Guanylyl cyclase/natriuretic peptide receptor-A gene disruption causes increased adrenal angiotensin II and aldosterone levels

Di Zhao, Elangovan Vellai chamy, Naveen K. Somanna, and Kailash N. Pandey

Department of Physiology, Tulane University Health Sciences Center School of Medicine, New Orleans, Louisiana

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Zhao D, Vellai chamy E, Somanna NK, Pandey KN. Guanylyl cyclase/natriuretic peptide receptor-A gene disruption causes increased adrenal angiotensin II and aldosterone levels. Am J Physiol Renal Physiol 293: F121–F127, 2007. First published March 27, 2007; doi:10.1152/ajprenal.00478.2006.—Disruption of the guanylyl cyclase-A/natriuretic peptide receptor-A (GC-A/NPRA) gene leads to elevated arterial blood pressure and congestive heart failure in mice lacking NPRA. This study was aimed at determining whether Npr1 (coding for GC-A/NPRA) gene copy number affects adrenal ANG II and aldosterone (Aldo) levels in a gene-dose-dependent manner in Npr1 gene-targeted mice. Adrenal ANG II and Aldo levels increased in 1-copy mice compared with 2-copy mice, but decreased in 3-copy and 4-copy mice. In contrast, renal ANG II levels decreased in 1-copy (25%), 3-copy (38%), and 4-copy (39%) mice compared with 2-copy mice. The high-salt diet suppressed adrenal ANG II and Aldo levels in 1-copy (46 and 29%) and 2-copy (38 and 17%) mice. On the other hand, the low-salt diet stimulated renal ANG II levels in 1-copy (45%), 2-copy (45%), 3-copy (59%), and 4-copy (48%) mice. However, the high-salt diet suppressed renal ANG II levels in 1-copy (28%) and 2-copy (27%) mice. In conclusion, NPRA signaling antagonizes adrenal ANG II and Aldo levels in a gene-dose dependent manner. Increased adrenal ANG II and Aldo levels may play an important role in elevated arterial blood pressure and progressive hypertension, leading to renal and vascular injury in Npr1 gene-disrupted mice.

salt diets; gene duplication

Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) constitute the natriuretic peptide family (6, 40, 45). ANP and BNP are mainly released from the heart (7, 27, 46), whereas CNP is produced in endothelial cells (45). Three distinct natriuretic peptide receptors have been identified and characterized by molecular cloning; these are natriuretic peptide receptor-A (NPRA), natriuretic peptide receptor-B (NPBR), and natriuretic peptide receptor-C (NPRC) (12, 38, 41). ANP and BNP bind to NPRA, a membrane-bound form of guanylyl cyclase also known as GC-A (10, 35). CNP binds to NPRB, which is also a membrane-bound form of guanylyl cyclase type of receptor called GC-B (12, 16, 41). All three of the natriuretic peptides also bind to NPRC, which lacks guanylyl cyclase activity (12, 22). ANP binding to NPRA increases intracellular second-messenger cGMP concentration by enhanced guanylyl cyclase activity (21, 33, 34, 42). The major effects of ANP-NPRA signaling are natriuretic, diuretic, vasodilatory, antifibrotic, and antihypertrophic (19, 30, 33, 48). Npr1 is the gene that codes for NPRA (13, 14). It has been reported that Npr1 expression affects arterial blood pressure in a gene-dose-dependent manner in Npr1 gene-targeted mice (30, 31, 44). Our previous studies have indicated that NPRA signaling exerts protective effects with respect to blood pressure and cardiovascular regulation and plays important roles in other physiological functions (30, 37, 43, 48).

The adrenal gland regulates blood volume homeostasis and blood pressure mainly by secreting aldosterone (Aldo). A shift has occurred in recent years from an emphasis on the function of the circulating renin-angiotensin system (RAS) to a focus on the local tissue RAS in the brain, heart, peripheral blood vessels, adrenal glands, and kidney (39). Sodium restriction has been found to increase adrenal renin activity and Aldo production in parallel (8) and to increase angiotensin II (ANG II) secretion from the zona glomerulosa cells (5). Nephrectomy has been shown to increase adrenal renin activity via increased serum potassium but to decrease plasma renin to undetectable levels (8, 9). Although some studies have shown that adrenal ANG II may be important in Aldo production through a paracrine or autocrine effect (5, 24), the function of the adrenal RAS in regulating adrenal function still awaits further study.

ANP exerts its biological activity by binding to NPRA, which is predominantly located in the renal glomeruli and adrenal zona glomerulosa (4). ANP inhibits adrenal ANG synthesis (1, 3, 11) and renal renin secretion (22). It is likely that the inhibitory effect of ANP on Aldo synthesis depends on the functionally active NPRA (32). Adrenal renin activity and mRNA expression were found to be increased in Npr1 gene-disrupted mice compared with wild-type mice (43). Since ANP-NPRA signaling counteracts the RAS in a tissue-specific manner (44), we investigated adrenal ANG II and Aldo levels in Npr1 gene-disrupted and gene-duplicated mice. We also studied the effect of a low- or high-salt diet on adrenal ANG II and Aldo levels in Npr1 gene-disrupted and gene-duplicated mice.

METHODS

Animals. Npr1 gene-targeted mice were generated by homologous recombination as previously described (30). All mice were littermate male or female mice 24–32 wk old. The following are the mice genotypes used: 0-copy (−/−) is a homozygous mutant allele; 1-copy (+/−) is a heterozygous allele; 2-copy (+/+) is a wild-type allele; 3-copy (+/−) is a gene-duplicated heterozygous allele; and 4-copy (+/+ +) is a gene-duplicated homozygous allele. Mice were maintained at a 12:12 alternating light-dark schedule (light from 6 AM to 6 PM)
at 25°C. They were given a low-salt (0.05% NaCl), normal-salt (0.3% NaCl), or high-salt (8% NaCl) diet along with tap water during the 3 wk of this study. All protocols were approved by the Institutional Animal Care and Use Committee at Tulane University Health Sciences Center.

Blood pressure measurement. We measured the systolic blood pressures of Npr1 gene-targeted mice every other day during the 3-wk experimental period by the noninvasive computerized tail-cuff method with Visitech 2000 (Apex, NC) as previously described (17, 43). An average systolic blood pressure level of 5 sessions/day was calculated for analysis after 7 days of training for blood pressure measurement.

Adrenal gland, kidney, and plasma collection. Animals under CO2 anesthesia were killed by cardiac puncture. Blood was collected with 10 μl 0.25 M EDTA. After centrifugation at 3,000 rpm for 30 min, plasma was separated and kept at −80°C until used. Adrenal glands and kidneys were removed, weighed, and frozen in liquid nitrogen and kept at −80°C until used.

Adrenal and renal ANG II extraction. Adrenal glands and kidneys were rinsed in 0.5 ml buffer and homogenized with a Polytron (Brinkman Instruments, Westbury, NY) at a setting of 10 (3 times) for 30 s at 4°C in 1.5 ml buffer containing 10 mM pyrophosphate, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 mM EDTA (36). The supernatants were collected after centrifugation at 40,000 g for 40 min at 4°C. We determined the protein concentrations of adrenal and renal extract using a protein assay reagent (Bio-Rad, Hercules, CA). We used 200-mg Sep-Pak C18 columns (Waters Associates, Milford, MA) for ANG II extraction; the columns were equilibrated with 3 ml 0.1% trifluoroacetic acid (TFA) and washed with 10 ml 0.1% TFA-1% NaCl. The 1 ml adrenal or renal extract was loaded and eluted with a 2-ml mixture of methanol:water:TFA (80:19:1). Eluates were dried overnight in a Speed-Vac centrifuge. The residues were kept at 20°C and for ANG II assay were dissolved into 250 μl 100 mM Tris-acetate buffer (pH, 7.5) containing 1 mM PMSF, 0.02% sodium azide, and 0.1% bovine serum albumin.

Adrenal Aldo extraction. A 0.5-ml adrenal extract and a 5-ml mixture of hexane:ethyl acetate (3:2) were pipetted into a glass tube and vortexed vigorously for 1 min. The 4-ml organic layer was transferred into another glass tube and dried overnight in a Speed-Vac centrifuge. The residues were kept at 20°C and for the Aldo assay were dissolved into 230-μl Aldo zero standards (Diagnostic System, Webster, TX).

ANG II and Aldo assay. We measured adrenal, renal, and plasma ANG II levels using radioimmunoassay (RIA) as previously reported (36, 43). Briefly, bound and free ANG II peptides were separated by dextran-coated charcoal after a 48 h of incubation at 4°C. The supernatants were counted by a gamma counter for 1 min. The results are presented as femtomoles per milligram of adrenal or renal protein content. Adrenal and plasma Aldo levels were assayed as described before (43) using the RIA Kit (Diagnostic System, Webster, TX) and are presented as picograms Aldo per milligram of protein content.

Statistical analysis. The results are expressed as means ± SE. The differences between groups were determined by ANOVA combined with Dunnett’s multiple comparison post hoc test using GraphPad PRISM (version 4.0; GraphPad Software, San Diego, CA). A value of \( P < 0.05 \) was used as the criterion to indicate a statistically significant difference.

RESULTS

Effect of different salt diets on systolic blood pressure. With mice receiving a normal-salt diet, systolic blood pressure (mmHg) increased in 1-copy mice (12%, 114.0 ± 1.9, \( P < 0.01 \)) compared with 2-copy mice (102.1 ± 2.0), but decreased in 3-copy (9%, 92.7 ± 1.8, \( P < 0.01 \)) and 4-copy mice (16%, 86.2 ± 2.2, \( P < 0.01 \)) (Fig. 1A). With a low-salt diet, systolic blood pressures increased in 1-copy mice (11%, 116.0 ± 4.4, \( P < 0.01 \)) compared with 2-copy mice (104.3 ± 2.1), but decreased in 3-copy (10%, 93.7 ± 1.8, \( P < 0.01 \)) and 4-copy mice (18%, 85.3 ± 2.6, \( P < 0.001 \)) (Fig. 1B). Similarly, with...
a high-salt diet, systolic blood pressures increased in 1-copy mice (13%, 126.9 ± 2.3, \( P < 0.05 \)) compared with 2-copy mice (112.2 ± 3.2), but decreased in 3-copy (16%, 94.7 ± 2.8, \( P < 0.01 \)) and 4-copy mice (24%, 85.9 ± 2.4, \( P < 0.001 \)) (Fig. 1C). We compared blood pressures in relation to different salt diets in the same \( \text{Npr1} \) gene copy number mice. The high-salt diet increased systolic blood pressures in 1-copy (11%, \( P < 0.01 \)) and 2-copy mice (10%, \( P < 0.05 \)) compared with a normal-salt diet (Fig. 1, A and C), whereas the low-salt diet did not alter systolic blood pressures in 1-copy, 2-copy, 3-copy or 4-copy mice (Fig. 1, A and B).

**Adrenal ANG II level with different salt diets.** With a normal-salt diet, adrenal ANG II levels (fmol/mg protein) increased in 1-copy mice (15%, 74.8 ± 2.7, \( P < 0.05 \)) compared with 2-copy mice (65.1 ± 2.7), but decreased in 3-copy (17%, 53.9 ± 2.7, \( P < 0.05 \)) and 4-copy mice (33%, 43.7 ± 4.7, \( P < 0.01 \)) (Fig. 2A). Similarly, adrenal ANG II levels increased with a low-salt diet in 1-copy mice (20%, 89.8 ± 4.7, \( P < 0.05 \)) compared with 2-copy mice (74.8 ± 3.0), but decreased in 3-copy (14%, 64.6 ± 2.2, \( P < 0.05 \)) and 4-copy mice (24%, 57.2 ± 3.1, \( P < 0.01 \)) (Fig. 2B). On the other hand, with a high-salt diet there were no statistical differences among adrenal ANG II levels in 1-copy (40.6 ± 3.3), 3-copy (40.9 ± 5.8), or 4-copy mice (39.6 ± 4.7) compared with 2-copy mice (40.1 ± 4.1) (Fig. 2C).

In \( \text{Npr1} \) mice with different numbers of gene copies, the low-salt diet increased adrenal ANG II levels in 1-copy mice (20%, \( P < 0.05 \)), 2-copy (15%, \( P < 0.05 \)), 3-copy (20%, \( P < 0.05 \)), and 4-copy mice (31%, \( P < 0.05 \)) compared with levels in those given a normal-salt diet (Fig. 2, A and B). In contrast, the high-salt diet decreased adrenal ANG II levels in 1-copy (46%, \( P < 0.001 \)) and 2-copy mice (38%, \( P < 0.01 \)), but not in 3-copy and 4-copy mice (Fig. 2, A and C).

**Adrenal Aldo level with different salt diets.** With a normal-salt diet, adrenal Aldo levels (pmol/mg protein) were increased in 1-copy mice (38%, 8.7 ± 0.8, \( P < 0.05 \)) compared with 2-copy mice (6.3 ± 0.2), but decreased in 3-copy (13%, 5.5 ± 0.3, \( P < 0.05 \)) and 4-copy mice (38%, 3.8 ± 0.4, \( P < 0.001 \)) (Fig. 3A). Similarly, with a low-salt diet, adrenal Aldo levels were increased in 1-copy mice (44%, 221.4 ± 19.6, \( P < 0.05 \)) compared with 2-copy mice (153.6 ± 20.9), but decreased in 3-copy (81%, 28.6 ± 4.0, \( P < 0.001 \)) and 4-copy mice (85%, 22.8 ± 3.7, \( P < 0.001 \)) (Fig. 3B). However, with a high-salt diet, adrenal Aldo levels in 1-copy (6.2 ± 0.7), 3-copy (4.8 ± 0.4), and 4-copy mice (4.2 ± 0.5) did not statistically differ from levels in 2-copy mice (5.2 ± 0.3) (Fig. 3C).

In \( \text{Npr1} \) mice with different numbers of gene copies, the low-salt diet increased adrenal Aldo levels compared with levels in those fed a normal-salt diet (Fig. 3, A and B). Specifically, the low-salt diet increased the Aldo level in 1-copy mice by 2,440.6%, or 24.4-fold (\( P < 0.001 \)); 2-copy mice by 2,338.9%, or 23.4-fold (\( P < 0.001 \)); 3-copy mice by 424.0%, or 4.2-fold (\( P < 0.001 \)); and 4-copy mice by 485.7%, or 4.9-fold (\( P < 0.001 \)). In contrast, the high-salt diet decreased adrenal Aldo levels in 1-copy (29.1%, \( P < 0.05 \)) and 2-copy mice (17.2%, \( P < 0.05 \)), but not in 3-copy or 4-copy mice (Fig. 3, A and C).

**Renal ANG II level with different salt diets.** With a normal-salt diet, renal ANG II levels (fmol/mg protein) were decreased in 1-copy (25%, 7.7 ± 0.8, \( P < 0.05 \)), 3-copy (38%, 6.4 ± 0.8, \( P < 0.01 \)), and 4-copy mice (39%, 6.3 ± 0.7, \( P < 0.01 \)) compared with 2-copy (10.3 ± 0.8) mice (Fig. 4A). Similarly, with a low-salt diet, renal ANG II levels were decreased in 1-copy (25%, 11.2 ± 1.0, \( P < 0.05 \)), 3-copy (32%, 10.1 ± 1.6, \( P < 0.05 \)), and 4-copy mice (38%, 9.2 ± 1.1, \( P < 0.01 \)) compared with 2-copy (14.9 ± 1.0) mice (Fig. 4B). With a high-salt diet, although renal ANG II levels were decreased in 1-copy mice (26%, 5.6 ± 0.6, \( P < 0.01 \)), only minor statistical differences were found in 3-copy (7.0 ± 0.9) and 4-copy (6.3 ± 0.7) mice compared with 2-copy mice (7.5 ± 0.6) (Fig. 4C).
In *Npr1* mice, the low-salt diet increased renal ANG II levels in 1-copy (45%, \(P < 0.05\)), 2-copy (45%, \(P < 0.05\)), 3-copy (59%, \(P < 0.05\)), and 4-copy mice (48%, \(P < 0.05\)) compared with levels when mice were fed a normal-salt diet (Fig. 4, A and B). However, the high-salt diet decreased renal ANG II levels in 1-copy (28%, \(P < 0.05\)) and 2-copy mice (27%, \(P < 0.05\)), but not in 3-copy or 4-copy mice (Fig. 4, A and C).

Plasma ANG II and Aldo levels with different salt diets. The levels of plasma ANG II and Aldo with different salt diets are shown, respectively, in Tables 1 and 2. With a normal-salt diet, plasma ANG II levels decreased in 1-copy (45%), 3-copy (25%), and 4-copy (32%) mice compared with 2-copy mice (Table 1). In contrast, plasma Aldo levels increased only in 1-copy mice (33%) compared with 2-copy mice, while levels in 3-copy (36%) and 4-copy (48%) mice decreased (Table 2). With a low-salt diet, plasma ANG II levels also decreased in 1-copy (20%), 3-copy (17%), and 4-copy (26%) mice compared with 2-copy mice. Conversely, plasma Aldo levels increased only in 1-copy (45%) mice compared with 2-copy mice, but decreased in 3-copy (25%) and 4-copy (30%) mice. In contrast, with a high-salt diet, plasma ANG II levels decreased in 1-copy (55%) mice compared with 2-copy mice; however, the plasma ANG II levels in 3-copy and 4-copy mice did not statistically differ from that in 2-copy mice. Similarly, plasma Aldo levels in 1-copy, 3-copy, and 4-copy mice did not statistically differ from that in 2-copy mice with a high-salt diet.
Table 1. Plasma angiotensin II levels in Npr1 gene-disrupted and gene-duplicated mice fed different salt diets

<table>
<thead>
<tr>
<th>Salt Diet</th>
<th>1-copy (+/-)</th>
<th>2-copy (+/+), wild-type allele</th>
<th>3-copy (+/+) gene-duplicated heterozygous allele</th>
<th>4-copy (+/+++) gene-duplicated homozygous allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt</td>
<td>47.1±3.9a</td>
<td>85.0±3.1</td>
<td>63.6±4.0b</td>
<td>57.6±3.8c</td>
</tr>
<tr>
<td>Low salt</td>
<td>87.5±5.7d</td>
<td>108.8±5.9c</td>
<td>90.7±5.4ae</td>
<td>80.9±8.4ad</td>
</tr>
<tr>
<td>High salt</td>
<td>24.3±2.0d</td>
<td>53.4±6.4e</td>
<td>52.2±3.3</td>
<td>49.6±5.5</td>
</tr>
</tbody>
</table>

Values are means ± SE (pmol/l) for the representative Npr1 mice genotypes, including 1-copy (+/-), gene-disrupted heterozygous allele; 2-copy (+/+), wild-type allele; 3-copy (+/+) gene-duplicated heterozygous allele; and 4-copy (+/+++) gene-duplicated homozygous allele. Comparisons were made among different Npr1 mice genotypes fed the same salt diet (\(P<0.05\), \(P<0.01\), \(P<0.001\)) as well as among mice fed diets with different amounts of salt in the same Npr1 gene copy number mice (\(P<0.05\), \(P<0.01\), \(P<0.001\)).

We also analyzed the effect of different salt diets on plasma ANG II and Aldo levels within the same Npr1 gene copy number mice. Compared with the levels obtained with a normal-salt diet, the low-salt diet increased plasma ANG II levels in 1-copy (86%), 2-copy (28%), 3-copy (43%), and 4-copy (41%) mice (Table 1). Aldo levels were also greatly increased in 1-copy (184%), 2-copy (159%), 3-copy (206%), and 4-copy (250%) mice fed with a low-salt diet. However, the high-salt diet decreased plasma ANG II levels in 1-copy (48%) and 2-copy mice (37%). Similarly, Aldo levels were also decreased in 1-copy (54%) and 2-copy mice (42%) kept on a high-salt diet (Table 2).

**DISCUSSION**

The present results demonstrate that adrenal ANG II and Aldo levels are increased in 1-copy mice lacking one Npr1 allele compared with levels in 2-copy mice, but that ANG II and Aldo levels are decreased in gene-duplicated 3-copy and 4-copy mice in a gene-dose dependent manner. In line with these findings, our previous studies have also shown that the adrenal renin activity is increased in Npr1 null mutant mice (43). Previous studies have shown that adrenal RAS greatly influences the production of Aldo both in vitro and in vivo (23, 24). The same studies indicated that antagonism of ANP-NPRA signaling on the adrenal RAS may lead to the inhibition of adrenal Aldo production and secretion. This may explain, at least in part, our finding that compared with levels in wild-type mice, adrenal Aldo levels were increased in Npr1 gene-disrupted mice but decreased in Npr1 gene-duplicated mice.

In the present study, a low-salt diet stimulated adrenal ANG II and Aldo levels in 1-, 2-, 3-, and 4-copy mice compared with the levels in mice given a normal-salt diet. However, a high-salt diet suppressed adrenal ANG II and Aldo levels in 1- and 2-copy mice, but not in 3- and 4-copy mice compared with levels obtained in mice given a normal-salt diet. Indeed, we were surprised by the significant response of adrenal aldosterone levels to the low-salt diet. It is interesting that adrenal aldosterone levels were increased >20-fold in 1- and 2-copy mice given a low-salt diet, but increased only about 4-fold in 3- and 4-copy mice. This suggested that in mice fed the low-salt diet, the elevation of adrenal Aldo level was effectively attenuated by increased Npr1 gene copy number. These results suggested that the Npr1 gene copy number affects adrenal Aldo level, especially with a low-salt diet. Sodium restriction increases adrenal renin activity, ANG II release, and Aldo production (5, 8, 24). In a single-cell study, superfused zona glomerulosa cells from rats kept on a low-sodium diet secreted 1.5-fold higher ANG II than did cells from rats kept on a high-sodium diet (23). The increased adrenal ANG II concentration induced by salt restriction may have led to an increase in Aldo production. On the other hand, compared with a low-sodium diet, a high-sodium diet has been shown to decrease the percentage of secretory zona glomerulosa cells (23).

Adrenal Aldo levels were also affected by Npr1 gene copy number in mice given the high-salt diet. Specifically, adrenal Aldo levels in 1- and 2-copy mice were suppressed in response to the high-salt diet compared with levels in mice fed the normal-salt diet. However, this diet did not suppress adrenal Aldo levels in 3- and 4-copy mice. The underlying mechanism may be related to the elevated sodium excretion and urine output that occurs with relatively lower blood pressure in 3- and 4-copy mice than in 2-copy mice; thus, the inhibitory effect of the high-salt diet on adrenal Aldo levels was attenuated in 3- and 4-copy mice. Adrenal Aldo levels in 1- and 2-copy mice decreased to a similar level with 3- and 4-copy mice on a high-salt diet. Therefore, both the low-salt and high-salt data further confirmed that Npr1 gene copy number affects adrenal Aldo levels. Our findings further suggest that the adrenal gland is more sensitive to a low-salt diet than to a high-salt diet, which is consistent with the fact that the adrenal gland functions to retain salt to regulate blood volume homeostasis. Also, it is well known that a low-salt diet stimulates plasma ANG II and Aldo levels, whereas a high-salt diet inhibits them (18, 26).

We found that renal ANG II levels were decreased in 1-copy, 3-copy, and 4-copy mice compared with 2-copy mice. Interestingly, our previous studies have shown that renal ANG II levels increased in newborn pups of Npr1 gene-disrupted mice more than that of wild-type mice. However, renal ANG II levels decreased in both young (at 3 wk of age) as well as adult (at 16–26 wk of age) Npr1 gene-disrupted mice compared with wild-type mice (43). The mechanism may be due to the elevated blood pressure that decreases renal ANG II levels by inhibiting renin synthesis and release from the kidney juxtaglomerular cells as a negative feedback mechanism in Npr1 gene-disrupted mice (43). The significant changes in renal ANG II levels at different ages in Npr1 gene-disrupted mice indicated that renal ANG II levels may be regulated by ANP-NPRA signaling and also by additional factors, including salt diet, blood pressure, plasma and tissue RAS, and other hormonal milieu (2, 47, 49, 50). In 1-copy mice, adrenal ANG II and Aldo levels were higher than 2-copy mice. The mechanism that...
might be related to increased adrenal renin levels in 1-copy mice compared with 2-copy mice (43). It may indicate that the adrenal RAS is mainly regulated by local tissue factors other than circulating factors. Variations in dietary sodium intake are closely associated with changes in renal renin and ANG II contents (28). In our study, the low-salt diet stimulated renal ANG II levels in 1-copy, 2-copy, 3-copy, and 4-copy mice compared with ANG II levels with the normal-salt diet. The high-salt diet suppressed renal ANG II levels in 1-copy and 2-copy mice, but not in 3-copy and 4-copy mice. These data also further confirmed that variations in dietary salt intake affect renal ANG II levels.

Interestingly, plasma ANG II levels decreased in 1-copy Npr1 mice compared with 2-copy mice, whereas plasma Aldo levels increased in 1-copy mice. The increased plasma Aldo levels may be due to elevation in the adrenal renin level in 1-copy mice compared with 2-copy mice, as reported previously (43). Elevated arterial blood pressure decreases the plasma ANG II level by inhibiting renin synthesis; the release of renin from the kidney juxtaglomerular cells is a negative feedback mechanism in 1-copy mice but not in 2-copy mice (43). On the other hand, decreased plasma ANG II levels in Npr1 gene-duplicated 3- and 4-copy mice compared with levels in 2-copy mice seem to be mainly a consequence of antagonism of ANP/NPRA signaling to the RAS. Plasma ANG II levels in different Npr1 gene copy mice indicated that the levels of ANG II in plasma might be regulated not only by NPRA signaling but also by factors such as arterial blood pressure and other hormonal factors. Elevations in renal ANG II levels cause reductions in renal function and sodium excretion that contribute to progressive hypertension and lead to renal and vascular injury (29). In the present study, the low-salt diet stimulated plasma ANG II and Aldo levels in 1-, 2-, 3-, and 4-copy Npr1 mice compared with levels found in the same mice given a normal-salt diet. However, the high-salt diet inhibited plasma ANG II and Aldo levels in 1- and 2-copy mice, but not in 3- and 4-copy mice. Increased sodium excretion and urine output with decreased arterial blood pressure in 3- and 4-copy mice might attenuate the inhibitory effect of the high-salt diet on plasma ANG II and Aldo levels (44). Our findings suggest that NPRA signaling has a protective effect on blood volume homeostasis and blood pressure regulation in Npr1 gene-duplicated mice compared with Npr1 gene-disrupted mice.

We further delineated the effect of Npr1 gene copy number on blood pressure in a gene-dose dependent manner with the three salt diets. Intriguingly, the high-salt diet caused an increase in blood pressure in 1- and 2-copy mice, but did not exert a significant effect on blood pressure in 3- and 4-copy mice. Previously, it has been reported that blood pressure was decreased in 4-copy mice on a high-salt diet compared with blood pressure in 4-copy mice fed a low-salt diet (31). Earlier studies have also suggested that the Npr1 gene, like the gene coding for ANP, may directly affect the sensitivity of blood pressure to salt (15, 25). Our present results with Npr1 gene-targeted mice provide evidence that NPRA signaling has a protective effect on blood pressure regulation in response to high salt. However, in another Npr1 gene knockout mouse model, neither a low- nor high-salt diet affected systemic blood pressure, although mice lacking the Npr1 gene had elevated systolic blood pressure and the authors concluded that hypertension is salt resistant in that model (20). Both of these mouse models focus on the Npr1 gene, but the detailed gene-targeting method is different (20, 30). In the model we used, the gene-targeting method involves replacing exon 1 and intron 1 of the Npr1 gene with a DNA fragment containing a neomycin-selectable marker (30). The other model uses simple insertion of a neomycin-resistance gene into exon 4 of the Npr1 gene (20). Although a similar degree of hypertension has been confirmed in that model, the heart, kidneys, and vasculature of the mutant mice were normal after histological examination at <5 mo of age (20, 30). The contradictory results in these models regarding the sensitivity of blood pressure to salt is likely to be related to the greater difficulty of observing small genetic effects in F2 vs. F1 mice (32). However, we cannot exclude the possibility that the contradictory results also involve the use of different gene-targeting methods. Our findings suggest that NPRA signaling has a protective effect against high-salt in Npr1 gene-duplicated mice compared with Npr1 gene-disrupted mice.

The results obtained in the present study provide evidence that adrenal ANG II and Aldo levels are increased in Npr1 gene-disrupted mice and, moreover, this increase appears to participate in elevating systemic blood pressure in mice with decreasing Npr1 gene-copy numbers. On the other hand, adrenal ANG II and Aldo levels are decreased in Npr1 gene-duplicated mice. A low-salt diet stimulated adrenal ANG II and Aldo levels in all Npr1 gene-targeted mice, whereas a high-salt diet suppressed adrenal ANG II and Aldo levels in Npr1 gene-disrupted mice and wild-type mice, but not in Npr1 gene-duplicated mice. Together, the present results clearly demonstrate the importance of ANP-NPRA signaling on the regulation of adrenal ANG II and Aldo levels. The interaction between ANP-NPRA signaling and local independent tissue ANG II and Aldo levels seems to be critical in the regulation of blood pressure and cardiovascular disease states.

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