Long-Term Effects of Angiotensin-Converting Enzyme Inhibition on Renal Medullary Neutral Lipid in Spontaneously Hypertensive Rats

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Abstract—Short-term treatment of young spontaneously hypertensive rats (SHR) with angiotensin-converting enzyme (ACE) inhibitors reduces systolic blood pressure. Renal medullary neutral lipids (RMNLs) have vasodilator properties that may explain the effects of ACE inhibition. We measured RMNL levels of SHR treated between 6 and 10 weeks of age with (1) vehicle, (2) ramipril 1 mg · kg\(^{-1}\) · d\(^{-1}\), (3) the bradykinin B\(_2\) receptor antagonist icatibant 0.5 mg · kg\(^{-1}\) · d\(^{-1}\), or (4) icatibant 0.5 mg · kg\(^{-1}\) · d\(^{-1}\) plus ramipril 1 mg · kg\(^{-1}\) · d\(^{-1}\). RMNLs were quantified by oil red O fluorescence at 10 and 20 weeks of age. Systolic blood pressure (BP) was measured by tail-cuff plethysmography. Ramipril reduced BP at 10 weeks of age and increased RMNLs compared with controls (0.99 ± 0.07% versus 0.56 ± 0.06%, P<0.01). Icatibant alone had no significant effect on RMNLs (0.55 ± 0.04%) but attenuated the increase in RMNLs by ramipril (0.81 ± 0.05%). In control SHR, the increase in BP between 10 and 20 weeks of age was associated with a significant increase in RMNLs (0.79 ± 0.09%). SHR that had received ramipril had significantly lower BP than controls at 20 weeks of age, but RMNL was not significantly different (0.92 ± 0.10%). Therefore, in young SHR, ACE inhibition increases RMNLs and reduces blood pressure, an effect that appears to depend on bradykinin. The changes in RMNLs at the age of 10 weeks paralleled long-term BP effects and may be involved in setting the BP track in SHR. (Hypertension. 1999;33:1214-1217.)

Key Words: bradykinin • kidney medulla • lipids • interstitial cells

Angiotensin-converting enzyme (ACE) inhibitors decrease blood pressure (BP) and reduce cardiac and vascular hypertrophy in spontaneously hypertensive rats (SHR).\(^1\) Brief ACE inhibitor treatment of young SHR causes persistent hypotensive effects.\(^1\) Reduction of angiotensin II (Ang II) formation is considered central in the antihypertensive actions of ACE inhibitors. However, these effects seem to depend also on increased bradykinin levels during the treatment phase. Previous studies have demonstrated that administration of the bradykinin B\(_2\) receptor antagonist icatibant with an ACE inhibitor prevented the long-term reduction in systolic BP found after ACE-inhibitor treatment of young SHR.\(^2\)

The kidney seems to be an important factor for the long-term effects of ACE inhibition. Transplantation studies suggest that the level of BP follows the kidney after ACE-inhibitor treatment.\(^3\) Other evidence indicates that the renal medulla may be of particular importance to BP effects of ACE inhibition. In doses that have no effect when injected intravenously, ACE inhibitor injected directly into the renal medulla of SHR increases medullary blood flow,\(^4\) with an associated decrease in arterial pressure. Chemical renal papillotectomy of young SHR during ACE-inhibitor treatment prevents long-term reduction in BP.\(^5\) We have also demonstrated reduced size of the renal medulla after ACE-inhibitor treatment in young SHR, and bradykinin seemed an important factor in this phenomenon.\(^6\)

In the renal medulla, bradykinin binds to renal medullary interstitial cells (RMICs)\(^7\) that produce renal medullary neutral lipids (RMNLs).\(^8\) RMNLs have important effects on BP through vasodilator and sympatholytic activities.\(^8\),\(^9\) There is potential for interaction between the renin-angiotensin and RMNL production because there are abundant Ang II receptors on RMICs.\(^10\)

Electron-dense lipid droplets within RMICs are thought to contain the substrate triglycerides required for the formation of RMNLs.\(^11\),\(^12\) The number of cytoplasmic lipid droplets within RMICs correlates with arterial pressure in certain hypertensive states. Deficiencies in RMIC lipid droplets are found in hypertension in the deoxycorticosterone acetate-salt,\(^13\) post-salt,\(^14\) Goldblatt,\(^15\) and post-Goldblatt\(^16\) models. Morphological studies of the hypertensive Dahl salt-sensitive rat have shown that RMICs in these rats are smaller, fewer in number, and have fewer lipid droplets than in Dahl salt-resistant normotensive control rats.\(^17\) In the SHR, Mandal et al\(^18\) demonstrated that RMICs seem to be smaller, with reduced...
average electron-dense fatty droplets per RMIC than in normotensive Wistar-Kyoto rats. Given the possible role of RMNLs in BP control, the present study was designed to investigate the effects of ACE inhibition and bradykinin receptor antagonism on RMNL content in SHR. We used a specific histological stain to label and quantify neutral lipid within the renal medulla of SHR.

**Methods**

The present experiment is a detailed study of tissues obtained from animals involved in experiments published previously.² The treatment regimes have been described in detail before.² Briefly, male SHR were divided into 4 groups to receive 1 of the following treatments: (1) water by gavage (n=13), (2) the ACE inhibitor ramipril (1 mg·kg⁻¹·d⁻¹ by gavage; n=13), (3) the specific bradykinin B₂ receptor antagonist icatibant (0.5 mg·kg⁻¹·d⁻¹ by subcutaneous osmotic minipump; n=11), and (4) icatibant (0.5 mg·kg⁻¹·d⁻¹) plus ramipril (1 mg·kg⁻¹·d⁻¹; n=12). Treatment was for 4 weeks, between 6 and 10 weeks of age. The dose of icatibant used has been demonstrated previously to be biologically active.²,¹⁹ Systolic BP was measured continuously between 6 and 20 weeks of age.² However, in this study, we only refer to average values at 10 and 20 weeks of age.

At 10 and 20 weeks of age, rat kidneys were perfusion-fixed with 10% buffered formalin. Each left kidney was then cut into 1-mm slices, and every second slice was sampled. The frozen sections from the sampled slices were cut at 30 μm. Once dry, these sections were stained with oil red O and counterstained with Mayer’s hematoxylin and Scott’s tap water and coverslipped.²⁰ This procedure is specific for staining neutral lipids.²¹,²² Approximately 30 sections were cut from each slice. These were classified into thirds; front, middle and final. Two sections from each third were then randomly selected.

Kidney sections were viewed with a BioRad MRC-1000 confocal microscope. Oil red O stain maximally absorbed light when excited by green light of a wavelength from 500 to 550 nm.²³ The argon laser was set to emit light of 514 nm. Images were captured as light intensities, digitized with an image analysis system, and converted into corresponding intensities on the gray-scale range of pixels between 0 and 256, where 0 is black and 256 is white. For this study, the optimal fluorescence of the oil red O stain, where background is minimal, was between 180 and 256 pixels on the gray scale. Observations were made on the extent of oil red O staining throughout the kidney. The renal cortex showed no specific lipid staining, but oil red O staining was clearly evident in the medulla, especially the inner medulla. The renal inner medulla was compared in all groups, and RMNL content was estimated from the ratio (expressed as a percentage) of number of fluorescent pixels (Ntfp) to the total number of pixels in the inner medulla (Ntkp). Descriptive statistics presented are mean and SEM. Differences between groups were compared with the use of 1-way ANOVA and Student-Newman-Keuls range test. Statistical significance was accepted when P<0.05.

**Results**

**Ten Weeks of Age**

As reported previously,² ramipril decreased systolic BP substantially (144±9 mm Hg) at 10 weeks of age compared with control (188±2 mm Hg, P<0.001). Ramipril treatment induced a significant increase in RMNL content (Figure 1) in the young SHR compared with control. Icatibant alone had no effect on systolic BP (182±3 mm Hg) or on RMNL content (Figure 1). The combination of icatibant plus ramipril treatments resulted in RMNL levels that were significantly higher than control values (P<0.01) and significantly lower than those in SHR that received ramipril alone (P<0.01). The systolic BP of these animals was slightly less than controls (133±7 mm Hg, P<0.05).

**Twenty Weeks of Age**

SHR that had received ramipril showed a significant and persistent reduction in systolic BP (175±4 mm Hg) at 20 weeks of age compared with the control group (208±2 mm Hg, P<0.05). SHR that received icatibant plus ramipril treatment had intermediate systolic BP levels (196±4 mm Hg, P<0.05 versus control and ramipril groups).

By 20 weeks of age, the RMNL content of control SHR (Figure 2) was higher than values observed in younger SHR. RMNL content in the ramipril group was not significantly different from that in control animals. Rats treated with icatibant had significantly (P<0.01) reduced RMNL content compared with ramipril-treated animals.
Discussion

We found that in young SHR, the RMNL content was increased during ACE-inhibitor treatment and associated with a substantial reduction in systolic BP. Although the exact nature of the RMNLs is uncertain, they are known to cause significant reductions in BP. One interpretation of these findings is that the ACE-inhibitor treatment exerts its antihypertensive action in part through the stimulation of the release and production of RMNLs. This possibility is consistent with the general idea that the renal medulla is involved in BP control and specifically in relation to the antihypertensive action of ACE inhibitors.

In some models of hypertension, including renoprival hypertension and partial nephrectomy–salt hypertension, either RMICs are absent or their function is impaired and ACE inhibitors are ineffective in lowering pressure. However, in other hypertensive models in which RMICs are functional, administration of an ACE inhibitor is capable of reducing blood pressure. Therefore, the increase in RMNLs in 10-week-old SHR receiving ramipril may be an important component of the antihypertensive effect. Other studies of RMIC lipids have shown an increase in the number and size of lipid droplets with ACE-inhibitor treatment and a reduction in lipid droplets in Ang II–induced hypertension.

ACE-inhibitor effects, however, extend well beyond the period of treatment, and this effect seems to parallel the changes in RMNLs at 10 weeks of age. The magnitude of increase in RMNLs in the young SHR parallels the long-term reduction in systolic BP that follows treatment, with the greatest long-term reduction in systolic BP in ramipril-treated rats and intermediate systolic BP reduction in rats treated with icatibant plus ramipril. The implication of this study is that the mechanism responsible for the long-term reduction in systolic BP of SHR after ACE-inhibitor treatment during youth is related, in part, to an elevation in RMNL content during treatment in the young SHR. How these changes reset long-term blood pressure is unclear. It certainly does not involve significant long-term effects on RMNL content.

There was no simple relationship between RMNL content at 20 weeks of age and systolic BP at that time. However, there may be a number of confounding and conflicting influences on RMNLs in the established phase of hypertension. Changes in BP per se may be important. The increase in RMNLs associated with the age-related rise in BP in untreated SHR may be indicative of an attempted homeostatic response to counter the genetically determined increase in BP. There may also be other developmental stage–specific changes in the relationship between RMNL levels and the alterations induced after exposure to ACE inhibition and/or bradykinin receptor blockade in early life. Whatever the explanation, there is no consistent relationship between RMNLs and BP in older SHR.

The possible short- and long-term effects of bradykinin are of potential importance. In the short term, the increase in RMNL content with ACE-inhibitor treatment was significantly attenuated by concomitant blockade of bradykinin B1 receptors with icatibant, which suggests that increased bradykinin levels may affect RMIC neutral lipid content. The presence of bradykinin receptors on RMICs has been demonstrated previously. In the long term, the greatest contrast in RMNL levels was seen between rats exposed to the highest (ramipril treatment) and lowest (icatibant treatment) activity of bradykinin between 6 and 10 weeks of age.

In summary, these studies provide further insights into the mechanisms responsible for the long-term effects of ACE-inhibitor treatment in young SHR. The results emphasize the importance of the renal medulla and the neutral lipid from RMICs.

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