ANP-mediated inhibition of distal nephron fractional sodium reabsorption in wild-type and mice overexpressing natriuretic peptide receptor

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Submitted 19 August 2009; accepted in final form 5 November 2009

Zhao D, Pandey KN, Navar LG. ANP-mediated inhibition of distal nephron fractional sodium reabsorption determined after blockade of the two major distal nephron sodium transporters with amiloride (5 μg/g body wt) plus bendroflumethiazide (12 μg/g body wt) in male anesthetized C57/B6L and natriuretic peptide receptor-A gene (Npr1) targeted four-copy mice. The lower dose of ANP (0.1 ng/g body wt−1·min−1, n = 6) increased distal sodium delivery (DSD, 2.4 ± 0.4 vs. 6.6 ± 0.2 μeq/min, P < 0.05) but did not change fractional reabsorption of DSD compared with control (86.3 ± 2.0 vs. 83.9 ± 3.6%, P > 0.05), thus limiting the magnitude of the natriuresis. In contrast, the higher dose (0.2 ng/g body wt−1·min−1, n = 6) increased DSD (2.8 ± 0.3 μeq/min, P < 0.01) and also decreased fractional reabsorption of DSD (67.4 ± 4.5%, P < 0.01), which markedly augmented the natriuresis. In Npr1 gene-duplicated four-copy mice (n = 6), the lower dose of ANP increased urinary sodium excretion (0.6 ± 0.1 vs. 0.3 ± 0.1 μeq/min, P < 0.05) and decreased fractional reabsorption of DSD compared with control (72.2 ± 3.4%, P < 0.05) at similar mean arterial pressures (91 ± 6 vs. 92 ± 3 mmHg, P > 0.05). These results provide in vivo evidence that ANP-mediated increases in DSD alone exert modest effects on sodium excretion. For the present study, we determined the effects of ANP on the possible changes in tubular sodium reabsorption in vivo. The present experiments were performed in both wild-type and gene-targeted mice overexpressing natriuretic peptide receptor-A gene (Npr1) to obtain in vivo evidence regarding the natriuretic actions of ANP.

The distal nephron segments are ultimately responsible for the fine regulation of sodium excretion, whereas the proximal tubule is responsible for reabsorbing the bulk of the GFR (18, 44). In distal nephron segments, sodium reabsorption is mainly mediated by amiloride (AM) sensitive-epithelial sodium channels (ENaC) and thiazide-sensitive Na+-Cl− cotransporter (NCC). During treatment with AM plus bendroflumethiazide (BFTZ) to block most of sodium transport in distal nephron segments, sodium excretion can be used as a collective measure of sodium delivery to distal nephron segments (18, 21, 49, 50). Sodium reabsorption in distal nephron segments can thus be determined from the difference between urinary sodium excretion during distal blockade and control urinary sodium excretion. For the present study, we determined the effects of two doses of ANP on sodium reabsorption in distal nephron segments using the method previously described (49, 50). The approach utilized provides a noninvasive collective assessment of distal nephron sodium reabsorption that may be particularly useful in screening studies using gene-targeted mice.

METHODS

Animals. Studies were performed on 9- to 12-wk-old male C57/B6L (Jackson Laboratory, Bar Harbor, ME) and Npr1 gene-duplicated mice (24, 28, 51) that were maintained on a 12:12-h light-dark schedule (6:00 A.M. to 6:00 P.M.) at 25°C in the vivarium at Tulane University Health Sciences Center. The protocol was approved by the...
Institutional Animal Care and Use Committee of Tulane University

Table 1. Time control

<table>
<thead>
<tr>
<th>Period</th>
<th>AP, mmHg</th>
<th>GFR, ml/min</th>
<th>UF, µl/min</th>
<th>UNaV, µeq/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1</td>
<td>93 ± 2</td>
<td>0.18 ± 0.03</td>
<td>3.4 ± 0.8</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Period 2</td>
<td>92 ± 2</td>
<td>0.20 ± 0.06</td>
<td>3.5 ± 0.9</td>
<td>0.19 ± 0.06</td>
</tr>
<tr>
<td>Period 3</td>
<td>91 ± 2</td>
<td>0.18 ± 0.04</td>
<td>3.7 ± 0.3</td>
<td>0.19 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 mice in each group. AP, arterial pressure; GFR, glomerular filtration rate; UF, urine flow; UNaV, urinary sodium excretion.

Experimental protocol. On the day of experiment, mice were anesthetized with Inactin (thiobutabarbital sodium) injected intraperitoneally at 200 µg/g body wt. Supplemental doses of anesthesia were administrated as required to maintain a stable plane of anesthesia. Once a stable level of anesthesia was obtained, judged by heart rate and lack of toe reflex, mice were placed on a surgical table (37°C) with servo-control of temperature to maintain body temperature at 37°C. A tracheostomy was performed with PE-90 tubing, and mice were allowed to breathe air enriched with O2 by placing the exterior end of the tracheal cannula inside a small plastic chamber into which humidified 95% O2-5% CO2 was continuously passed. The right carotid artery was cannulated with PE-10 tubing connected to PE-50 tubing for fluid infusion. During surgery, an isotonic saline solution containing 6% BSA (Sigma Chemical, St. Louis, MO) was infused at a rate of 4 µl/min to maintain euvoolemia. The bladder was catheterized with PE-90 tubing via a suprapubic incision for urine collections. After completion of surgery, the intravenous infusion solution was changed to isotonic saline containing 1% BSA and 4.5% polyfructosan (Inutest; Laevosan, Linz/Donau, Austria) and was infused at 4 ml/min, which have a gene-duplicated four-copy mice (35, 51). Only the lower dose was used because of hypotension caused by higher doses. The body weights in each group of mice were 25.7 ± 0.8 (control), 24.0 ± 0.5 (low-dose ANP), 25.3 ± 0.6 (high-dose ANP), and 26.8 ± 0.9 (Npr1 4-copy) g. Terminal arterial blood samples were collected from the arterial catheter at the end of the experiment for measurements of plasma insulin and sodium concentrations. To avoid the hypotension that results in mice when even small blood samples are taken, blood was collected only one time at the end of the experiment. In a control group of mice, stability of renal function was assessed. AP, GFR, urine flow, and urinary sodium excretion were measured during three clearance periods without addition of the diuretics after period 2. As shown in Table 1, these values remained stable during the three collection periods. In initial experiments, we compared AP and GFR between mice treated with AM + BFTZ (n = 12) and mice not treated with AM + BFTZ (n = 5). There were no significant differences between untreated control mice and diuretic-treated mice in AP (93 ± 2 vs. 90 ± 3 mmHg, P > 0.05) and GFR (0.19 ± 0.02 vs. 0.20 ± 0.04 ml/min, P > 0.05). Importantly, in each mouse, the ANP infusion was started at the onset of the experiment, and, except for the diuretics given after period 2, no other manipulations were made during the collection periods. Also, there were not any significant differences in GFR in control mice before and after treatment with AM + BFTZ (0.22 ± 0.04 vs. 0.21 ± 0.03 ml/min, P > 0.05).

Urine and plasma inulin measurements. Urine and plasma inulin concentrations were measured using standard colorimetric techniques as reported previously (35, 49, 50). GFR was calculated as the ratio of urinary and plasma inulin concentrations times urine flow. GFR was calculated three times during the three collection periods based on the inulin excretion rates during each period.

Urine and plasma sodium measurements. Urine output was determined gravimetrically assuming a density of 1 g/ml. Urine and plasma sodium concentrations were measured using flame photometry (Flame Photometer IL 973; Instrumentation Laboratory, Lexington, MA).

Calculations. UNaVc is the urinary sodium excretion during control periods (the average of periods 1 and 2). UNaVAM+BFTZ (distal sodium delivery) indicates the urinary sodium excretion following administration of AM + BFTZ (period 3). Sodium reabsorption in distal nephron segments (distal sodium reabsorption) was determined from the UNaVAM+BFTZ - UNaVc. Fractional reabsorption of distal sodium delivery was then determined from (UNaVAM+BFTZ - UNaVc)/UNaVc.

Statistical analysis. The statistical analysis was performed by one-way ANOVA followed by Bonferroni’s multiple-comparison test using the GraphPad PRISM program (GraphPad, San Diego, CA). The results are presented as means ± SE. Significance was set at P < 0.05.

RESULTS

Arterial pressure. As shown in Fig. 1, there were no significant differences in AP among the control (92 ± 3 mmHg) and ANP-infused groups before administration of AM + BFTZ (92 ± 6 mmHg, P > 0.05; 97 ± 3 mmHg, P > 0.05). There were no significant differences in AP among the control (91 ± 3 mmHg) and ANP-infused groups during administration of
AM + BFTZ (93 ± 6 mmHg, P > 0.05; 97 ± 4 mmHg, P > 0.05). In addition, AP in Npr1 gene-duplicated four-copy mice under anesthesia before (91 ± 6 mmHg, P > 0.05) and after (88 ± 6 mmHg, P > 0.05) administration of AM + BFTZ appeared to trend slightly lower, but the differences were not statistically significant. AP remained stable during the three clearance periods.

**GFR.** As shown in Fig. 2, GFR values during low-dose ANP (0.22 ± 0.01 ml/min, P > 0.05) or high-dose ANP (0.26 ± 0.05 ml/min, P > 0.05) were not significantly different from each other or from GFR in control mice (0.22 ± 0.04 ml/min). Although slightly lower, GFR was not significantly different in Npr1 gene-duplicated four-copy mice compared with control (0.16 ± 0.02 ml/min, P > 0.05).

**Urine flow and urinary sodium excretion.** As shown in Table 2, urine flow values before and during administration of AM + BFTZ during low-dose ANP (1.9 ± 0.4 µl/min, P > 0.05; 1.8 ± 0.3 µl/min, P > 0.05; 6.9 ± 0.9 µl/min, P > 0.05) or the higher ANP dose (3.5 ± 0.5 µl/min, P > 0.05; 3.4 ± 0.4 µl/min, P > 0.05; 9.2 ± 0.8 µl/min, P > 0.05) were not significantly different from urine flow in control mice (2.4 ± 0.3 µl/min; 2.6 ± 0.3 µl/min; 6.3 ± 0.7 µl/min). Compared with control mice, urine flows were not significantly different in Npr1 gene-duplicated four-copy mice before and during administration of AM + BFTZ (3.9 ± 0.9 µl/min, P > 0.05; 3.3 ± 0.6 µl/min, P > 0.05; 6.5 ± 0.9 µl/min, P > 0.05). Urinary sodium excretion rates in the low-dose ANP group before administration of AM + BFTZ (0.38 ± 0.13 µeq/min, P > 0.05; 0.28 ± 0.06 µeq/min, P > 0.05) were not significantly different from urinary sodium excretion rates in control mice (0.31 ± 0.07 and 0.25 ± 0.05 µeq/min); however, urinary sodium excretion rates were significantly higher in the group infused with the higher-dose ANP before administration of AM + BFTZ (0.82 ± 0.14 µeq/min, P < 0.01; 0.95 ± 0.16 µeq/min, P < 0.001). In Npr1 gene-duplicated four-copy mice, urinary sodium excretion rate was significantly higher before administration of AM + BFTZ (0.65 ± 0.12 µeq/min, P < 0.05; 0.57 ± 0.09 µeq/min, P < 0.05). In response to AM + BFTZ, sodium excretion increased significantly in all four groups.

**Distal sodium delivery and sodium reabsorption in distal nephron segments.** As shown in Fig. 3, estimated distal sodium delivery values were higher in both groups infused with ANP compared with control [2.4 ± 0.4 (P < 0.05) and 2.8 ± 0.3 (P < 0.01) vs. 1.6 ± 0.2 µeq/min], suggesting similar degrees of inhibition in the segments proximal to the distal nephron. However, distal nephron sodium reabsorption rate was significantly higher in the low-dose ANP group (2.0 ± 0.3 µeq/min, P < 0.05), but not in the high-dose ANP group (1.9 ± 0.3 µeq/min, P > 0.05) compared with control (1.3 ± 0.2 µeq/min). In Npr1 gene-duplicated four-copy mice, distal sodium delivery (1.9 ± 0.1 µeq/min, P > 0.05) and distal nephron sodium reabsorption rate were not significantly higher (1.4 ± 0.1 µeq/min, P > 0.05). Importantly, low-dose ANP did not change fractional reabsorption of distal sodium delivery (86.3 ± 2.0%, P > 0.05) compared with control (83.9 ± 3.6%). However, fractional reabsorption of distal sodium delivery was significantly lower in the group infused with the higher-dose ANP (67.4 ± 4.5%, P < 0.01) and in Npr1 gene-duplicated four-copy mice (72.2 ± 3.4%, P < 0.05).

**DISCUSSION**

Although the natriuretic effects of ANP are well established, the responses have been attributed to inhibition of sodium reabsorption at proximal nephron segments (14, 15, 32, 43) and/or decreases in the collecting duct segments (1, 3, 12, 31, 37, 39, 46). However, in vivo evidence regarding the segment predominantly responsible for the actual natriuretic response has remained uncertain. The present study provides in vivo evidence that inhibition of fractional reabsorption of distal sodium delivery is requisite in mediating substantive ANP-induced natriuresis.

Previous studies using the lithium clearance method have shown that ANP inhibits proximal sodium reabsorption and limits the increase in proximal reabsorption associated with increases in filtered load in anaesthetized rats (6, 14). At physiological concentrations, ANP decreases proximal reabsorption, in part, by counteracting angiotensin-stimulated sodium reabsorption (15). ANP can produce a natriuresis in the

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**Table 2. UF and UNaV**

<table>
<thead>
<tr>
<th></th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3 (A + B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UF, µl/min</td>
<td>UNaV, µeq/min</td>
<td>UF, µl/min</td>
</tr>
<tr>
<td>Control</td>
<td>2.4±0.3</td>
<td>0.31±0.07</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Low-dose ANP</td>
<td>1.9±0.4</td>
<td>0.38±0.13</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>High-dose ANP</td>
<td>3.5±0.5</td>
<td>0.82±0.14*</td>
<td>3.4±0.4‡</td>
</tr>
<tr>
<td>Npr1 4-copy</td>
<td>3.9±0.9</td>
<td>0.65±0.12†</td>
<td>3.3±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANP, atrial natriuretic peptide; Npr1, gene coding for natriuretic peptide receptor-A. Shown are data for UF and UNaV in control, ANP-infused mice, and Npr1 gene-duplicated 4-copy mice before and during administration of amiloride plus bendroflumethiazide (A + B) during period 3. Compared with control, †P < 0.05, *P < 0.01, and §P < 0.001. Compared with low-dose ANP, ‡P < 0.05.

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Fig. 3. Effects of acute ANP infusions on distal sodium delivery (DSD), distal sodium reabsorption (DSR), and fractional reabsorption of DSD (FRDSD) in control and ANP-infused mice. Values are means ± SE. *P < 0.05 and **P < 0.01.

In the present study, acute ANP infusions at doses of 0.1 or 0.2 ng·g body wt·min⁻¹ did not significantly influence AP. Higher doses of ANP were not used to avoid the confounding effects of decreases in AP that occur with higher doses. At these doses, we did not observe significant differences in GFR between control and ANP-infused groups. We also did not find any effects of AM and BFTZ on GFR. Thus ANP inhibited net sodium reabsorption without increasing whole kidney GFR (23). Paul et al. (30) reported that ANP-(8–33) infusion (~0.15 μg·kg⁻¹·min⁻¹) led to natriuresis without affecting systemic blood pressure or renal hemodynamics in dogs. Furthermore, ANP infusions increased GFR at AP around 80 mmHg (30). ANP infusions also led to the dilation of afferent arterioles (40), which may be the major mechanism for increased GFR. Caron and Kramp (9) found that GFR was transiently elevated in rats after an intravenous injection of 1 μg ANP. Wang et al. (42) reported that GFR did not change during acute ANP infusions (0.5 ng·g body wt·min⁻¹) in rats, but there was a significant decrease in systemic blood pressure. Likewise, rat pro-ANP-(1–30) infusions (30 ng/min after a bolus of 10 ng) for 240 min did not change GFR in rats (11). Thus most of the data suggest that effects of ANP on GFR may be related to the dose and administration method, but that the natriuretic effects are not dependent on increases in GFR.

Although both ANP doses used in the present study increased distal sodium delivery, we found that urinary sodium excretion was significantly augmented only in the group given the higher dose of ANP. In Npr1 gene-duplicated four-copy mice, urinary sodium excretion was also significantly higher with low-dose ANP infusions, suggesting that the overexpression of Npr1 augments natriuresis induced by ANP infusions. Paul et al. (29) reported that rat ANP-(103–126) infusions (0.2 μg·kg⁻¹·min⁻¹) led to natriuresis in rats, and a positive relationship was observed between urinary sodium excretion and plasma ANP concentration. However, it was reported that acute ANP infusions (0.5 ng·g body wt·min⁻¹) induced a rapid increase in urine flow that peaked 10 min after the initiation of ANP infusion and returned to the baseline level by 25 min after the initiation of ANP infusion (42). Furthermore, acute ANP infusions significantly increased urinary sodium excretion and peaked 15 min after the onset of ANP infusion and declined gradually after the peak but remained elevated compared with control (42). Of note, several studies have shown that diuresis and natriuresis induced by ANP are transient when ANP is given as continuous infusions or as repeated bolus injections in humans and animals because resistance to the diuretic and natriuretic actions of ANP develops after relatively brief periods of ANP infusion (7, 13, 41). This phenomenon may be related to the mechanism that the subcellular localization of ENaC increases after 90 min of ANP infusion (42). It is also known that ANP binding to Npr1 increases cellular cGMP production, which activates cGMP-dependent protein kinases, cGMP-dependent phosphodiesterases, and cyclic nucleotide-gated ion channels as effector molecules (26, 27). The activation of these three effector molecules elicits vasodilation and inhibits sodium reabsorption (27). Increased intracellular cGMP may regulate sodium, and perhaps calcium, uptake in nephron segments proximal to the IMCD (22). Luminal ANP also decreases chloride reabsorption in mouse cortical thick ascending limb and medullary thick ascending limb (2). Inoue et al. (17) reported that ANP causes
diuresis and natriuresis, at least in part by inhibiting the vasopressin 2 receptor-mediated action of vasopressin in the collecting ducts. Inhibition of basolateral Na\(^+\)-K\(^+\)-ATPase, probably via the stimulation of PGE\(_2\) synthesis, may also be involved in the natriuresis induced by ANP (45).

In the present study, sodium reabsorption in distal nephron segments was effectively inhibited during administration of AM + BFTZ although we cannot be certain that we elicited complete inhibition of the distal sodium transporters. The fractional urinary sodium excretion rates varied from <1% during control conditions to >4% during dual distal nephron blockade with AM + BFTZ, suggesting effective blockade of distal nephron transport function. This value is consistent with current concepts regarding the sodium load to distal nephron segments. In our original experiments, we tested various doses and determined the maximal dose that would inhibit distal sodium reabsorption without also increasing the distal sodium delivery to values suggestive of proximal inhibition. We also considered the possibility that our dose of AM might cause inhibition of Na\(^+\)/H\(^+\) exchanger (NHE) 3. However, the dose of AM in this study would be expected to lead to plasma concentrations much lower than those shown to inhibit NHE3. Furthermore, if the dose of AM used also inhibited the NHE isoform, NHE3, in proximal tubules, we would have expected much greater increases in urinary sodium excretion. In other studies using AM, AM did not affect Li\(^+\) clearance in rats (36) and slightly increased fractional Li\(^+\) excretion in dogs (34). However, the effect of AM to increase Li\(^+\) excretion may be because of inhibition of distal Li\(^+\) uptake in rats (19). These data suggest that the dose of AM used does not affect proximal sodium reabsorption. Furthermore, BFTZ did not increase Li\(^+\) clearance in rats (38). From these data, it can be concluded that inhibition of sodium reabsorption in response to AM + BFTZ occurs primarily at distal nephron segments. Therefore, sodium excretion during dual blockade provides a collective measure of distal sodium delivery to distal nephron segments (21, 49, 50). We found that the low-dose ANP increased distal sodium delivery, indicating inhibition at earlier nephron segments but also increased distal nephron sodium reabsorption in response to the increased load compared with control. In contrast, high-dose ANP increased distal sodium delivery and blunted the increase in distal nephron sodium reabsorption. Thus the low-dose ANP did not affect fractional reabsorption of distal sodium delivery, which limited the magnitude of the natriuresis, whereas the high-dose ANP decreased fractional reabsorption of distal sodium delivery and led to the augmented natriuresis in this group. These results help explain the lack of sustained natriuresis in response to low-dose ANP and the differences between the effects of low-dose and high-dose ANP. In Npr1 gene-duplicated four-copy mice, the low-dose ANP decreased fractional reabsorption of distal sodium delivery, suggesting that the augmented natriuresis induced by ANP infusions is mediated by overexpression of Npr1 via inhibition of distal sodium reabsorption. Importantly, while we agree that previous studies have shown actions of ANP on multiple nephron segments, the present results provide the hierarchy of these actions and provide in vivo evidence to support the critical role of reduced fractional sodium reabsorption in distal nephron segments in the mediation of ANP-induced increases in sodium excretion.

The present study describes an in vivo approach to obtain a collective and quantitative index of distal nephron sodium reabsorption that allows overall assessment of distal nephron transport in intact kidneys. Although we are providing only an estimated index of distal nephron sodium reabsorption, it is also a quantitative index of the collective distal sodium delivery and distal sodium reabsorption of the total nephron population, which is not possible from micropuncture or isolated perfused tubule experiments. By minimizing the extent of surgical manipulations, more robust values for renal function can be obtained. This technique is particularly applicable to studies in transgenic mice that may have alterations in sodium transport in distal nephron segments that need to be quantified (47). This technique will be helpful to elucidate the role of increased distal nephron sodium reabsorption in the pathophysiology of hypertension. Furthermore, this approach may be useful in translation studies by providing a technique to assess distal transport function in patients with disorders of sodium excretion. Such data would thus provide the rationale for considering the use of these diuretics that primarily inhibit distal nephron sodium reabsorption such as those used in the present study.

ACKNOWLEDGMENTS

We thank Edward Au and Subhankar Das for providing Npr1 gene-duplicated four-copy mice.

GRANTS

This work was supported by National Institutes of Health Grants HL-18426, HL-62147, and P20RR-0117659 from the Institutional Development Award Program of NCRR.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


AJP-Renal Physiol • VOL 298 • JANUARY 2010 • www.ajprenal.org

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ANP EFFECTS IN WT AND NPRA-OVEREXPRESSING MICE


