# Intrarenal Aminopeptidase N Inhibition Restores Defective Angiotensin II Type 2–Mediated Natriuresis in Spontaneously Hypertensive Rats

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***Abstract***—The preferred ligand of angiotensin (Ang) II type 2 ($AT_2R$)–mediated natriuresis is Ang III. The major enzyme responsible for the metabolism of Ang III is aminopeptidase N, which is selectively inhibited by compound PC-18. In this study, urine sodium excretion rates ($U_{Na}V$), fractional excretion of sodium, fractional excretion of lithium, glomerular filtration rate, and mean arterial pressures were studied in prehypertensive and hypertensive spontaneously hypertensive rats (SHRs) and compared with age-matched Wistar-Kyoto rats (WKYs). Although renal interstitial infusion of Ang II type 1 receptor blocker candesartan increased $U_{Na}V$ in WKYs from a baseline of $0.05\pm0.01$ to $0.17\pm0.04$ $\mu$mol/min ($P<0.01$), identical infusions failed to increase $U_{Na}V$ in hypertensive SHRs. Coinfusion of $AT_2R$ antagonist PD-123319 abolished the natriuretic responses to candesartan in WKYs, indicating an $AT_2R$-mediated effect. $AT_2R$-mediated natriuresis was enabled in hypertensive SHRs by inhibiting the metabolism of Ang III with PC-18 ($0.05\pm0.01$ to $0.11\pm0.03$ $\mu$mol/min; $P<0.05$). The defects in sodium excretion were present before the onset of hypertension in SHRs, because young WKYs demonstrated double the $U_{Na}V$ of SHRs ($0.04\pm0.006$ versus $0.02\pm0.003$ $\mu$mol/min; $P<0.01$) at baseline. The increased $U_{Na}V$ of young WKYs was attributed to reduced renal proximal tubule sodium reabsorption, because increases in fractional excretion of sodium were paralleled by increases in fractional excretion of lithium. Renal interstitial PC-18 infusion ameliorated defective $AT_2R$-mediated natriuresis in young SHRs by increasing fractional excretion of sodium and fractional excretion of lithium without changing the glomerular filtration rate. Thus, increased renal proximal tubule sodium retention is observed before the onset of hypertension in SHRs, and inhibition of the metabolism of Ang III ameliorates this pathophysiologic defect in sodium excretion. **(*Hypertension*. 2010;55[part 2]:474-480.)**

**Key Words:** natriuresis ■ angiotensin receptors ■ hypertension ■ angiotensin III ■ aminopeptidase N ■ SHR

Spontaneously hypertensive rats (SHRs) develop hypertension at ≈6 weeks of age and are widely used as a model to study the development and maintenance of human genetic hypertension.[1] One of the proposed mechanisms of the initiation of hypertension in SHRs involves a primary defect in renal sodium ($Na^+$) excretion.[2–8] Over time, this defect necessitates an increase in renal perfusion pressure, an adaptation that becomes central to the development and maintenance of hypertension.[9,10]

In normal rodents, both the intrarenal renin-angiotensin (Ang) system and the renal dopaminergic system play important roles in renal proximal tubule $Na^+$ handling. Basal $Na^+$ excretion rates are generally determined by the activity of the intrarenal renin-Ang system, whereas the dopaminergic system regulates $Na^+$ excretion in response to high-salt intake.

Although an acute sodium load or rise in blood pressure increases sodium excretion in normal rodents, the following 3 significant pharmacological manipulations also induce natriuresis: (1) blockade of intrarenal Ang II type 1 receptors ($AT_1Rs$); (2) stimulation of renal dopamine $D_1$-like receptors; and (3) activation of intrarenal Ang II type 2 receptors ($AT_2Rs$). Recent studies have shown that natriuresis, resulting from both $AT_1R$ blockade and $D_1$-like receptor stimulation, are mediated, at least in part, by $AT_2R$ activation, because concomitant blockade of renal $AT_2Rs$ in these situations abolishes the natriuresis.[11,12] Regarding direct renal $AT_2R$-induced natriuresis, renal interstitial (RI) Ang III, but not Ang II, results in increased $Na^+$ excretion when $AT_1Rs$ are blocked systemically.[11] This effect is also abolished by concomitant infusion of a selective $AT_2R$ antagonist, highlighting the important direct role of renal $AT_2R$ activation by Ang III in natriuresis.[11]

In the kidney, aminopeptidase A (APA), an enzyme normally expressed on the brush border of renal proximal tubule

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cells, is responsible for converting Ang II to Ang III. Ang III is subsequently degraded to Ang IV by aminopeptidase N (APN). Inhibition of intrarenal APN results in augmented natriuretic responses to Ang III in the presence of systemic $AT_1R$ blockade,[13] and natriuresis engendered by inhibition of APN is abolished by concomitant inhibition of APA.[14] Taken together, these data suggest that renal $AT_2Rs$ mediate natriuresis engendered by $D_1$-like receptor activation and $AT_1R$ blockade and that Ang III, and not Ang II, is the preferred agonist of this response.

Thus far, studies regarding the etiology of increased $Na^+$ reabsorption in young SHRs have focused on alterations in renal dopaminergic and $AT_1R$-mediated effects.[15–18] However, as mentioned previously, $D_1$-like receptor–mediated natriuresis and natriuresis because of $AT_1R$ blockade are dependent, at least in part, on renal $AT_2Rs$.[11,12] Thus, we hypothesize that rapid metabolism of Ang III, the preferred ligand of $AT_2R$-mediated natriuresis, leads to abnormal sodium reabsorption demonstrated previously to occur in association with the development of hypertension in the SHR. The results indicate that renal $AT_1R$ blockade fails to induce natriuresis in hypertensive SHRs unless the degradation of Ang III is inhibited and that this defect is present before, rather than as a consequence of, established hypertension. Amelioration of $AT_2R$-mediated natriuresis in prehypertensive SHRs is achieved through inhibition of renal APN activity, and this effect is mediated by $AT_2Rs$ of the renal proximal tubule.

## Methods

### Animal Preparation

The experiments, which were approved by the University of Virginia Animal Care and Use Committee, were conducted in 4- and 12-week–old female Wistar-Kyoto rats (WKY; Harlan) and SHRs (Taconic), in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Rats were placed under general anesthesia with pentobarbital (50 mg/mL) given 5 mg/100 g of body weight IP. A tracheostomy was performed, and arterial access was achieved by direct cannulation of the right carotid artery. Intravenous access was obtained via cannulation of the right internal jugular vein. Renal cortical interstitial infusion catheters were placed, as reported previously.[11–14] When >1 substance was simultaneously infused into the kidney, separate interstitial catheters were used.

Rats were housed under controlled conditions (temperature: 21±1°C; humidity: 60±10%; light: 8 to 20 hours). Experiments were initiated at the same time each day to prevent any effect of diurnal variation in blood pressure. Mean arterial pressure (MAP) was measured by the direct intracarotid method with the use of a blood pressure analyzer (Micromed Inc). MAP was recorded every 5 minutes and averaged for each of the control and experimental periods.

### Pharmacological Agents

Candesartan, a specific, potent insurmountable inhibitor of $AT_1R$ ($IC_{50}$ $>1\times10^{-5}$ mol/L and $2.9\times10^{-8}$ mol/L for $AT_2Rs$ and $AT_1Rs$, respectively), was used for $AT_1R$ blockade. PD-123319 (PD; Parke-Davis), a specific $AT_2R$ antagonist ($IC_{50}$ $2\times10^{-8}$ mol/L and $>1\times10^{-4}$ mol/L for $AT_2Rs$ and $AT_1Rs$, respectively), was used to block $AT_2Rs$. A specific APN inhibitor, PC-18 (2-amino-4-methylsulfonyl-butane-thiol; inhibition constant: 8.0±1.7 nM),[19,20] was provided by M.-C.F.-Z. and B.P.R. and infused interstitially to block the metabolism of Ang III to Ang IV. PC-18 has been shown to increase the half-life of endogenous Ang III in vivo.[19]

### Measurement of Glomerular Filtration Rate, Fractional Excretion of Sodium, and Fractional Excretion of Lithium

Urinary and plasma $Na^+$ and $Li^+$ concentrations were measured using a flame photometer (Instrumentation Laboratory 943). Glomerular filtration rate (GFR) was measured by inulin clearance using a method described previously.[21] Tubular $Na^+$ reabsorption was determined by calculating the fractional excretion of sodium ($FE_{Na}$), and renal proximal tubule $Na^+$ reabsorption was estimated using fractional excretion of lithium ($FE_{Li}$), as published previously.[22]

### Effects of RI $AT_1R$ Blockade, RI $AT_1R$ Blockade+APN Inhibition, and RI $AT_1R$ Blockade+APN Inhibition+$AT_2R$ Blockade on $U_{Na}V$, GFR, $FE_{Na}$, $FE_{Li}$, and MAP

Four- and 12-week–old WKYs and SHRs were studied on normal $Na^+$ intake (N=6 per group) 72 hours after uninephrectomy. The remaining kidney was then infused for 1 hour with 5% dextrose in water ($D_5W$), designated as the control period in Figure 1. After the control period, the kidney was infused with 1 of the following: (1) $D_5W$ at 2.5 μL/min; (2) candesartan (0.01 mg/kg per minute); (3) candesartan+PD (10 μg/kg per minute); (4) candesartan+PC-18 (25 μg/min); or (5) candesartan+PC-18+PD directly into the RI space during 3 consecutive 1-hour experimental periods. Inulin and lithium chloride in $D_5W$ were infused throughout the study via an internal jugular catheter. $U_{Na}V$, GFR, $FE_{Na}$, $FE_{Li}$, and MAPs were calculated and/or recorded for each period.

### Statistical Analysis

Comparisons among vehicle, $AT_1R$ blocker (candesartan), $AT_2R$ blocker (PD), and PC-18 (APN inhibitor) were estimated by ANOVA, including a repeated-measures term, by using the general linear models procedure of SAS (version 9.1; SAS Institute, Inc). Multiple comparisons of individual pairs of effect means were conducted by the use of a least-square means pooled variance. Data are expressed as mean±1 SE. Statistical significance was identified at $P<0.05$.

## Results

### Effects of RI Candesartan Infusion and Candesartan+PD Infusion on $U_{Na}V$ and MAP in 12-Week–Old WKYs and SHRs

As demonstrated in Figure 1A, in WKYs, RI candesartan increased $U_{Na}V$ from a baseline of 0.05±0.01 to 0.16±0.03 μmol/min ($P<0.0001$) during experimental period 2 and to 0.17±0.04 μmol/min ($P<0.01$) during experimental period 3. PD coinfusion abolished the natriuretic responses to RI candesartan in WKYs. In 12-week–old SHRs, however, identical infusions of candesartan failed to increase $U_{Na}V$ (baseline: 0.04±0.01 to 0.03±0.01 μmol/min after 3 hours of candesartan infusion; $P$ value not significant). As illustrated in Figure 1B, SHRs had higher MAP values compared with WKYs at baseline, but RI candesartan infusion did not significantly alter baseline MAP values in WKYs or SHRs. Similarly, coinfusion of PD with candesartan did not influence MAP in WKYs.

### Effects of RI Candesartan±APN Inhibition on $U_{Na}V$ and MAP in 12-Week–Old Hypertensive SHRs

Figure 2A demonstrates that candesartan+PC-18 infusion increased $U_{Na}V$ from a baseline value of 0.05±0.01 to 0.10±0.02 μmol/min ($P<0.05$) during experimental period 1 to 0.12±0.02 μmol/min ($P<0.01$) during experimental pe-

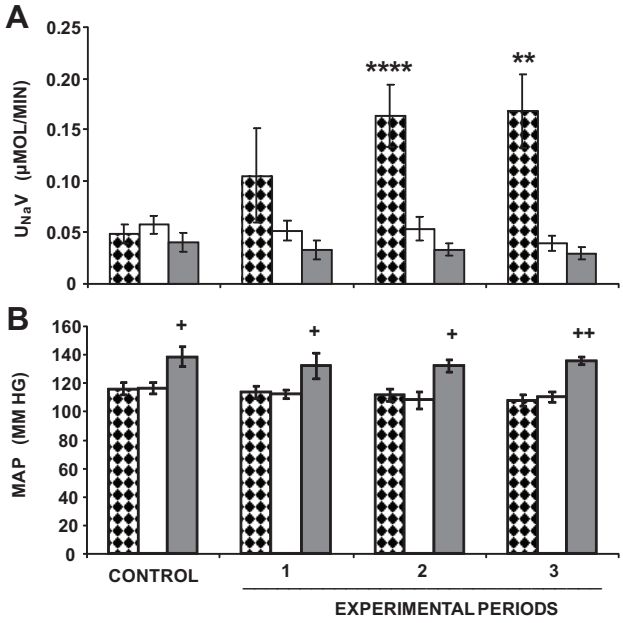


**Figure 1.** Direct RI infusion of candesartan, an $AT_1R$ antagonist, induces natriuresis that is blocked by the coinfusion of PD, a selective $AT_2R$ antagonist, in 12-week–old WKYs but not SHRs. MAP responses are significantly higher in SHRs than in WKYs and remain unchanged in response to any of the experimental infusions. A, During the control, only 5% $D_5W$ is infused in each animal. During the experimental periods, ▩ (n=7) indicates $U_{Na}V$ in WKYs in response to RI candesartan infusion, ■ (n=6) indicates $U_{Na}V$ in WKYs in response to RI infusion of candesartan+PD, and ■ (n=6) indicates $U_{Na}V$ in SHRs in response to RI infusion of candesartan. B, MAP responses to the conditions in A. Data represent mean±1 SE; $^{**}P<0.01$ and $^{****}P<0.0001$ from respective control period, and $+P<0.05$ and $++P<0.01$ between WKYs and SHRs.

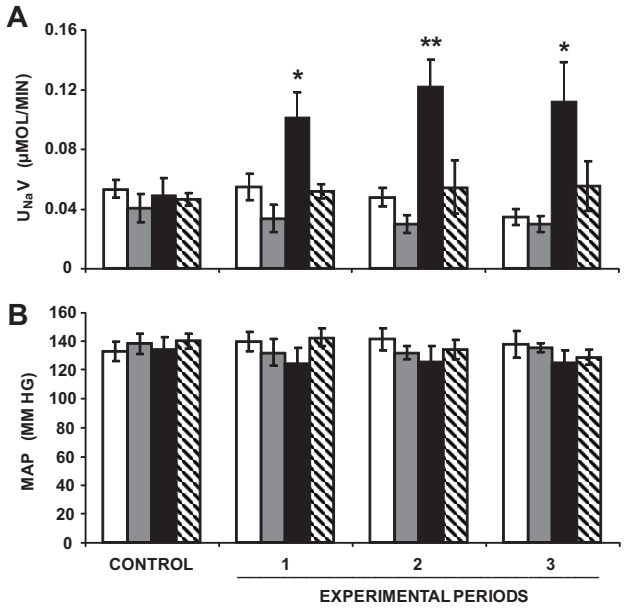


**Figure 2.** Direct RI infusion of candesartan, an $AT_1R$ antagonist, with and without PC-18, an inhibitor of APN, induces natriuresis that is blocked by infusion of PD, an $AT_2R$-antagonist, in 12-week–old SHRs. A, □ (n=6) indicates $U_{Na}V$ in response to RI $D_5W$ infusion for the entire duration of the experiment. ■ (n=6) indicates $U_{Na}V$ in response to RI infusion of candesartan, after a 1-hour control period, when only $D_5W$ was infused. ■ (n=6) indicates $U_{Na}V$ in response to RI coinfusion of candesartan+PC-18, after a 1-hour control period, when only $D_5W$ was infused. ▨ (n=7) indicates $U_{Na}V$ in response to RI coinfusion of candesartan+PC-18+PD after a 1-hour control period, when only $D_5W$ was infused. B, MAP responses to the conditions in A. Data represent mean±1 SE; $^{*}P<0.05$ and $^{**}P<0.01$ from the respective control period.

riod 2 and to 0.11±0.03 μmol/min ($P<0.05$) during experimental period 3. The addition of PD, an $AT_2R$ antagonist, abolished PC-18–engendered natriuresis in hypertensive SHRs. Neither $D_5W$- nor candesartan-infused kidneys demonstrated a significant change in $U_{Na}V$ across the duration of the experiment in these animals. MAP values remain unchanged in response to any of the pharmacological infusions in 12-week–old SHRs (Figure 2B).

## Baseline Renal Function Studies on 4-Week–Old WKYs and Prehypertensive SHRs

Figure 3A demonstrates reduced $U_{Na}V$ in 4-week–old prehypertensive SHRs compared with WKYs after 4 hours of vehicle infusion with RI $D_5W$ (0.02±0.003 versus 0.04±0.006 μmol/min, respectively). MAP values were not significantly different between 4-week–old WKYs and SHRs, with average values over 4 hours of 120.3±4 and 120.0±4 mm Hg, respectively (Figure 3B). Figure 4A demonstrates that 4-week–old WKYs and SHRs have similar GFRs after 4 hours of vehicle infusion with RI $D_5W$ (0.51±0.04 and 0.45±0.08 mL/min per gram of kidney weight, respectively). However, compared with 4-week–old SHRs, age-matched WKYs demonstrated significantly higher $FE_{Na}$ (0.16±0.02% versus 0.09±0.01%; $P<0.05$; Figure 4B) and $FE_{Li}$ (11.7±0.9% versus 7.7±0.9%; $P<0.01$; Figure 4C).

## Effects of RI Candesartan±APN Inhibition on $U_{Na}V$ and MAP in 4-Week–Old WKYs and Prehypertensive SHRs

After RI candesartan infusion, 4-week–old WKYs demonstrated an increase in $U_{Na}V$ from a baseline value of 0.05±0.01 to 0.15±0.02 μmol/min ($P<0.05$) after 3 hours of candesartan infusion (Figure 5A). The increase in $U_{Na}V$ was abolished by coinfusion of PD. In 4-week–old SHRs, RI candesartan infusion failed to increase $U_{Na}V$. However, as demonstrated in Figure 5A, RI infusion of PC-18, an inhibitor of APN, enabled natriuretic responses to RI candesartan in 4-week–old SHRs by increasing $U_{Na}V$ from a baseline value of 0.020±0.002 to 0.100±0.020 μmol/min ($P<0.01$). RI $AT_2R$ blockade with PD abolished PC-18–enabled natriuresis in 4-week–old SHRs. MAP values remained unchanged in 4-week–old WKYs or SHRs in response to RI candesartan± PC-18±PD (Figure 5B).

## Renal Function Studies in 4-Week–Old WKYs and Prehypertensive SHRs in Response to Natriuretic Stimuli

In 4-week–old WKYs, RI candesartan increased $FE_{Na}$ (Figure 6B) and $FE_{Li}$ (Figure 6C) from baseline values of 0.16±0.02% and 12.40±1.10% to 0.31±0.03% ($P<0.01$) and 26.00±2.40% ($P<0.001$), respectively. RI $AT_1R$ blockade failed to induce changes in GFR (Figure 6A) in these

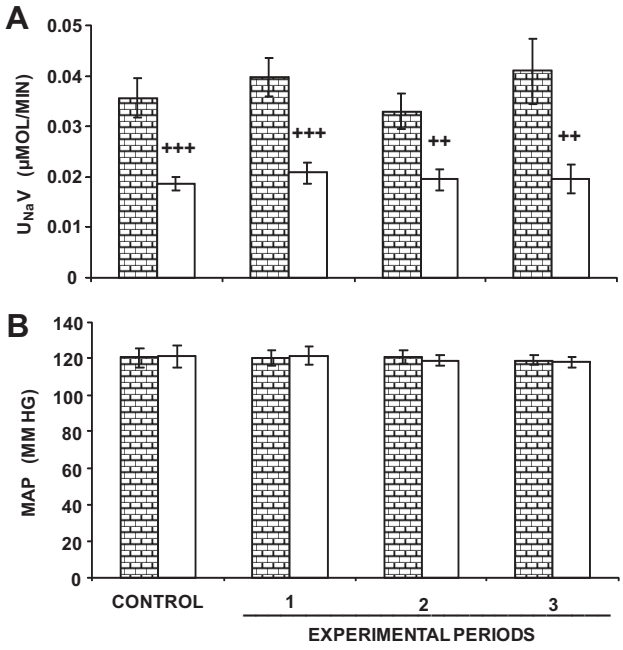


**Figure 3.** Baseline renal function studies on 4-week–old WKYs and SHRs. A, ▦ (n=9) indicates $U_{Na}V$ in WKYs in response to RI infusion of vehicle $D_5W$. □ (n=8) indicates $U_{Na}V$ in SHRs in response to RI infusion of vehicle $D_5W$. B, MAP responses to the conditions in A. Data represent mean±1 SE; $++P<0.01$ and $+++P<0.001$ from the respective WKY period.

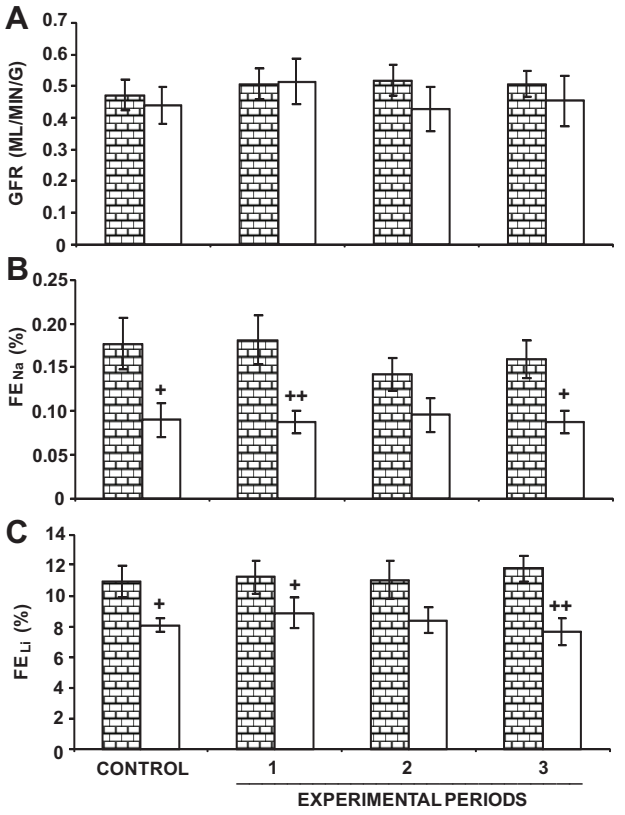


**Figure 4.** Baseline renal function studies on 4-week–old WKYs and SHRs. A, ▦ (n=9) indicates GFR in WKYs in response to RI infusion of vehicle $D_5W$. □ (n=8) indicates GFR in SHRs in response to RI infusion of vehicle $D_5W$. B, $FE_{Na}$ responses to conditions in A. C, $FE_{Li}$ responses to conditions in A. Data represent mean±1 SE; $+P<0.05$ and $++P<0.01$ from WKYs.

animals. In 4-week–old SHRs, RI candesartan infusion alone did not alter $FE_{Na}$, $FE_{Li}$, or GFR significantly. However, the addition of PC-18 to RI candesartan infusion increased the $FE_{Na}$ (Figure 6B) and the $FE_{Li}$ (Figure 6C) from 0.09±0.02% and 8.60±0.70% to 0.28±0.04% ($P<0.001$) and 29.30±4.20% ($P<0.0001$) after 3 hours. GFR remained unaffected after the addition of PC-18 to candesartan in 4-week–old SHRs (Figure 6A).

## Discussion

One of the proposed mechanisms of the initiation of hypertension in SHRs and humans involves a fundamental defect in the capacity of the kidney to excrete $Na^+$. Over time, a compensatory increase in renal perfusion pressure permits proper $Na^+$ excretion but also renders the animal hypertensive. Supporting this theory are the observations that transplantation of prehypertensive kidneys from SHRs to WKYs produces hypertension in WKYs[23] and that human subjects with genetic hypertension[24] and SHRs[25,26] excrete less $Na^+$ and water than normotensive controls when renal perfusion pressure is lowered to normotensive levels. Chronic relationships between arterial pressure and urinary $Na^+$ and water output are also shifted toward higher pressures in SHRs compared with WKYs, reflecting the kidney's adaptation to a higher perfusion pressure.[27]

In the present study, we hypothesized that $AT_2R$-mediated natriuresis is dysfunctional in SHRs because of rapid inactivation of the preferred ligand, Ang III. The major results provide insight into both the site and mechanisms of defective natriuresis in SHRs and are summarized as follows: (1) although selective intrarenal $AT_1R$ blockade induces significant $AT_2R$-mediated natriuresis in 12-week–old WKYs, identical infusions fail to do so in age-matched hypertensive SHRs; (2) defective natriuresis is present in 4-week–old SHRs before the onset of hypertension, and this occurs at the level of the renal proximal tubule; (3) inhibition of the activity of APN, the enzyme responsible for the degradation of Ang III, permits $AT_2R$-mediated natriuresis in both 4- and 12-week–old SHRs; and (4) in 4-week–old SHRs, the natriuresis engendered by PC-18 occurs at the level of the renal proximal tubule.

Previous studies have shown that RI $AT_1R$ blockade with candesartan induces natriuresis that is mediated by renal $AT_2Rs$ in 12-week–old Sprague-Dawley rats.[11] These results are not specific for the Sprague-Dawley strain, because the present study demonstrates similar $AT_2R$-mediated natriuresis in response to RI candesartan infusion in WKYs. The absence of MAP changes during RI $AT_1R$ blockade in WKYs indicates that the observed natriuresis is because of direct intrarenal, and not systemic hemodynamic, factors. Low-dose candesartan has been reported previously to increase $U_{Na}V$ without affecting MAP values in WKYs when administered systemically,[28] and the results of this study demonstrate that direct RI candesartan infusion at low doses has the same effect.

In comparison, 12-week–old SHRs fail to demonstrate an increase in $U_{Na}V$ after RI candesartan infusion. To investi-

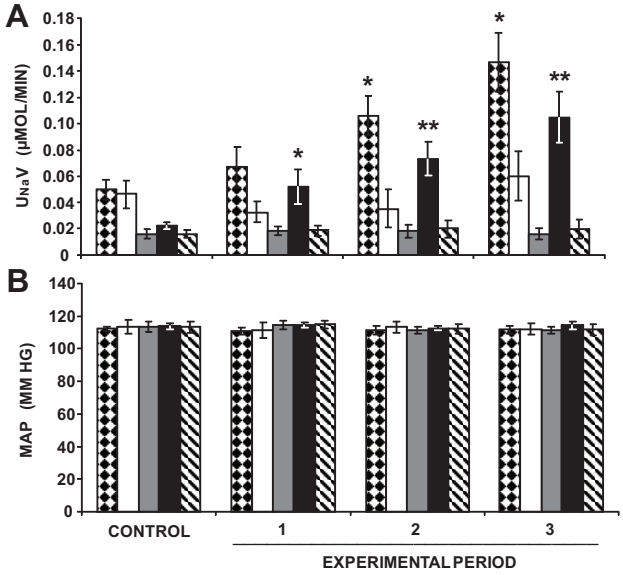


**Figure 5.** Direct RI infusion of candesartan, an $AT_1R$ antagonist, induces natriuresis in 4-week–old WKYs but not SHRs. The natriuresis is blocked by PD, an $AT_2R$ antagonist. RI coinfusion of candesartan+PC-18, an inhibitor of APN, engenders natriuresis in 4-week–old SHRs, and this is also blocked by PD. A, ▩ (n=6) indicates $U_{Na}V$ in WKYs in response to RI infusion of candesartan. □ (n=8) indicates $U_{Na}V$ in WKYs in response to RI coinfusion of candesartan+PD. ■ (n=7) indicates $U_{Na}V$ in SHRs in response to RI infusion of candesartan. ■ (n=8) indicates $U_{Na}V$ in SHRs in response to RI coinfusion of candesartan+PC-18. ▨ (n=8) indicates $U_{Na}V$ in SHRs in response to RI coinfusion of candesartan+PC-18+PD. B, MAP responses to the conditions in A. Data represent mean±1 SE; **P*<0.05 and ***P*<0.01 from the respective control period.

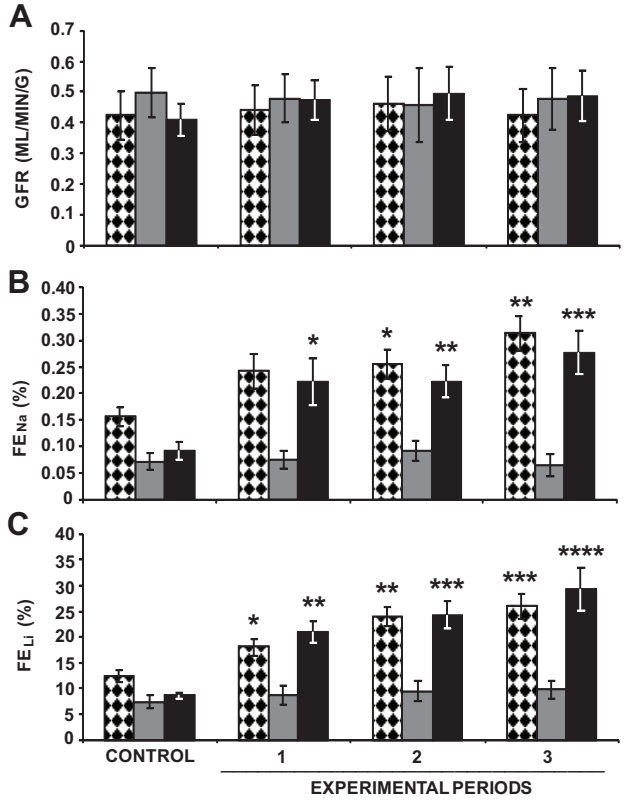


**Figure 6.** Renal function studies on 4-week–old WKYs and SHRs after the RI infusion of candesartan with and without PC-18. A, ▩ (n=11) indicates GFR in WKYs in response to RI infusion of candesartan. ■ (n=10) indicates GFR in SHRs in response to RI infusion of candesartan. ■ (n=10) indicates GFR in SHRs in response to RI coinfusion of candesartan+PC-18, an inhibitor of APN. B, $FE_{Na}$ responses to conditions in A. C, $FE_{Li}$ responses to conditions in A. Data represent mean±1 SE; **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001 from the respective control period.

gate whether the lack of response was a consequence of established hypertension, both basal and stimulated natriuretic responses were assessed in young, 4-week–old prehypertensive SHRs. Baseline $U_{Na}V$ was significantly reduced in young SHRs compared with age-matched WKYs, a finding that has been reported previously.[6,26] However, in the present study, a defect in stimulated natriuresis, that is, in response to $AT_1R$ blockade, was also observed in young SHRs. Thus, not only is baseline $Na^+$ excretion impaired before hypertension is established, but beneficial natriuretic responses mediated by renal $AT_2Rs$ are also compromised before hypertension develops in these animals.

The preferred ligand of $AT_2R$-mediated natriuresis in normal rodents is Ang III, not Ang II.[11,14] In the systemic circulation, Ang III is metabolized 2 to 4 times more rapidly than Ang II.[29,30] APN is the major enzyme responsible for the metabolism of Ang III in the kidney[31] and is expressed on brush border (apical) membranes of renal proximal tubule cells and enterocytes.[31] One of the first in vivo studies using PC-18 to inhibit the activity of APN was conducted in mice,[19] during which intracerebroventricular administration of PC-18 resulted in a 3.9-fold increase in the half-life of Ang III compared with control. The in vitro specificity of PC-18 toward APN, APA, and aminopeptidase B, 3 zinc metalloproteases with significant identity between their amino acid sequences, was also tested.[19] The inhibition constant values of this compound for APN were in the nanomolar range (inhibition constant: 8.0±1.7 nM), but PC-18 was 2150 and 125 times less active on APA and aminopeptidase B, respectively.[19] Thus, the infusion of PC-18 into the RI compartment in the present study allowed for examination of the effects mediated by Ang III within the intrarenal renin-angiotensin system.

In the present study, RI PC-18 infusion enabled natriuretic responses to $AT_1R$ blockade in both 4- and 12-week–old SHRs. Thus, the decreased availability of intrarenal Ang III, whether because of increased degradation by APN or decreased formation by APA, appears to be an important determinant of acute sodium excretion in SHRs. Previous reports have suggested that SHRs have increased renal proximal tubule cell APN protein expression compared with WKYs, although APN mRNA levels are similar between the 2 strains.[32] The increased expression of the major enzyme responsible for Ang III degradation may contribute to the lack of available Ang III for effective $AT_2R$-mediated natriuresis in SHRs, especially because natriuresis is ameliorated by APN inhibition. Furthermore, an inhibitory effect on APN during chronic $AT_1R$ blockade may provide an additional mechanism by which $AT_1R$ blockers improve hypertension in this strain. Chronic valsartan treatment has been reported to

reduce renal APN activity in renovascular hypertension,[33] and chronic ARB administration may influence APN activity in SHRs as well.

The nephron site at which $AT_2R$-mediated natriuresis is stimulated in 4- and 12-week–old WKYs and is defective in SHRs is the renal proximal tubule. Previous studies have validated the use of the $FE_{Li}$ as a marker of renal proximal tubule $Na^+$ transport in both WKYs[5] and SHRs.[5,34] In all of our studies, tubule events distal to the renal proximal tubule would have been detected by changes in $FE_{Na}$ that were not accompanied by parallel changes in $FE_{Li}$. However, this was not the case in the baseline sodium excretion rates in young SHRs or the stimulated natriuresis in WKYs or SHRs.

Thus far, studies regarding the mechanisms of increased renal proximal tubule $Na^+$ reabsorption in young SHRs have focused on alterations in renal dopaminergic and $AT_1R$-mediated effects. In the renal proximal tubule, increased activities of apical membrane sodium-hydrogen exchanger 3 and basolateral membrane sodium-potassium ATPase are associated with increased $Na^+$ reabsorption. In young SHRs, the ability of the dopamine $D_1$-like receptor to inhibit sodium-hydrogen exchanger 3 or sodium-potassium ATPase is impaired because of an uncoupling of the $D_1$-like receptor from its G-protein/effector complex.[15–17] Furthermore, increased renal proximal tubule $AT_1R$ expression,[18] elevated renal Ang II content,[35,36] and increased Ang II-$AT_1R$–mediated activation of sodium-hydrogen exchanger 3[37–39] have also been suggested as possible contributors to the excess $Na^+$ retention of young SHRs. However, as mentioned previously, $D_1$-like receptor-mediated natriuresis and natriuresis because of $AT_1R$ blockade are mediated, at least in part, by renal $AT_2Rs$.[11,12] Thus, the direct characterization of the natriuretic role of renal proximal tubule $AT_2Rs$ in this study, both in normal rodents and SHRs, where excess sodium reabsorption actually contributes to the pathogenesis of the disease, permits a deeper understanding of the mechanisms underlying the initiation of hypertension in this model. The provision of APN as a potential therapeutic target for the amelioration of hypertension in SHRs will be addressed in future studies.

## Perspectives

In both SHRs and hypertensive humans, increased $Na^+$ reabsorption contributes to the eventual onset of genetic hypertension. To date, the only published studies examining the increased $Na^+$ reabsorption of young prehypertensive SHRs have focused on 2 defects, elevated renal Ang II content causing increased $Na^+$ retention via the $AT_1R$ and functional hyposensitivity of renal proximal tubule cells to dopamine resulting in decreased $Na^+$ excretion. The recently elucidated roles of the renal $AT_2R$ and Ang III in the natriuretic responses of nonhypertensive rodents have become important to our understanding of the mechanisms that permit $Na^+$ excretion in normal animals. The present study investigated the role of $AT_2Rs$ in natriuresis in young SHRs and identified a potentially compelling therapeutic target to overcome early defects in renal proximal tubule $Na^+$ excretion in the initiation of hypertension.

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## Disclosures

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