Contribution of cytochrome P-450 ω-hydroxylase to altered arteriolar reactivity with high-salt diet and hypertension

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Frisbee, Jefferson C., John R. Falck, and Julian H. Lombard. Contribution of cytochrome P-450 ω-hydroxylase to altered arteriolar reactivity with high-salt diet and hypertension. Am J Physiol Heart Circ Physiol 278: H1517–H1526, 2000.—The present study evaluated the contribution of cytochrome P-450 ω-hydroxylase in modulating the reactivity of cremaster muscle arterioles in normotensive rats on high-salt (HS) and low-salt (LS) diet and in rats with reduced renal mass hypertension (RRM-HT). Changes in arteriolar diameter in response to ACh, sodium nitroprusside (SNP), ANG II, and elevated O₂ were measured via television microscopy under control conditions and following cytochrome P-450 ω-hydroxylase inhibition with 17-octadecynoic acid (17-ODYA) or N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS). In normotensive rats on either LS or HS diet, resting tone was unaffected and arteriolar reactivity to ACh or SNP was minimally affected by cytochrome P-450 ω-hydroxylase inhibition. In RRM-HT rats, cytochrome P-450 ω-hydroxylase inhibition reduced resting tone and significantly enhanced arteriolar dilation to ACh and SNP. Treatment with 17-ODYA or DDMS inhibited arteriolar constriction to ANG II and O₂ in all the groups, although the degree of inhibition was greater in RRM-HT than in normotensive animals. These results suggest that metabolites of cytochrome P-450 ω-hydroxylase contribute to the altered reactivity of skeletal muscle arterioles to vasoconstrictor and vasodilator stimuli in RRM-HT.

Previous studies by Lombard and colleagues (6–8, 15–17, 30) and by others (3, 27, 31) have demonstrated that high-salt diet (with no change in blood pressure) and hypertension are associated with alterations in the reactivity of skeletal muscle resistance arteries and arterioles to vasoactive stimuli. Dilator responses of skeletal muscle arterioles to agonists acting throughout the signal transduction pathways of vascular relaxation are impaired with either reduced renal mass (RRM)-hypertension or high-salt diet alone (3, 6–8, 27). Constrictor responses of skeletal muscle arteries and arterioles to ANG II are also enhanced in normotensive rats on high-salt diet (30) and in rabbits with two-kidney, one-clip renovascular hypertension (31), and arteriolar responses to elevated PO₂ are enhanced in RRM-hypertensive rats (17) and spontaneously hypertensive rats (15, 16). Finally, a hallmark of developing hypertension is an increased basal arteriolar tone, requisite in the autoregulation of tissue blood flow (28). Taken together, these observations suggest that hypertension and high-salt diet may be associated with enhanced constrictor influences on skeletal muscle arteries and arterioles, which could contribute to an elevated basal tone, an enhanced response to constrictor stimuli, and an impaired relaxation in response to dilator stimuli.

Recent studies indicate that vascular smooth muscle cells metabolize arachidonic acid via a cytochrome P-450 ω-hydroxylase-dependent pathway to produce 20-hydroxyeicosatetraenoic acid (20-HETE), a potent constrictor of cerebral arteries (11), renal arteries (20), renal arterioles (13), and skeletal muscle arterioles (12). The presence of cytochrome P-450 ω-hydroxylase has been demonstrated in skeletal muscle, where it appears to play an important role in regulating arteriolar diameter during changes in O₂ availability by generating 20-HETE in an O₂-dependent manner (12). Finally, several studies have indicated that cytochrome P-450 ω-hydroxylase expression and vascular sensitivity to 20-HETE are elevated in some tissues with hypertension (2, 9, 26). On the basis of these prior investigations, it is important to evaluate the role of P-450 ω-hydroxylase in contributing to the alterations in the regulation of vascular tone that develop with high-salt diet and RRM-hypertension.

Materials and methods

Renal mass reduction. Male Sprague-Dawley rats were anesthetized with a 9:2 mixture of 100 mg/ml ketamine and 10 mg/ml acepromazine (0.1 ml/100 g body wt). As described previously (17), the rats underwent a two-stage procedure in which their total renal mass was reduced by ~75%. After recovering from the procedures, all rats with RRM were placed on a high-salt diet (4.0% NaCl; Dyets, Bethlehem, PA) for 3 days to produce RRM-hypertension.

Animal groups and preparation. The present studies were conducted on RRM-hypertensive rats and two groups of normotensive Sprague-Dawley controls. In these experiments, rats not undergoing renal mass reduction were maintained on either a low-salt diet (0.4% NaCl) or a high-salt diet for 3 days. All rats drank tap water ad libitum. A 3-day period
for high-salt diet and RRM-hypertension was chosen because previous studies demonstrated that this time period allows for significant alterations to vascular reactivity while minimizing any structural narrowing of arterioles (7, 8). Body weights of normotensive rats maintained on the low-salt and high-salt diets were not different, whereas body weight for RRM-hypertensive rats was significantly less than that in normotensive rats on either diet (Table 1).

On the day of the experiment, rats were anesthetized with an intraperitoneal injection (60 mg/kg) of pentobarbital sodium (Veterinary Laboratories, Lenexa, KS), and the trachea was cannulated to ensure a patent airway. A femoral artery and an external jugular vein were cannulated for arterial pressure recording and infusion of supplemental anesthetic, respectively. After this initial surgery, the in situ cremaster muscle was prepared for television microscopy, as described previously (17). Once the initial surgical preparation was completed, the tissue was continuously superfused with physiological salt solution (PSS) equilibrated with a 5% CO2-95% N2 gas mixture and maintained at 34–35°C as it flowed over the muscle. The ionic composition of the PSS was as follows (in mM): 119.0 NaCl, 4.7 KCl, 1.6 CaCl2, 1.18 NaH2PO4, 1.17 MgSO4, 24.0 NaHCO3, and 0.03 disodium EDTA. Arteriolar diameter was determined with a videomicroscope that was accurate to ±1 µm (17).

The selection criteria for choosing arterioles for study were similar to those described in earlier studies (7). Arterioles used in the present study were just proximal to the capillaries in a clearly visible region of the cremaster muscle that was located away from any incision. Vessels chosen for study had clearly discernible walls, a brisk flow velocity, and active tone, as indicated by the occurrence of a brisk dilation in response to topical application of 10-4 M adenosine.

Inhibition of cytochrome P-450 ω-hydroxylase. To assess the role of cytochrome P-450 ω-hydroxylase in regulating vascular reactivity of distal arterioles, we inhibited the enzyme with either 17-octadecynoic acid (17-ODYA) or β-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS) before evaluating reactivity. Two inhibitors of ω-hydroxylations were used because 17-ODYA also inhibits arachidonic acid epoxidation, which inhibits not only the formation of 20-HETE but also the formation of eicosatetraenoic acids (EETs; Ref. 33). DDMS is a more selective inhibitor of ω-hydroxylation (and therefore of 20-HETE production) and has minimal effects on EET formation via arachidonic acid epoxidation (29). Because 17-ODYA irreversibly inhibits cytochrome P-450 ω-hydroxylase and treatment with DDMS is reversible only over an extended time frame (29), each animal in the present study did not serve as its own control. Thus the animals were divided into three treatment groups: 1) control (receiving no inhibition of cytochrome P-450 ω-hydroxylase), 2) 17-ODYA (receiving cytochrome P-450 ω-hydroxylase inhibition with 17-ODYA), and 3) DDMS (receiving cytochrome P-450 ω-hydroxylase inhibition with DDMS). Baseline data describing each of the nine total experimental groups are summarized in Table 1.

The topical application procedures for applying 17-ODYA and DDMS to the cremaster muscle were similar to those described previously (12, 18). Briefly, while normal PSS superfusion was interrupted, 10 ml of 10 µM 17-ODYA or DDMS (in PSS) were superfused over the cremaster muscle over 30 min. After topical application of either cytochrome P-450 ω-hydroxylase inhibitor, superfusion with normal PSS was restored. To control for any time-dependent effects in control animals, we maintained the cremaster muscle of the animals in the control group under PSS superfusion for a period of time identical to that required for 17-ODYA and DDMS treatment with no experimental manipulation.

Determination of vascular reactivity. After treatment with either cytochrome P-450 ω-hydroxylase inhibitor or completion of the time-control procedures, arteriolar diameter was measured before and after challenge with 1) ACh (10-8-10-6 M), 2) sodium nitroprusside (SNP; 10-8-10-6 M), 3) ANG II (10-5-10-7 M), and 4) elevated superfusate O2 concentration (0, 5, 10, and 21%). Maximum arteriolar diameter was assessed by measuring the vascular response to superfusion with Ca2+-free PSS containing 10-4 M adenosine. One arteriole was selected for analysis in each rat, and each arteriole was exposed to all challenges. In an additional group of normotensive rats on low-salt diet (n = 5), the cremaster muscle was superfused with PSS containing a subthreshold dose of 20-HETE (10-10 M; Ref. 11) such that no constriction from baseline was identified. During this superfusion period, arteriolar responses to topical application of ACh (10-4 M) and SNP (10-6 M) were evaluated.

Arteriolar diameters were measured after the vascular response to the individual agonists had stabilized. This occurred after ~30 s for the agonist challenges and after ~5 min for the O2 challenge. Successive agonist challenges were applied only after the vessel had returned to its original diameter following application of the preceding agonist. Successive O2 challenges were imposed immediately following determination of the new steady-state diameter of the arteriole. Challenges with the individual stimuli were randomized to prevent the occurrence of ordering effects and to control for...
any time-dependent changes in vascular reactivity during the course of the experiment. After the initial inhibition of 20-HETE production or the time-control period, the total experimental duration was ~60 min.

Data and statistical analyses. All data are expressed as means ± SE. ANOVA with Tukey’s post test hoc was used to determine differences between experimental groups for mean arterial pressure, body weight, and resting and maximum arteriolar diameter. Statistical evaluation of the effects of cytochrome P-450 ω-hydroxylase inhibition on arteriolar responses to the challenges in individual rat groups (i.e., normotensive rats on low- or high-salt diet and RRM-hypertensive rats) used repeated-measures ANOVA with Tukey’s test post hoc. The statistical evaluation of the effects of superfusion of the cremaster muscle with 20-HETE on microvessel reactivity to ACh and SNP employed Student’s t-test.

To determine the degree of alteration in the vasoconstrictor reactivity to ANG II and O2 with 17-ODYA and DDMS, we fitted the following regression equation to the dose-response curves (ordinary least-squares analysis; \( r^2 > 0.90 \)): \( y = \alpha + \beta x \), where \( y \) represents the change in arteriolar diameter from rest to challenge (expressed as % resting diameter) with either ANG II or O2 at a specific concentration, \( \alpha \) is an intercept term, \( x \) represents the individual stimulus concentration, and \( \beta \) represents the rate of change in arteriolar diameter for a change in stimulus concentration, with \( \beta \) representing the slope of the equation. The alteration of the vasoconstrictor reactivity after application of either 17-ODYA or DDMS was evaluated by determining the difference between \( \beta \)-coefficients (and the pooled variance surrounding that difference) describing the dose-response data between control rats and rats receiving cytochrome P-450 ω-hydroxylase inhibition in each animal group (23). The statistical analysis of differences in \( \beta \)-coefficients employed ANOVA and Tukey’s test post hoc. Throughout all analyses, a probability level of \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Mean arterial pressure and resting and maximum arteriolar diameter. At the time of the experiment, mean arterial pressure was significantly higher in the RRM-hypertensive rats than in either normotensive group (which were not different from each other). Resting diameter of arterioles in rats not receiving cytochrome P-450 ω-hydroxylase inhibition was not different across the three rat groups. Inhibition of cytochrome P-450 ω-hydroxylase did not alter arteriolar diameter in either normotensive rat group, although treatment with either 17-ODYA or DDMS increased arteriolar diameter in RRM-hypertensive rats. The maximum diameter of cremasteric arterioles was not different between the three rat groups. These data are summarized in Table 1.

Responses to ACh. Figure 1 presents the effects of high-salt diet on the response of cremasteric arterioles to ACh in normotensive and RRM-hypertensive rats. Compared with low-salt diet and normotension in rats, both high-salt diet and RRM-hypertension exhibited reduced microvessel dilation in response to 10^{-6} M ACh. In RRM-hypertensive rats, the arteriolar response to 10^{-6} M ACh was also significantly less than that in normotensive rats on high-salt diet.

![Fig. 1. Responses of cremasteric arterioles (n = 7 for each group) to ACh in normotensive rats on low- or high-salt diet and in reduced renal mass (RRM)-hypertensive rats. Data are presented as mean ± SE). ANOVA with Tukey’s post test hoc was used to determine differences between experimental groups for mean arterial pressure, body weight, and resting and maximum arteriolar diameter. Statistical evaluation of the effects of cytochrome P-450 ω-hydroxylase inhibition on arteriolar responses to the challenges in individual rat groups (i.e., normotensive rats on low- or high-salt diet and RRM-hypertensive rats) used repeated-measures ANOVA with Tukey’s test post hoc. The statistical evaluation of the effects of superfusion of the cremaster muscle with 20-HETE on microvessel reactivity to ACh and SNP employed Student’s t-test.](http://ajpheart.physiology.org/)

Figure 2 summarizes the effects of cytochrome P-450 ω-hydroxylase inhibition on arteriolar responses to ACh. In normotensive rats on low-salt diet (Fig. 2A), inhibition of 20-HETE production with 17-ODYA had no significant effect on arteriolar reactivity to ACh, although treatment with DDMS significantly increased microvessel dilation to 10^{-6} and 10^{-7} M ACh (Fig. 2A). In normotensive rats on high-salt diet, application of 17-ODYA had no significant effect on arteriolar responses to ACh, and cremasteric arteriolar reactivity to ACh was significantly increased following DDMS treatment at 10^{-6} M ACh only (Fig. 2B). However, in RRM-hypertensive rats (Fig. 2C), treatment of the cremaster muscle with either 17-ODYA or DDMS significantly increased arteriolar responses to ACh at each agonist concentration.

Responses to SNP. Figure 3 summarizes cremasteric arteriolar responses to SNP in normotensive rats on low- or high-salt diet and in rats with RRM-hypertension. The response of cremasteric arterioles to SNP was not different between the two normotensive rat groups, although vascular reactivity to SNP was significantly reduced with RRM-hypertension.

Figure 4 summarizes the effects of cytochrome P-450 ω-hydroxylase inhibition on these arteriolar responses to SNP in the three experimental groups. In normotensive rats on low- (Fig. 4A) or high-salt diet (Fig. 4B), treatment of the muscle with either 17-ODYA or DDMS had no significant effect on arteriolar dilation to SNP. In RRM-hypertensive rats, blockade of cytochrome P-450 ω-hydroxylase significantly increased vascular reactivity to all concentrations of SNP used in the present study (Fig. 4C).
Responses to ANG II. Figure 5 summarizes the response of cremasteric arterioles to ANG II in the three rat groups in the present study. In normotensive rats on low- or high-salt diet, there were no differences in the ANG II-induced microvessel constriction, with the exception of the response at $10^{-7}$ M ANG II, where microvessels of rats on the high-salt diet constricted significantly more than those of rats on the low-salt diet ($P < 0.05$). In RRM-hypertensive rats, arteriolar constriction to $10^{-8}$ and $10^{-7}$ M ANG II was significantly increased compared with that identified for either group of normotensive rats.

Figure 6 summarizes the effects of cytochrome P-450 $\omega$-hydroxylase inhibition on the response of microvessels to ANG II. In normotensive rats on low- (Fig. 6A) or high-salt diet (Fig. 6B) and in RRM-hypertensive rats (Fig. 6C), blockade of cytochrome P-450 with either 17-ODYA or DDMS significantly reduced cremasteric arteriolar responses to ANG II throughout the agonist concentration range in the present study. In the RRM-hypertensive rats, pharmacological inhibition of cytochrome P-450 $\omega$-hydroxylase with 17-ODYA or DDMS reduced cremasteric arteriolar responses to ANG II to...
levels that were not different from those determined in the normotensive controls.

Responses to elevated superfusate O₂ concentration. Figure 7 presents the cremasteric arteriolar response to elevated superfusate O₂ concentration in the various animal groups. Arterioles of normotensive rats on low- or high-salt diet exhibited similar constrictor responses at all levels of superfusate O₂ concentration. However, the O₂-induced constriction of arterioles in RRM-hypertensive rats was significantly greater than that of normotensive rats on low-salt diet at all superfusate O₂ concentrations and was significantly greater than that of normotensive rats on high-salt diet at 10 and 21% O₂.

Figure 8 summarizes the effects of cytochrome P-450 ω-hydroxylase inhibition on arteriolar responses to elevated superfusate O₂ in the various experimental groups. In normotensive rats on either low- (Fig. 8A) or high-salt diet (Fig. 8B), inhibition of cytochrome P-450 ω-hydroxylase significantly reduced the arteriolar constriction to 21% O₂ only. In RRM-hypertensive rats, treatment of the cremaster muscle with either cytochrome P-450 ω-hydroxylase inhibitor significantly reduced arteriolar responses to each superfusate O₂ level (Fig. 8C). No significant differences were evident in the

Fig. 4. Responses of cremasteric arterioles (n = 7 for each group) to sodium nitroprusside in normotensive rats on low-salt diet (A), normotensive rats on high-salt diet (B), and RRM-hypertensive rats (C) under control conditions and following treatment of cremaster muscle with either 17-ODYA or DDMS to inhibit cytochrome P-450 ω-hydroxylase. Data are presented as mean (±SE) responses to sodium nitroprusside, expressed as a percentage of maximum possible dilation determined during superfusion with Ca²⁺-free PSS containing 10⁻⁴ M adenosine. ‡Significant increase (P < 0.05) in response to sodium nitroprusside compared with that determined under control conditions.

Fig. 5. Responses of cremasteric arterioles (n = 7 for each group) to ANG II in normotensive rats fed low-salt or high-salt diets and in RRM-hypertensive rats. Data are expressed as mean (±SE) decreases in arteriolar diameter (normalized to rest diameter). *Significant difference (P < 0.05) in constrictor response compared with normotensive rats on low-salt diet. †Significant difference (P < 0.05) in constrictor response compared with normotensive rats on high-salt diet.
degree of the inhibition of $O_2$-induced constriction following treatment with either 17-ODYA or DDMS in normotensive rats on low- and high-salt diet. However, in RRM-hypertensive rats, depression of the $O_2$-induced vasoconstriction following inhibition of cytochrome P-450 $\nu$-hydroxylase was significantly greater than in either normotensive rat group (assessed by comparing the difference in $\beta$-coefficients in the various groups), and arteriolar responses to elevated $P_2$ in the presence of the inhibitor were not significantly different from those measured in the normotensive control groups.

Effects of superfusion with 20-HETE on dilator reactivity. To determine whether exogenous application of 20-HETE alters the reactivity of cremasteric arterioles to ACh and SNP, we added a subthreshold dose of 20-HETE directly to the superfusate before evaluating arteriolar reactivity to these agonists. Figure 9 presents data describing the effects of superfusion of the cremaster muscle of normotensive rats on low-salt diet with $10^{-10}$ M 20-HETE (in PSS) on microvessel reactivity to ACh ($10^{-6}$ M) and SNP ($10^{-6}$ M). In response to superfusion with 20-HETE, arteriolar responses to both agonists were significantly reduced compared with responses determined during superfusion with normal PSS.

DISCUSSION

Previous studies indicated that the reactivity of skeletal muscle arterioles to both dilator and constrictor stimuli is significantly altered with high-salt diet.
and RRM-hypertension (3, 6–8, 17, 27, 30). Other studies demonstrated that cytochrome P-450 ω-hydroxylase expression is elevated in many tissues with hypertension (2, 10, 26), resulting in an increased production of 20-HETE, a potent constrictor of skeletal muscle arterioles (12). On the basis of those previous investigations, the goal of the present study was to evaluate the potential contribution of cytochrome P-450 ω-hydroxylase products (presumably 20-HETE) to the altered reactivity of skeletal muscle arterioles in normotensive rats on high-salt diet and in RRM-hypertensive rats.

Effects of cytochrome P-450 ω-hydroxylase inhibition on basal arteriolar tone. Inhibition of cytochrome P-450 ω-hydroxylase with either 17-ODYA or DDMS had no effect on the resting diameter of cremasteric arterioles in normotensive rats on low- or high-salt diet, although cytochrome P-450 inhibition significantly increased arteriolar diameter, i.e., decreased basal tone, in RRM-hypertensive rats. The lack of an effect on active tone in skeletal muscle arterioles of normotensive rats with 17-ODYA is consistent with the results of previous studies (12, 18). However, the lack of an effect on basal tone in normotensive rats on high-salt diet and the significant reduction of active tone in arterioles of RRM-hypertensive rats was not identified previously.

It was previously demonstrated that 20-HETE inhibits Ca²⁺-activated potassium (K_{Ca}) channels in renal arterioles (32) and activates L-type Ca²⁺ channels in cerebral arteries (9). These effects would increase constrictor influences on vascular smooth muscle, leading to a predisposition toward increased vascular tone. The lack of an effect on basal tone of arterioles following cytochrome P-450 ω-hydroxylase inhibition suggests that the effects of 20-HETE produc-
tion on these channels may not be of primary importance in regulating resting tone of the vascular smooth muscle in these vessels. However, in RRM-hypertensive rats, the increased arteriolar diameter following inhibition of P-450 \(\omega\)-hydroxylase suggests that enhanced 20-HETE production or an increased sensitivity of the arteriolar smooth muscle cells to this compound may contribute to the commonly identified increases in vascular tone with developing hypertension (28). An increase in resting tone was proposed to be an essential step in the transition from the high cardiac output stage of RRM-hypertension to the established stage of hypertension, where peripheral vascular resistance is elevated via local autoregulatory mechanisms (17). The present study suggests that metabolites of the cytochrome P-450 system may contribute to this transition to a maintained elevation in vascular resistance in this volume-expanded form of hypertension.

Effects of cytochrome P-450 \(\omega\)-hydroxylase inhibition on ACh- and SNP-induced vasodilatation. The results of the present study are consistent with previous observations that cremaster arterioles of normotensive rats on high-salt diet and RRM-hypertensive rats rapidly develop an impaired reactivity to ACh and SNP (within 3 days following exposure to high-salt diet) compared with responses of arterioles in normotensive rats on low-salt diet (7, 8). The present study demonstrates that treatment of the cremaster muscle with the cytochrome P-450 \(\omega\)-hydroxylase inhibitors 17-ODYA or DDMS had disparate effects on ACh- and SNP-induced arteriolar dilation across the three rat groups. In normotensive rats on low- or high-salt diet, cytochrome P-450 \(\omega\)-hydroxylase inhibition had little effect on cremaster arteriolar reactivity to ACh and SNP. These observations for ACh are comparable to previous observations in which pharmacological inhibition of cytochrome P-450 \(\omega\)-hydroxylase in normotensive rats fed standard rat chow had no significant effect on cremaster arteriolar reactivity to ACh and SNP (18). However, in RRM-hypertensive rats, cytochrome P-450 \(\omega\)-hydroxylase inhibition significantly enhanced arteriolar reactivity to ACh and SNP such that the responses to these agonists were similar to those in normotensive rats on low-salt diet. These findings represent previously unidentified relationships and suggest that the reduced vascular reactivity to ACh and SNP with RRM-hypertension may be partially a function of elevated 20-HETE production or an increased vascular sensitivity to 20-HETE. Because 20-HETE inhibits \(K_Ca\) channels (32) in vascular smooth muscle, blocking the production of this compound could alleviate its influence on \(K_Ca\) channels of microvessels and restore the reduced ACh- and SNP-induced dilator responses in the RRM-hypertensive rats to normal levels. This speculation is supported by the results of the studies summarized in Fig. 9 showing that superfusion of the cremaster muscle with a subthreshold dose of 20-HETE blunted the dilation of the arterioles in response to ACh and SNP in a manner similar to that observed during high-salt diet (for ACh) and RRM-hypertension (for both agonists). Clearly, additional investigation is warranted to further delineate the role of 20-HETE in the depressed vascular reactivity to ACh and SNP that develops under these conditions.

Effects of cytochrome P-450 \(\omega\)-hydroxylase inhibition on ANG II-induced vasoconstriction. The present study indicates that both RRM-hypertension and high-salt diet alone increase the responsiveness of cremaster arterioles to ANG II. These observations are in agreement with the previous study demonstrating an increased reactivity to ANG II in skeletal muscle resistance arteries of normotensive rats on high-salt diet (30). In the present study, treatment of the cremaster muscle with either 17-ODYA or DDMS reduced the vasoconstriction to ANG II in all rats, suggesting that some portion of the ANG II effect on vascular smooth muscle cells may be mediated via the cytochrome P-450 system and 20-HETE. Although further investigation is necessary to more fully establish the contribution of 20-HETE in mediating vasoconstrictor responses to ANG II, this speculation is supported by previous studies demonstrating a role for products of cytochrome P-450 \(\omega\)-hydroxylase, including 20-HETE, in regulating renal (4) and placental (14) vascular tone and renal tubular transport (1, 4, 19) following challenge with ANG II.

Effects of cytochrome P-450 \(\omega\)-hydroxylase inhibition on O\(_2\)\(_2\)-induced vasoconstriction. The present study indicates that O\(_2\)\(_2\)-induced constriction of cremaster muscle arterioles of normotensive rats is not altered by high-salt diet alone. However, development of RRM-hypertension is associated with a significantly increased microvessel constrictor sensitivity to elevated O\(_2\) levels. This latter observation is in agreement with previous studies by Lombard et al. (15–17) and by Rafi and Boegehold (25), in which the O\(_2\)-induced constriction of skeletal muscle microvessels in RRM-hypertensive rats, spontaneously hypertensive rats, and hypertensive Dahl salt-sensitive rats was increased compared with that observed in their normotensive controls. Taken together, the results of these studies and the present experiments clearly indicate that the development of hypertension is associated with a significant increase in microvascular sensitivity to increased O\(_2\) availability.

In addition to previous studies implicating endothelium-derived products as regulators of microvessel tone during changes in ambient O\(_2\) levels (5, 21, 22, 24), recent studies by Harder et al. (12) and Lombard et al. (18) implicated 20-HETE production by cytochrome P-450 \(\omega\)-hydroxylase as an important mediator of O\(_2\)-induced constriction of arterioles in rat and hamster skeletal muscle, respectively. Harder et al. (12) demonstrated that 20-HETE production from renal microsomes was highly O\(_2\) dependent and that 20-HETE production is accelerated as ambient O\(_2\) levels increase (0–150 mmHg). In the studies of both Harder et al. (12) and Lombard et al. (18), treatment of skeletal muscle arterioles with cytochrome P-450 \(\omega\)-hydroxylase inhibitors blunted vasoconstriction to elevated O\(_2\) tension. The results of these studies, when integrated with the present experiments, suggest that endogenous produc-
tion of 20-HETE plays a significant role in mediating O2-induced constriction of arterioles in skeletal muscle.

Inhibition of vasoconstrictor responses between animal groups with 17-ODYA and DDMS. The major goal of the present study was to determine the role of endogenously produced 20-HETE in regulating vascular reactivity in skeletal muscle microvessels of normotensive animals on high-salt diet and of RRM-hypertensive rats. Inhibition of 20-HETE production reduced the arteriolar constriction to ANG II in all rats, although this effect was exacerbated with RRM-hypertension, as indicated by a significant difference in the β-coefficient in the RRM-hypertensive rats versus the normotensive control groups. These results suggest that cytochrome P-450 and the production of 20-HETE may play a more significant role in mediating arteriolar responses to ANG II in RRM-hypertensive rats than in normotensive animals.

The comparable inhibition of the vascular O2 response following treatment of the cremaster muscle with either 17-ODYA or DDMS in normotensive rats on high- and low-salt diet suggests that the role of 20-HETE in regulating O2-induced changes in vascular tone is not altered with high-salt diet. However, the greater inhibition of O2-induced constriction with RRM-hypertension suggests that 20-HETE production via cytochrome P-450 ω-hydroxylase may play a major role in contributing to the enhanced response of arterioles to elevated O2 tension in hypertensive rats compared with normotensive controls.

In conclusion, results from the present study suggest that 20-HETE production via cytochrome P-450 ω-hydroxylase may play a central role in regulating 1) resting microvascular tone in RRM-hypertensive rats, 2) enhanced arteriolar constrictor responses to ANG II and O2, and 3) reduced dilator responses with RRM-hypertension and, to a lesser extent, high-salt diet alone. Addressing the role of cytochrome P-450 metabolites of arachidonic acid in regulating vascular reactivity under normal physiological conditions and during the development of different pathological conditions represents an exciting area for future investigation.

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