Natriuretic peptides buffer renin-dependent hypertension

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Demerath T, Staffel J, Schreiber A, Valletta D, Schweda F. Natriuretic peptides buffer renin-dependent hypertension. Am J Physiol Renal Physiol 306: F1489–F1498, 2014. First published April 9, 2014; doi:10.1152/ajprenal.00668.2013.—The renin-angiotensin-aldosterone system and cardiac natriuretic peptides [atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP)] are opposing control mechanisms for arterial blood pressure. Accordingly, an inverse relationship between plasma renin concentration (PRC) and ANP exists in most circumstances. However, PRC and ANP levels are both elevated in renovascular hypertension. Because ANP can directly suppress renin release, we used ANP knockout (ANP−/−) mice to investigate whether high ANP levels attenuate the increase in PRC in response to renal hyperperfusion, thus buffering renovascular hypertension. ANP−/− mice were hypertensive and had reduced PRC compared with that in wild-type ANP+/+ mice under control conditions. Unilateral renal artery stenosis (2-kidney, 1-clip) for 1 wk induced similar increases in blood pressure and PRC in both genotypes. Unexpectedly, plasma BNP concentrations in ANP−/− mice significantly increased in response to two-kidney, one-clip treatment, potentially compensating for the lack of ANP. In fact, in mice lacking guanylyl cyclase A (GC-A−/− mice), which is the common receptor for both ANP and BNP, renovascular hypertension was markedly augmented compared with that in wild-type GC-A+/+ mice. However, the higher blood pressure in GC-A−/− mice was not caused by disinhibition of the renin system because PRC and renal renin synthesis were significantly lower in GC-A−/− mice than in GC-A+/+ mice. Thus, natriuretic peptides buffer renal vascular hypertension via renin-independent effects, such as vasorelaxation. The latter possibility is supported by experiments in isolated perfused mouse kidneys, in which physiological concentrations of ANP and BNP elicits renal vasodilatation and attenuated renal vasoconstriction in response to angiotensin II.

The physiological effects of the RAAS that result in the conservation of extracellular volume and stabilization of blood pressure are counterbalanced by the natriuretic peptides: atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). ANP and BNP are mainly released from the heart and provide a hormonal link between the heart and kidney (4, 22, 24). ANP and BNP both activate guanylyl cyclase-A (GC-A), which is also referred to as natriuretic peptide receptor-A, leading to cGMP production (30). ANP and BNP also bind to natriuretic peptide receptor-C, which mainly serves as a clearance receptor via receptor-mediated internalization and degradation of natriuretic peptides (30). GC-A is widely expressed in the body and is critically involved in the regulation of blood pressure and renal function. In addition to other effects, GC-A activation by either ANP or BNP induces vasorelaxation and marked increases in natriuresis and diuresis (5, 11, 15, 19, 21, 26, 31, 37), thus counterbalancing the main effects of the RAAS. Moreover, ANP directly suppresses renin secretion from juxtaglomerular cells (16), and, in vivo, ANP and BNP can reduce plasma renin activity (1, 12, 33).

Plasma levels of natriuretic peptides and renin are inversely related in most circumstances. For instance, plasma ANP and BNP concentrations are elevated under conditions of high salt intake, hypervolemia, and DOCA-salt hypertension, whereas plasma renin activity is suppressed in these situations (3, 10, 18, 23, 44). This inverse relationship does not exist in pathological situations, such as heart failure, in which both the natriuretic peptide system and RAAS are upregulated. The increase in the plasma renin concentration (PRC) in experimental heart failure is exaggerated in GC-A knockout (GC-A−/−) mice (25), and pharmacological blockade of natriuretic peptide receptors induces an increase in plasma renin activity in heart failure (41). Therefore, natriuretic peptides appear to buffer the increase in renin in this situation, thus ameliorating disease progression. As in the case of heart failure, renin and ANP levels are both elevated in renovascular hypertension, which is the most frequent form of secondary hypertension (6, 17, 40, 43). Moreover, pharmacological doses of ANP are capable of suppressing plasma renin activity in rats with two-kidney, one-clip (2K1C) renovascular hypertension (8, 9). Whether the increase in endogenous ANP in response to renal artery stenosis attenuates the stimulation of renin release and thus buffers renovascular hypertension is unknown. To address this question and to gain further insights into the complex interrelation between natriuretic peptides and the RAAS, experiments were performed in ANP knockout (ANP−/−) mice and GC-A−/− mice subjected to experimental renal artery stenosis.

METHODS

Animals. Male 16- to 24-wk-old mice were used in this study. Breeder pairs of ANP−/− mice (13) on a C57BL/6 background were purchased from the Jackson Laboratory. Breeder pairs of GC-A−/− mice (20) on a mixed genetic background (SV129/C57BL/6) were kindly provided by Prof. Michaela Kuhn (University of Würzburg, Würzburg, Germany). To avoid background-related effects, offspring of heterozygous breeder pairs were used throughout the study.

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C57BL/6 mice were purchased for isolated perfused kidney experiments from Charles River (Sulzfeld, Germany).

**Unilateral renal artery stenosis.** Unilateral renal artery stenosis (2K1C) was induced as previously described in detail (42). Before surgery, 20 μl of blood were collected from conscious mice by submandibular venipuncture to determine baseline PRC. Thereafter, mice were anesthetized with sevoflurane (Abbott), the left kidney was exposed by a small flank incision, the renal artery was dissected from the renal vein, and a U-shaped silver clip (0.11-mm inner diameter) was placed around the renal artery. The same surgical procedure, except for the use of a clip, was performed in sham-operated (sham) mice. Seven days after mice had been clipped, another 20-μl blood sample was collected to determine PRC. Thereafter, mice were anesthetized using sevoflurane, and trunk blood was collected by decapitation to determine plasma ANP and BNP concentrations. Due to the high plasma volume needed, repetitive blood sampling by submandibular puncture in the same mice was not possible for ANP and BNP determinations.

**Blood pressure measurement by the tail-cuff method.** Mice were conditioned by placing them in the holding devices for 7 consecutive days before the first measurement was performed. Subsequently, eight blood pressure and heart rate values per mouse were determined per day and averaged for a total of 5 consecutive days after renal artery stenosis. Daily values from each individual mouse were averaged for days 4–7 for the analysis.

**Determination of plasma ANP and BNP concentrations and PRC.** ANP and BNP levels were determined using the respective enzyme immunoassays (Phoenix Pharmaceuticals). Briefly, blood was collected in tubes containing EDTA and aprotinin, and peptides were extracted by C18-Sep columns. ANP and BNP enzyme immunoassays were performed according to the manufacturer’s instructions. Measurements of PRC in plasma samples from conscious mice were based on the generation of ANG I after the addition of plasma from bilaterally nephrectomized male rats as excess renin substrate. The generated ANG I (in ng·ml⁻¹·h⁻¹) was determined by a radioimmunoassay (DiaSorin) (34).

**Determination of plasma volume.** Plasma volume was determined in conscious ANP⁻/⁻ mice (only under baseline conditions) and GC-A⁻/⁻ mice (baseline and 6 days after renal artery clipping) by the Evans blue dilution method as previously described (29). In brief, 30 μl of an Evans blue solution (5 mg/ml in sterile saline) was injected retrobulbarly under light anesthesia (isoflurane). Blood samples (5 μl) were taken from the tail vein before (baseline) and 10 and 30 min after the injection of Evans blue. After centrifugation of the blood, absorbance at 620 nm was determined, and the Evans blue concentration was calculated according to a standard curve. Plasma volume was calculated using a linear regression model.

**Determination of mRNA expression by real-time PCR.** Total RNA was isolated from frozen kidneys using TRIzol reagent (Life Technologies). After reverse transcription (Moloney murine leukemia virus reverse transcriptase, Superscript, Invitrogen), real-time RT-PCR was performed to assess renin (sense: 5'-ATGAGGGGAGGTGCTTTGTTYGGGGG-3' and antisense: 5'-ATGGGGGAGGTGGGCACCT3') and β-actin (sense: 5'-CGGAGTCCCCGCTAGGACGCAACGGGTGTTGTTGAAAGGTCCTCAAG-3') expression using a LightCycler Instrument (Roche Diagnostics).

**Determination of renal renin content.** Renal renin content was determined by measuring the capacity of homogenized kidneys to generate ANG I in the presence of excess renin substrate, as previously described (35).

**Immunofluorescence for renin.** Kidneys were fixed by perfusion with paraformaldehyde (4%), embedded in paraffin, and sectioned in 5-μm sections. Stainings were performed as previously described (32) using an antibody directed against prorenin and renin (AF4277, R&D Systems).

**Isolated perfused mouse kidneys.** Kidneys of C57BL/6 mice or GC-A⁻/⁻ mice were perfused ex situ at a constant perfusion pressure (100 mmHg) as previously described in detail (35). The perfusion medium consisted of a modified Krebs-Henseleit buffer supplemented with BSA (6 g/100 ml) and human erythrocytes (10% hematocrit). The renal vein was cannulated, and samples of the venous perfusate were collected every 2 min to determine renal blood flow and PRC. Five samples were taken during each experimental period, and the last two values of each experimental period were averaged for statistical analysis. To determine the pressure-dependent regulation of renin secretion from the kidneys of GC-A⁻/⁻ mice, renin secretion rates were prestimulated with the β-adrenoceptor agonist isoproterenol (3 mmol/l), and perfusion pressure was changed in a stepwise manner from 40 to 140 mmHg.

**Statistics.** Values are presented as means ± SE. Differences between the groups or different time points within a group were analyzed by one-way or two-way ANOVA followed by the Bonferroni post hoc test if necessary. In the isolated perfused kidney experiments, the last two values obtained within an experimental period were averaged for statistical analysis. All statistical analyses were performed using GraphPad Prism software. P values of < 0.05 were considered to be statistically significant.

**Declarations.** All animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health and were approved by local authorities.

**RESULTS**

Renovascular hypertension in ANP⁻/⁻ mice. As previously reported (13), ANP⁻/⁻ mice were hypertensive during baseline conditions (Fig. 1A) and had normal heart rates (Fig. 1B) and an elevated plasma volume [ANG I: 4.10 ± 0.18% of body weight and wild-type (ANG I⁻/⁻) mice: 3.57 ± 0.08% of body weight, P < 0.05, n = 6 mice/genotype]. Moreover, PRC was significantly lower in ANP⁻/⁻ mice than in their ANP+/+ littersmates (Fig. 1C). To test whether endogenous ANP attenuates the increase in PRC in response to renal hypoperfusion, left-sided renal artery stenosis (2K1C) was used in ANP⁻/⁻ mice. In fact, the plasma ANP concentration was doubled in 2K1C ANP+/+ mice compared with that in sham mice (Fig. 2A). Systolic blood pressure, heart rate, and PRC were not significantly altered in sham ANP⁻/⁻ and ANP+/+ mice compared with baseline values (Fig. 1, A–C). In contrast, renal artery stenosis induced marked increases in blood pressure in ANP+/+ and ANP⁻/⁻ mice to a similar extent in both genotypes (ANG I⁻/⁻: +17.7 ± 4.5 mmHg and ANP⁻/⁻ mice: +20.9 ± 3.5 mmHg vs. baseline; P = 0.6, ANP+/+ vs. ANP⁻/⁻ mice). Due to the higher ANP⁻/⁻ blood pressure at baseline, the absolute value of systolic blood pressure was higher in ANP⁻/⁻ mice than in ANP+/+ mice after renal artery stenosis (Fig. 1A). When related to baseline values before renal artery clipping, the relative blood pressure increase was essentially the same in ANP+/+ mice (1.16 ± 0.04-fold) and ANP⁻/⁻ mice (1.16 ± 0.03-fold). Heart rates were not affected by renal artery clipping (Fig. 1B). PRC was stimulated in response to 2K1C in ANP+/+ mice from 158 ± 35 ng ANG I·h⁻¹·ml⁻¹ (baseline) to 615 ± 94 ng ANG I·h⁻¹·ml⁻¹ and in ANP⁻/⁻ mice from 79 ± 12 ng ANG I·h⁻¹·ml⁻¹ (baseline) to 262 ± 54 ng ANG I·h⁻¹·ml⁻¹. Whereas PRC in clipped ANP+/+ mice was significantly higher compared with sham ANP+/+ mice, there was only a strong trend for higher PRC in ANP⁻/⁻ clipped mice compared with sham control mice (Fig. 1C). Absolute values of PRC were significantly lower in ANP⁻/⁻ mice than in
A operation (sham) on systolic blood pressure (mmHg), heart rate (beats/min; B), and plasma renin concentration (PRC; C) in atrial natriuretic peptide (ANP) knockout (ANP−/−) mice (right) and their wild-type (ANP+/+) littermates (left). n = 12 mice/genotype for 2K1C and n = 5 mice/genotype for sham operation. *P < 0.05 and #P < 0.001 vs. ANP+/+ mice in the same treatment group.

Fig. 2. Effects of renal artery stenosis (2K1C) or sham operation on plasma ANP (A) and B-type natriuretic peptide (BNP; B) concentrations in ANP−/− mice and their ANP+/+ littermates. n = 12 mice/genotype for 2K1C and 12 mice/genotype for sham operation.

ANP+/+ mice not only under baseline conditions but also after renal artery clipping. The relative increases in PRC in response to 2K1C did not differ between ANP+/+ mice (3.9-fold) and ANP−/− mice (3.3-fold, P = 0.2 vs. ANP+/+ mice). Renal renin mRNA expression and renin contents were lower in sham ANP−/− mice than in sham ANP+/+ mice (Fig. 3, A and B). Renin synthesis, reflected by renin mRNA abundance and renin content, was stimulated by renal artery stenosis in the affected left kidney, whereas it was suppressed in the un-implicated contralateral kidney in both genotypes (Fig. 3). The relative changes in renin synthesis were similar in ANP+/+ and ANP−/− mice, and inhibition of the renin system was not detected in ANP−/− mice. Importantly, the plasma BNP concentration was very similar in sham ANP+/+ and ANP−/− mice but was significantly elevated in response to renal artery clipping in ANP−/− mice (sham: 38.3 ± 6.2 pmol/l and 2K1C: 71.6 ± 6.2 pmol/l, P < 0.01; Fig. 2B). In ANP+/+ mice, BNP levels tended to be elevated in response to 2K1C without reaching statistical significance (sham: 38.1 ± 5.0 pmol/l and 2K1C: 54.7 ± 7.9 pmol/l, P = 0.1; Fig. 2B). Because BNP and ANP signaling both occur via the activation of one receptor, GC-A, it appeared reasonable to speculate that the increase in BNP concentration in response to renal artery stenosis might compensate for the lack of ANP in ANP−/− mice.

**Augmented renovascular hypertension in GC-A−/− mice.** To prevent the compensation for the lack of ANP by BNP, we repeated the 2K1C experiments in GC-A−/− and their wild-type (GC-A+/+) littermates. Systolic blood pressure was higher in GC-A−/− mice than in GC-A+/+ mice at baseline and was significantly elevated after 2K1C in both genotypes compared with baseline blood pressure or with sham mice (Fig. 4A). However, in contrast to the results in ANP−/− mice, the blood pressure increase in response to renal artery clipping was approximately twice as high in GC-A−/− mice relative to GC-A+/+ mice (compared with baseline: +39.7 ± 3.7 mmHg in GC-A−/− mice and +18.7 ± 1.8 mmHg in GC-A+/+ mice, P = 0.001; compared with sham mice: +35.6 mmHg in GC-A−/− mice and +16.3 mmHg in GC-A+/+ mice; Fig. 4A).
Thus, the relative increase in arterial blood pressure (after/ before renal artery clipping) was significantly pronounced in GC-A+/ mice (1.30 ± 0.03) compared with GC-A+/ mice (1.16 ± 0.02, P < 0.01). Actually, this marked difference was presumably underestimated because four GC-A+/ mice that had very high blood pressure on day 3 after renal artery clipping died between days 3 and 5; therefore, these mice were excluded from analysis. No mouse died in any of the other groups or genotypes. No differences in heart rates were detected between any of the groups (Fig. 4B). PRC was stimulated 10-fold by 2K1C in GC-A+/ mice but only 2.8-fold in GC-A+/ mice (P = 0.006 vs. GC-A+/ mice); thus, PRC was markedly lower in GC-A+/ mice than in GC-A+/ mice (Fig. 4C). Similarly, stimulation of renin synthesis by renal artery stenosis was attenuated in GC-A+/ mice because renal renin mRNA expression and renin content only tended to be elevated in 2K1C relative to sham mice, without reaching statistical

Fig. 3. Effects of renal artery stenosis (2K1C) or sham operation on renal renin mRNA expression (A) and renal renin content (B) in the left (clipped) and right (untouched) kidneys of ANP+/ mice and their ANP−/− littermates. n = 12 mice/genotype for 2K1C and 5 mice/genotype for sham operation. *P < 0.05 vs. ANP+/ mice in the same treatment group.

Fig. 4. Effects of renal artery stenosis (2K1C) or sham operation on systolic blood pressure (A), heart rate (B), and PRC (C) in guanylyl cyclase A (GC-A) knockout (GC-A−/−) mice (right) and their wild-type (GC-A+/+) littermates (left). n = 13 GC-A+/+ mice and 9 GC-A+/+ mice in the 2K1C group and 10 GC-A+/+ mice and 9 GC-A+/+ mice in the sham group. *P < 0.05 vs. GC-A+/+ mice in the same treatment group; #P < 0.001 vs. GC-A+/+ mice in the same treatment group.
Moreover, renin synthesis in the untouched right kidney was adequately suppressed in clipped mice compared with sham-operated mice of both genotypes. Immunofluorescence staining of renin showed a very similar pattern (Fig. 6). In sham mice, renin staining occurred at the regular position in renin-producing juxtaglomerular cells in both genotypes, whereas the stained area and number of positive cells appeared to be lower in GC-A/−/− mice compared with GC-A+/+ mice. Renal artery stenosis resulted in a marked increase in renin staining in the clipped kidney in GC-A+/+ mice, and recruitment of renin-producing cells in the renal blood vessels was regularly observed (example shown in Fig. 6). Whereas in some glomeruli of the clipped kidney of GC-A/−/− mice renin expression was slightly stimulated, there were others in which no increase in renin immunofluorescence was detectable compared with sham mice (example shown in Fig. 6).

The plasma volume was higher in GC-A−/− mice (3.53 ± 0.07% of body weight, n = 10) compared with GC-A+/+ mice (3.24 ± 0.09% of body weight, n = 9, P < 0.05) under baseline conditions (Fig. 7A) but did not further increase in response to renal artery stenosis or sham operation in any of the genotypes. The plasma ANP concentration was not different between GC-A−/− and GC-A+/+ mice after sham operation and increased to similar levels after 2K1C in both genotypes (Fig. 7B). Plasma BNP levels were not different between sham mice of both genotypes. Similar to what we observed in ANP−/− mice, BNP levels were significantly elevated in response to renal artery clipping in GCA−/− mice, whereas there

**Fig. 6.** Renin immunofluorescence stainings of right and left kidneys of GC-A−/− mice and their GC-A+/+ littermates. Top: sham mice; bottom: effects of left-sided renal artery stenosis (2K1C). Bars = 50 μm.
was only a trend to higher BNP levels in GC-A\(+/\+\) mice without reaching statistical significance (Fig. 7C).

**Experiment in isolated perfused kidneys.** As described above, PRC was reduced in GC-A\(-/-\) mice relative to that in their respective wild-type littermates at baseline and in response to renal artery stenosis in vivo. This may be caused by either a systemic phenotype of GC-A\(-/-\) mice that suppresses renin synthesis and release or it may be the consequence of an inadequate response of renin secretion to physiological regulators of renin release in GC-A-deficient kidneys. To address the latter hypothesis, experiments using isolated perfused kidneys from GC-A\(-/-\) and GC-A\(+/\+) mice were performed in which confounding systemic influences on renin secretion, such as hypervolemia or arterial hypertension, were excluded.

Under prestimulation of renin secretion rates using isoproterenol (3 nmol/l), renal perfusion pressure was reduced to 40 mmHg and subsequently elevated in a stepwise manner up to 140 mmHg. As shown in Fig. 8A, the pressure-dependent regulation of renin release was virtually the same in both genotypes, indicating an intact pressure-dependent regulation of renin release at the organ level in GC-A\(-/-\) mice. Moreover, when perfused with a standard perfusate devoid of natriuretic peptides, GC-A\(-/-\) kidneys did not show any signs of an altered responsiveness to ANG II since renal vasoconstriction (Fig. 8B) and inhibition of renin secretion (Fig. 8C) by ANG II were not different between GC-A\(-/-\) and GC-A\(+/\+) kidneys. Congruently, renal ANG II type 1 (AT1) receptor mRNA expression was similar in both genotypes (GC-A\(+/\+) mice: 0.86 ± 0.06 AT1 receptor/β-actin mRNA, \(n = 8\), and GC-A\(-/-\) mice: 0.90 ± 0.05 AT1 receptor/β-actin mRNA, \(n = 10\), \(P = 0.6\)).

Infusion of ANP and BNP at relevant in vivo concentrations resulted in concentration-dependent increases in perfusate flow in isolated perfused wild-type kidneys, indicating renal vasodilatation (Fig. 9A). Thus, both ANP and BNP at a concentration of 100 pmol/l enhanced renal perfusate flow (Fig. 9A). Concomitant application of ANG II induced a marked decrease in renal perfusion (Fig. 9A). Neither ANP nor BNP up to a concentration of 1 nmol/l suppressed renin secretion rates from isolated perfused kidneys (Fig. 9B), whereas ANG II potently suppressed renin release, indicating the functionality of the preparation (Fig. 9B). Because renin secretion rates from isolated perfused mouse kidneys were rather low under control conditions, an inhibitory effect of ANP or BNP on renin release may have been difficult to detect. Therefore, renin secretion rates were prestimulated with the β-adrenoreceptor agonist isoproterenol. As shown in Fig. 9C, even with prestimulated renin secretion rates, neither of the natriuretic peptides exerted a significant effect on the renin secretion rate, whereas ANG II potently inhibited it.

To get further insights into the mechanisms by which natriuretic peptides buffer ANG II-dependent renovascular hypertension, the renal vascular reactivity to ANG II was investigated in the absence or presence of a combination of ANP and BNP (Fig. 9, D and E). As expected from the results shown in Fig. 9A, infusion of the natriuretic peptides enhanced renal perfusate flow. Subsequent infusion of ANG II in increasing concentrations reduced renal perfusion in a concentration-dependent manner. However, renal perfusion at the respective ANG II concentrations was higher in the presence of natriuretic peptides compared with the kidneys without concomitant application of natriuretic peptides (Fig. 9D). Even when the perfusate flow values in response to ANG II were related to baseline values (with/without natriuretic peptides, respectively; Fig. 9E), the dose-response curve was shifted to the right by natriuretic peptides, indicating that the renal vasculature is more sensitive to ANG II-induced vasoconstriction in the absence of natriuretic peptide signaling.

**DISCUSSION**

The RAAS and the natriuretic peptide system, consisting of the cardiac natriuretic peptides ANP and BNP and their common receptor GC-A, are two opposing systems that control blood pressure. Both the RAAS and natriuretic peptide system target the kidney, thus indirectly regulating blood pressure via controlling extracellular volume, and they target the cardiovascular system, including direct effects on the vasculature. An infusion of ANP and BNP induces natriuresis and diuresis and,
moreover, suppresses plasma renin levels, thus contributing to a reduction in blood pressure (1, 12). The inhibition of renin release can occur directly at renin-producing juxtaglomerular cells (16) and may involve indirect regulation of the renin system via the tubular or endocrine effects of natriuretic peptides (33). In renovascular hypertension, the increase in plasma renin activity, which causes the increase in blood pressure, is accompanied by high plasma levels of ANP (6, 17, 40, 43). Accordingly, these elevated ANP levels may attenuate the upregulation of renin synthesis and release and may thereby buffer the renovascular hypertension.

In fact, the data from our study clearly show that natriuretic peptides buffer renal hypertension but that the augmentation of the blood pressure response to renal artery stenosis in the absence of natriuretic peptide signaling does not result from a disinhibition of renin release or synthesis. Both ANP−/− and GC-A−/− mice were already hypertensive under baseline conditions, as has been previously demonstrated (13, 14, 20). As in rats (43), plasma ANP levels were significantly elevated in ANP+/+ mice and in GC-A+/+ and GC-A−/− mice in response to renal artery stenosis. Despite the lack of ANP, renal artery stenosis induced blood pressure increases of comparable magnitude in ANP−/− mice and their ANP+/+ littermates, and no signs of a disinhibited renin synthesis and release were observed. Thus, PRC was lower in ANP−/− mice at baseline and did not increase to excessive high levels in response to renal artery clipping, as would be expected if ANP buffered the stimulation of renin release in renovascular hypertension. This conclusion is corroborated by the regulation of renal renin mRNA and renin content, which also did not show signs of disinhibition in ANP−/− mice.

As in a previous report (39), ANP−/− mice had normal plasma BNP concentrations at baseline conditions. In rats, BNP has been reported to be rapidly upregulated within the first 2 days after 2K1C, and BNP levels returned back to baseline values by day 4 (43). Plasma BNP levels only tended to be elevated 7 days after renal artery clipping in the wild-type mice in our study; in contrast, in ANP−/− and GC-A−/− mice, plasma BNP levels were significantly higher in response to 2K1C than in sham mice. ANP and BNP exert their biological functions via the activation of GC-A, and BNP suppresses PRC and reduces blood pressure (12, 26). Accordingly, BNP may compensate for the lack of ANP in ANP−/− mice. In fact, the increase in blood pressure in response to 2K1C was markedly augmented in GC-A−/− mice, which lack both ANP and BNP signaling. Moreover, 4 of 13 GC-A−/− mice died in the first week after renal artery stenosis and were excluded from analysis. These four mice had very high blood pressure values before death. Therefore, although no detailed autopsy was performed, it appears likely that these mice died from hypertensive complications, further underscoring the importance of natriuretic peptide signaling in this situation. The marked increase in blood pressure of the GC-A−/− mice did not result from an overactive renin system. In contrast, PRC was only mildly stimulated by renal artery clipping in GC-A−/− mice, and the stimulation of renin synthesis in the clipped kidney that was observed in GC-A+/+ mice was markedly attenuated in GC-A−/− mice. These data indicate that either stimulation of the renin system by a low renal perfusion pressure is defective in GC-A−/− mice or that other factors, such as the exaggerated arterial hypertension in these mice, properly suppress renin secretion. To test the first possibility, we performed experiments using an isolated perfused mouse kidney model. This model allows the study of renal renin release and renal perfusion under controlled conditions, i.e., without confounding systemic phenotypes. We have previously shown that the major intrarenal control systems of renin release, such as the
renal baroreceptor, endogenous nitric oxide, prostaglandin, and adenosine formation, are intact in our model (2, 34, 35). Moreover, hormonal regulation of renin secretion, such as by ANG II, occurs in the physiological concentration range (42). In fact, the pressure-dependent regulation of renin release was completely intact in GC-A−/− mice, as indicated by the inverse relationship between perfusion pressure and renin secretion rates. Moreover, renin secretion was normally stimulated by the β-adrenoceptor agonist isoproterenol and properly inhibited in response to ANG II in GC-A−/− kidneys. Accordingly, the reduced PRC in GC-A−/− mice in vivo is most likely not caused by an intrinsic renal defect in the regulation of renin.
secretion in GC-A−/− mice but is an indirect consequence of the systemic GC-A−/− phenotype. In fact, ANP−/− and GC-A−/− mice have higher plasma volumes and arterial hypertension compared with wild-type mice, which both suppress renin synthesis and release.

To our knowledge, no data are available on the direct effects of natriuretic peptides on renin release from mouse kidneys. Therefore, to investigate whether the observed increases in plasma ANP and BNP concentrations in response to renal artery clipping were capable of suppressing renin release at all, we performed experiments using the isolated perfused kidney model. The infusion of ANP and BNP did not suppress the renin secretion rate at physiological concentrations that we had determined in mice in vivo. Even when the renin secretion rate was prestimulated by activation of β-adrenoceptors, no inhibition of the renin secretion rate by ANP or BNP was detectable; in contrast, physiological concentrations of ANG II markedly suppressed the renin secretion rate. It is noteworthy that the majority of previous studies using different experimental models have shown an inhibition of renin secretion by exogenous ANP at concentrations near or above the upper limit of physiological levels, whereas in some reports, ANP did not suppress renin release (33). Since ANP can not only directly influence renin secretion at the cellular level but can also indirectly regulate renin release, for instance, by its tubular and cardiovascular effects, it is plausible that the effect of exogenously applied natriuretic peptides on renin secretion critically depends on the experimental model, the method of application, and the dose of ANP (33). Accordingly, our data obtained in isolated perfused mouse kidneys do not exclude the possibility that natriuretic peptides have an inhibitory effect on renin secretion at higher supraphysiological concentrations, in other experimental models or in vivo. However, since all other known regulators of renin secretion that we have tested so far (i.e., catecholamines, PGE2, PGI2, nitric oxide, ANG II, perivascular mechanisms) are known regulators of renin secretion that we have tested so far (i.e., catecholamines, PGE2, PGI2, nitric oxide, ANG II, perivascular mechanisms)

The infusion of ANP and BNP did not suppress the renin secretion rate at physiological concentrations that we had determined in mice in vivo. Even when the renin secretion rate was prestimulated by activation of β-adrenoceptors, no inhibition of the renin secretion rate by ANP or BNP was detectable; in contrast, physiological concentrations of ANG II markedly suppressed the renin secretion rate. It is noteworthy that the majority of previous studies using different experimental models have shown an inhibition of renin secretion by exogenous ANP at concentrations near or above the upper limit of physiological levels, whereas in some reports, ANP did not suppress renin release (33). Since ANP can not only directly influence renin secretion at the cellular level but can also indirectly regulate renin release, for instance, by its tubular and cardiovascular effects, it is plausible that the effect of exogenously applied natriuretic peptides on renin secretion critically depends on the experimental model, the method of application, and the dose of ANP (33). Accordingly, our data obtained in isolated perfused mouse kidneys do not exclude the possibility that natriuretic peptides have an inhibitory effect on renin secretion at higher supraphysiological concentrations, in other experimental models or in vivo. However, since all other known regulators of renin secretion that we have tested so far (i.e., catecholamines, PGE2, PGI2, nitric oxide, ANG II, perfusion pressure, and tubular salt transport) exerted profound effects on renin secretion rates in our isolated perfused mouse kidney model, the absence of an effect of ANP and BNP on renin secretion rates suggests that their direct effect at the organ level is, if at all, rather small. This conclusion is compatible with the reduced PRC in ANP−/− and GC-A−/− mice compared with their respective wild-type littermates. If natriuretic peptides per se had an overriding direct inhibitory effect on renal renin release, PRC in ANP−/− and GC-A−/− mice should be elevated and not reduced under control conditions. The fact that this outcome was not the case in our present and previous studies in vivo clearly indicates that the direct effect of natriuretic peptides on renin release in mice is either absent or is inferior to the effects of other systemic regulators of renin secretion, such as the extracellular volume or blood pressure, which are both elevated in ANP−/− and GC-A−/− mice, as has been shown in the present study and previously demonstrated in GC-A−/− mice (36) and suggested for ANP−/− mice (13).

In contrast to their ineffectiveness on renin secretion, both ANP and BNP concentration dependently induced renal vasorelaxation in isolated perfused mouse kidneys, which is an effect that has been observed in other species and might well explain the blood pressure-buffering capacity of natriuretic peptides. Moreover, the combination of ANP and BNP in relevant concentrations mitigated the ANG II-dependent vasoconstriction in isolated perfused kidneys. Since ANP and BNP both exert their effects via activation of GC-A, this anticontractile effect of natriuretic effects might not occur in GC-A−/− mice in vivo. Therefore, it may be speculated that the vascular sensitivities toward ANG II is enhanced in GC-A−/− mice compared with wild-type mice. Together with the other prohypertensive phenotypes of GC-A−/− mice, such as the elevated plasma volume, the enhanced vascular reactivity to ANG II can explain the marked increase in arterial blood pressure in response to renal artery clipping in GC-A−/− mice despite the rather mild activation of the renin-angiotensin system.

As mentioned above, the role of natriuretic peptides in blood pressure control has been clearly shown in gene-targeted mice. With effects similar to those of pharmacological elevation of plasma ANP and BNP levels, genetic overexpression of ANP and BNP and their receptor GC-A results in hypertension (26, 28, 37). Consistent with these results, GC-A−/− and ANP−/− mice are also hypertensive (13, 14, 20, 27). While ANP−/− mice largely resemble the hypertensive phenotype of GC-A−/− mice, BNP deletion does not result in hypertension (13, 38). These data indicate that BNP is not critically involved in blood pressure regulation under physiological conditions, and its function appears to be more important in pathological situations, such as congestive heart failure, that lead to high plasma BNP concentrations (24). In our study, plasma ANP levels were significantly elevated in wild-type mice in renal hypertension, indicating that ANP is released in response to an increase in blood pressure even in otherwise healthy mice. BNP levels only tended to be elevated in 2K1C wild-type mice compared with sham mice but were significantly stimulated by renal artery clipping in ANP−/− and GC-A−/− mice, which have arterial hypertension and high plasma volume already under control conditions. Thus, our data further underline the assumption that BNP is more relevant in severe pathophysiological situations, whereas ANP is the dominant peptide in situations of minor disturbances of fluid homeostasis or blood pressure. Moreover, our results clearly allow the conclusion that natriuretic peptide signaling via GC-A is an important protective yet renin-independent mechanism for buffering renovascular hypertension.

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AUTHOR CONTRIBUTIONS

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