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INTRARENAL AMINOPEPTIDASE N INHIBITION RESTORES DEFECTIVE AT_2R-MEDIATED NATRIURESIS IN SHR

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Abstract

The preferred ligand of AT_2R-mediated natriuresis is Ang III. The major enzyme responsible for the metabolism of Ang III is aminopeptidase N (APN), which is selectively inhibited by compound PC-18. In this study, urine sodium excretion rates ($U_{Na}V$), fractional excretion of sodium (FE_{Na}) and lithium (FE_{Li}), GFR, and mean arterial pressures (MAP) were studied in pre-hypertensive and hypertensive SHR, and compared to age-matched WKY. While renal interstitial (RI) infusion of AT_1R blocker candesartan increased $U_{Na}V$ in WKY from a baseline of 0.05±0.01 to 0.17±0.04 μmol/min (P<0.01), identical infusions failed to increase $U_{Na}V$ in hypertensive SHR. Co-infusion of AT_2R antagonist PD-123319 abolished the natriuretic responses to candesartan in WKY, indicating an AT_2R-mediated effect. AT_2R-mediated natriuresis was enabled in hypertensive SHR by inhibiting the metabolism of Ang III with PC-18 [0.05±0.01 to 0.11±0.03 μmol/min (*P*<0.05)]. The defects in sodium excretion were present prior to the onset of hypertension in SHR, since young WKY demonstrated double the $U_{Na}V$ of SHR (0.04±0.006 vs. 0.02±0.003 μmol/min, P<0.01) at baseline. The increased $U_{Na}V$ of young WKY was due to reduced renal proximal tubule sodium reabsorption, since increases in FE_{Na} were paralleled by increases in FE_{Li}. RI PC-18 infusion ameliorated defective AT_2R-mediated natriuresis in young SHR by increasing FE_{Na} and FE_{Li}, without changing GFR. Thus, increased renal proximal tubule sodium retention is observed prior to the onset of hypertension in SHR, and inhibition of the metabolism of Ang III ameliorates this pathophysiologic defect in sodium excretion.

Keywords

Natriuresis; angiotensin receptors; hypertension; angiotensin III; aminopeptidase N; SHR

INTRODUCTION

Spontaneously hypertensive rats (SHR) develop hypertension at approximately 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 SHR involves a primary defect in renal sodium (Na^+) excretion[2-8]. Over time, this defect necessitates an increase in renal perfusion pressure, an adaptation which becomes central to the development and maintenance of hypertension[9, 10].

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In normal rodents, both the intrarenal renin-angiotensin 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-angiotensin system, while the dopaminergic system regulates Na^+ excretion in response to high salt intake.

While an acute sodium load or rise in blood pressure increase sodium excretion in normal rodents, the following three significant pharmacological manipulations also induce natriuresis: (1) blockade of intrarenal angiotensin type-1 receptors (AT_1Rs), (2) stimulation of renal dopamine D_1-like receptors and (3) activation of intrarenal angiotensin 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, since concomitant blockade of renal AT_2Rs in these situations abolishes the natriuresis[11, 12]. Regarding direct renal AT_2R-induced natriuresis, renal interstitial (RI) angiotensin III (Ang III), but not angiotensin II (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 cells, is responsible for converting Ang II to Ang III. Ang III is subsequently degraded to angiotensin IV (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 SHR 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 due to 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 previously demonstrated 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 SHR unless the degradation of Ang III is inhibited, and that this defect is present prior to, rather than as a consequence of, established hypertension. Amelioration of AT_2R-mediated natriuresis in pre-hypertensive SHR 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- and12-week-old female WKY (Harlan) and SHR (Taconic), in accordance with the NIH 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 intraperitoneally. 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 more than one substance was simultaneously infused into the kidney, separate interstitial catheters were employed.

Rats were housed under controlled conditions (temperature: 21±1°C; humidity: 60±10%; and light: 8 to 20 h). 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 blodd pressure analyzer (Micromed Inc). MAP was recorded every 5 min and averaged for each of the control and experimental periods.

Pharmacological Agents

Candesartan, a specific, potent insurmountable inhibitor of AT_1R (IC_{50} >1×10^{-5} mol/L and 2.9×10^{-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} 2×10^{-8} mol/L and >1×10^{-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, K_i=8.0±1.7 nM)[19, 20], was provided by Drs. Fournie-Zaluski and Roques, 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 GFR, FE_{Na}, and FE_{Li}

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 utilizing a previously described method[21]. Tubular Na^+ reabsorption was determined by calculating FE_{Na} and renal proximal tubule Na^+ reabsorption was estimated using FE_{Li} as previously published[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

4- and 12-week-old WKY and SHR were studied on normal Na^+ intake (N=6 per group) 72 h following uninephrectomy. The remaining kidney was then infused for one hour with 5% dextrose in water (D_5W), designated as the 'Control Period' in the Figures. Following the 'Control Period', the kidney was infused with one of the following: 1) D_5W at 2.5 μL/ min, 2) candesartan (0.01 mg/kg/min), 3) candesartan + PD (10 μg/kg/min), 4) candesartan + PC-18 (25 μg/min), or 5) candesartan + PC-18 + PD directly into the RI space during three consecutive 1 h 'Experimental Periods'. Inulin and lithium chloride in D_5W were infused throughout the study via 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 the Statistical Analysis System. Multiple comparisons of individual pairs of effect means were conducted by the use of 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 WKY and SHR

As demonstrated in Figure 1A, in WKY, 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 co-infusion abolished the natriuretic responses to RI candesartan in WKY. In 12-week-old SHR, 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 h of candesartan infusion; P=NS). As illustrated in Figure 1B, SHR had higher MAP values

compared to WKY at baseline, but RI candesartan infusion did not significantly alter baseline MAP values in WKY or SHR. Similarly, co-infusion of PD with candesartan did not influence MAP in WKY.

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

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 period 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 SHR. 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 SHR (Figure 2B).

Baseline Renal Function Studies on 4-Week-Old WKY and Pre-Hypertensive SHR

Figure 3A demonstrates reduced $U_{Na}V$ in 4-week-old pre-hypertensive SHR compared to WKY following 4 h of vehicle infusion with RI D_5W (0.02±0.003 vs. 0.04±0.006 μmol/min, respectively). MAP values are not significantly different between 4-week-old WKY and SHR, with average values over 4 h of 120.3±4 and 120.0±4 mmHg, respectively (Figure 3B). Figure 4A demonstrates that 4- week-old WKY and SHR have similar GFRs following 4 h of vehicle infusion with RI D_5W (0.51±0.04 and 0.45±0.08 mL/min/g of kidney weight, respectively). However, compared to 4-week-old SHR, age-matched WKY demonstrate significantly higher FE_{Na} (0.16±0.02 vs. 0.09±0.01%, P<0.05, Figure 4B) and FE_{Li} (11.7±0.9 vs. 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 WKY and Pre-Hypertensive SHR

Following RI candesartan infusion, 4-week-old WKY 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) following 3 h of candesartan infusion (Figure 5A). The increase in $U_{Na}V$ was abolished by co-infusion of PD. In 4-week-old SHR, 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 SHR by increasing $U_{Na}V$ from a baseline value of 0.02±0.002 to 0.10±0.02 μmol/min (P<0.01). RI AT_2R blockade with PD abolished PC-18 enabled natriuresis in 4-week-old SHR. MAP values remained unchanged in 4-week-old WKY or SHR in response to RI candesartan ± PC-18 ± PD (Figure 5B).

Renal Function Studies in 4-Week-Old WKY and Pre-Hypertensive SHR In Response To Natriuretic Stimuli

In 4-week old WKY, RI candesartan increased FE_{Na} (Figure 6B) and FE_{Li} (Figure 6C) from baseline values of 0.16±0.02% and 12.4±1.1%, to 0.31±0.03% (P<0.01) and 26.0±2.4% (P<0.001), respectively. RI AT_1R blockade failed to induce changes in GFR (Figure 6A) in these animals. In 4-week-old SHR, 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 8.6±0.7% to 0.28±0.04% (P<0.001) and 29.3±4.2% (P<0.0001) after 3 h. GFR remained unaffected following the addition of PC-18 to candesartan in 4-week-old SHR (Figure 6A).

DISCUSSION

One of the proposed mechanisms of the initiation of hypertension in SHR and humans involves a fundamental defect in the kidney's capacity 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 pre-hypertensive kidneys from SHR to WKY produces hypertension in WKY[23], and that human subjects with genetic hypertension[24] and SHR[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 SHR compared to WKY, reflecting the kidneys adaptation to a higher perfusion pressure[27].

In the present study, we hypothesized that AT_2R-mediated natriuresis is dysfunctional in SHR due to rapid inactivation of the preferred ligand, Ang III. The major results provide insight into both the site and mechanisms of defective natriuresis in SHR, and are summarized as follows: (1) while selective intrarenal AT_1R blockade induces significant AT_2R-mediated natriuresis in 12-week-old WKY, identical infusions fail to do so in age-matched hypertensive SHR; (2) defective natriuresis is present in 4-week-old SHR prior to 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 SHR; and (4) in 4-week old SHR, 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, since the present study demonstrates similar AT_2R-mediated natriuresis in response to RI candesartan infusion in WKY. The absence of MAP changes during RI AT_1R blockade in WKY indicates that the observed natriuresis is due to direct intrarenal, and not systemic hemodynamic, factors. Low-dose candesartan has previously been reported to increase $U_{Na}V$ without affecting MAP values in WKY 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 SHR fail to demonstrate an increase in $U_{Na}V$ following RI candesartan infusion. To investigate whether the lack of response was a consequence of established hypertension, both basal and stimulated natriuretic responses were assessed in young, 4-week-old pre-hypertensive SHR. Baseline $U_{Na}V$ was significantly reduced in young SHR compared to age-matched WKY, a finding which has been reported previously[6, 26]. However, in the present study, a defect in stimulated natriuresis, i.e., in response to AT_1R blockade, was also observed in young SHR. 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 (APB), three zinc metalloproteases with significant identity between their amino acid sequences, was also

tested[19]. The K_i values of this compound for APN were in the nanomolar range (K_i=8.0±1.7 nM), but PC-18 was 2150 and 125 times less active on APA and APB, 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 SHR. Thus, the decreased availability of intrarenal Ang III, whether due to increased degradation by APN, or decreased formation by APA, appears to be an important determinant of acute sodium excretion in SHR. Previous reports have suggested that SHR have increased renal proximal tubule cell APN protein expression compared to WKY, even though APN mRNA levels are similar between the two 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 SHR, especially since 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 SHR as well.

The nephron site at which AT_2R-mediated natriuresis is stimulated in 4- and 12-week-old WKY and is defective in SHR, 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 WKY[5] and SHR[5, 34]. In all of our studies, tubule events distal to the renal proximal tubule would have been detected by changes in FENa that were not accompanied by parallel changes in FELi. However, this was not the case in the baseline sodium excretion rates in young SHR, or the stimulated natriuresis in WKY or SHR.

Thus far, studies regarding the mechanisms of increased renal proximal tubule Na^+ reabsorption in young SHR 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 (NHE-3) and basolateral membrane sodium-potassium ATPase (NKA) are associated with increased Na^+ reabsorption. In young SHR, the ability of the dopamine D_1-like receptor to inhibit NHE-3 or NKA is impaired due to 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 NHE-3 [37-39] have also been suggested as possible contributors to the excess Na^+ retention of young SHR. However, as mentioned previously, D_1-like receptor mediated natriuresis and natriuresis due to 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 SHR, 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 SHR will be addressed in future studies.

PERSPECTIVES

In both SHR 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 pre-hypertensive SHR have focused on two defects: (1) elevated renal Ang II content causing increased Na^+ retention via the AT_1R and (2) functional hyposensitivity of renal proximal tubule cells to dopamine resulting in decreased Na^+ excretion. The recently elucidated role of the renal AT_2R and Ang III in the natriuretic responses of non-hypertensive rodents has become important to our understanding of the

mechanisms which permit Na^+ excretion in normal animals. The present study investigated the role of AT_2Rs in natriuresis in young SHR, 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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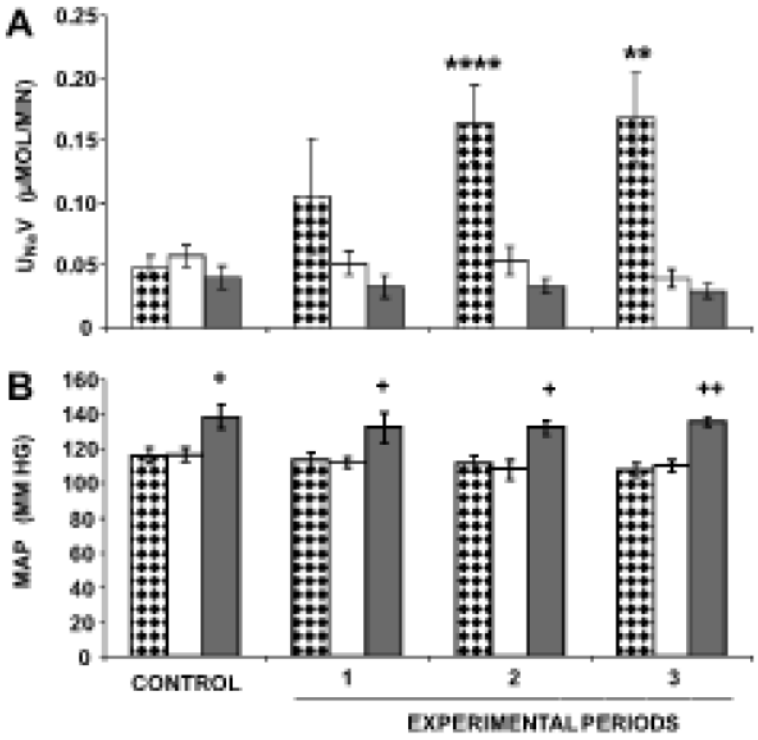

FIGURE 1.

Direct renal interstitial (RI) infusion of candesartan, an AT_1R antagonist, induces natriuresis that is blocked by the co-infusion of PD, a selective AT_2R antagonist, in 12-week-old WKY, but not SHR. Mean arterial pressure (MAP) responses are significantly higher in SHR than WKY and remain unchanged in response to any of the experimental infusions. **Panel A.** During the "Control", only 5% dextrose in water (D_5W) is infused in each animal. During the "Experimental Periods", ✠, (N=7) indicates urine sodium excretion rate ($U_{Na}V$) in WKY in response to RI candesartan infusion, □, (N=6) indicates $U_{Na}V$ in WKY in response to RI infusion of candesartan + PD, and ■, (N=6) indicates $U_{Na}V$ in SHR in response to RI infusion of candesartan. **Panel B.** Mean arterial pressure (MAP) responses to the conditions in **Panel 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 WKY and SHR.

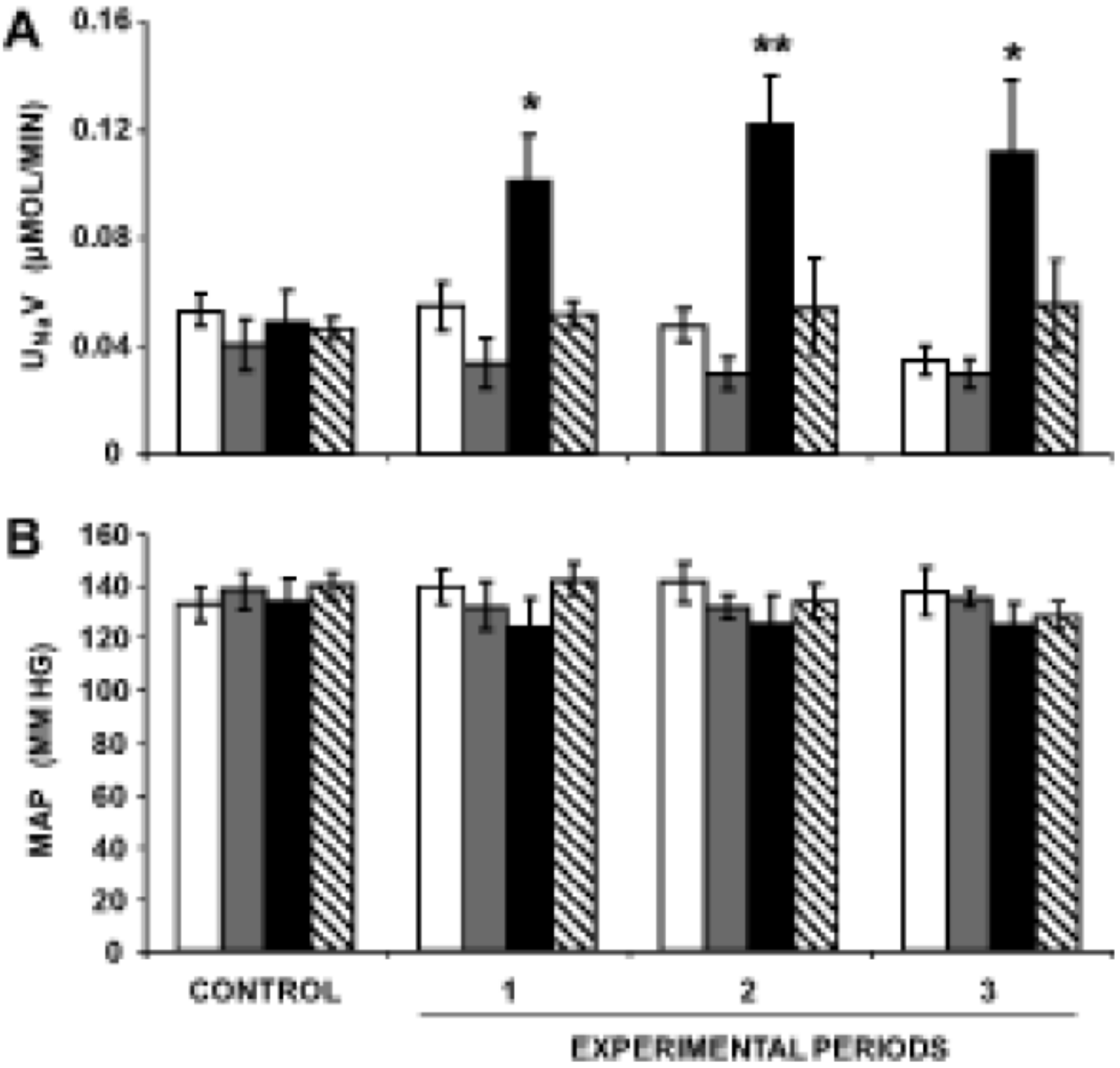

FIGURE 2.

Direct renal interstitial (RI) infusion of candesartan, an AT_1R antagonist, with and without PC-18, an inhibitor of aminopeptidase N (APN), induces natriuresis that is blocked by infusion of PD, an AT_2R-antagonist, in 12-week-old SHR. **Panel A.** □, (N=6) indicates urine sodium (Na^+) excretion rate ($U_{Na}V$) 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, following a 1 h control period, when only D_5W was infused. ■, (N=6) indicates $U_{Na}V$ in response to RI co-infusion of candesartan + PC-18, following a 1 h control period, when only D_5W was infused. ▨, (N=7) indicates $U_{Na}V$ in response to RI co-infusion of candesartan + PC-18 + PD, following a 1 h control period, when only D_5W was infused. **Panel B.** Mean arterial pressure (MAP)

responses to the conditions in **Panel A.** Data represent mean ± 1 SE; *P<0.05 and **P<0.01 from respective control period.

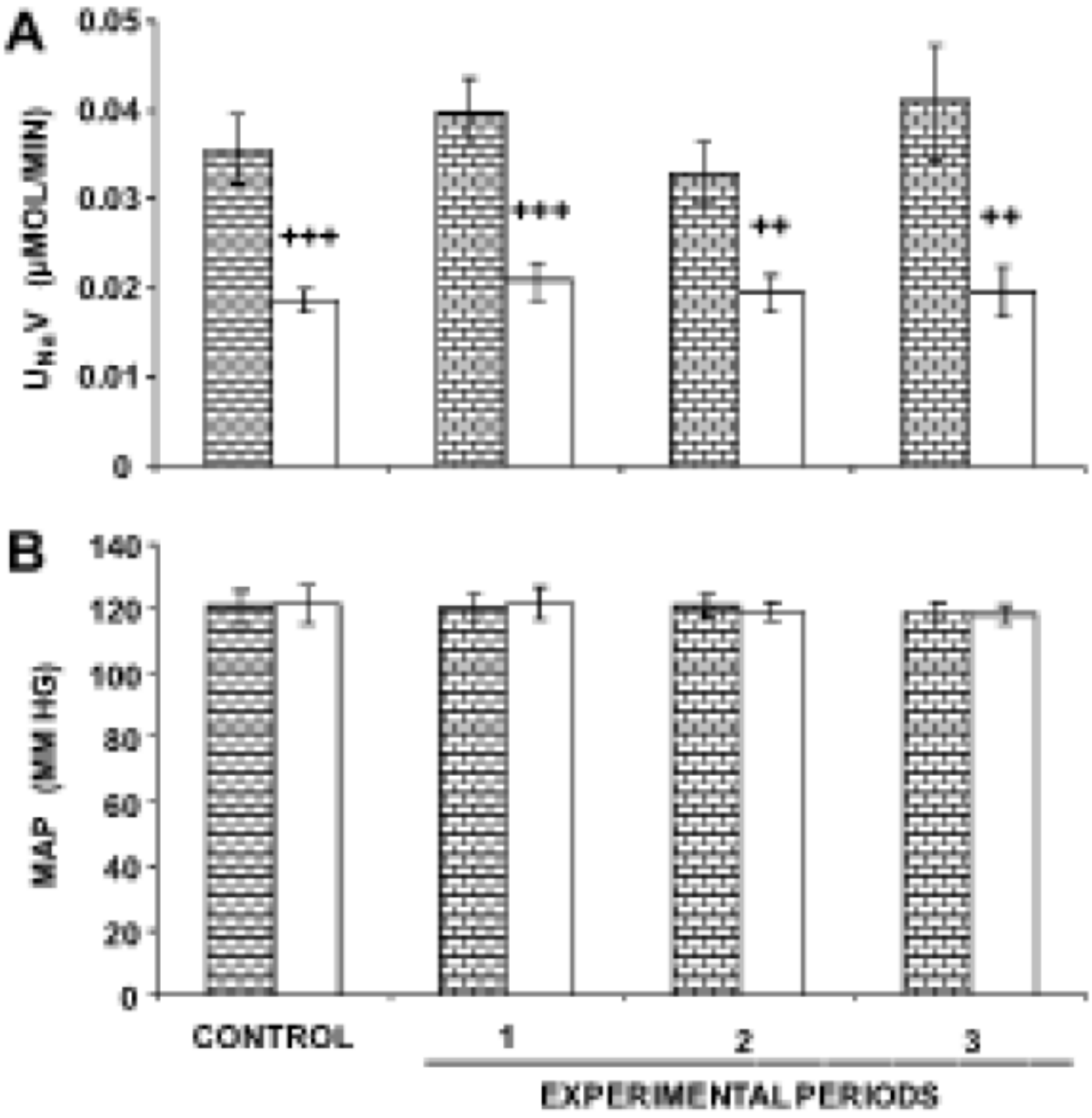

FIGURE 3.

Baseline renal function studies on 4-week-old WKY and SHR. **Panel A.** ▤, (N=9) indicates urine sodium excretion rate ($U_{Na}V$) in WKY in response to RI infusion of vehicle D_5W. □, (N=8) indicates $U_{Na}V$ in SHR in response to RI infusion of vehicle D_5W. **Panel B.** Mean arterial pressure (MAP) responses to the conditions in **Panel A.** Data represent mean ± 1 SE; ++P<0.01 and +++P<0.001 from respective WKY period.

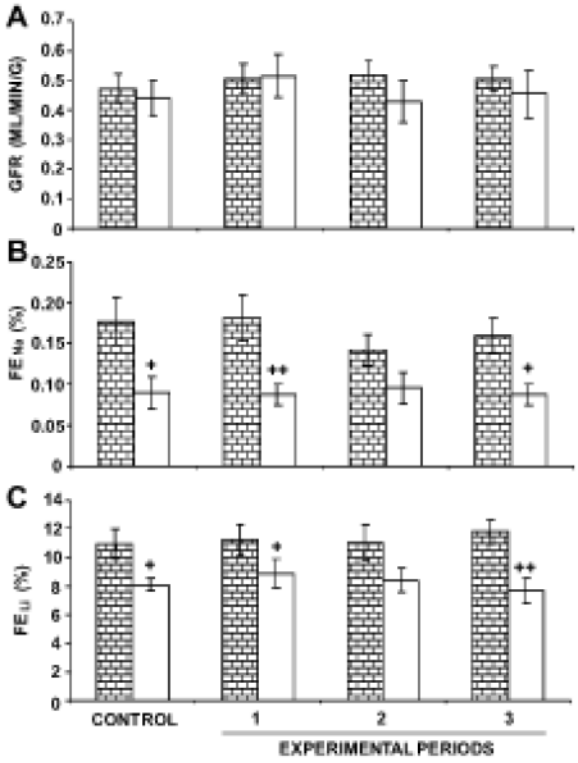

FIGURE 4.

Baseline renal function studies on 4-week old WKY and SHR. **Panel A.** ▤, (N=9) indicates glomerular filtration rate (GFR) in WKY in response to RI infusion of vehicle D_5W. □, (N=8) indicates GFR in SHR in response to RI infusion of vehicle D_5W. **Panel B.** Fractional excretion of sodium (FE_{Na}) responses to conditions in **Panel A. Panel C.** Fractional excretion of lithium (FE_{Li}) responses to conditions in **Panel A.** Data represent mean ± 1 SE; +P<0.05 and ++P<0.01 from WKY.

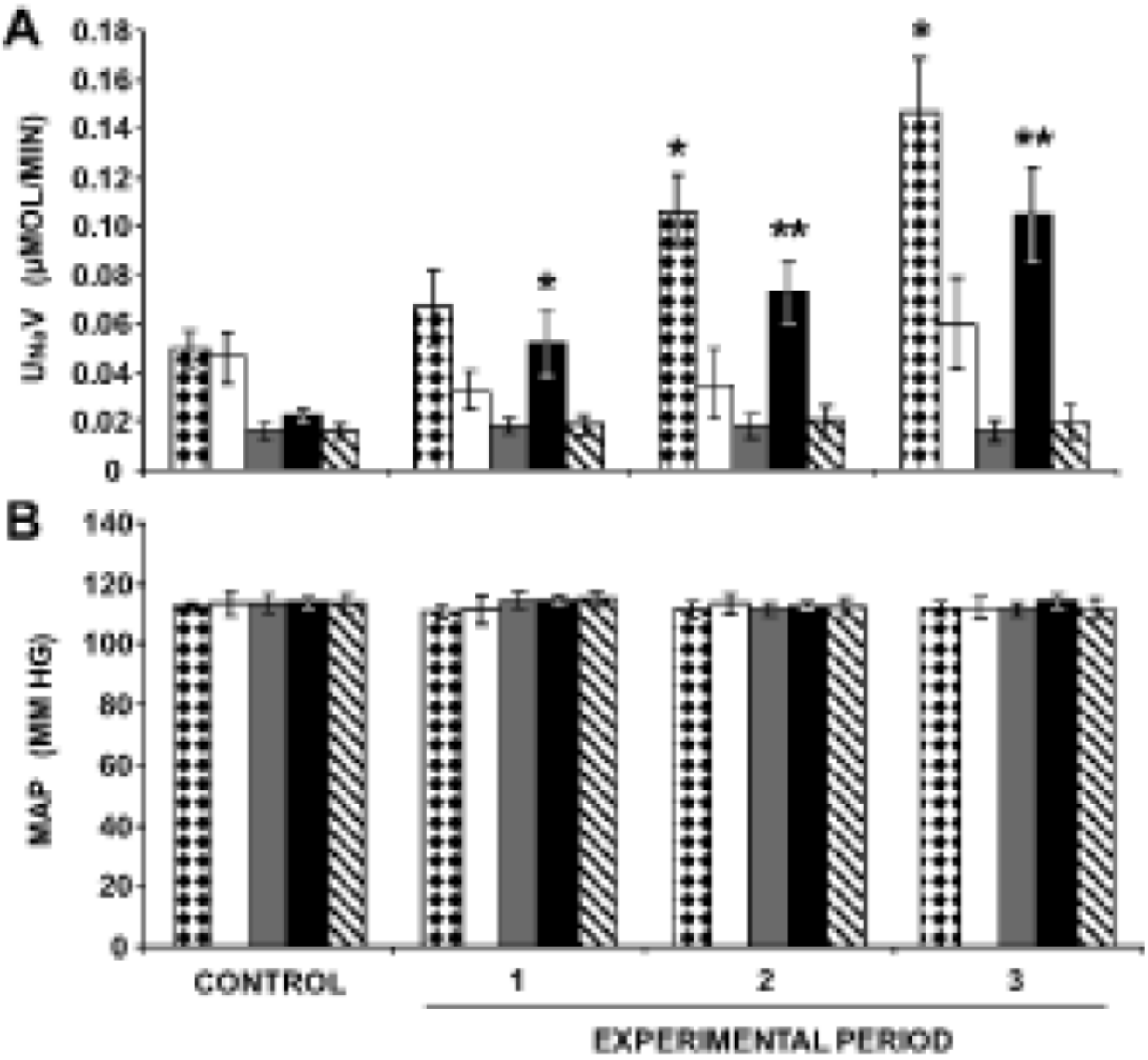

FIGURE 5.

Direct renal interstitial (RI) infusion of candesartan, an AT_1R antagonist induces natriuresis in 4-week-old WKY, but not SHR. The natriuresis is blocked by PD, an AT_2R antagonist. RI co-infusion of candesartan + PC-18, an inhibitor of aminopeptidase N (APN), engenders natriuresis in 4-week-old SHR, and this is also blocked by PD. **Panel A.** ⊡, (N=6) indicates urine sodium excretion rate ($U_{Na}V$) in WKY in response to RI infusion of candesartan. □, (N=8) indicates $U_{Na}V$ in WKY in response to RI co-infusion of candesartan + PD. ▩, (N=7) indicates $U_{Na}V$ in SHR in response to RI infusion of candesartan. ■, (N=8) indicates $U_{Na}V$ in SHR in response to RI co-infusion of candesartan + PC-18. ▨, (N=8) indicates $U_{Na}V$ in SHR in response to RI co-infusion of candesartan + PC-18 + PD. **Panel B.** Mean arterial pressure (MAP) responses to the conditions in **Panel A.** Data represent mean ± 1 SE; *P<0.05 and **P<0.01 from respective control period.

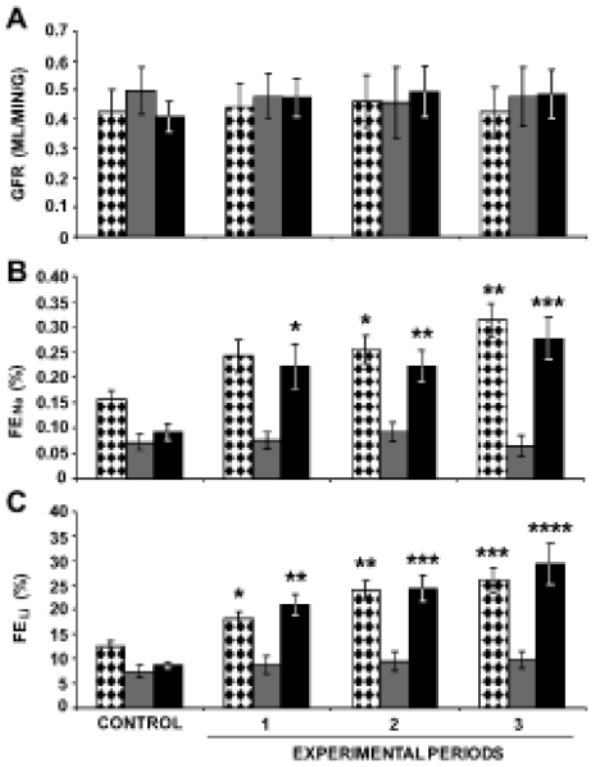

FIGURE 6.

Renal function studies on 4-week-old WKY and SHR following the RI infusion of candesartan with and without PC-18. **Panel A.** ⊡, (N=11) indicates glomerular filtration rate (GFR) in WKY in response to RI infusion of candesartan. ▩, (N=10) indicates GFR in SHR in response to RI infusion of candesartan. ■, (N=10) indicates GFR in SHR in response to RI co-infusion of candesartan + PC-18, an inhibitor of APN. **Panel B.** Fractional excretion of sodium (FE_{Na}) responses to conditions in **Panel A. Panel C.** Fractional excretion of lithium (FE_{Li}) responses to conditions in **Panel A.** Data represent mean ± 1 SE; *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 from respective control period.