Kidney Aminopeptidase A and Hypertension, Part I
Spontaneously Hypertensive Rats
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Abstract—Tissue and plasma levels of aminopeptidase A (APA), the principal enzyme that hydrolyzes angiotensin II (Ang II) to angiotensin III, were measured in spontaneously hypertensive rats (SHR) and their normotensive control strain at 3 different ages corresponding to prehypertensive (4 weeks), developing (8 weeks), and established (16 weeks) phases of hypertension. Plasma APA activity was significantly but modestly elevated in SHR at all 3 ages compared with normotensive Wistar-Kyoto rats. Likewise, levels of APA in brain, heart, and adrenal gland were generally, but again only moderately, elevated in SHR at all ages. However, a large increase in APA activity was seen within the kidney in which APA levels were elevated 41%, 51%, and 68% in SHR at 4, 8, and 16 weeks of age, respectively. Kidney APA levels were also significantly increased in immunoblots from 8- and 16-week-old SHR. Glomeruli isolated from 16-week-old SHR had 57% higher APA activity and increased immunoreactivity compared with Wistar-Kyoto rats. To determine whether the increase in kidney APA activity in SHR was related to Ang II levels, SHR were treated for 2 weeks with the angiotensin-converting enzyme inhibitor captopril. Captopril treatment reduced blood pressure to normotensive values and resulted in a 25% reduction in kidney APA activity. These results suggest that APA expression in the kidney may be regulated by activity of the renin-angiotensin system. If so, this would further suggest that upregulation of APA during conditions in which Ang II levels were elevated would have a protective effect against Ang II–mediated cardiovascular diseases, whereas a decrease in APA expression or a failure to upregulate would exacerbate such conditions. (Hypertension. 1999;33:740-745.)

Key Words: aminopeptidases ■ angiotensin II ■ angiotensin III ■ hypertension ■ kidney ■ kidney glomerulus

The renin-angiotensin system (RAS) seems to play a role in the development of hypertension in spontaneously hypertensive rats (SHR). Angiotensin-converting enzyme (ACE) inhibitors and angiotensin type I (AT1) antagonists or, more recently, delivery of antisense oligonucleotides or complementary RNA against angiotensinogen or AT1 receptors reduce or prevent hypertension in SHR.1,2 On the other hand, plasma renin activity and plasma angiotensin II (Ang II) levels are not consistently elevated during any given stage of development in SHR.3–7 Likewise, although plasma Ang II is elevated in stroke-prone SHR, plasma renin, angiotensin II (Ang II) levels are not consistently elevated during any given stage of development in SHR.3–7,23 It is now recognized that the tissue levels of components of the RAS are more reliable indices of functional activity than are plasma levels.10–13 Whereas plasma levels of Ang II may be unchanged or actually increased after ACE inhibition, a reduction in tissue Ang II content is well correlated with a reduction in blood pressure after ACE inhibition.3,6,11,14–17

Transplantation studies indicate that the hypertension follows the SHR kidneys.18 Likewise, the renal vasculature of SHR have been shown to be hypersensitive to Ang II.19–22 There is no agreement, however, as to whether Ang II levels4,5,7,23 or Ang II receptors19,24–28 are altered during the development or maintenance of the hypertension. Young SHR have elevated proximal tubule sodium reabsorption that can be reduced by ACE inhibition, whereas sodium reabsorption from adult SHR proximal tubules is reduced.29 Because young SHR have been reported to have elevated kidney content of Ang II7 and increased Ang II receptors,7,30 the increased sodium reabsorption may be Ang II dependent. Kidney Ang II content is reduced in adult SHR,7 and proximal tubule AT1 receptor levels in adult SHR are comparable to levels in Wistar-Kyoto rats (WKY).30 Interestingly, the potency of Ang II in stimulating sodium reabsorption from proximal tubules is decreased in young SHR and increased in adult SHR,31 just the opposite of kidney Ang II content7 and perhaps reflecting receptor desensitization and supersensitivity, respectively. Thus, alterations in Ang II–mediated vascular reactivity and tubular sodium reabsorption in young animals may contribute to the development of hypertension in SHR.

Although the local concentration of Ang II is dependent both on its rate of synthesis and its rate of degradation, the role that degradation of Ang II plays in regulating Ang II activity within tissues has been relatively unexplored. A

Received June 29, 1998; first decision August 5, 1998; revision accepted October 6, 1998.
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decrease in the degradation of Ang II and prolongation of its half-life within its local environment would result in an increase in Ang II–mediated effects. Indeed, inhibition of peptidases that degrade angiotensins potentiate their effects on blood pressure.32,33 Moreover, the cardiovascular effects due to an increase in the half-life of Ang II could, over time, be indistinguishable from the effects due to elevated synthesis of Ang II. A decrease in Ang II degradation in the kidneys from young SHR would be consistent with observations that young SHR are hypersensitive to Ang II injected into the renal artery.20,21 And that kidneys of young SHR have normal4 or elevated7 Ang II levels with depressed or normal, respectively, plasma renin activity. On the other hand, the levels of Ang II within the kidney have been reported to be lower in adult SHR relative to normotensive rats.4,7 Elevated degradation of Ang II within the kidney would be expected to decrease Ang II levels. Thus, decreased degradation of Ang II may be a contributing factor to the development of hypertension in SHR, whereas increased degradation of Ang II in SHR may be a mitigating factor.

The aim of the present study was to monitor changes in the level of the principal enzyme that degrades Ang II throughout the development of hypertension in SHR. The predominant route for Ang II degradation in the peripheral circulation and brain is hydrolysis of its amino terminal aspartyl residue by aminopeptidase A ([APA], glutamyl aminopeptidase EC 3.4.11.7) to produce des-Asp1-Ang II or angiotensin III (Ang III).34,35 The half-life of Ang III is less than the half-life of Ang II such that the hydrolysis of Ang II to Ang III by APA is the rate-limiting step in Ang II degradation.32 APA is an ectoenzyme that is widely expressed.36 The highest levels of APA are found in the kidney and small intestine, with lower levels of APA associated with vascular elements found throughout the body. In the kidney, APA is expressed in proximal tubule brush border,37 and glomerular mesangial, and epithelial cells.37–40

Therefore, in the present study, we sought to determine whether tissue and plasma APA levels are altered during the development of hypertension in SHR compared with its normotensive control strain, WKY. Our findings indicate that the major difference between strains seems to be elevated activity of APA within SHR kidney at all stages of hypertension.

Methods

General
Male SHR and WKY were obtained from Charles River Breeding Laboratories (Wilmington, MA) and housed on a 12-hour light/dark schedule and allowed free access to food and water. Blood pressure of the 8- and 16-week-old animals was measured with a tail-cuff sphygmomanometer (IITC Inc) 2 days before the animals were killed. Animals were killed at 4, 8, and 16 weeks of age by asphyxiation with CO2 and decapitation. Truncal blood was collected in heparinized syringes and washed with D-PBS. Samples were conducted as previously described.37,41 Briefly, samples were separated on a 10% SDS-PAGE gel and transferred to an Immobilon membrane (Millipore). The membranes were washed and incubated with primary antiserum (1:3000 dilution) overnight at 4°C. The membranes were then washed and visualized by chemiluminescence (ECL Western blotting, Amersham). Quantification of the immunoblots was performed by an Agfa Arcus flatbed scanner interfaced to a computer running ImageQuant (Molecular Dynamics) densitometry software.

Isolation of Glomeruli
The isolation of rat kidney glomeruli was conducted as previously described.37,41 Briefly, 16-week-old SHR and WKY were killed and their kidneys were immediately removed and placed in ice-cold D-PBS, pH 7.4, containing (in mmol/L): NaCl 137, KCl 2.7, Na2HPO4 8.1, KH2PO4 1.5, CaCl2 0.9, MgCl2 0.49, and glucose 5.6. Six kidneys from separate animals were pooled for isolation of glomeruli. The medulsa were dissected away and the cortex was minced and suspended in cold D-PBS. The suspension was passed successively through 200- and 150-μm sieves and glomeruli were collected on a 50-μm sieve and washed with D-PBS. Samples were monitored microscopically after each wash until they were considered free of tubulular contamination.

Enzyme Activity
APA enzyme activity was measured in crude membrane preparations from various tissues as previously reported.37 APA activity was measured by use of α-glutamyl-2-naphthylamide (Bachem Bio-science) as substrate. Enzyme-specific activity was expressed as μmol/mg protein per hour. Plasma APA specific activity was measured as μmol·mL⁻¹·h⁻¹.

Immunoblots
Immunoblots of kidney membranes (40 μg per sample) and isolated glomeruli (20 μg per sample) were conducted as previously described.37,41 Briefly, samples were separated on a 10% SDS-PAGE gel and transferred to an Immobilon membrane (Millipore). The membranes were washed and incubated with primary antiserum (1:3000 dilution) overnight at 4°C. The membranes were then washed and visualized by chemiluminescence (ECL Western blotting, Amersham). Quantification of the immunoblots was performed by an Agfa Arcus flatbed scanner interfaced to a computer running ImageQuant (Molecular Dynamics) densitometry software.

Results

Blood Pressure
Blood pressures of 8- and 16-week-old SHR and WKY were measured via tail cuff. Eight-week-old SHR had slightly higher blood pressures but were not significantly different from 8-week-old WKY (Figure 1). Sixteen-week-old SHR had significantly higher blood pressures than age-matched WKY (Figure 1).

Enzyme Activity
APA enzyme activity was measured in plasma from SHR and WKY at all 3 ages (Figure 2). Plasma APA activity from SHR was significantly increased at all 3 ages although the increases were modest: 21%, 16%, and 16% at 4, 8, and 16 weeks of age, respectively. APA enzyme activity was measured in brain, heart, adrenal gland, liver, and kidney from SHR and WKY at all 3 ages. In general, there was a tendency for APA activity to be increased in tissues from SHR at all 3 ages although significant increases were modest (Figure 3). Significant increases in APA activity were seen in SHR brain at 8 and 16 weeks of age, in heart at 16 weeks of age, and in...
adrenal gland at 4 and 8 weeks of age (Figure 3). There were no significant differences in APA activity within the liver. Kidney APA activity was higher in both strains compared with other tissues (Figure 4). In addition, SHR kidneys had significantly higher APA activity compared with WKY kidneys at 4, 8, and 16 weeks of age: 41%, 51%, and 68%, respectively.

Immunoblots
The increase in APA activity could arise from either an increase in the specific activity of APA or an increase in the levels of APA. To test which of these possibilities was more likely, immunoblots of kidney APA from individual SHR and WKY were compared. APA immunoreactivity was significantly increased in kidney membranes from SHR at 8 and 16 weeks of age (Figure 5). Densitometric analysis indicated a 1.7-fold increase (P<0.01) in APA immunoreactivity at 8 weeks and a 3.3-fold increase (P<0.01) at 16 weeks in SHR compared with WKY; APA levels were increased 23% in 4-week SHR kidney samples but did not reach significance.

Isolated Glomeruli
Because previously we had reported that APA activity is high within glomeruli,37 we isolated glomeruli from 6 kidneys pooled separately from 16-week-old SHR and WKY and compared APA activity and immunoreactivity. APA activity was increased in glomeruli from SHR by 57% (29.4 μmol·

Captopril Treatment
Several studies report that Ang II levels in the kidney are elevated in SHR.7,23 Because SHR had higher APA activity, we sought to determine whether inhibition of Ang II synthesis by administration of an ACE inhibitor would influence APA activity. Although the effects of ACE inhibitors on plasma Ang II are variable,3,6,7,10,14–17,23 ACE inhibition consistently lowers kidney Ang II levels.7,10,11,14–17,23 If APA activity stayed high in treated SHR, then it would argue that the increase in APA activity was strain related and not regulated by Ang II. A reduction in APA activity would argue that either Ang II had a positive influence on APA expression or that APA expression was sensitive to changes in blood pressure. Captopril was administered in the drinking water to 14-week-old SHR for a period of 2 weeks. The concentration in the water was adjusted so that animals received 100 mg of captopril per day. Blood pressure in captopril-treated SHR was reduced to normotensive levels (Figure 6A). Plasma APA activity was not significantly different between treated and nontreated animals (Figure 6B). Kidney APA activity,
Discussion

Tissue and plasma levels of APA tended to be slightly elevated at all 3 ages. The single exception among the tissues tested was the kidney, in which APA activity was elevated ∼50% at all 3 ages. To begin to determine whether expression of APA was related to the levels of angiotensin or whether there is a simple strain difference between WKY and SHR, we determined whether ACE inhibition would alter APA activity. Although ACE inhibition has variable effects on plasma Ang II levels, kidney Ang II levels are consistently decreased after ACE treatment. Captopril treatment (≈100 mg·kg⁻¹·d⁻¹) of SHR for a period of 2 weeks reduced blood pressure to normotensive levels and reduced APA activity 25%. These results suggest that the elevated expression of APA in SHR kidney may be related to elevated levels or turnover of Ang II. Although we cannot rule out that the reduction in kidney APA activity was secondary to a reduction in blood pressure caused by ACE inhibition, the fact that APA activity was elevated in kidneys from prehypertensive SHR would argue against this possibility.

Plasma levels of APA were slightly elevated in SHR at all 3 ages studied. Because plasma levels of renin or Ang II do not always reflect accurately the level of activity within tissues, it is not clear whether there is any functional significance to the increase in plasma APA in SHR. Further, the molecular nature of this activity has not been determined. APA has a single transmembrane-spanning domain. APA can be solubilized and retains activity when clipped from the membrane by mild enzymatic digestion, much like what has been shown for ACE. It is not known whether plasma APA is generated in such a fashion or whether it represents a secreted form or some other APA-like enzyme.

Among extrarenal tissues, the biggest increase in APA activity was in brains from 16-week-old SHR in which APA activity was increased 52%. These results support recent findings by Zini et al that showed that APA activity was higher in SHR brain compared with age-matched WKY. Young SHR have been reported to have elevated turnover and levels of Ang II in the brain. SHR have also been reported to be hyperresponsive to the effects of intracerebroventricular Ang II and Ang III. In membrane preparations from SHR and WKY brains, Ang II is metabolized more rapidly by SHR than by WKY but Ang III metabolism is similar. This may be indicative of enhanced generation of Ang III within the brain. This is interesting because recent studies indicate that Ang III may be the active peptide in the central nervous system (CNS). Zini et al reported that inhibition of APA centrally blocks the formation of Ang III from Ang II and also blocks Ang II–mediated increases in vasopressin release. We have reported that intracerebroventricular administration of APA antiserum with anticatalytic activity blocked the drinking and blood pressure effects of Ang II but not of Ang III administered intracerebroventricularly. These findings are consistent with the finding that APA activity was elevated in SHR brains.

Figure 6. Effects of the ACE inhibitor captopril on blood pressure, plasma APA activity, and kidney APA activity from 16-week-old SHR. Fourteen-week-old SHR were treated with captopril (≈100 mg·kg⁻¹·d⁻¹) for a period of 2 weeks. Two days before the SHR were killed, tail-cuff blood pressures were taken. Panel A, Systolic blood pressure in captopril-treated SHR (hatched bars) was significantly lower compared with untreated littermates (solid bars). Panel B, Plasma APA activity was unchanged. Panel C, Kidney APA activity was significantly reduced in the captopril-treated animals. A minimum of 8 animals were included in each group. *P<0.05, ***P<0.001, Student’s t test.
consistent with earlier studies from Wright and Harding\(^5^0\) that suggest that Ang III is the active peptide in the brain. Enhanced generation of Ang III in peripheral tissues may shorten the effects of Ang II, but if Ang III is the active peptide in the brain, an increase in Ang III formation within the brain may contribute to hypertension. If so, conversion of Ang II to Ang III may be an important step in the regulation of brain angiotensin activity, and enhanced generation of Ang III would exacerbate, not ameliorate, hypertension in SHR.

SHR have been shown to be hypersensitive to Ang II injected intrarearily.\(^1^9\)–\(^2^2\) Likewise, transplantation studies have determined that hypertension follows the SHR kidney.\(^1^8\) Although we speculated initially that a reduction in APA activity within the kidneys of SHR may contribute to observed hyperresponsiveness to Ang II, the elevated levels of APA seen here in kidneys of SHR at all ages suggest that this is not the case. Whether these changes in APA activity result in an alteration in the half-life or content of Ang II within the kidney is not clear at this time because there is some disagreement as to whether Ang II levels are increased or decreased in SHR kidney. Matsushima et al\(^9\) reported that Ang II content is increased in kidneys from 4-week-old SHR but decreased at 20 weeks of age. Campbell et al\(^9\) found that Ang II levels were unaltered at 6 weeks of age but decreased in SHR kidneys at 10 and 20 weeks of age. Meng et al\(^9\) reported that kidney Ang II levels were increased in 10-week-old SHR kidneys. Thus, it is not clear whether the change in APA activity within the kidney influences Ang II levels in the kidney.

The demonstration here that APA levels in SHR kidney are influenced by activity of the renal RAS is supported by several other studies. Wolf et al\(^5^1,5^2\) reported that APA activity was increased in partial remnant kidneys. Ang II levels are known to be elevated in this model, and ACE inhibition prevented the increase in kidney APA levels. Johnson et al\(^5^3\) reported that subcutaneous infusion of pressor levels of Ang II into rats increased APA activity within glomeruli. Thus, APA expression in the kidney and, in particular, within glomeruli may be regulated in part by activity of the RAS.

Does APA activity play a role in hypertension? Early reports conflicted as to whether plasma angiotensinase levels were different between normal and hypertensive patients.\(^5^4,5^5\) Based on preceding discussions about the functional relevance of the levels of the components of the RAS in the plasma, tissue measurements of APA would be more meaningful; however, there have been no reports on tissue levels of APA in essential hypertensive subjects. That APA exerts a tonic effect on Ang II levels can be surmised from studies that show that aminopeptidase inhibitors produce transient increases in blood pressure and potentiate the pressor effects of angiotensins.\(^3^2,3^3\) Conversely, intravenous administration of purified APA reduces blood pressure in SHR in a dose-dependent manner.\(^5^6\) Thus, one could speculate that a reduction in APA activity may contribute to the development of Ang II–dependent hypertension, whereas an increase in APA activity may play a protective role in counteracting the adverse effects of chronic Ang II stimulation. In this regard, the increased APA activity seen in SHR within Ang II–targeted tissues such as the kidney may be interpreted as being a homeostatic mechanism that counterbalances Ang II overstimulation. If so, one could further speculate that a defect in APA metabolism of Ang II within humans or a failure to upregulate APA activity in the presence of elevated Ang II synthesis may be considered a permissive factor for human essential hypertension.

Acknowledgment

This work was supported by grant HL42585 from the National Institutes of Health.

References


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Hypertension. 1999;33:740-745
doi: 10.1161/01.HYP.33.2.740

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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