Cytochrome P-450 epoxygenase products contribute to attenuated vasoconstriction after chronic hypoxia

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Earley, Scott, Andrzej Pastuszyn, and Benjimen R. Walker. Cytochrome P-450 epoxygenase products contribute to attenuated vasoconstriction after chronic hypoxia. Am J Physiol Heart Circ Physiol 285: H127–H136, 2003. First published March 6, 2003; 10.1152/ajpheart.01052.2002.—The systemic vasculature exhibits attenuated vasoconstriction following chronic hypoxia (CH) that is associated with endothelium-dependent vascular smooth muscle (VSM) cell hyperpolarization. We hypothesized that increased production of arachidonic acid metabolites such as the cyclooxygenase product prostacyclin or cytochrome P-450 (CYP) epoxygenase-derived epoxygenase products epoxyeicosatrienoic acids (EETs) contributes to VSM cell hyperpolarization following CH. VSM cell resting membrane potential (E_{m}) was measured in superior mesenteric artery strips isolated from rats with control barometric pressure (P_b, =630 Torr) and CH (P_b, 380 Torr for 48 h). VSM cell E_{m} was normalized between groups following administration of the CYP inhibitors 17-octadecynoic acid and SKF-525A. VSM cell hyperpolarization after CH was not altered by cyclooxygenase inhibition, whereas the selective CYP2C9 inhibitor sulfaphenazole normalized VSM cell E_{m} between groups. Iberiotoxin also normalized VSM cell E_{m}, which suggests that large-conductance, Ca^{2+}-activated K^{+} (BK_{Ca}) channel activity is increased after CH. Sulfaphenazole administration restored phenylephrine-induced and myogenic vasoconstriction and Ca^{2+} responses of mesenteric resistance arteries isolated from CH rats to control levels. Western blot experiments demonstrated that CYP2C9 protein levels were greater in mesenteric arteries from CH rats. In addition, 11,12-EET levels were elevated in endothelial cells from CH rats compared with controls. We conclude that enhanced CYP2C9 expression and 11,12-EET production following CH contributes to BK_{Ca} channel-dependent VSM cell hyperpolarization and attenuated vasoreactivity.

Chronic hypoxia (CH) results from prolonged residence at high altitude as well as pathological conditions that impair oxygenation. Several studies report that systemic vasoconstrictor responsiveness is attenuated following exposure to both normobaric and hypobaric hypoxia (1, 7, 20). Altered responsiveness to vasoconstrictor stimuli persists on restoration of normoxic conditions (7), which demonstrates that the consequences of long-term exposure to hypoxia are distinct from vasodilation resulting from acute exposure to this stimulus (19). In addition, prolonged arterial hypoxemia in humans is associated with persistent vasodilation of the forearm circulation and blunted constriction in response to lower body negative pressure (4, 22), which indicates that obstructive pulmonary diseases may also result in attenuated vasoconstriction. Our laboratory has recently shown (8, 9) that attenuated agonist-induced and myogenic vasoconstriction following prolonged hypoxic exposure is associated with persistent endothelium-dependent vascular smooth muscle (VSM) cell hyperpolarization and decreased intracellular Ca^{2+} concentration ([Ca^{2+}]_{i}). VSM cell hyperpolarization after CH is independent of nitric oxide synthase (NOS) activity (8). This suggests that increased production of a non-nitric oxide (NO) endothelial-derived hyperpolarizing factor (EDHF) may account for this response.

Endothelial-derived metabolites of arachidonic acid are potent vasodilator and VSM cell hyperpolarizing factors (11, 33). For example, the cyclooxygenase product prostacyclin activates ATP-sensitive K^{+} (K_{ATP}) channels to alter VSM resting membrane potential (E_{m}; Ref. 17), whereas the cytochrome P-450 (CYP) epoxygenase products epoxygenase products epoxyeicosatrienoic acids (EETs) activate large-conductance Ca^{2+}-activated K^{+} (BK_{Ca}) channels (3). We hypothesized that increased cyclooxygenase and/or CYP epoxygenase activity contribute to endothelium-dependent VSM cell hyperpolarization and attenuated vasoconstrctor reactivity following CH. Our findings suggest that enhanced expression of a CYP isoform 2C9 (CYP2C9)-like protein and increased EET production contribute to endothelium-dependent VSM cell hyperpolarization and blunted sensitivity to vasoconstrictor stimuli following prolonged hypoxic exposure.

Methods
Animals. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine. Size-matched male Sprague-Dawley rats (Harlan Industries) were provided with...
fresh bedding, rat chow, and drinking water, and a 12:12-h light-dark cycle was maintained. CH rats were exposed to hypobaric hypoxia at a barometric pressure (Pb) of 380 Torr for 48 h, whereas control rats were housed in identical cages at ambient Pb (∼630 Torr). Our laboratory previously reported that the arterial PO2 for rats under control conditions was 73 ± 1 compared to 44 ± 3 Torr under hypoxic conditions (20). The duration of hypoxic exposure employed for this study was selected based on previous reports that demonstrate attenuated vascular reactivity following 48-h hypoxic exposure for the renal and mesenteric circulations in vivo (20, 23). In addition, attenuated reactivity of mesenteric and diaphragmatic small arteries isolated from rats exposed to hypoxia for 48 h has also been reported (8, 9, 20, 38). Immediately before experimentation, rats were deeply anesthetized with pentobarbital sodium (32.5 mg ip); after vessels were harvested, rats were killed by exsanguination. VSM cell resting \( E_m \). VSM cell \( E_m \) values were recorded from superior mesenteric artery (SMA) strips using glass intracellular microelectrodes. The chests and abdomens of anesthetized rats were opened, and heparin (100 U in 0.1 ml) was injected into the hearts through the aorta. The arterioles were pinned out, isolated, and excised, and artery strips were secured in an organ bath with the luminal surfaces exposed. Strips were superfused (5 ml/min) with physiological saline solution (PSS) that contained (in mM) 129.8 NaCl, 5.4 KCl, 0.83 MgSO4, 19 NaHCO3, 1.8 CaCl2, and 5.5 glucose warmed to 37°C and aerated with a normoxic gas mixture that consisted of 21% O2-6% CO2-73% N2. VSM cells were impaled with microelectrodes (tip resistance, ∼100 MΩ) filled with 3 M KCl inserted into the artery strip through the endothelial surface. A Neuroprobe model 1600 amplifier (A-M Systems) was used to record membrane potential. Analog output from the amplifier was low-pass filtered at 1 kHz and routed to a Tektronix RM502A oscilloscope and a Gould chart recorder. Criteria for acceptance of \( E_m \) recordings were 1) an abrupt negative deflection of potential as the microelectrode was advanced into a cell, 2) stable \( E_m \) for at least 3 min, and 3) an abrupt change in potential to −0 mV after the electrode was retracted from the cell. Generally, recordings from several VSM cells were made for each animal. The mean potential of all VSM cells recorded for a particular rat was considered as a single replicate for statistical purposes. VSM cell \( E_m \) values were recorded for vessels isolated from control and CH rats in the presence of vehicle, the nonselective CYP inhibitors 17-odeacycnoic acid (17-ODYA, 1 μM) and SKF-525A (5 μM), the cyclooxygenase inhibitor meclofenamate (10 μM), or the selective CYP2C9 inhibitor sulfaphenazole (10 μM); \( n = 5 \) animals for each group. In addition, experiments, \( E_m \) measurements from VSM cells in SMAs isolated from control and CH rats were recorded in the presence of the BKCα channel inhibitor iberiotoxin (50 nM) or vehicle (\( n = 5 \) animals for both control and CH groups). SKF-525A and 17-ODYA were dissolved in ethanol, whereas sulfaphenazole, meclofenamate, and iberiotoxin were dissolved in PSS. All experiments were performed under normoxic conditions to eliminate the hyperpolarizing effects of acute hypoxia (19).

Isolated vessel preparation. Mesenteric resistance arteries from control and CH rats were isolated and pressurized. The mesenteric arcade was excised and transferred to ice-cold dissecting solution that contained 3 mM MOPS (pH 7.4), 15 mM NaCl, 5 mM KCl, 1 mM MgSO4, 2.5 mM CaCl2, 1 mM KH2PO4, 0.02 mM EDTA, 2 mM pyruvate, 5 mM glucose and 1% bovine serum albumin. The arcade was secured in a Silastic-coated petri dish that contained dissecting solution. Resistance-artery branches were cleaned of adipose tissue and transferred to a beaker of dissecting solution. Fourth- or fifth-order vessel segments [passive inner diameter (ID) at 70 Torr, 100–200 μm] were dissected from the cleaned branches, transferred to a vessel chamber (Living Systems), cannulated with glass micropipettes, and secured with liga-tures and blood was gently rinsed from the lumen. Vessels were slowly pressurized to 70 Torr with PSS using a servo-controlled peristaltic pump (Living Systems) and were superfused (5 ml/min) with warmed (37°C) PSS aerated with a normoxic gas mixture (21% O2-6% CO2-73% N2). After a 30-min equilibration period, intraluminal pressure was slowly increased to 120 Torr, vessels were stretched to remove bends, and pressure was reduced to 70 Torr for an additional 30-min equilibration period. Pressurized resistance arteries were loaded with the cell-permeant ratiometric Ca2+-sensitive fluorescent dye fura 2-AM (Molecular Probes). Fura 2-AM was dissolved in anhydrous DMSO at a concentration of 1 mM. Immediately before loading, fura 2-AM was mixed with 0.5 volumes of a 20% solution of Pluronic acid in DMSO, and this mixture was diluted with dissecting solution to yield a final concentration of 2 μM fura 2-AM for each group. Vessels were loaded with fura 2-AM at this solution for 45 min at room temperature in the dark. Administration of fura 2 to the abluminal surfaces of pressurized arterioles has been shown to preferentially load VSM cells (25). Before experimentation, vessels were equilibrated for 20 min with warmed, aerated PSS following the loading period to wash out excess dye and allow for esterification of AM groups. Myogenic and agonist-induced vasoconstrictor and Ca2+ responsiveness. Myogenic and phenylephrine (PE)-induced vasoconstrictor responses were determined for small mesenteric arteries isolated from control and CH rats. Fura-loaded vessels were exposed to a series of pressure steps between 20 and 120 Torr, and spontaneous myogenic tone was allowed to develop at each step for 3 min. After pressure-response curves were completed, vessels were reequilibrated at 70 Torr for 10 min and were then superfused with PSS that contained increasing concentrations of PE (0.05–50 μM). The ID was continuously monitored using video microscopy and edge-detection software (IonOptix). Fura-loaded vessels were alternatively excited at 340 and 380 nm at a frequency of 10 Hz, and the respective 510-nm emissions were quantified using a photomultiplier tube and recorded using IonWizard software (ver. 4.4; IonOptix). Vessel wall [Ca2+]i was calculated at each pressure as the mean F340/F380 ratio from the background-subtracted 510-nm signal collected over ~3 min. After the pressure-response and PE curves were completed, intraluminal pressure was maintained at 70 Torr, and vessels were superfused for 1 h with Ca2+-free PSS that contained (in mM) 129.8 NaCl, 5.4 KCl, 0.83 MgSO4, 19 NaHCO3, 5.5 glucose, and 3 EGTA. Another pressure-response curve was completed for vessels under Ca2+-free conditions to obtain passive responses. Myogenic tone was calculated as the percent difference of ID observed for Ca2+-containing vs. Ca2+-free PSS at each pressure. PE-induced vasoconstriction was calculated as the percent change in ID relative to the ID in Ca2+-free PSS at an intraluminal pressure of 70 Torr. Vasoconstrictor and Ca2+ responses were determined for arteries from control and CH rats in the presence of sulfaphenazole (10 μM) or vehicle (\( n = 6 \) animals for each group). All experiments were performed under normoxic conditions to eliminate the vasodilatory effects of acute hypoxia (18).

Effects of exogenous 11,12-EET administration. Fura-loaded mesenteric small arteries were preconstricted with PE to ~30% of basal diameter, and the effects of 11,12-EET
(1 μM) administration on diameter and vessel wall [Ca2+] were determined.

**Western blot analysis for CYP2C9.** Arteries from the entire mesenteric arcade were isolated from control and CH rats and were snap-frozen in liquid nitrogen. Vessels were homogenized on ice in 10 mM Tris-HCl buffer (pH 7.4) that contained 255 mM sucrose, 2 mM EDTA, 12 mM pepstatin A, 1 μM aprotinin, and 2 mM PMSF (all from Sigma). Homogenates were centrifuged at 13,400 g at 4°C for 10 min to remove tissue debris, and the microsomal fraction was collected by centrifugation at 100,000 g for 1 h. Protein concentrations of samples were determined using the Bradford method (Bio-Rad protein assay). Microsomal proteins were resolved on 7.5% SDS-PAGE minigels (Bio-Rad). In addition to the samples, each gel included both molecular mass (Bio-Rad) and a CYP2C-positive control that consisted of microsomal proteins from rat liver (16, 35). Separated proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) and blocked with 5% nonfat milk, 3% BSA (Sigma), and 0.05% Tween 20 (Bio-Rad) in Tris-buffered saline solution [TBS; 10 mM Tris-HCl and 50 mM NaCl (pH 7.5)]. The membranes were incubated overnight at room temperature with an anti-CYP2C9 antibody (R&D Systems) diluted 1/200 and an anti-rabbit IgG (1:5,000; StressGen) in TBS, which was followed by a horseradish peroxidase-conjugated goat antibody homologous to the human epoxygenase CYP2C9. Immunoblotting was performed using Scion Image software (version 4.0.2). Numerical densitometry units were compared between groups. Membranes were stained with Coomassie brilliant blue following immunoblotting procedures to confirm equal protein loading per lane.

**EET levels in vascular tissue.** SMAs were isolated from control and CH rats. Endothelial cells were harvested from these vessels after intraluminal incubation with dispase (2.4 U/ml) for 45 min at 37°C (31). Endothelial cells recovered from two arteries (from different rats) were pooled and then homogenized in 0.2 M KCl that contained 0.01% butylated hydroxytoluene (BHT; Ref. 13). PGB2 (100 ng) was added as an internal standard to each sample to control for extraction efficiency (24). Endothelial cell homogenates were extracted three times with an equal volume of diethyl ether that contained 5% nonfat milk. According to the supplier, this antibody binds to the rat CYP2C isoform that is homologous to the human epoxygenase CYP2C9. Immunochromatography was achieved by incubation for 1 h at room temperature with a horseradish peroxidase-conjugated goat antibody homologous to the human epoxygenase CYP2C9. Immunoblotting was performed using Scion Image software (version 4.0.2). Numerical densitometry units were compared between groups. Membranes were stained with Coomassie brilliant blue following immunoblotting procedures to confirm equal protein loading per lane.

**Effects of CYP and cyclooxygenase inhibition on VSM cell Em.** In agreement with a previous report (8), VSM cells in SMAs from CH rats were hyperpolarized compared with controls (Fig. 1). Inhibition of CYP activity with both 17-ODYA and SKF-525A normalized VSM cell Em between the groups (Fig. 1). Although 17-ODYA was without effect on VSM cell Em values in control
tissue, VSM cells in control vessels treated with SKF-525A were slightly depolarized compared with vehicle-treated control arteries (Fig. 1). Furthermore, in the presence of the selective CYP2C9 inhibitor sulfa-phenazole, VSM cell $E_m$ values of arteries from CH rats were not different from those of sulfaphenazole-treated vessels from control animals (Fig. 2). In contrast, VSM cells in vessels isolated from CH rats remained hyperpolarized compared with controls in the presence of the cyclooxygenase inhibitor meclofenamate (Fig. 2).

Effects of BK$_{Ca}$ channel inhibition on VSM cell membrane potential. Previous studies have demonstrated that CYP-derived EETs elicit VSM cell hyperpolarization by activating BK$_{Ca}$ channels (3). We therefore examined the effects of the specific BK$_{Ca}$ channel inhibitor iberiotoxin on VSM cell $E_m$ in SMAs from control and CH rats. Iberiotoxin administration did not alter VSM cell $E_m$ values in arteries from control rats (Fig. 3). However, VSM cells in iberiotoxin-treated vessels isolated from CH rats were depolarized compared with VSM cells in vehicle-treated vessels from CH rats (Fig. 3). In addition, VSM cell $E_m$ values did not differ between arteries from control and CH rats following iberiotoxin administration (Fig. 3).

Effects of CYP2C9 inhibition on myogenic responsiveness. Consistent with previously reported findings (9), myogenic vasoconstrictor responsiveness was blunted in small arteries obtained from CH rats compared with controls at intraluminal pressures $>20$ Torr (Fig. 4). In addition, myogenic responsiveness of vessels from control rats treated with sulfaphenazole was not different from that of vehicle-treated vessels (Fig. 4A). In contrast, myogenic vasoconstriction of sulfaphenazol-ole-treated vessels obtained from CH rats was greater than vehicle-treated vessels from this group (Fig. 4B). Furthermore, pressure-induced constritor responses of vessels from CH rats were not different from those of control arteries after CYP2C9 inhibition (Fig. 4).

These experiments also demonstrated that vessel wall [Ca$^{2+}$]$_i$ was diminished in vessels obtained from CH rats compared with controls at intraluminal pressures $>20$ Torr (Fig. 5). Vessel wall [Ca$^{2+}$]$_i$ of arteries from control rats that were administered sulfaphenazole was not different from vehicle-treated vessels from control rats (Fig. 5A), whereas vessel wall [Ca$^{2+}$]$_i$ of sulfaphenazole-treated arteries isolated from CH rats was greater than that of vehicle-treated ves-sels from CH rats (Fig. 5B). These data also showed that vessel wall [Ca$^{2+}$]$_i$ did not differ between arteries from control and CH rats following CYP2C9 inhibition (Fig. 5C)
isolated from CH and control rats in the presence of sulfaphenazole (Fig. 5).

**Effects of CYP2C9 inhibition on PE-induced vasoconstriction.** PE-induced vasoconstriction was attenuated for vessels isolated from CH rats compared with controls (Fig. 6; Ref. 8). In addition, PE-induced vasoconstriction was not different between sulfaphenazole- and vehicle-treated arteries isolated from control rats (Fig. 6A). However, PE-induced vasoconstriction of arteries from CH rats treated with sulfaphenazole was greater than that of vehicle-treated vessels isolated from CH rats (Fig. 6B). Furthermore, PE-induced vasoconstriction of CYP2C9-inhibited vessels from CH rats was not different from that of vehicle- or sulfaphenazole-treated control arteries.

Changes in vessel wall $[Ca^{2+}]_i$ that resulted from PE administration were not different for arteries isolated from control rats treated with sulfaphenazole or vehicle (Fig. 7A). In contrast, PE-induced vessel wall $[Ca^{2+}]_i$ responses were greater for sulfaphenazole-treated arteries from CH rats compared with vehicle-treated vessels from CH rats (Fig. 7B). Vessel wall $[Ca^{2+}]_i$ did not differ between arteries isolated from control and CH rats in the presence of sulfaphenazole.

**Effects of exogenous 11,12-EET administration.** In agreement with an earlier report (27), 11,12-EET administration (1 µM) elicited dilation of PE-constricted mesenteric arteries. PE-induced constriction was reversed by $47.0 \pm 13.6\%$ (n = 3). In addition, 11,12-EET reversed PE-induced increases in vessel wall $[Ca^{2+}]_i$ by $30.9 \pm 11.4\%$ (n = 3). The vehicle for 11,12-EET (ethanol) was without effect.

**CYP2C9 protein levels.** A protein homologous to CYP2C9 was detected in microsomes prepared from mesenteric arteries isolated from both control and CH rats as a single major band of $\sim 56$ kDa (Fig. 8A). The electrophoretic mobility of this band was identical to that of a band detected in a gel lane loaded with microsomes from rat liver, a tissue that has been shown to express CYP2C proteins (16, 35). CYP2C9-band density was greater in lanes loaded with microsomes from CH rats compared with controls (Fig. 8B).

**EET levels in vascular tissue.** A peak that is consistent with the retention time and spectra characteristics of the 11,12-EET standard was detected in chromatograms of endothelial cells harvested from vessels isolated from both control and CH rats (Fig. 9, B and C). The area of the 11,12-EET peak normalized to the PGB$_1$ internal standard for tissue from CH rats was greater than that of controls (Fig. 9E). In addition,
These experiments demonstrated that the area of the 11,12-EET peak for vessels treated with sulfaphenazole after isolation from CH rats (Fig. 9D) was less than that of vehicle-treated vessels from CH rats (Fig. 9E).

Effects of sulfaphenazole and ZnPPIX on CYP2C9 activity. A previous report from our laboratory demonstrated that VSM cell $E_m$ values in SMAs from CH rats were normalized to control levels by administration of the heme oxygenase (HO) inhibitor ZnPPIX (8). The current study reports similar findings for the CYP2C9 inhibitor sulfaphenazole (see Fig. 2). Because CYPs are heme-containing enzymes and may be inhibited by ZnPPIX, an in vitro assay was employed to examine the potential inhibitory effects of ZnPPIX and sulfaphenazole administration on CYP2C9 activity (Fig. 10). In contrast, CYP2C9 activity was not altered by ZnPPIX (500 nM) administration (Fig. 10).

DISCUSSION

This study examined the contribution of endothelial arachidonic acid metabolites to VSM cell hyperpolarization and blunted myogenic and agonist-induced vasoconstrictor responsiveness associated with prolonged hypoxic exposure. The major findings of this study are 1) CYP inhibition normalizes VSM cell $E_m$ between arteries isolated from control and CH rats, 2) BK$_{Ca}$ channel blockade reverses CH-induced VSM cell hyperpolarization, 3) administration of a selective CYP2C9 inhibitor restores myogenic and PE-induced vasoconstrictor and Ca$^{2+}$ responsiveness of arteries isolated from CH rats to control levels, 4) expression of a rat CYP2C9-immunoreactive protein is greater in mesenteric arteries isolated from CH rats compared with controls, and 5) 11,12-EET levels are greater in mesenteric endothelial cells harvested from CH rats compared with controls. These findings suggest that enhanced vascular CYP2C9 expression and endothelial 11,12-EET production following CH contribute to BK$_{Ca}$-dependent VSM hyperpolarization and attenuated vasoconstrictor responsiveness.

Earlier reports from our laboratory indicate that blunted vasoconstrictor responsiveness following CH is associated with endothelium-dependent VSM cell hyperpolarization (8, 9). These previous findings also suggest that VSM cell hyperpolarization associated with prolonged hypoxic exposure is NOS independent (8), whereas the current study demonstrates that altered VSM cell $E_m$ following CH is also independent of cyclooxygenase activity (see Fig. 2). Endothelium-dependent VSM cell hyperpolarization that is independent of NOS and cyclooxygenase is attributed to EDHF (6). Various studies have reported that EDHF-type responses result from release of K$^+$ from endothelial cells (10), myoendothelial gap junctional communication (5, 34, 37), and CYP epoxygenase activity (21). The current findings demonstrate that VSM cell $E_m$ is normalized between control and CH groups by inhibition of CYP activity (see Fig. 1), which suggests that products of this pathway contribute to VSM cell hyperpolarization that is associated with prolonged hypoxic exposure. Furthermore, administration of the selective CYP2C9 inhibitor sulfaphenazole also eliminated differences in VSM cell $E_m$ values between groups (see
Fig. 2), which indicates that this isoform plays an important role in CH-associated VSM cell hyperpolarization. This observation is consistent with reports that show that agonist-induced NO- and prostacyclin-independent VSM cell hyperpolarization in porcine coronary arteries is mediated by CYP2C9-dependent production of EETs (2, 12–15, 21). Given that EETs can elicit VSM cell hyperpolarization through activation of BKCa channels (3), we examined the effects of BKCa channel blockade on VSM Em following CH. Selective inhibition of BKCa channels with iberiotoxin normalized VSM cell Em between control and CH rats (see Fig. 3), which suggests that hyperpolarization after prolonged hypoxia results from increased activity of this channel. Taken together, these findings support the hypothesis that enhanced CYP2C9 activity following CH contributes to VSM cell hyperpolarization via increased BKCa channel conductance.
VSM cell $E_m$ largely regulates vascular tone by determining the activity of voltage-dependent Ca$^{2+}$ channels (28). In addition, vasoconstriction can be influenced by the sensitivity of the VSM cell contractile apparatus to [Ca$^{2+}$]$_i$ (30). Previous findings from our laboratory have demonstrated that VSM cell hyperpolarization and attenuated vasoconstrictor responsiveness following CH are associated with decreased vessel wall [Ca$^{2+}$], which demonstrates that blunted constriction results from diminished VSM cell [Ca$^{2+}$]i rather than from altered Ca$^{2+}$ sensitivity (8, 9). In agreement with these findings, the current study also shows that attenuated myogenic vasoconstriction is associated with decreased vessel wall [Ca$^{2+}$]. In addition, CYP2C9 inhibition enhances both myogenic and PE-induced constrictor responsiveness of vessels from CH rats without altering vasoconstriction of vessels from control rats. Furthermore, enhanced reactivity of vessels from CH rats was associated with increased vessel wall [Ca$^{2+}$] following sulfaphenazole administration, whereas neither vasoconstriction nor vessel wall [Ca$^{2+}$] of control vessels were significantly altered by CYP2C9 inhibition. Therefore, we conclude that a CYP2C9-dependent decrease in VSM cell [Ca$^{2+}$]i rather than altered Ca$^{2+}$ sensitivity contributes to blunted reactivity following CH. These findings suggest that CYP2C9 activity does not greatly influence vascular tone or vessel wall [Ca$^{2+}$] of arteries isolated from control rats.

Our findings demonstrate that CYP2C9 inhibition influences VSM cell $E_m$ and contractility of vessels isolated from CH rats without altering these variables in arteries from control rats. These preferential effects of CYP2C9 inhibition observed in vessels from CH rats may be due to increased expression or activity of CYP2C9 that result in elevated production of EETs or alternatively may be due to enhanced sensitivity of the vasculature to these compounds. To address these possibilities, we performed Western blots on mesenteric vasculature to these compounds. To address these possibilities, we performed Western blots on mesenteric vasculature to these compounds. To address these possibilities, we performed Western blots on mesenteric vasculature to these compounds. To address these possibilities, we performed Western blots on mesenteric vasculature to these compounds. To address these possibilities, we performed Western blots on mesenteric vasculature to these compounds.
increased EET production. Investigations of potential interactions between these two pathways are needed to elucidate the mechanism that is responsible for VSM cell hyperpolarization after CH.

In summary, we have demonstrated that CYP2C9 activity is associated with BKCa-dependent VSM cell hyperpolarization and attenuated vasoconstriction and Ca2+ responses following CH. In addition, our findings show that vascular CYP2C9 protein and EET levels are increased after prolonged hypoxic exposure. We conclude that elevated EET production that results from increased CYP2C9 expression contributes to endothelium-dependent VSM cell hyperpolarization and attenuated vasoreactivity following CH.

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REFERENCES


27. Newton DJ, Wang RW, and Lu AY. Cytochrome P450 inhib-


