The Blood Pressure-Lowering Effect of 20-HETE Blockade in Cyp4a14(−/−) Mice Is Associated with Natriuresis


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ABSTRACT

20-Hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) has been linked to pro-hypertensive and anti-hypertensive actions through its ability to promote vasoconstriction and inhibit Na transport in the ascending limb of the loop of Henle, respectively. In this study, we assessed the effects of 20-HETE blockade on blood pressure, renal hemodynamics, and urinary sodium excretion in Cyp4a14(−/−) male mice, which display androgen-driven 20-HETE-dependent hypertension. Administration of 2,5,8,11,14,17-hexaoxanonadecan-19-yl-20-hydroxyicos-a-6(Z),15(Z)-dienoate (20-SOLA), a water-soluble 20-HETE antagonist, in the drinking water normalized the blood pressure of male Cyp4a14(−/−) hypertensive mice (±124 vs. ±153 mmHg) while having no effect on age-matched normotensive wild-type (WT) male mice. Hypertension in Cyp4a14(−/−) male mice was accompanied by decreased renal perfusion and reduced glomerular filtration rates, which were corrected by treatment with 20-SOLA. Interestingly, Cyp4a14(−/−) male mice treated with 20-SOLA displayed increased urinary sodium excretion that was paralleled by the reduction of blood pressure suggestive of an antinatriuretic activity of endogenous 20-HETE in the hypertensive mice. This interpretation is in line with the observation that the natriuretic response to acute isosmotic saline loading in hypertensive Cyp4a14(−/−) male mice was significantly impaired relative to that in WT mice; this impairment was corrected by 20-SOLA treatment. Hence, endogenous 20-HETE appears to promote sodium conservation in hypertensive Cyp4a14(−/−) male mice, presumably, as a result of associated changes in renal hemodynamics and/or direct stimulatory action on tubular sodium reabsorption.

Introduction

20-Hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) is a metabolite of arachidonic acid produced by the cytochrome P450ω-hydroxylases including members of the CYP4A and CYP4F gene families (Capdevila et al., 1992). This eicosanoid affects vascular and renal functions in a manner consistent with a potential involvement in the implementation of both prohypertensive and anti-hypertensive mechanisms (Williams et al., 2010; Wu et al., 2014). Previous studies documented that arterial hypertension is associated with derangement in 20-HETE production at vascular and/or renal tubular sites in various experimental models. For example, the development of hypertension in animals displaying augmentation of 20-HETE biosynthesis at vascular sites was attributed to one or more actions of this eicosanoid promoting increased reactivity to myogenic and neurohormonal stimuli (Miyata and Roman, 2005; Hoopes et al., 2015), endothelial dysfunction (Wang et al., 2006; Singh et al., 2007; Cheng et al., 2008), and/or upregulation of angiotensin-converting enzyme (ACE) (Sodhi et al., 2010; Garcia et al., 2015, 2016), all of which are actions capable of bringing about vasoconstriction and blood pressure elevation. On the other hand, the salt-sensitive hypertension featured by transgenic mice overexpressing the human 20-HETE synthase CYP4A11 was attributed to enhanced retention of sodium resulting from 20-HETE–dependent and angiotensin II–dependent upregulation of the sodium chloride cotransporter (NCC) (Savas et al., 2016). Interestingly, salt-sensitive hypertension also was shown to develop in animals with depressed renal medullary synthesis of 20-HETE, which conditions retention of sodium in the ascending limb of the loop of Henle (Roman et al., 1993, 1997; Stec et al., 1997; Ito and Roman, 1999). Inasmuch as 20-HETE contributes to pro-hypertensive mechanisms via vascular and tubular actions that foster vasoconstriction and/or conservation of sodium, as well as to anti-hypertensive mechanisms via actions that facilitate sodium excretion, it is expected that the blood pressure effect of interventions that interfere with 20-HETE actions is conditioned by

ABBREVIATIONS: ACE, angiotensin-converting enzyme; FITC, fluorescein isothiocyanate; GFR, glomerular filtration rate; 20-HETE, 20-Hydroxy-5,8,11,14-eicosatetraenoic acid; NCC, sodium chloride cotransporter; NHE3, sodium/hydrogen exchanger type; RBF, renal blood flow; 20-SOLA, 2,5,8,11,14,17-hexaoxanonadecan-19-yl-20-hydroxyicos-6(Z),15(Z)-dienoate; UNaV, urinary volume and sodium excretion; WT, wild type.

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the mix of the various vascular and renal blood pressure regulatory mechanisms involving this eicosanoid.

The present study was undertaken to examine this notion using Cyp4a14(−/−) male mice, which develop 20-HETE–dependent hypertension linked, sequentially, to the upregulation of plasma androgen levels and enhanced renal expression of CYP4A12, the dominant 20-HETE synthase in mice (Holla et al., 2001; Wu et al., 2013; Gangadhariah et al., 2015). First, we contrasted male Cyp4a14(−/−) hypertensive mice with corresponding 129/SVE normotensive wild-type (WT) controls in terms of the effects of a newly developed water-soluble 20-HETE antagonist, 2,5,8,11,14,17-hexaoxanodecan-19-yl-20-hydroxyeicosa-6(Z),15(Z)-dienoate (20-SOLA), on systolic blood pressure, in relation to associated effects on renal blood perfusion, glomerular filtration rate (GFR), urine volume, and sodium excretion. Second, we compared male Cyp4a14(−/−) hypertensive mice and 129/SVE normotensive WT mice undergoing treatment with 20-SOLA or its vehicle control only in terms of the ability to excrete sodium load. The results suggest that in addition to its effects on renal hemodynamics, 20-HETE promotes sodium conservation that also contributes to hyper-tension in this model.

Materials and Methods

20-SOLA was synthesized as a PEG-conjugate of 20-6,15-hydroxyeicosadienoic acid, an effective 20-HETE antagonist (Alonso-Galicia et al., 1999; Ding et al., 2013). The 20-HETE antagonistic activity of 20-SOLA was examined by its ability to prevent 20-HETE–mediated sensitization of the constrictor activity of phenylephrine in renal interlobar arteries as previously described (Zhang et al., 2001). Briefly, cumulative concentration-response curves to phenylephrine (1 × 10−9 to 1 × 10−4 M)–induced contraction of renal interlobar arterial rings mounted on a myograph were constructed in the presence of DDMS (30 μM) to inhibit endogenously generated 20-HETE, with and without exposure to 20-HETE (1 μM) alone or to 20-HETE (1 μM) + 20-SOLA (1 μM). Phenylephrine elicited concentration-dependent increases of isometric tension as seen in Fig. 1A. In vessels bathed in media containing 20-HETE, the concentration-dependent increase in tension produced by phenylephrine was shifted to the left revealing a major decrease in the EC50 of phenylephrine (Fig. 1B). Coaddition of 20-SOLA prevented 20-HETE–mediated sensitization of phenylephrine-induced vasoconstriction (Fig. 1). Fluorescein isothiocyanate (FITC)-inulin was obtained from Sigma-Aldrich (St. Louis, MO).

Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee. The Cyp4a14(−/−) mouse model has been previously characterized (Holla et al., 2001). Male but not female Cyp4a14(−/−) mice show a marked induction of Cyp4a12, the sole 20-HETE synthase in the mouse, and exhibit 20-HETE–dependent hypertension (Wu et al., 2013; Gangadhariah et al., 2015). Male Cyp4a14(−/−) mice and age- and sex-matched background WT 129/SVE mice (WT, 8–10 weeks old) were used.

Experimental Protocols

Protocol 1. Mice were housed in metabolic cages with free access to food and drinking water containing or not containing 20-SOLA (10 mg/kg/day) for 10 days. Food and water intake and urinary output were monitored daily. No differences were observed in food and water intake between Cyp4a14(−/−) and WT mice receiving 20-SOLA (data not shown). For blood pressure measurements, mice were acclimated to the tail-cuff measurement for 7 days prior to the start of experiments. In brief, mice were placed on an infrared heating pad for 7–10 minutes. Systolic blood pressure was recorded using the CODA noninvasive blood pressure system (Kent Scientific, Torrington, CT) as previously described (Wu et al., 2013). Urinary sodium concentration was determined by flame photometry, and the daily excretion rate was calculated. Renal blood flow (RBF) and GFR were measured at the end of the 10-day experimental protocol, as follows.

RBF was evaluated under anesthesia by using laser Doppler flowmetry (Shi et al., 2007). Briefly, a midline incision was made to access the kidneys. A laser Doppler scanner was used to obtain a pseudocolored scan of renal blood perfusion in live anesthetized mice. A laparotomy was performed to expose the kidneys followed by assessment of cortical renal blood perfusion values using the laser Doppler perfusion monitoring probes, image scanner, and accompanying software (PeriScan PIM II, PeriFlux system 5000, and LDFwin version 2.6.1, respectively; Perimed Inc., Ardmore, PA). The correct positioning of laser Doppler probes was verified after completion of each experiment.

GFR was measured by the FITC-inulin clearance method (Rieg, 2013). Briefly, FITC-inulin (5% in 0.85% NaCl) was dialyzed against 0.85% NaCl for 24 hours, resulting in an ~2% solution that was used to establish the standard curve. The dialyzed FITC-inulin solution was sterile filtered and injected into the retro-orbital plexus (3.74 μg/l of b.wt.) during brief isoflurane anesthesia. At 3, 7, 10, 15, 35, 55, and 75 minutes
after injection, blood was collected from the tail into a Na+ heparinized 70 μl capillary tube. After centrifugation, 10 μl of plasma was diluted 1:5 in 500 mM HEPES (pH 7.4), and fluorescence was determined on a 96-well plate using Synergy HT (Biotek) with 485 nm excitation and 538 nm emission. GFR was calculated using a two-compartment model of two-phase exponential decay (GraphPad Prism; GraphPad, San Diego, CA).

**Protocol 2.** WT and Cyp4a14(−/−) mice undergoing pretreatment with either vehicle or 20-SOLA (10 mg/kg/day) for 72 hours were placed in metabolic cages for urine collection before and 4 hours after intraperitoneal injection of isotonic saline (10% v/w). The amount of sodium excreted and the ratio of injected to excreted sodium were assessed (Trott et al., 2014).

**Statistical Analysis**

Data are expressed as the mean ± S.E.M. Significance of difference in mean values was determined by using two-tailed t test for comparison between two groups or one-way ANOVA for multiple-group comparisons, followed by the Tukey-Kramer post hoc test. \( P < 0.05 \) was considered to be significant.

**Results**

**20-SOLA Normalizes Blood Pressure in Cyp4a14(−/−) Male Hypertensive Mice.** The Cyp4a14(−/−) mouse is a model of androgen-driven male-specific 20-HETE–dependent hypertension; Cyp4a14(−/−) male mice are hypertensive whereas corresponding females are normotensive (Holla et al., 2001; Wu et al., 2013). As previously reported, the administration of the 20-HETE antagonists 20-6,15-hydroxyeicosadecaenoic acid or 20-SOLA normalizes blood pressure in the male Cyp4a14(−/−) mice without affecting renal or vascular levels of 20-HETE (Wu et al., 2013; Gangadhariah et al., 2015). As seen in Fig. 2, administration of 20-SOLA (10 mg/kg/day) in the drinking water to Cyp4a14(−/−) male mice significantly reduced systolic blood pressure within 4 days of the onset of treatment and normalized the blood pressure after 10 days of treatment. In contrast, the same treatment had no effect on systolic blood pressure in age-matched WT mice (Fig. 2).

**Impaired Renal Hemodynamic Function Is Corrected by Treatment with 20-SOLA in Cyp4a14(−/−) Mice.** We contrasted male WT and Cyp4a14(−/−) mice treated with vehicle or 20-SOLA in terms of renal blood perfusion and GFR. As seen in Fig. 3A, renal blood perfusion in hypertensive Cyp4a14(−/−) mice was significantly lower compared with WT controls. Treatment with 20-SOLA for 10 days did not affect renal perfusion in WT controls but increased renal perfusion in the hypertensive Cyp4a14(−/−) mice to the level observed in the WT controls (Fig. 3A). The GFR of hypertensive Cyp4a14(−/−) mice was surpassed by that of WT mice (1.83 ± 0.15 vs. 2.41 ± 0.12 μl/min/mg kidney weight; \( P < 0.05 \) vs. corresponding WT). Treatment with 20-SOLA did not affect the GFR of WT mice but increased the GFR of Cyp4a14(−/−) mice (2.38 ± 0.04 μl/min/mg; \( P < 0.05 \)) to levels similar to those of WT mice (Fig. 3B). Hence, 20-HETE appears to be a critical determinant not only of the"
hypertension featured by Cyp4a14(+/−) male mice but also of the accompanying alterations in renal hemodynamics, namely, a reduction in renal blood perfusion and GFR.

20-SOLA Treatment Increases Urinary Sodium Excretion in Male Cyp4a14(+/−) Mice. The urinary volume of 7 × 24-hour collections over 10 days was significantly lower in Cyp4a14(+/−) mice when compared with corresponding WT mice (55.90 ± 1.89 μl/g.b.wt./d vs. 60.29 ± 3.14 μl/g.b.wt./day, n = 7, P = 0.02). Likewise, UNaV (average of 7 × 24 hour collections over 10 days) was lower in Cyp4a14(+/−) compared with WT (5.77 ± 0.25 vs. 7.17 ± 0.22 μmol/g b.wt./day; n = 7; P = 0.0006). Urinary volume and sodium excretion (UNaV) in WT mice were unaffected by treatment with 20-SOLA (Fig. 4A and C). On the other hand, treatment of male Cyp4a14(+/−) mice with 20-SOLA elicited increases in UNaV, beginning on day 2 after the onset of treatment and lasting until day 6 (Fig. 4B and D), at which time blood pressure is appreciably decreased (Fig. 2). There were no changes in body weight between treated and untreated Cyp4a14(+/−) mice with 20-SOLA during the course of the study (e.g., 19.3 ± 1.8 vs. 20.5 ± 0.2 g in Cyp4a14(+/−) mice treated with vehicle and 20-SOLA for 10 days, respectively). Urinary protein was also unchanged (e.g., 2.64 ± 0.66 vs. 3.38 ± 0.44 mg/g b.wt./day in Cyp4a14(+/−) mice treated with vehicle and 20-SOLA for 10 days, respectively). Likewise, no changes in these parameters were observed between WT mice treated with 20-SOLA and untreated mice (data not shown).

Natriuretic Response to a Salt Challenge Is Attenuated in Cyp4a14(+/−) Mice. Next, we contrasted WT and Cyp4a14(+/−) male mice pretreated for 72 hours with vehicle or 20-SOLA (10 mg/kg/day) in terms of their ability to dispose of Na in response to acute isotonic salt loading (10% v/w, 0.15 mol/l NaCl). As shown in Fig. 5A, acute salt loading caused urinary sodium excretion to increase significantly (P < 0.05) relative to the corresponding basal value in both WT and Cyp4a14(+/−) male mice pretreated for 72 hours with 20-SOLA or vehicle only. Of note, the natriuretic response to salt loading was comparable in WT male mice pretreated with 20-SOLA or vehicle only, but was depressed in vehicle-pretreated Cyp4a14(+/−) male mice relative to corresponding values in similarly treated WT mice. Importantly, the impaired natriuretic response to salt loading noted in Cyp4a14(+/−) male mice pretreated with vehicle only was prevented in Cyp4a14(+/−) mice pretreated with 20-SOLA. Assessment of the fraction of the sodium load excreted in the urine during the first 4 hours after saline administration also showed that 20-SOLA pretreatment corrects the impaired natriuretic response observed in Cyp4a14(+/−) male mice.

Fig. 4. UNaV in WT (A and C) and Cyp4a14(+/−) (B and D) mice treated with vehicle or 20-SOLA for 10 days (n = 6/group; *P < 0.05 vs. vehicle-treated Cyp4a14(+/−) mice).
that overexpress CYP4A12, a 20-HETE biosynthetic enzyme, but is without effect on the natriuretic response to the salt loading in WT mice that do not overexpress Cyp4a12 (Fig. 5B).

Discussion

This study demonstrates that systolic blood pressure and indices of renal hemodynamic function, renal blood perfusion and GFR, in hypertensive Cyp4a14(/−/) male mice and corresponding 129/SVE normotensive WT controls are dissimilarly impacted by treatment with 20-SOLA. That the 20-HETE antagonist is without effect on blood pressure and renal hemodynamics in normotensive WT mice but lowers blood pressure and increases renal blood perfusion and GFR in hypertensive Cyp4a14(/−/) mice is attributable to differences in the status of 20-HETE synthesis among the strains. This interpretation derives support from previous reports documenting androgen-driven upregulation of CYP4A12, the main 20-HETE synthase in mice, and 20-HETE generation in vascular and renal structures of hypertensive Cyp4a14(/−/) mice (Holla et al., 2001; Wu et al., 2013). Since 20-HETE supports pro-hypertensive mechanisms relying on enhanced vasoconstriction (Williams et al., 2010; Hoopes et al., 2015; Fan et al., 2016), the heightened blood pressure and depressed renal perfusion featured by Cyp4a14(/−/) male mice are viewed as manifestations of increased 20-HETE generation (Wu et al., 2013). Conversely, the antihypertensive effect of 20-SOLA in Cyp4a14(/−/) hypertensive mice is attributable to its ability to antagonize 20-HETE–mediated vasoconstriction. Of note, recently we reported that a 20-HETE antagonist related to 20-SOLA, namely, N-(20-hydroxyeicosa-6(Z), 15(Z)- dienoyl)glycine (20-6,15-HEDGE), prevents the binding of 20-HETE to an orphan G-protein–coupled receptor identified as GPR75 (Garcia et al., 2017). Since the pairing of 20-HETE and GPR75 is an essential feature of the above-referenced vasoconstrictor-pressor actions of 20-HETE (Garcia et al., 2017), it is plausible that the antihypertensive response to the treatment of Cyp4a14(/−/) mice with 20-SOLA results from interference of the latter with the binding of 20-HETE to GPR75, its putative receptor.

Our study also demonstrates that the treatment with 20-SOLA affects urinary sodium excretion and urine volume differently in hypertensive Cyp4a14(/−/) mice and corresponding WT normotensive mice. The 20-HETE antagonist had no effect on sodium excretion or urine volume in normotensive mice, but in hypertensive Cyp4a14(/−/) mice it caused slight elevation of urine volume and a robust increase of urinary sodium excretion. Since the natriuretic effect of 20-SOLA in Cyp4a14(/−/) hypertensive mice precedes by 2 days the onset of blood pressure reduction, it is conceivable that the enhanced sodium loss prompted by the 20-HETE antagonist is a primary contributor to the slightly delayed hypotensive effect, presumably via a mechanism involving the reduction of extracellular fluid volume with attendant reduction of cardiac output (Williams et al., 2010). This interpretation is not necessarily incongruent with the established concept that urinary sodium excretion is negatively affected by an associated reduction of blood pressure (Reckelhoff et al., 1998). Indeed, in our study, the natriuretic effect of 20-SOLA in Cyp4a14(/−/) hypertensive mice had largely waned on days 8 and 10 after the onset of treatment, at which time the hypertension had nearly subsided. Based on these considerations, it would appear that the antihypertensive effect of treatment with 20-SOLA in Cyp4a14(/−/) mice results from antagonism of the pro-hypertensive actions of 20-HETE at vascular and perhaps at renal tubular sites promoting vasoconstriction and conservation of sodium, respectively.

The notion that the overproduction of 20-HETE contributes to excessive renal conservation of sodium in Cyp4a14(/−/) hypertensive mice fits well with our current findings confirming that these animals are impaired in their ability to increase urinary sodium excretion after an acute saline load (Fidelis et al., 2010). That treatment with 20-SOLA results in complete normalization of the natriuretic response to acute saline loading in Cyp4a14(/−/) hypertensive mice is strong evidence that in these animals the overproduction of 20-HETE and the accompanying impairment to excrete sodium are causally related. Enhanced activity of 20-HETE–driven sodium-conserving mechanisms in Cyp4a14(/−/) hypertensive mice may be attributed to the actions of this eicosanoid targeting renal vascular structures to reduce renal perfusion and glomerular filtration and/or tubular structures to directly increase sodium reabsorption (Evans et al., 2005; Williams et al., 2007). Quigley et al. (2009) have documented the
augmentation of renal proximal convoluted tubule transport in Cyp4a14(–/–) hypertensive mice along with enhanced brush border proximal tubule expression of the sodium/hydrogen exchanger type 3 (NHE3), the major sodium transporter in this segment of the nephron (McDonough, 2010). Upregulation of renal 20-HETE synthesis, along with increased proximal tubule volume reabsorption and NHE3 protein abundance in kidney brush border membranes, also has been documented in rats treated with dihydrotestosterone (Quigley, 2008), which increases blood pressure via mechanisms supported by 20-HETE (Wu et al., 2013) and angiotensin II (Quan et al., 2004; Yanes et al., 2009) that foster vasoconstriction and sodium conservation. However, the concept that 20-HETE–driven sodium reabsorption in animals with androgens-dependent hypertension relies on excessive sodium retention that fosters direct activation of renal NHE3. On the other hand, it would be premature to exclude from consideration a more complex mechanism for the engagement of 20-HETE in the promotion of sodium conservation, namely, a mechanism that includes an early step whereby 20-HETE of tubular origin stimulates the expression of components of the renin-angiotensin system intrinsic to renal tubular structures, ACE and/or angiotensinogen, with attendant elevation of angiotensin levels, which, in turn, augment sodium reabsorption via activation of NHE3 and/or NCC (Dos Santos et al., 2004; Sodhi et al., 2010; Cheng et al., 2012; Savas et al., 2016). Experimental support for this concept includes reports documenting that 20-HETE–dependent induction of ACE leads to the elevation of angiotensin II tissue levels (Sodhi et al., 2010; Cheng et al., 2012), that NHE3 is subject to stimulatory regulation by angiotensin II (McDonough, 2010), and that hypertension in transgenic mice overexpressing human CYP4A11 relies on excessive sodium retention that is dependent on 20-HETE–dependent and angiotensin II–dependent upregulation of NCC (Savas et al., 2016). Further experiments are required to examine the activity of NHE3 in proximal tubule and NCC in distal convoluted tubule in Cyp4a14(–/–) mice.

In summary, this study underscores the significance of excessive 20-HETE production to the hypertensive phenotype displayed by Cyp4a14(–/–) male mice, which is associated with diminished renal blood perfusion and GFR and impaired ability to excrete an acute sodium load. That this phenotype is corrected by treatment with 20-SOLA, an antagonist of 20-HETE, implicates endogenous 20-HETE in the mechanisms underlying the development of hypertension, renal hypoperfusion, reduced glomerular filtration, and excessive sodium conservation in Cyp4a14(–/–) male mice. There is a wealth of information on the mechanisms underlying the vascular mechanisms mediating the actions of 20-HETE, which implement the elevation of blood pressure and decrease RBF and glomerular filtration (Williams et al., 2010). On the other hand, information is limited on 20-HETE–mediated tubular actions that promote sodium conservation.

### 20-HETE–Mediated Na Retention in Cyp4a14(–/–) Mice

#### Authorship Contributions

Participated in research design: Falck, Naslletti, Wang, Schwartzman.

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