Low-Dose Angiotensin II Reduces Urinary Cyclic AMP Excretion in Spontaneously Hypertensive, But Not Normotensive, Rats: Independence from Hypertension and Renal Hemodynamic Effects of Angiotensin

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ABSTRACT

The purpose of this study was to determine whether the greater inhibitory effect of angiotensin II (Ang II) on urinary cAMP excretion in spontaneously hypertensive rats (SHRs) compared with normotensive Wistar-Kyoto (WKY) rats is secondary to hypertension and/or renal hemodynamic changes induced by Ang II. SHRs and WKY rats were treated chronically from conception, 6 weeks of age, or 10 weeks of age (n = 8–10) with the angiotensin-converting enzyme inhibitor captopril (100 mg/kg/day). A fourth group was not treated chronically with captopril (n = 7). At ~13 weeks of age, all rats were anesthetized, given a bolus of captopril (30 mg/kg), and received an intrarenal infusion of a low dose of Ang II (1 ng/min). SHRs compared with WKY rats were normotensive, mildly hypertensive, and moderately hypertensive when treated with captopril from conception, 6 weeks of age, and 10 weeks of age, respectively, whereas untreated SHRs were severely hypertensive. In SHRs, Ang II decreased urinary cAMP excretion (p < .001), and this effect was independent of duration of captopril pretreatment (p = .696). In WKY rats, Ang II did not affect urinary cAMP excretion. Low-dose Ang II caused small and similar changes in renal blood flow and glomerular filtration rate in SHRs versus WKY rats and did not affect urine volume in either strain. We conclude that the greater effect of Ang II on urinary cAMP excretion in SHRs is not due to hypertension or to the renal hemodynamic effects of Ang II, but most likely to a greater effect of Ang II on some compartment of renal adenylyl cyclase activity in SHRs.

Angiotensin II (Ang II) has an enhanced ability to increase renal vascular resistance and decrease glomerular filtration rate and sodium excretion in spontaneously hypertensive rats (SHRs) compared with normotensive Wistar-Kyoto (WKY) rats (Li and Jackson, 1989; Arendshorst et al., 1990; Chatziantoniou et al., 1990; Chatziantoniou and Arendshorst, 1991; Kost and Jackson, 1993, 1994; Jackson, 1994; Vyas and Jackson, 1995). This renal abnormality is apparently genetically determined, as opposed to hypertension-induced, because the renal response to Ang II is enhanced in adult SHRs maintained normotensive by daily administration of captopril from 4 weeks of age until the time of study (Li and Jackson, 1989; Kost and Jackson, 1993) as well as in young (6 weeks of age) “prehypertensive” SHRs (Arendshorst et al., 1990; Chatziantoniou et al., 1990; Vyas and Jackson, 1995). Although the exact molecular defect remains elusive, some progress has been made in illuminating the mechanism of the enhanced renal sensitivity to Ang II in genetic hypertension. Several lines of evidence point to an abnormality involving dysregulation of renal cAMP metabolism (formation and/or catabolism). For instance, renal blood-flow studies demonstrate that the ability of prostacyclin, as well as other adenylyl-cyclase-activating agents, to attenuate Ang II-induced renal vasoconstriction is diminished in SHRs (Chatziantoniou and Arendshorst, 1992; Jackson and Herzer, 1993; Chatziantoniou et al., 1993, 1995; Jackson and Herzer, 1994). Because the ability of a lipophilic cAMP analog to inhibit renal vascular responses to Ang II is not diminished in SHRs (Chatziantoniou et al., 1993), the signal transduction defect appears not to involve biochemical systems activated by cAMP, thus suggesting a defect in renal cAMP metabolism.

Along these lines, we recently found that intrarenal artery infusions of Ang II decreased urinary cAMP excretion in young (6 weeks of age) SHRs and WKY and that this effect
was more pronounced in SHRs (Vyas and Jackson, 1995). Although our findings suggested a greater ability of Ang II to inhibit some compartment of renal adenyl cyclase in the SHR kidney, other explanations were possible. For instance, the strain-dependent effect of Ang II on urinary cAMP excretion could have been secondary, rather than primary, to hypertension because even at 6 weeks of age the arterial blood pressures were higher in SHRs. Moreover, in our previous study, the infusion rate of Ang II that caused a strain-dependent effect on urinary cAMP excretion also caused a strain-dependent effect on glomerular filtration and renal excretory function. Therefore, the greater effect of Ang II on urinary cAMP excretion in SHRs could have been secondary to the greater effects of Ang II on renal function.

The objective of the present study was to determine whether the greater inhibitory effect of Ang II on urinary cAMP excretion in SHRs is secondary to hypertension and/or renal hemodynamic changes induced by Ang II. To achieve this goal, we examined the effects of low-dose intrarenal infusions of Ang II on urinary cAMP excretion and renal hemodynamic parameters in SHRs and WKY rats that had been treated from either conception, 6 weeks of age, or 10 weeks of age with the angiotensin-converting enzyme (ACE) inhibitor captopril, as well as in SHRs and WKY rats that were not pretreated chronically with captopril. With the use of a low dose of Ang II, we were able to avoid strain-dependent effects of Ang II on renal hemodynamics, and with the use of rats treated for various lengths of time with captopril, we were able to determine whether the greater effects of Ang II on urinary cAMP in SHRs correlated with the degree of hypertension.

**Materials and Methods**

Female SHRs and WKY breeders (Taconic Farms, Germantown, NY) were treated with captopril (100 mg/kg/day in drinking water) for 1 week before conception and continuing through gestation and lactation. At 4 weeks of age, the male offspring of the captopril-treated dams were weaned and treated with captopril (100 mg/kg/day in drinking water) from 4 weeks of age until 13 weeks of age when the studies were performed. Other male SHR and WKY rats were treated with captopril (100 mg/kg/day in drinking water) from either 6 weeks of age or 10 weeks of age until 13 weeks of age when the studies were performed. Yet other male SHRs and WKY rats were not chronically pretreated with captopril and were studied at 13 weeks of age. Institutional guidelines for animal welfare were followed at all times. Rats were housed in the animal care facility at the University of Pittsburgh and kept in a 12-h light/dark cycle (7 AM to 7 PM) at an ambient temperature of 22°C and relative humidity of 55% and were fed Wayne Rodent Blox 8604 (sodium, 135 mM, pH 9.3) and 20 μl of a 10-μM solution of internal standard 9-β-D-arabinofuranosyladenine were added to 0.2 ml of urine, and the sample was cleaned with a C18 Sep-Pak cartridge (Waters, Milford, MA) by washing the column with 5 ml of 0.5 mM ammonium sulfate (pH 9.3) followed by 2 ml of 10% methanol in 10 mM phosphoric acid. Forty microliters of 0.5 M acetate buffer (pH 4.8) and 40 μl of 50% chloroacetalddehyde in water were added to the last 1.5 ml of eluant from the Sep-Pak cartridge, and the sample was incubated for 1 h at 80°C. This affected derivatization of cAMP to N6-etheno-cAMP and of internal standard to N6-etheno-9-D-arabinofuranosyladenenine. Derivatized samples (40 μl) were injected into an ISCO (Lincoln, NE) HPLC system (pump model 2350, gradient program model 2360, 4.6 × 250 mm C18 reversed-phase column with 5-μm particle size; ChemiResearch Data Management System). Fluorometric detection was achieved at an excitation wavelength of 275 nm and emission wavelength of 420 nm with a Waters 470 fluorescence detector. The mobile phase was composed of 95.5% citrate-phosphate buffer (0.014 M citric acid and 0.017 M Na2PO4) with 4.5% acetonitrile and was run isocratically at 1.2 ml/min. A standard curve for cAMP was constructed with the ratio of areas of cAMP with that of the internal standard. This method achieved a detection sensitivity of 0.12 pmol/injection.

Glomerular filtration rate was calculated by dividing urinary inulin excretion by the midpoint plasma inulin concentration, and cAMP excretion was calculated by multiplying the urine volume by the urine cAMP concentration.

Data were analyzed with a three-factor ANOVA with repeated measures (factor A, onset of captopril treatment (four levels: conception, 6 weeks of age, 10 weeks of age, or no chronic pretreatment); factor B, rat strain (two levels: SHR and WKY); and factor C, intrarenal infusion (two repeated levels: vehicle and Ang II)) with the Number Cruncher Statistical System (version 6.0; Kaysville, UT). Post hoc tests were conducted only if a main effect or interaction effect was significant. In this regard, if no interaction effects were significant, yet a main effect was significant, post hoc comparisons (Fisher’s least-significant difference (LSD) test) were conducted on the main effect if the main effect had more than two levels. If a two-way interaction, but not a three-way interaction, was significant, post hoc comparisons were performed on the two-way interac-
tion effects (Fisher’s LSD test), and post hoc comparisons on the involved main effects were not performed. If a three-way interaction was significant, post hoc comparisons were performed on the three-way interaction effects by conducting an appropriate subset of two-factor ANOVAs. For significant three-way interactions, post hoc comparisons involving two-way interactions and main effects were not performed. The criterion for significance was \( p < .05 \). All data in figures and text are presented as mean \( \pm \) S.E.

**Results**

In animals not pretreated chronically with captopril, MABP was much greater in SHRs compared with WKY rats (before the acute bolus of captopril: 171 ± 8 versus 110 ± 7 mm Hg, respectively, \( p < .001 \); 1 h after the acute bolus of captopril: 115 ± 5 versus 93 ± 6 mm Hg, respectively, \( p < .001 \)). In animals treated chronically with captopril from 10 weeks of age, MABP was moderately higher in SHRs versus WKY rats (before the acute bolus of captopril: 124 ± 3 versus 107 ± 2 mm Hg, respectively, \( p < .001 \); 1 h after the acute bolus of captopril: 114 ± 2 versus 99 ± 2 mm Hg, respectively, \( p = .001 \)). In animals treated chronically with captopril from 6 weeks of age, MABP was modestly higher in SHRs versus WKY rats (before the acute bolus of captopril: 104 ± 2 versus 95 ± 3 mm Hg, respectively, \( p = .031 \); 1 h after the acute bolus of captopril: 100 ± 3 versus 91 ± 3 mm Hg, respectively, \( p = .043 \)). In animals treated chronically with captopril from conception, MABPs in SHRs versus WKY rats were numerically similar and not significantly different (before the acute bolus of captopril: 98 ± 4 versus 99 ± 4 mm Hg, respectively; 1 h after the acute bolus of captopril: 92 ± 1 versus 87 ± 4 mm Hg, respectively). The younger the animals when captopril was initiated, the less the absolute differences in average MABPs between SHRs and WKY rats. Before the acute bolus of captopril, the differences in average MABPs between SHRs and WKY rats were 61, 17, 9, and −1 mm Hg when chronic captopril was not administered and when chronic captopril was initiated at 10 weeks of age, 6 weeks of age, or conception, respectively. After the acute bolus of captopril, the difference in average MABPs between SHRs and WKY rats was 22, 15, 9, and 5 mm Hg when chronic captopril was not administered and when chronic captopril was initiated at 10 weeks of age, 6 weeks of age, or conception, respectively.

Figure 1 illustrates the effects of low-dose intrarenal infusions of Ang II (1 ng/min) on urinary cAMP excretion in SHRs and WKY rats not chronically pretreated with captopril and in SHRs and WKY rats pretreated with captopril from 10 weeks of age, 6 weeks of age, or conception. For urinary cAMP excretion, a three-factor ANOVA indicated a significant effect of Ang II (\( p < .001 \)), a significant interaction between rat strain and Ang II (\( p < .001 \)), but a nonsignificant interaction between level of chronic captopril pretreatment (none, 10 weeks of age, 6 weeks of age, or from conception), rat strain, and Ang II. This analysis indicates that Ang II reduced urinary cAMP; however, the reduction was dependent on rat strain regardless of whether chronic captopril was administered and regardless of the duration of chronic captopril pretreatment. A post hoc analysis (Fisher LSD test) indicated that Ang II significantly decreased urinary cAMP excretion in SHRs, but not in WKY rats.

As shown in Fig. 2, low-dose intrarenal infusions of Ang II slightly but significantly (\( p < .001 \)) reduced renal blood flow. This effect was not statistically dependent on rat strain (i.e., neither the strain × Ang II nor the captopril × strain × Ang II interaction was significant). However, the ability of Ang II to reduce renal blood flow was significantly (\( p < .001 \)) dependent on chronic captopril treatment. In this regard, Ang II significantly decreased renal blood flow in all groups treated chronically with captopril but not in animals not pretreated chronically with captopril.

By three-factor ANOVA, Ang II (\( p = .046 \)) and captopril (\( p < .001 \)) independently affected glomerular filtration rate; however, these effects were mild and independent of rat strain; i.e., neither the strain × Ang II, captopril × strain, nor the captopril × strain × Ang II interaction was significant (Fig. 3). Post hoc analysis demonstrated that captopril treatment from conception lowered glomerular filtration rate compared with all other groups. Low-dose intrarenal artery infusions of Ang II did not affect urine volume, and the strain × Ang II and captopril × strain × Ang II interactions were not significant (Fig. 4).

As shown in Fig. 5, intrarenal infusions of Ang II were associated with very small increases in MABP. In this regard, there was a significant three-way interaction among captopril pretreatment, rat strain, and Ang II. This interaction was further explored by subjecting each captopril-pre-treatment paradigm group to a two-factor ANOVA. This analysis indicated that the effects of Ang II on MABP were no different in SHRs versus WKY rats not chronically pretreated with captopril and were no different in SHRs versus WKY rats pretreated with captopril from 6 weeks of age. In SHRs treated with captopril from 10 weeks of age, the pressor response to Ang II was greater versus comparably treated WKY rats, and in SHRs treated with captopril from conception, the pressor response to Ang II was lesser versus comparably treated WKY rats.

**Discussion**

The importance of the enhanced sensitivity of the SHR kidney to Ang II, with respect to Ang II-induced changes in renal vascular resistance and excretory function, is that it may explain in part the pathophysiology of hypertension in this animal model of genetic hypertension. Numerous studies demonstrate that chronic blockade of the renin-angiotensin system with pharmacological agents prevents the development of hypertension in young SHRs (Ferrone et al., 1979; Hefti et al., 1986; Bunkenburg et al., 1991) and normalizes arterial blood pressure in adult SHRs (Antonaccio et al., 1979), despite the fact that the renin-angiotensin system does not appear to be overly activated in SHRs (Koletsky et al., 1970; Sen et al., 1972; Shiono and Sokabe, 1976; Campbell et al., 1995). Furthermore, renal transplantation studies in SHRs clearly demonstrate that the mechanism responsible for genetic hypertension in SHRs resides in the kidney (Rettig et al., 1991; Rettig and Unger, 1991). An enhanced renal response to Ang II in SHRs, with respect to Ang II-induced changes in renal vascular resistance and excretory function, would explain why Ang II is critical to the development and maintenance of hypertension in SHRs and would explain why hypertension in SHRs tracks the SHR kidney. It is important, therefore, to elucidate the renal biochemical defect that gives rise to enhanced ability of Ang II to affect
renovascular resistance and excretory function in the SHR kidney.

In a previous study, we demonstrated that in isolated perfused kidneys from adult SHRs and WKY rats, Ang II inhibited renal venous cAMP secretion induced by isoproterenol in SHRs, but not in WKY rats (Vyas et al., 1996). In another study, we found that urinary cAMP excretion in young (6 weeks of age) SHRs and WKY rats was decreased by intrarenal infusions of Ang II and that the Ang II-induced decrease in urinary cAMP was greater in SHRs compared with WKY rats (Vyas and Jackson, 1995). These data suggest that some renal compartments of adenylyl cyclase (e.g., vascular, tubular, and/or interstitial) are more sensitive to Ang II-induced inhibition in SHRs compared with WKY rats. Although suggestive of a genetically determined defect, our previous studies do not rule out the possibility that the greater effects of Ang II on cAMP metabolism in the SHR kidney are secondary, rather than primary, to hypertension because blood pressure in SHRs is already elevated by 6 weeks of age. To test this possibility, we examined the effects of intrarenal infusions of Ang II on renal urinary cAMP excretion in SHRs and WKY rats treated chronically for different periods of time with captopril.

Chronic administration of ACE inhibitors such as captopril prevents the development of hypertension in young SHRs (Ferrone et al., 1979; Hefti et al., 1986) and dramatically lowers blood pressure in adult SHRs (Antonaccio et al., 1979), with the degree of antihypertensive effect roughly dependent on the age at which ACE inhibition is initiated as well as on the duration of treatment. By either not pretreating with captopril or by treating rats with captopril from conception, 6 weeks of age, or 10 weeks of age until 13 weeks of age, we were able to effect different degrees of blood pressure status in the SHRs. SHRs not pretreated with captopril were severely hypertensive, and SHRs treated from 10 weeks of age with captopril were moderately hypertensive. In contrast, SHRs treated from 6 weeks of age were borderline hypertensive, and SHRs treated from conception were clearly hypertensive, and SHRs treated from conception were clearly hypertensive.
normotensive, in fact “equitensive” with WKY rats treated from conception with captopril.

In the present study, we observed a greater reduction in urinary cAMP excretion in response to intrarenal infusions of a low dose of Ang II in 13-week-old SHRs compared with age-matched WKY rats. Importantly, unlike arterial blood pressure, the effects of Ang II on urinary cAMP excretion were not dependent on the duration of captopril administration. All groups of SHRs displayed statistically indistinguishable reductions in urinary cAMP excretion, whereas Ang II did not influence urinary cAMP excretion in WKY rats regardless of how long they had received captopril. Thus, the greater effect of Ang II on urinary cAMP excretion in SHRs is not secondary to the development of hypertension.

In our previous study (Vyas and Jackson, 1995), we demonstrated a large difference between SHRs and WKY rats with regard to Ang II-induced inhibition of urinary cAMP excretion, indicating the potential importance of this phenomenon for the pathophysiology of hypertension. However, the dose of Ang II that decreased urinary cAMP excretion in SHRs also caused a much greater reduction in glomerular filtration rate and urine volume in SHRs compared with WKY rats. Although Ang II usually causes a greater reduction in renal hemodynamic and excretory parameters in SHRs compared with WKY rats, in the present study, by using a very low dose of Ang II, we were able to avoid statistically significant strain-dependent differences in the renal responses to Ang II. Therefore, the greater effects of Ang II on urinary cAMP excretion in SHRs cannot be attributed to differential effects of Ang II on renal hemodynamics or excretory function. Although the low dose of Ang II avoided strain-dependent differences in the renal responses to Ang II, the changes in cAMP excretion in SHRs induced by the low dose of Ang II were modest. In this regard, our previous study (Vyas and Jackson, 1995) and present study are complementary. Our previous study (Vyas and Jackson, 1995) demonstrates the magnitude of the effects of Ang II on urinary cAMP excretion in SHRs, and the present study
demonstrates that the effects of Ang II on urinary cAMP excretion can be dissociated from Ang II-induced changes in renal hemodynamics or excretory function. Although more experiments may have revealed significant differences in the renal hemodynamic or excretory responses to Ang II, given the numerical similarity of renal responses in SHRs and WKY rats in the present study, it is highly unlikely that any differences, even if statistically significant, could account for the greater effects of Ang II on urinary cAMP excretion in SHRs.

The greater effects of Ang II on urinary cAMP excretion in SHRs cannot be attributed to differential effects of intrarenal infusions of Ang II on MABP. The acute intrarenal infusions of Ang II increased MABP in both SHRs and WKY rats. In two groups, these mild pressor effects were similar in SHRs versus WKY rats, whereas in one group the pressor effect was greater in WKY rats compared with SHRs. In yet another group, the pressor effect was greater in SHRs compared with WKY rats. Thus, although Ang II always reduced urinary cAMP excretion in SHRs more so than in WKY rats, the mild pressor effects of intrarenal infusions of Ang II were variably associated with strain, depending upon the exact captopril-pretreatment paradigm. Therefore, it is unlikely that variable differential changes in blood pressure induced by Ang II account for the consistent strain-dependent effects of Ang II on urinary cAMP.

Is it possible that chronic captopril treatment resulted in alterations in Ang II receptor populations in the kidney, particularly in the SHRs, contributing to the observed differences in the effects of exogenous Ang II on urinary cAMP excretion? Wu et al. (1994) treated SHRs and WKY rats from conception with captopril in their drinking water. At 7 days of age, specific $^{125}$I-$[\text{Sar}^1,\text{Ile}^8]\text{angiotensin II}$ binding in kidney membranes was similar in control WKY rats, control SHRs, captopril-treated WKY rats, and captopril-treated SHRs. At 4 months of age, specific $^{125}$I-$[\text{Sar}^1,\text{Ile}^8]\text{angiotensin$}$
II binding in kidney membranes was somewhat lower in captopril-treated SHRs versus control SHRs. Receptor affinities were not altered by captopril treatment, regardless of the duration of treatment. Thus, the studies by Wu et al. (1994) support the conclusion that the enhanced inhibitory effect of Ang II on urinary cAMP excretion in SHRs pretreated chronically with captopril cannot be attributed to up-regulation of Ang II receptors in SHR kidneys by the chronic captopril pretreatment. This conclusion is further supported by our observation that Ang II decreased urinary cAMP excretion in SHRs, but not in WKY rats, regardless of whether the animals were chronically treated with captopril from conception, 6 weeks of age, or 10 weeks of age, and regardless of whether the animals were chronically pretreated with captopril or not.

The observations of the current study are consistent with our previous study (Vyas and Jackson, 1995), which demonstrated a greater reduction in urinary cAMP in response to intrarenal infusions of Ang II in SHRs that were 6 weeks of age, and are consistent with a genetically determined dysregulation of renal cAMP metabolism. It is not possible, however, to infer from the present study whether this defect in renal cAMP metabolism resides in the renal circulation, tubular epithelial cells, or renal interstitial cells. A renovascular site would be most consistent with our hypothesis that an abnormality in renovascular cAMP metabolism explains the greater renovascular response to Ang II in SHRs. However, counter to this argument is our in vitro results in cultured preglomerular vascular smooth muscle cells from SHRs and WKY rats, demonstrating that Ang II enhances, rather than inhibits, cAMP production in cells from both strains (Mokkapatti et al., 1998). However, we do not know whether these results in cell culture reflect the true response of preglomerular vascular smooth muscle cells in vivo. Nonetheless, the results of our current and previous (Vyas et al., 1996) studies demonstrate that in the intact kidney the net effect of Ang II on renal cAMP metabolism is negative and more so in SHR kidneys. Additional studies are warranted to determine which renal compartments of adenylyl cyclase are more sensitive to the inhibitory effects of Ang II and to determine whether this increased sensitivity of renal adenylyl cyclase to Ang II actually contributes to the enhanced

![Graph showing urinary volume in SHRs and WKY rats](image1)

**Fig. 4. Effects of Ang II on urine volume in SHRs and WKY rats.** Rats were either not chronically pretreated with captopril or were pretreated with captopril from either 10 weeks of age, 6 weeks of age, or conception until ~13 weeks of age, at which time Ang II was infused into the renal artery at 1 ng/min. Values represent means ± S.E. for the indicated number of animals.
renal sensitivity to Ang II and to the pathophysiology of genetic hypertension.

In summary, in SHRs and WKY rats either not pretreated chronically with captopril or pretreated from either conception, 6 weeks of age, or 10 weeks of age with an antihypertensive dose of captopril, Ang II exerted an enhanced ability to reduce urinary cAMP excretion. This greater effect on urinary cAMP excretion could not be attributed to the status of arterial blood pressure or the renal response to Ang II. Therefore, SHRs appear to have a genetically determined increased sensitivity to Ang II with regard to inhibiting at least some compartments of adenylyl cyclase activity. Which renal compartments of adenylyl cyclase are more sensitive to the inhibitory effects of Ang II, whether this effect is observed with chronic administration of Ang II, and whether this genetically determined feature of SHRs contributes to the pathophysiology of essential hypertension will be the subject of future investigations.

References


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