Expanded Materials & Methods

Expanded Results section

Supplemental tables 1 and 2

Supplemental, online Figures I – IX

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Legends to figures

Figure 1

Concentration-response curves generated from the measured tension as induced by increasing concentrations of (A) potassium (KPSS in log[mol × L^{-1}]) and (B) phenylephrine in order to

determine EC_{70} values (in log[mol × L^{-1}]). The number of individual experiments contributing to each curve is given in each panel. Data are presented relative to tension induced by 40 mM K^+ during viability test as mean ± SEM.

Figure 2

Changes in active isometric wall tension during cumulative increase in concentration of BNP and ANP (in log[mol × L^{-1}]) in precontracted intrarenal arterial segments from humans (40 mM K^+, left column) and in renal arterial segments from wildtype mice (1 µM phenylephrine, right column). Tension is expressed relative to stabile precontraction level.

BNP induced concentration dependent relaxation in (A) human and (B) murine segments. This was also the case for ANP where responses were more potent both in (C) human (n=2 at 0.1-1 µM) and (D) murine segments. (E) BNP concentration-response with and without pre-incubation with 1 µmol/L of the NPR-C inhibitor (inhib) AZ12107657 in murine intrarenal arterial segments (non significant). Time-tension curves for (F) KPSS ($P<0.01$ BNP vs time control) and (G) phenylephrine ($P<0.05$ BNP vs time control) during time-control experiments compared with cumulative concentration-responses to BNP. The number of individual experiments contributing to each curve is given in each panel. Data are presented relative to tension for precontraction for (A to E) and of maximal induced contraction by the respective agonist as mean ± SEM. Data were compared by two-way analysis of variance with Bonferroni's multiple comparisons test. Results were interpreted as significant if $P<0.05$.

Figure 3

Changes in active isometric wall tension during cumulative increase in concentrations of BNP (in log[mol × L^{-1}]) in precontracted arterial segments during L-NAME treatment (human, left) or eNOS knock-out (murine, right). (A) Human segments were precontracted by 40 mM K^+, incubated with 100 µM L-NAME following which endothelium-dependent relaxation was exhausted by 10 µM acetylcholine. This did not affect BNP-induced relaxation in human (n=1 at 1 µM BNP, P=non significant) intrarenal arteries. (B) Murine renal segmental arterial segments from wildtype and eNOS -/- mice were precontracted with 1 µM phenylephrine which yielded a stabile contraction. Responses to BNP were unaffected by eNOS knock-out as compared to wildtype mice (P=non significant). In (A) the BNP concentration response curve is superimposed from figure 1. (C) same dataset as (A), but in N/m. Relaxation to acetylcholine is impaired by L-NAME seen as the curve

difference at E_{min} ($P<0.05$), but responses to BNP are unaffected. (D) same dataset as (B), but in N/m. Contraction to phenylephrine is attenuated in eNOS -/- mice ($P<0.05$), but responses to BNP are unaffected. (E) Effect of L-NAME on acetylcholine -induced relaxation in human segments as compared with control (CON, normalized to 100%, $P<0.05$). (F) Effect of eNOS -/- on acetylcholine-induced relaxation in murine segments as compared with responses in wildtype segments (WT, normalized to 100%, $P<0.05$). The number of individual experiments contributing to each curve is given in each panel. Tension is expressed in absolute units or relative to stabile precontraction level as mean ± SEM. Curves are compared for E_{min}, E_{max}, and IC_{50} by extra sum-of-squares F-test for A-D and by *Student's* t-test for E and F. Differences were interpreted as significant if $P<0.05$.

Figure 4

Cumulative concentration-response curves generated from isometric tension development in precontracted arterial segments (A and B) to BNP (in log[mol × L^{-1}]) in the absence or presence of the phosphodiesterase-V-inhibitor sildenafil in human (A and C) intrarenal arterial segments and in murine (B and D) intrarenal arterial segments from wildtype animals or (E and F) to sildenafil in corresponding (E) human and (F) murine segments. Tension is expressed relative to stabile precontraction level. Sildenafil dose-dependently relaxed human and murine segments, but incubation with a non-relaxing dose of sildenafil did not potentiate the response to BNP (P=non significant for curve comparisons in figures A to D). The number of individual experiments contributing to each curve is given in each panel. Data are presented relative to tension for precontraction as mean ± SEM. Curves are compared for differences in E_{max} and IC_{50} by F-test. In case of large errors in estimates of E_{max} and IC_{50}, curves were compared by two-way ANOVA and Bonferroni's multiple comparisons test.

Figure 5

Changes in active isometric wall tension during cumulative increase in concentrations of the sGC activator BAY 60-2770 and stimulator BAY 41-2272 and of BNP during sGC stimulation (in log[mol × L^{-1}]) in precontracted (40 mM K^+) intrarenal arterial segments from humans and in renal segmental arterial segments from wildtype mice (1 µM phenylephrine for prestimulation). Tension is expressed relative to stabile precontraction level. Concentration dependent relaxation was

induced by the sGC activator in both (A) human and (B) murine segments. Similar responses were found for the sGC stimulator in (C) human and (D) murine segments. (E) Incubation with the sGC stimulator prior to relaxation with BNP by cumulatively increasing concentration, did not affect relaxation to BNP in murine segments ($P>0.05$). The number of individual experiments contributing to each curve is given in each panel. Data are presented relative to tension for precontraction as mean ± SEM. Curves were compared for statistical differences in E_{max} and IC_{50} by F-test.

Figure 6

Concentration-tension curves generated from cumulatively increasing concentrations of (A) norepinephrine (NE, n=3 at highest concentrations 32 µM – 100 µM) with and without 0.1 µM BNP pre-incubation in human intrarenal arterial segments ($P>0.05$ between curves). (B) shows a representative trace of the oscillatory response of tension in response to norepinephrine (NE) at the indicated concentrations in human intrarenal segments. In A, curves show concentration (log [mol x L^{-1}]-response curves relative to tension induced by 40 mM K^+ during viability test. Curves are compared for statistical differences in E_{max} and EC_{50} by F-test.

Figure 7

Expression of NPRA and –C by PCR, immunohistochemistry [IHC] and in situ hybridization by RNAscope. (A and B) PCR on microvessels [MV1-3], renal artery [RA], cortex [C], outer medulla [OM] and glomeruli [G1-3] and without reverse transcriptase [-RT]. Housekeeping gene was (A) TFIIB and (B) RPL22. (C) Location of NPRA in kidney tissue artery, glomerulus and vasa recta by IHC as compared to negative control (no NPRA antibody) visualized by DAP or flourescence. Endothelial cells [E], smooth muscle cells [SM] (D) In situ hybridization for NPRA RNA in glomerulus, the Capsule of Bowmann [BC] and in intrarenal artery in endothelial [E] and smooth muscle cells [SM]. (E and F) Merged IHC images stained for synaptopodin [SYNPO] and NPRA showing NPRA expression in podocytes. Scalebar is 50 µm.