Journal of the American Society of Hypertension 10(4) (2016) 346–351

Research Article

Renal intramedullary infusion of tempol normalizes the blood pressure response to intrarenal blockade of heme oxygenase-1 in angiotensin II–dependent hypertension

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Manuscript received November 2, 2015 and accepted January 24, 2016

Abstract

Previous studies have demonstrated that intramedullary inhibition of heme oxygenase-1 (HO-1) increases the blood pressure and superoxide production response to angiotensin II (Ang II) infusion. The present study was designed to test the hypothesis that increased renal medullary superoxide production contributes to the increase in blood pressure in response to blockade of renal medullary HO-1 in Ang II–induced hypertension. Male C57BL/6J mice (16–24 weeks of age) were implanted with chronic intrarenal medullary interstitial (IRMI) and infused with: saline, tempol (6 mM), the HO-1 inhibitor QC-13 (25 μM), or a combination of tempol + QC-13. Tempol treatment was started 2 days before infusion of QC-13. After 2 days, Ang II was infused subcutaneously at a rate of 1 μg/kg/min for 10 days. Blood pressures on days 7–10 of Ang II infusion alone averaged 150 ± 3 mm Hg in mice receiving IRMI infusion of saline. IRMI infusion of QC-13 increased blood pressure in Ang II–treated mice to 164 ± 2 ($P < .05$). Renal medullary superoxide production in Ang II–treated mice was significantly increased by infusion of QC-13 alone. Ang II–treated mice receiving IRMI infusion of tempol had a blood pressure of 136 ± 3 mm Hg. Ang II–treated mice receiving IRMI infusion of tempol and QC-13 had a significantly lower blood pressure (142 ± 2 mm Hg,$P < .05$) than mice receiving QC-13 alone. The increase in renal medullary superoxide production was normalized by infusion of tempol alone or in combination with QC-13. These results demonstrate that renal medullary interstitial blockade of HO-1 exacerbates Ang II–induced hypertension via a mechanism that is dependent on enhanced superoxide generation and highlight the important antioxidant function of HO-1 in the renal medulla. J Am Soc Hypertens 2016;10(4):346–351.

Keywords: Bilirubin; blood pressure; carbon monoxide; superoxide.

Introduction

Heme oxygenase (HO) is the enzyme responsible for the breakdown of heme into carbon monoxide (CO), biliverdin, and free iron. HO is present in two isoforms in the kidney. HO-2 is the constitutively expressed isoform of HO in the kidney, and the main isoform found under basal conditions.[1] HO-1 is the inducible isoform of HO in the kidney.[2] Several studies have demonstrated the importance of renal HO-1 in the regulation of blood pressure in response to high salt diet, angiotensin II infusion, and in genetic models of hypertension such as the spontaneously hypertensive rat.[3–6]

Within the kidney, HO-1 expression in the renal medulla has been shown to play a critical role in the regulation of blood pressure and kidney function. For example, induction of HO-1 specifically in the renal medulla via intrarenal medullary interstitial (IRMI) infusion of the HO-1 inducer cobalt protoporyphrin prevented the development of angiotensin II–dependent hypertension.[7] Moreover, HO-1 overexpression specifically in thick ascending loop of Henle (TALH) cells in the kidney attenuated the development of angiotensin

Conflict of interest: The authors have no conflicts of interest to declare.

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1933-1711/$ - see front matter
http://dx.doi.org/10.1016/j.jash.2016.01.023

II–induced hypertension by altering the levels of the sodium, potassium, and 2 chloride (NKCC2) cotransporter.[8]

Previous studies from our group have demonstrated that renal medullary specific inhibition of HO-1 exacerbates angiotensin II–induced hypertension.[9] The effect of renal medullary HO-1 inhibition on blood pressure in response to angiotensin II–dependent hypertension is similar to the blood pressure effect of HO-1 inhibition observed in response to a high sodium diet.[3]

Previous studies have demonstrated that alterations in renal medullary HO activity are associated with an increase in reactive oxygen species (ROS) production and decreases in antioxidant proteins.[5,9] Additional studies in cultured TALH cells have also demonstrated that overexpression of HO-1 attenuates angiotensin II–dependent ROS production.[10] Despite the evidence demonstrating a link between renal medullary HO activity and ROS production, the antioxidant role of HO-1 in the regulation of blood pressure in response to angiotensin II–dependent hypertension is not known. The goal of this study was to determine the role of increased ROS production to the enhanced blood pressure effect of renal medullary HO-1 inhibition in response to angiotensin II–dependent hypertension. ROS production was blocked by IRMI infusion of the superoxide dismutase mimetic tempol in the presence and absence of inhibition of HO activity.[11,12]

Methods

Animals

Experiments were performed on 16- to 24-week-old C57BL/6J mice purchased from Jackson Labs (Bar Harbor, ME). The mice were fed a standard diet containing 0.29% NaCl and were provided water ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center and performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Implantation of Intrarenal Medullary Interstitial Catheters

All mice underwent unilateral nephrectomy of the right kidney to remove potential contributions of the noninfused kidney to the blood pressure response to experimental manipulations. After 7 days, intramedullary interstitial catheters were implanted 1.5–2 mm into the left kidney as previously described.[7,13] Saline was then infused through the catheter for a period of 3 days after which time the infusion was switched to Tempol (6 mM in saline) or QC-13,(2*R*,4*R*)-2-[2-(4-chlorophenyl)ethyl]-2-[(1*H*-imidazol-1-yl)methyl]-4-methyl-1,3-dioxolane hydrochloride, (25 μM, in saline), in some mice. Previous studies in rats and dogs served as the basis for the concentration of tempol use for chronic intramedullary interstitial infusion.[14,15] The concentration of QC-13 use for IRMI infusion was based on previous studies demonstrating a significant decrease in renal medullary HO activity.[9,13] After 2 days, some mice receiving tempol were additionally infused for with QC-13. Infusions were continued throughout the entire experimental protocol. Mice received their final combination of QC-13 and tempol for 2 days after which osmotic minipumps delivering either vehicle (saline) or Ang II at a rate of 1 μg/kg/min were implanted subcutaneously.

Blood Pressure

Blood pressure was directly measured via microrenathane catheters implanted into the carotid artery using aseptic surgical technique after implantation of angiotensin II osmotic minipumps as previously described.[5] This method is consistent with recommendations of the American Heart Association for measuring blood pressure in conscious animals.[16] The mice were allowed 2 days to recover from surgery and then mean arterial blood pressure was recorded from conscious, freely moving mice 5 hours per day for three consecutive days. Blood pressures are presented as the daily average pressure over the entire 3-day recording period. Mice were euthanized after blood pressure measurement at which time body and organ weights were measured. Tissues were frozen in liquid nitrogen and stored at −80°C until use.

Measurement of Renal Medullary Superoxide Production

Superoxide production in the renal medulla was measured using the lucigenin technique as previously described.[5] Briefly, infused kidneys were removed and separated into renal cortex and medulla. The medulla was then homogenized (1:8 wt/vol) in radioimmunoprecipitation assay buffer (phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and a protease inhibitor cocktail; Sigma Chemical). The samples were centrifuged at 12,000 *g* for 20 minutes at 4°C. The supernatant was incubated with lucigenin at a final concentration of 5 μM and samples were allowed to equilibrate for 3 minutes in the dark, and luminescence was measured every second for 5–15 minutes with a luminometer (Berthold, Oak Ridge, TN). Luminescence was recorded as relative light units per minute. After the initial measurement, nicotinamide adenine dinucleotide phosphate (NADPH) was added to a final concentration of 100 μM and measurements repeated as previously mentioned to give the basal plus NADPH-mediated superoxide production. An assay blank with no homogenate but containing lucigenin was subtracted from the reading before transformation of the data. The protein concentration was measured using a Bio-Rad protein assay with BSA standards. The data are expressed as relative light units per minute per milligram protein.