Acute Angiotensin II Infusions Elicit Pressure Natriuresis in Mice and Reduce Distal Fractional Sodium Reabsorption

Di Zhao, L. Gabriel Navar

Abstract—Acute angiotensin II (Ang II) infusions into mice increase arterial pressure (AP) and elicit pressure natriuresis. We used this model of pressure natriuresis to delineate the distal nephron responses to AP-mediated increases in distal sodium delivery. In the first group, we measured changes in urinary sodium excretion (UNa\textsubscript{V}) in male C57/BL6 anesthetized mice (n=9) before and during acute Ang II infusions (5 ng/g of body weight per minute). Acute Ang II infusions increased AP (98±3 to 126±5 mm Hg; P<0.001), urine flow (2.7±0.5 to 6.0±0.8 μL/min; P<0.01), and UNa\textsubscript{V} (0.6±0.2 to 1.3±0.2 μEq/min; P<0.05). There were significant relationships between UNa\textsubscript{V} and urine flow (y=0.207x+0.030; P<0.0001) and between UNa\textsubscript{V} and AP (y=0.027x−2.100). In a separate series, distal sodium delivery and fractional reabsorption of distal sodium delivery were determined in control (n=12) and Ang II–infused mice (n=8) by comparing UNa\textsubscript{V} before and after blockade of the 2 major distal nephron sodium transporters with amiloride (5 mg/kg of body weight) plus bendroflumethiazide (12 mg/kg of body weight). A positive relationship was found between UNa\textsubscript{V} and urine flow (y=0.015x−1.100; P<0.0001) or distal sodium delivery (y=0.027x−0.900; P<0.0001) and AP. An inverse relationship was found between fractional reabsorption of distal sodium delivery and AP (y=−0.511x+128.300; P<0.01). These data indicate that Ang II–mediated pressure natriuresis involves an increase in distal sodium delivery combined with a reduced distal nephron fractional sodium reabsorption, suggesting that increased AP prevents the distal nephron transport mechanisms from accommodating the increased distal delivery. (Hypertension. 2008;52:137-142.)

Key Words: Ang II ■ arterial pressure ■ renal sodium reabsorption ■ distal nephron segments ■ amiloride ■ bendroflumethiazide ■ fractional distal sodium reabsorption

It is well known that increases in arterial pressure (AP) lead to the increases in renal sodium excretion, a phenomenon commonly referred to as pressure natriuresis.\textsuperscript{1–8} The changes in renal sodium excretion in response to changes in AP provide a link between the mechanisms regulating sodium excretion and those regulating AP.\textsuperscript{1} Because glomerular filtration rate (GFR) and filtered sodium load are autoregulated over the same AP range, the mechanism of pressure natriuresis involves decreased tubular sodium reabsorption in response to increased AP;\textsuperscript{3} however, the relative contributions of the various nephron segments responsible have not been fully defined.\textsuperscript{5,9–12} Acute angiotensin II (Ang II) infusions in mice and rats increase AP and elicit pressure natriuresis, suggesting that Ang II infusion is a useful model to delineate the relative contributions of the various nephron segments to increases in AP.

The development of a mouse model with minimal surgical interventions to study pressure natriuresis would allow evaluation of the roles of specific tubular transport systems by using various gene targeted models. Although several studies have focused on the AP effects on proximal nephron segments,\textsuperscript{13,14} the distal nephron segments consisting of distal convoluted tubule, connecting tubule, and cortical and medullary collecting ducts are ultimately responsible for the fine regulation of sodium excretion.\textsuperscript{15} Sodium reabsorption at distal nephron segments is mainly mediated by amiloride (AM)-sensitive epithelial sodium channels and bendroflumethiazide (BFTZ)-sensitive Na\textsuperscript{+}–Cl\textsuperscript{−} cotransporters (NCCs). In the presence of AM plus BFTZ to block most of the sodium transport at distal nephron segments, sodium excretion provides a collective measure of sodium delivery to distal nephron segments.\textsuperscript{9,16} Sodium reabsorption by distal nephron segments can, thus, be determined from the differences between urinary sodium excretion (UNa\textsubscript{V}) during distal blockade and UNa\textsubscript{V} in the absence of blockade of epithelial sodium channels and NCCs. Although it has been shown clearly that increases in AP elicit increases in distal sodium delivery, the final effect of such changes on sodium excretion would be minimized by the predictable ability of the distal nephron transport mechanisms to accommodate the increased sodium delivery by increasing the reabsorption rate.\textsuperscript{17} However, a failure to accommodate the increased distal sodium delivery would augment the natriuretic responses.\textsuperscript{9,12}

In this study, we hypothesized that the pressure natriuresis elicited by acute Ang II infusion depends not only on the...
increased distal sodium delivery but also on a failure of the distal nephron segments to accommodate the increased delivery by augmenting the reabsorption rate. We determined the relationships among AP and UNaV, distal sodium delivery, and fractional reabsorption of the sodium delivered to distal nephron segments during acute Ang II infusions in mice. We recognized that the direct intrarenal effects of Ang II stimulate tubular sodium reabsorption and reduce sodium excretion and, thus, may blunt the responses to the increases in AP.15,18–21 This is particularly true during chronic Ang II infusions, which elevate intrarenal Ang II levels.20,22 For the present study, Ang II infusions were selected so that the natriuretic effects of the elevated AP predominated over any direct antinatriuretic effects.21–23

Methods

Animals
Studies were performed on 9- to 12-week-old male C57BL6 mice (Jackson Laboratory, Bar Harbor, Maine) that were maintained at a 12:12-hour light-dark schedule (6 AM to 6 PM) at 25°C in the vivarium at Tulane University Health Sciences Center. A normal salt diet along with tap water was provided. The protocol was approved by the institutional animal care and use committee of Tulane University Health Sciences Center.

Experimental Protocol
In the first series of mice (n = 9), we measured changes in sodium excretion in male C57BL6 anesthetized mice before and during acute Ang II (Phoenix Pharmaceuticals) infusions at 2 doses of 5 and 10 ng/g of body weight (BW) per minute.18 On the day of the experiment, mice were anesthetized with inactin (thiobutabarbital sodium) injected IP at 200 mg/kg of BW. Supplemental doses of anesthesia were administered 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). After shaving the incision site, a tracheostomy was performed with polyethylene (PE)-90 tubing with the exterior end of the tracheal cannula placed 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. Atropine was administered as required to maintain a stable plane of anesthesia. After surgery (in the left decubitus position), the IV infusion solution was changed to isotonic saline containing 1% albumin and was infused at 4 mL/min. After a 30-minute recovery period, urine was collected for 30 minutes as the control (period 1). Ang II was infused after period 1. A 5-minute period was allowed after which a urine sample was collected during acute Ang II infusion (period 2). In the second series, renal plasma flow (RPF) and GFR were assessed by a renal clearance protocol in mice reported previously18 in a control group (n = 12) and a group (n = 8) continuously infused with Ang II for the duration of the experiment. After surgery, the intravenous infusion solution was changed to isotonic saline containing 1% albumin, 4.5% polyfructosan (InuTest, Laevosan), and 1.5% para-aminophenurate-PAH; Merck Sharp & Dohme) and was infused at 4 mL/min. The Ang II–infused mice also received Ang II at 5 ng/g of BW per minute. After a 60-minute equilibration period, two 30-minute control urine samples were collected. After the 2 control periods (periods 1 and 2), a combination of AM (5 mg/kg of BW) to block epithelial sodium channels and BFTZ (12 mg/kg of BW) to block NCCs was administered IV as suggested previously,9,16 and a 15-minute period was allowed after which a 30-minute urine sample was collected (period 3). The sodium excretion during blockade was used as an estimate of sodium delivery to the distal nephron segments. When compared with sodium excretion measured during periods 1 and 2, distal nephron sodium reabsorption was determined. Terminal arterial blood samples were collected from the arterial catheter at the end of the experiment for measurements of plasma PAH, inulin, and sodium concentrations.

Urine, Plasma PAH, and Inulin Measurements
Urine and plasma PAH and inulin concentrations were measured using standard colorimetric techniques, as reported previously, adapted for a plate reader.24 RPF was estimated from PAH clearance calculated as the ratio of urine and plasma PAH Concentrations times urine flow. GFR was calculated as the ratio of urine and plasma inulin concentrations times urine flow.

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). UNaV was normalized by 30 minutes (urine collection period).

Calculations
UNaVc indicates the absolute UNaV during control periods (the average of periods 1 and 2). UNaVAM-BFTZ (distant sodium delivery) indicates the absolute UNaV during AM+BFTZ (period 3). Filtered BFTZ sodium indicates the GFR×plasma sodium concentration. Fractional distal sodium delivery indicates the distal sodium delivery/ filtered sodium (control period). Absolute sodium reabsorption at distal nephron segments (distant sodium reabsorption) indicates UNaVAM-BFTZ–UNaVc. Fractional reabsorption of sodium delivery indicates (UNaVAM-BFTZ–UNaVc)/UNaVAM-BFTZ.

Statistical Analysis
The statistical analysis was performed by paired t test using the GraphPad Prism program (GraphPad) in the same individual mouse data (the first series) and unpaired t test between the control group and acute Ang II–infusion group. The relationships among AP and UNaV, distal sodium delivery, and fractional reabsorption of sodium delivery were analyzed by linear regression, respectively. The results are presented as means±SE. Significance was set at P<0.05.

Results

Relationship Between UNaV and Urine Flow or AP
Acute Ang II infusions (5 ng/g of BW per minute) increased AP from 98±3 to 126±5 mm Hg (P<0.001) and increased urine flow from 2.7±0.5 to 6.0±0.8 μL/min (P<0.01) and UNaV from 0.6±0.2 to 1.3±0.2 μEq/min (P<0.001). Higher doses did not significantly increase urine flow or UNaV, suggesting direct antinatriuretic effects that opposed the natriuretic effects of increased AP. As shown in Figure 1A and 1B, there were significant relationships between UNaV and urine flow (y=0.207x+0.030; P<0.0001) and between UNaV and AP (y=0.027x–2.100).

Effects of Acute Ang II Infusions on AP, RPF, GFR, Urine Flow, and UNaV
As shown in the Table, AP was higher in the Ang II–infused group as compared with the control group (126±5 versus 90±3 mm Hg; P<0.001). The Ang II–infused group had reduced RPF (0.8±0.1 versus 1.3±0.2 mL/min; P<0.05), but GFR values were similar to the control group (0.21±0.01 versus 0.20±0.04 mL/min; P>0.05), reflecting the direct
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Figure 1. The relationships between UNaV and urine flow (A) and between AP and UNaV (B).

![Diagram](image)

The present study provides evidence that acute Ang II infusions to mice elicit pressure natriuresis because of the increases in distal sodium delivery coupled with a reduced fractional reabsorption of distal sodium delivery. However, the sodium excretion responses to Ang II infusions are complex and depend on the dose and duration of the Ang II infusions.

Discussion

The present study provides evidence that acute Ang II infusions to mice elicit pressure natriuresis because of the increases in distal sodium delivery coupled with a reduced fractional reabsorption of distal sodium delivery. However, the sodium excretion responses to Ang II infusions are complex and depend on the dose and duration of the Ang II infusions.
infusions, as well as the magnitude of the blood pressure responses. In this study, Ang II infusion increased AP, urine flow, and UNaV. We found a significant relationship between UNaV and AP, suggesting that the natriuresis was attributable primarily to the associated changes in AP. In the study by Leong et al,19 there was no increase of urine output with acute infusions of captopril and Ang II in the absence of a change in AP. However, urine output increased with a 50- to 60-mm Hg elevation of AP by arterial constriction, although the effects were reduced 50% as compared with control that was without the Ang II clamp. These data indicate that the natriuresis with acute Ang II infusion is primarily because of the increases in pressure, whereas the direct effects of Ang II partially counteract the pressure natriuresis.

The effects of Ang II on renal function result from a combination of Ang II type 1 and Ang II type 2 receptor–mediated events, with the Ang II type 1 receptor–mediated effects generally predominant.25 Thus, Ang II administration results in net renal vasoconstriction.25 In this study, RPF decreased during acute Ang II infusions, whereas GFR was not significantly altered. Cervenka et al18 showed that RPF and GFR increased during volume expansion. Interestingly, the increases in RPF and GFR were inhibited during concurrent Ang II infusion with volume expansion.18 Mattson et al26 showed similar results in volume-expanded rats. It was also reported that the Ang II type 1 receptor antagonist increased RBF by 21%, whereas AP fell by only 4%, suggesting that the renal vasculature is already under substantial influence mediated by endogenous Ang II.27 Ang II blockade increased GFR, indicating that the kidney is under a substantial Ang II–mediated influence even in normal control conditions. Thus, acute systemic Ang II infusions that raise AP probably do not make a significant impact on the intrarenal Ang II–mediated effects, and the major effects of acute Ang II infusions are mainly because of the associated changes in AP, thus explaining the natriuretic effects of acute Ang II infusions as contrasted with the antinatriuretic effect of chronic Ang II infusions.20 In the recent study by Sandberg et al,28 acute Ang II infusions restored the apical localization of NCCs that had been reduced by angiotensin-converting enzyme inhibition but did not increase apical membrane localization above that in control nonangiotensin-converting enzyme–treated rats.

Blockade of the 2 major sodium transporters in the distal nephron segments with AM plus BFTZ leads to a marked increase in sodium excretion, which then provides a collective measure of sodium delivery to distal nephron segments. In this study, sodium delivery to distal nephron segments increased during acute Ang II infusions. The increase in distal sodium delivery indicates decreased sodium reabsorption in the earlier nephron segments, because filtered sodium was not significantly altered. Although the technique used in this study cannot delineate differences in sodium reabsorption between the proximal convoluted tubule and the loop of Henle, various studies in the rat have shown that inhibition of proximal fluid reabsorption is a primary response to acute hypertension and contributes to pressure diuresis.5,29 The mechanisms are related to both endocytic removal of apical Na+/H+ exchangers (NHEs) and basolateral Na+ pumps, as well as decreased total Na+ pump activity.30 More recently, it has been shown that NHE3 is redistributed to a domain at the base of the microvilli rather than endocytosed.31 Chou and Marsh3 further demonstrated that the loop of Henle does not contribute to pressure natriuresis but actually partially compensates, because there was a 40% increased flow out of the proximal tubule and only a 13% increased flow into the distal convoluted tubule. These studies suggest that the loop of Henle may play a compensatory role during the process of pressure natriuresis. In contrast, Roman11 reported that alterations in medullary hemodynamics participate in the pressure-natriuretic response by inhibiting tubular reabsorption in the proximal tubule and the thin descending limb of the loop of Henle (or both) of juxtamedullary nephrons. Thus, changes in renal perfusion pressure within the autoregulatory range may elicit changes in tubular sodium reabsorption in multiple nephron segments.32 Renal interstitial pressure could alter sodium reabsorption in the proximal fluid reabsorption is a primary response to acute hypertension and contributes to pressure diuresis.5,29 The mechanisms are related to both endocytic removal of apical Na+/H+ exchangers (NHEs) and basolateral Na+ pumps, as well as decreased total Na+ pump activity.30 More recently, it has been shown that NHE3 is redistributed to a domain at the base of the microvilli rather than endocytosed.31 Chou and Marsh3 further demonstrated that the loop of Henle does not contribute to pressure natriuresis but actually partially compensates, because there was a 40% increased flow out of the proximal tubule and only a 13% increased flow into the distal convoluted tubule. These studies suggest that the loop of Henle may play a compensatory role during the process of pressure natriuresis. In contrast, Roman11 reported that alterations in medullary hemodynamics participate in the pressure-natriuretic response by inhibiting tubular reabsorption in the proximal tubule and the thin descending limb of the loop of Henle (or both) of juxtamedullary nephrons. Thus, changes in renal perfusion pressure within the autoregulatory range may elicit changes in tubular sodium reabsorption in multiple nephron segments.32 Renal interstitial pressure could influence tubular sodium reabsorption directly or indirectly through the release of medullary humoral factors.33 Preventing renal interstitial hydrostatic pressure from increasing in response to increases in renal perfusion pressure attenuates pressure natriuresis, and increases in renal interstitial hydro-
static pressure decrease proximal sodium reabsorption. The exact mechanism whereby renal interstitial hydrostatic pressure influences tubular reabsorption is unknown but may be related to alterations in tight junctional permeability to sodium in proximal tubules, redistribution of apical sodium transporters, and/or release of renal autocooids such as prostaglandin E2. Increases in AP stimulate increased intrarenal NO levels, which have also been implicated in mediating pressure natriuresis. The present data also show a positive relationship between AP and distal sodium delivery, indicating that sodium reabsorption in nephron segments proximal to the distal nephron decrease with elevations of AP. However, Majid and Navar observed that the slope of the relation between renal AP and distal sodium delivery was markedly attenuated at renal AP >100 mm Hg but not <100 mm Hg during treatment with AM plus BFTZ in dogs. This study suggests that, at AP >100 mm Hg, distal sodium delivery is relatively stable, and the changes in sodium excretion are mediated primarily by changes in distal sodium reabsorption.

In our study, fractional UNaV rates varied from <1% during control conditions to ~4% during dual distal nephron blockade with AM plus BFTZ, suggesting predominant blockade of distal nephron transporters, epithelial sodium channels, and NCCs. If our dose of AM also inhibited the NHE isoform NHE3 in proximal tubules, we would have expected much greater increases in UNaV. Although proximal nephron effects cannot be ruled out, AM did not affect Li+ clearance in rats and slightly increased fractional Li+ excretion in dogs. However, the effect of AM to increase Li+ excretion may be because of inhibition of distal Li+ uptake in rats. BFTZ did not increase Li+ clearance in rats, and there was actually a decrease in Li+ clearance with BFTZ treatment.

Our key novel finding is that fractional reabsorption of distal sodium delivery decreased in response to the Ang II–mediated increases in AP, and an inverse relationship was found between AP and fractional reabsorption of distal sodium delivery. These findings indicate that there is not a compensatory increase in distal sodium reabsorption, which, thus, allows the effects of increased distal sodium delivery to elicit proportionately greater increases in sodium excretion. We also emphasize that the slope of the inverse relationship between AP and fractional reabsorption of distal sodium delivery is not trivial but quite substantial, going from 83% to 63%, which translates to a large change in sodium excretion. These data suggest that the pressure-natriuresis mechanism is mediated by angiotensin and are consistent with the concept that increases in AP inhibit sodium reabsorption by the main distal nephron sodium transporters, which are related to increased intrarenal NO levels in response to increases in AP.

Perspectives

Recent studies of monogenetic diseases have identified mutations of distal sodium transporters involving gain of function associated with hypertension. The hypertension could be related to impaired pressure natriuresis because of an inability to inhibit distal nephron sodium transport. The high efficacy of thiazide-like diuretics could be because of their ability to enhance sodium excretion via inhibition of pressure-natriuresis mechanisms. The present study develops a mouse model to study pressure natriuresis that will allow evaluation of the roles of specific tubular transport systems in mediating AP-dependent changes in net tubular sodium reabsorption.

Disclosures

None.

References

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