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Earley, Scott, Jay S. Naik, and Benjimen R. Walker. 48-h Hypoxic exposure results in endothelium-dependent systemic vascular smooth muscle cell hyperpolarization. Am J Physiol Regulatory Integrative Comp Physiol 283: R79–R85, 2002. First published March 22, 2002; 10.1152/ajpregu.00104.2002.—Chronic hypoxia (CH) results in reduced sensitivity to vasoconstrictors in conscious rats that persists upon restoration of normoxia. We hypothesized that this effect is due to endothelium-dependent hyperpolarization of vascular smooth muscle (VSM) cells after CH. VSM cell resting membrane potential was determined for superior mesenteric artery strips isolated from CH rats (Pb = 380 Torr for 48 h) and normoxic controls. VSM cells from CH rats studied under normoxia were hyperpolarized compared with controls. Resting vessel wall intracellular Ca2+ concentration ([Ca2+]i) and pressure-induced vasoconstriction were reduced in vessels isolated from CH rats compared with controls. Vasoconstriction and increases in vessel wall [Ca2+]i in response to the α1-adrenergic agonist phenylephrine (PE) were also blunted in resistance arteries from CH rats. Removal of the endothelium normalized resting membrane potential, resting vessel wall [Ca2+]i, pressure-induced vasoconstrictor responses, and PE-induced constriction and Ca2+ responses between groups. Whereas VSM cell hyperpolarization persisted in the presence of nitric oxide synthase inhibition, heme oxygenase inhibition restored VSM cell resting membrane potential in vessels from CH rats to control levels. We conclude that endothelial derived CO accounts for persistent VSM cell hyperpolarization and vasoconstrictor hyporeactivity after CH.

48-h Hypoxic exposure results in endothelium-dependent systemic vascular smooth muscle cell hyperpolarization

Chronic Hypoxic Exposure Results in Blunted Systemic Vasoconstrictor Responses in Conscious Rats (6) That Persists Upon Restoration of Normoxia (11), Which Demonstrates That the Consequences of Chronic Hypoxia (CH) Are Distinct From Acute Responses to This Stimulus (5). Persistently Blunted Vasoconstriction After CH Has Been Observed in Response to Both Receptor-dependent (6, 10) and Receptor-independent Stimuli (23). Studies Demonstrating Blunted Vasoactivity Following CH in Isolated Vessel Preparations such as Aortic (2, 4) and Uterine Artery Rings (10, 25) as Well as Isolated Diaphragmatic Resistance Arteries (23) Suggest That This Effect Is an Inherent Vascular Property and Is Not Mediated by Circulating Factors or by Altered Sympathetic Nervous System Activity. Interestingly, Chronic Obstructive Pulmonary Disease Patients Exhibit Chronic Vasodilation of the Forearm Circulation That is Proportional to the Degree of Hypoxemia Present (3), Which Suggests That Attenuated Vasoconstriction May Be Associated With This Pathology.

Ionic Conductances Regulate the Contractile State and Reactivity of Blood Vessels. For Example, Voltage-dependent Ca2+ Channels (VDCCs) Account for a Significant Portion of Ca2+ Influx Into Vascular Smooth Muscle (VSM) Cells (8). VSM Resting Membrane Potential (RMP) Largely Determines the Open Probability (Po) of VDCCs (18) Thereby Determining Free Intracellular Ca2+ Concentration ([Ca2+]i) and, Ultimately, Vascular Tone (12). Endothelial Derived Factors Can Influence Vascular Tone by Altering the Membrane Potential and [Ca2+]i of VSM Cells. Furthermore, the Synthesis and Release of These Factors Can Be Modulated by Environmental Stimuli. For Example, the Production of Vasoactive Endothelial Derived Factors Such as Endothelin-1 (7), Nitric Oxide (NO; Ref. 14), and Carbon Monoxide (CO; Ref. 15) May Be Regulated by Hypoxia. The Goal of This Study Was to Investigate a Causal Role for Endothelial Derived Factors in Blunted Vasoreactivity Following CH. We Hypothesized That Chronic Hypoxic Exposure Results in Persistent, Endothelium-dependent Hyperpolarization of VSM Cells. Furthermore, We Postulated That CH-induced VSM Cell Hyperpolarization Leads to Decreased Ca2+ Influx and That the Resultant Reduction in VSM Cell [Ca2+]i Attenuates Vasoconstrictor Responses. To Test This Hypothesis, We Measured VSM Cell RMP in Endothelium-intact and Endothelium-denuded Superior Mesenteric Artery (SMA) Strips Isolated From Normoxic Control and CH Rats Acutely Returned to Normoxia. In Addition, Pressure- and Agonist-induced Vasoconstriction and Vessel Wall [Ca2+]i Responses of Endothelium-intact and Endothelium-denuded Mesenteric Resistance Arteries Isolated From Control and CH Rats Were Determined Under Normoxic Conditions. Further Experiments Were Performed to Elucidate Which

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endothelial derived factors might be responsible for VSM cell hyperpolarization after CH.

**METHODS**

**Animals**

Before experimentation, male Sprague-Dawley rats (Harlan Industries) were deeply anesthetized with pentobarbital sodium (32.5 mg ip). Animals were humanely killed by exsanguination after vessels were harvested according to a protocol approved by the Institutional Animal Care and Use Committee of the University of New Mexico School of Medicine in accordance with United States Department of Agriculture and National Institutes of Health guidelines. Rats 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 of 380 Torr for 48 h, whereas control rats were housed in identical cages at ambient barometric pressure (~630 Torr).

**General Methods**

VSM cell RMP. VSM cell RMP was recorded from SMA strips using glass intracellular microelectrodes. The chest and abdomen of anesthetized rats were opened, and heparin (100 U in 0.1 ml) was injected into the heart to prevent clotting. SMAs were isolated and excised, and SMA strips were secured in an organ bath with the luminal surface exposed. SMA strips were superfused (5 ml/min) with physiologial saline solution (PSS) warmed to 37°C and aerated with a normoxic gas mixture consisting of 21% O2-6% CO2-73% N2. VSM cells were impaled with microelectrodes filled with 3 M KCl (tip resistance 20–50 MΩ) inserted into the artery strip through the endothelial surface. A Neuroprobe amplifier (model 1600, A-M Systems) was used for recording 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 membrane potential recordings included 1) an abrupt negative deflection of potential as the microelectrode was advanced into a cell, 2) stable membrane potential 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.

Resting vessel wall [Ca2+]. Mesenteric resistance arteries from control and CH rats were isolated and pressurized. The mesenteric arcade was excised and from control and CH rats were isolated and pressurized. The segments (ID = 100–200 μm; n = 20) were dissected from the cleaned branches, transferred to a vessel chamber (Living Systems), cannulated with glass micropipettes, and secured with ligatures. Vessels were slowly pressurized to 60 Torr using a column filled with PSS and were superfused (5 ml/min) with aerated PSS warmed to 37°C.

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 PSS to yield a final concentration of 2 μM fura 2-AM and 0.05% Pluronic acid. Vessels were incubated in this solution for 45 min at room temperature in the dark. Administration of fura 2-AM to the abluminal surfaces of pressurized arterioles has been shown to preferentially load VSM cells (13). The diluted fura 2-AM solution was aerated with normoxic gas mixture during the loading period. Vessels were equilibrated for 20 min with warmed, aerated PSS after the loading period to wash out excess dye and to allow for esterification of AM groups. Ratiometric images were collected using a Nikon Diaphot 300 microscope equipped with a ×10 Nikon Fluor objective (numeric aperture = 0.30). Fura-loaded vessels were alternatively excited at 340 and 380 nm, and images of the respective 510-nm emissions were collected at a rate of ~0.3 Hz using MetaFluor software (Universal Imaging). Resting vessel wall [Ca2+], was calculated as the mean 340:380 ratio from background-subtracted images for a selected region (generally the whole vessel) collected over ~3 min. The 340:380 ratio is linearly related to the true molar [Ca2+], assuming that the dissociation constant of fura 2-AM does not differ between treatment groups.

**Pressure-induced vasoconstriction.** Bright-field images of resistance arteries pressurized to 60 Torr were obtained immediately before fura 2-AM loading. At the end of the experiment, vessels were reequilibrated for 30 min, treated with the vasodilator papaverine (100 μM), and another bright-field image was obtained. The internal and external diameters of the pre-fura-loaded and maximally dilated vessel images were measured using MetaMorph 4.5 software (Universal Imaging) that was calibrated using a stage micrometer. Pressure-induced vasoconstriction was calculated as the percent change in internal diameter before fura 2-AM loading versus the internal diameter after papaverine administration. Some vessels were further treated with the NO donor S-nitroso-N-acetylpentillamine (SNAP, 10 μM), the VDC blocker nifedipine (1 μM), or were superfused with Ca2+-free PSS [which contained (in mM) 129.8 NaCl, 5.4 KCl, 0.83 MgSO4, 19 NaHCO3, 1.8 CaCl2, and 5.5 glucose] aerated with a normoxic gas mixture consisting of 21% O2-6% CO2-73% N2. The arcade was secured in a Silastic-coated petri dish containing cold, aerated PSS. Veins were removed, and resistance artery branches were cleaned of adipose tissue and transferred to a beaker of cold, aerated PSS. Vessel segments (ID = 100–200 μm; n = 20) were dissected from the cleaned branches, transferred to a vessel chamber (Living Systems), cannulated with glass micropipettes, and secured with ligatures. Vessels were slowly pressurized to 60 Torr using a column filled with PSS and were superfused (5 ml/min) with aerated PSS warmed to 37°C.

**Phenylephrine-induced constrictor and Ca2+ responses.** Pressurized resistance arteries were superfused with PSS containing increasing concentrations of phenylephrine (PE, 10 nM to 10 μM). Images were collected for 3 min for each PE dose, and the mean 340:380 ratio for the recording period was calculated and expressed as vessel wall [Ca2+]i. The outer diameters of the vessels were measured for every fifth image frame for each PE dose, and means were calculated and expressed as percent change from the maximally dilated state.

**Solutions.** Stock solutions of PE and ACh (Sigma) were prepared in water at a concentration of 1 M. Aliquots were stored at −20°C and were diluted in PSS on the day of use. Nω-nitro-L-arginine (L-NNA; Sigma) was dissolved in PSS on the day of experimentation. Papaverine (Sigma) was dissolved in PSS immediately before use. SNAP (Sigma) was dissolved in ethanol at a concentration of 0.1 M on the day of experimentation and then diluted with PSS before administration. Stock solutions of nifedipine (Sigma) were pre-
pared in DMSO, stored in aliquots at 4°C in light-proof containers, and diluted with PSS immediately before administration. The heme oxygenase (HO) inhibitor zinc protoporphyrin IX (ZnPPIX; Porphyrin Products) was dissolved in 10% ethanolamine at a concentration of 100 mg/ml. NaCl was slowly added to a final concentration of 0.72%, and the pH was adjusted to 7.6–8.0 with HCl. Spectrophotometric measurements were performed to verify the final ZnPPIX concentration. ZnPPIX solutions were prepared in the dark and experiments were performed in reduced light due to the light-sensitive nature of this compound.

**Experimental Protocols**

VSM cell RMP, myogenic and agonist-induced vasoconstrictor responses, and vessel wall [Ca\(^{2+}\)]\(_i\), of arteries from control and CH rats were determined for endothelium-intact and endothelium-denuded vessels. Additional experiments examined the effects of the NO synthase (NOS) inhibitor 1-NNa and the HO inhibitor ZnPPIX on VSM cell RMP in endothelium-intact vessels from control and CH rats.

**Endothelium-intact vessels.** VSM cell RMP was measured for endothelium-intact strips of SMA isolated from normoxic control and CH rats (control, n = 9; CH, n = 7). In addition, resting vessel wall [Ca\(^{2+}\)]\(_i\), pressure-induced vasoconstriction, and PE-induced vasoconstrictor and Ca\(^{2+}\) responses were determined for fura-loaded mesenteric resistance arteries (control and CH, n = 5).

**Endothelium-denuded vessels.** The endothelium was removed from SMA artery strips isolated from control and CH rats (control and CH, n = 6) by gentle rubbing with a cotton swab. Strips were superfused with PSS for 30 min after rubbing to wash out endothelial derived factors, and membrane potential was recorded. After completion of the recordings, the endothelium-dependent vasodilator ACh (10 μM) was administered to demonstrate removal of the endothelium. VSM membrane potential remained unchanged upon ACh administration to endothelium-denuded vessels, whereas VSM cells in endothelium-intact arteries were hyperpolarized by this treatment (Fig. 4, Table 1).

Resting vessel wall [Ca\(^{2+}\)]\(_i\), pressure-induced vasoconstriction, and PE-induced vasoconstrictor and Ca\(^{2+}\) responses were determined for resistance arteries after removal of the endothelium (control and CH, n = 5). For these experiments, endothelial cell integrity was demonstrated before removal by vasodilatory response to ACh (1 μM) in the presence of PE (10 μM). After a 30-min equilibration period following ACh administration, 1 ml of air was passed through the vessel lumen to inactivate the endothelium as described by others (23). Endothelium-denuded arteries were then perfused and superfused with warmed, aerated PSS for 30 min and represurized to 60 Torr. After a 30-min equilibration period, vessels were constricted with PE (10 μM), and ACh (1 μM) was administered to assess the efficacy of endothelium-removal procedures. Resistance arteries were loaded with fura 2-AM after a 30-min equilibration period following ACh administration. In contrast to endothelium-intact vessels, endothelial denuded resistance arteries further dilated upon SNAP administration after papaverine treatment. Therefore, for studies employing endothelial denuded arteries, the diameter measured after SNAP administration was used to calculate pressure and PE-induced vasoconstrictor responses.

**NOS inhibition.** VSM cell RMP was determined for endothelium-intact SMA strips isolated from control and CH rats in the presence of two concentrations of 1-NNa (100 μM; control and CH, n = 6) and (500 μM; control, n = 6; CH, n = 5). Previous experiments have demonstrated that PE-induced vasoconstriction of mesenteric arteries remains from CH rats blunted compared with controls during NOS inhibition (8).

**HO inhibition.** VSM cell RMP was recorded for endothelium-intact SMA strips isolated from control and CH rats in the presence of ZnPPIX (500 nM; control and CH, n = 6) or its vehicle (control, n = 7; CH, n = 5). A prior report from our laboratory has demonstrated that PE-induced responsiveness of small mesenteric arteries isolated from CH rats is enhanced by HO inhibition (8).

**Calculations and Statistics**

All data are expressed as means ± SE. Values of n refer to the number of animals in each group. Unpaired t-tests were used to make comparisons between control and CH groups for most experiments. Two-way ANOVA and subsequent Student-Newman-Keuls post hoc test were used to analyze data after HO inhibition. A probability ≤0.05 was accepted as statistically significant for all comparisons.

**RESULTS**

**Endothelium-Intact Vessels**

Consistent with our hypothesis, VSM cells in vessels from CH rats were persistently hyperpolarized compared with controls (Fig. 1, A-C). Furthermore, resting vessel wall [Ca\(^{2+}\)]\(_i\) was lower in resistance arteries isolated from CH rats compared with normoxic controls (Fig. 1D), which suggests that VSM cell hyperpolarization is associated with reduced Ca\(^{2+}\) influx. In addition, resistance arteries from CH rats exhibited decreased myogenic tone (Fig. 1E) and blunted PE-induced (Fig. 1F) vasoconstrictor and vessel wall [Ca\(^{2+}\)]\(_i\) responses (Fig. 1B). These data demonstrate that blunted myogenic and agonist-induced vasoconstrictor responses resulting from prolonged hypoxic exposure are associated with VSM cell hyperpolarization and decreased vessel wall [Ca\(^{2+}\)]\(_i\).

**Endothelium-Denuded Vessels**

VSM cell RMP was not different between endothelium-denuded arteries isolated from control and CH rats (Fig. 3A), which suggests that endothelial derived factors are responsible for VSM cell hyperpolarization after prolonged hypoxia. ACh administration to endothelium-denuded vessels did not alter VSM cell membrane potential (Table 1), which demonstrates that endothelium removal was effective. In contrast, administration of SNAP (100 μM) hyperpolarized VSM cells

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**Table 1. VSM cell resting \(E_m\) for endothelium-intact and endothelium-denuded vessels after \(\text{ACh}\) and \(\text{SNAP}\) administration**

<table>
<thead>
<tr>
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<th>(E_m), mV</th>
<th>(E_m) (ACh), mV</th>
<th>(E_m) (SNAP), mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>-47.8 ± 2.4 (9)</td>
<td>-59.4 ± 4.6 (8)</td>
<td>-61.6 ± 4.9 (5)</td>
</tr>
<tr>
<td>Denuded</td>
<td>-37.4 ± 2.9 (6)</td>
<td>-38.2 ± 4.9 (6)</td>
<td>-54.3 ± 4.5 (6)</td>
</tr>
<tr>
<td>Denuded CH</td>
<td>-36.1 ± 2.6 (6)</td>
<td>-34.5 ± 4.9 (6)</td>
<td>-52.6 ± 4.5 (6)</td>
</tr>
</tbody>
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Values are means ± SE; (n), no. of rats. \(P ≤ 0.05\) vs. untreated intact control; \(\dagger P ≤ 0.05\) vs. untreated denuded control; \(\ddagger P < 0.05\) vs. untreated denuded chronic hypoxia (CH). SNAP, S-nitroso-N-acetyl penicillamine; \(E_m\), membrane potential; VSM, vascular smooth muscle.
in these tissues (Table 1). Administration of both ACh and SNAP (Table 1) hyperpolarized VSM cells in endothelium-intact control vessels.

Similarly, removal of the endothelium abolished ACh-induced vasodilatory responses of mesenteric resistance arteries preconstricted with PE. ACh (10 μM) administration resulted in a 59.2 ± 8.8% reversal of PE (10 μM)-induced vasoconstriction of endothelium-intact vessels, whereas the reversal for endothelium-denuded arteries was 0.07 ± 1.1%. ACh-induced vasodilatory responses of control and CH vessels were not different. Resistance-artery resting vessel wall [Ca^{2+}]_{i} (Fig. 3B) and myogenic tone (Fig. 3C) were normalized by removal of the endothelium, which supports the possibility that an endothelial derived factor is responsible for both reduced vessel wall [Ca^{2+}]_{i} and blunted myogenic responsiveness resulting from extended hypoxic exposure. Consistently, PE-induced vasoconstrictor (Fig. 4A) and vessel wall [Ca^{2+}]_{i} (Fig. 4B) responses were also not different between endothelium-denuded vessels from control and CH rats.

**NOS Inhibition**

VSM cells in vessels from CH rats remained hyperpolarized compared with controls in the presence of NOS inhibition (Fig. 5). VSM cell RMP was not further altered when the L-NNA concentration was increased from 100 to 500 μM (Fig. 5), which suggests that the lower dose is effective in inhibiting NO production.
HO Inhibition

Administration of ZnPPIX normalized VSM cell RMP between vessels from control and CH rats, whereas VSM cells in vehicle-treated arteries from CH rats remained hyperpolarized compared with vehicle-treated controls (Fig. 6). VSM cells in control arteries treated with ZnPPIX were slightly depolarized compared with VSM cells in control, vehicle-treated vessels (Fig. 6).

DISCUSSION

The major findings of this study are 1) CH results in persistent hyperpolarization of VSM cells; 2) resting vessel wall $[\text{Ca}^{2+}]_{i}$ is decreased after prolonged hypoxic exposure; 3) agonist and pressure-induced vasoconstrictor responses of mesenteric resistance arteries are blunted as a result of extended hypoxia; 4) agonist-induced increases in vessel wall $[\text{Ca}^{2+}]_{i}$ are blunted after CH; 5) endothelium removal normalizes RMP, vasoconstrictor, and $[\text{Ca}^{2+}]_{i}$ responses for vessels isolated from control and CH rats; 6) VSM cells in vessels from CH rats remain hyperpolarized compared with controls during NOS inhibition; and 7) HO inhibition restores VSM cell RMP in arteries isolated from CH rats to control levels. These data suggest that blunted vasoreactivity after CH is associated with endothelium-dependent VSM cell hyperpolarization and decreased free $[\text{Ca}^{2+}]_{i}$. Considering that previous studies

Fig. 3. A: VSM cell RMP for endothelium-denuded arteries isolated from control ($n = 6$) and CH ($n = 6$) rats. B: resting vessel wall $[\text{Ca}^{2+}]_{i}$ of pressurized endothelium-denuded mesenteric resistance arteries isolated from control ($n = 5$) and CH ($n = 5$) rats. C: pressure-induced (60 Torr) vasoconstriction of endothelium-denuded resistance arteries isolated from control ($n = 5$) and CH ($n = 5$) rats.

Fig. 4. A: vasoconstriction in response to increasing doses of PE for endothelium-denuded vessels isolated from control and CH rats. B: changes in vessel wall $[\text{Ca}^{2+}]_{i}$ in response to increasing doses of PE for endothelium-denuded vessels isolated from control and CH rats.

Fig. 5. VSM cell RMP for vessels isolated from control and CH rats treated with the nitric oxide synthase inhibitor N$^\text{e}$-nitro-L-arginine (L-NNA): 0.1 mM L-NNA, control and CH, $n = 6$; 0.5 mM L-NNA, control, $n = 6$; CH, $n = 5$. *$P < 0.05$ vs. control, 0.1 mM L-NNA; #$P < 0.05$ vs. control, 0.5 mM L-NNA.
have also demonstrated attenuated vasoreactivity after normobaric hypoxic exposure (2, 23), this response is most likely a consequence of hypoxia rather than hypobaria. Furthermore, our results suggest that VSM cell hyperpolarization may result from increased production of CO by the endothelium after prolonged hypoxic exposure.

Vascular tone is largely dependent upon the free [Ca$^{2+}$] of VSM cells (17). Free [Ca$^{2+}$], within VSM cells is regulated by Ca$^{2+}$ influx through Ca$^{2+}$-specific (18) and nonselective cation (1) channels and through release of Ca$^{2+}$ from intracellular stores (22). Blockade of VDCCs attenuates receptor-dependent (18) and independent (21) VSM-cell Ca$^{2+}$ influx and subsequent vasoconstrictor responses, which demonstrate that these channels are the main pathway for Ca$^{2+}$ entry. Given that the $P_o$ of VDCCs is voltage dependent (18), free [Ca$^{2+}$], within VSM cells and therefore vascular tone are largely a function of RMP. Our findings demonstrate that VSM cell RMP after CH is hyperpolarized compared with controls (see Fig. 1, B-D) and that prolonged hypoxic exposure induces a parallel decrease in resting vessel wall [Ca$^{2+}$], (see Fig. 1E). These experiments also demonstrate that VSM cell membrane potential and [Ca$^{2+}$], remain altered after restoration of normoxia, which suggests that the effects of CH are distinct from those of acute hypoxia. Furthermore, reduced vessel wall [Ca$^{2+}$], after CH (see Fig. 1E) is well correlated with blunted pressure-induced (see Fig. 1F) and PE-induced vasoconstrictor responses (Fig. 2A). Thus attenuated vasoreactivity after extended hypoxic exposure likely results from VSM cell hyperpolarization and associated reduction of VSM [Ca$^{2+}$], that persists upon restoration of normoxia.

Removal of the endothelium from arteries eliminated differences in VSM cell RMP (Fig. 4A), resting vessel wall [Ca$^{2+}$], (Fig. 3B), pressure-induced vasoconstriction (Fig. 3C), and PE-induced vasoconstrictor and [Ca$^{2+}$], responses (Fig. 4, A and B) between control and CH groups, which suggests that VSM hyperpolarization and blunted vasoreactivity after CH may be mediated by an endothelium-derived influence. Although the endothelium may alter VSM cell RMP by direct electrical communication via myoendothelial gap junctions (20, 24), CH-induced, endothelium-dependent VSM cell hyperpolarization may also be due to increased synthesis or release of diffusible factors. Hypoxia may influence the production of a number of vasoactive endothelium-derived substances such as prostacyclin, cytochrome P-450 metabolites of arachidonic acid (16), NO (14), and CO (15). To elucidate the identity of potential CH-inducible hyperpolarizing factors, we examined the effects of NOS and HO inhibition on VSM cell RMP. Our findings demonstrate that the RMP of VSM cells in vessels from both control and CH rats is slightly depolarized by L-NNA administration. However, VSM cells from CH rats remained hyperpolarized compared with controls during NOS inhibition (see Fig. 5), which suggests that NO is not the hyperpolarizing factor that is persistently released after CH. These data are consistent with a previous report that demonstrates that PE-induced vasoconstriction of mesenteric resistance arteries from CH rats remains blunted compared with controls during NOS inhibition (9). In contrast to the effects of NOS inhibition, our findings (see Fig. 6) as well as previous reports from our laboratory strongly support a role for endothelial derived CO in the observed attenuation of vasoconstrictor responsiveness in arteries from CH rats. For example, HO inhibition normalizes VSM cell RMP between vessels isolated from control and CH rats (see Fig. 6). Although VSM cells in vessels from control rats were slightly depolarized compared with VSM cells in vehicle-treated control vessels, HO inhibition had a much more profound effect on membrane potential in vessels from CH rats (see Fig. 6). This finding is consistent with reports from our laboratory that demonstrate that HO inhibition enhances PE-induced vasoconstrictor responsiveness in mesenteric resistance arteries isolated from CH rats (9) and restores blunted PE-induced vasoconstrictor responses to endothelial-intact aortic rings after CH (4). Furthermore, molecular studies show that aortic protein (11) and mRNA levels (15) of the inducible isoform HO-1 are increased by hypoxic exposure. Increased vascular HO-1 gene expression and subsequent elevated CO production after CH are consistent with the persistent nature of the hyperpolarizing influence demonstrated by the current study. Furthermore, renal HO enzyme activity is increased after prolonged hypoxic exposure (19), which supports the hypothesis that increased CO production may exert a tonic vasodilatory effect. Taken together, these findings suggest that enhanced production of CO by the endothelium may be the most likely mechanism responsible for VSM cell hyperpolarization and attenuated vasoreactivity associated with prolonged hypoxia.

In summary, we have demonstrated that blunted vasoconstrictor responsiveness after CH are correlated with persistent VSM cell hyperpolarization and decreased resting vessel wall [Ca$^{2+}$]. These effects appear to be mediated by endothelium-derived CO acting as a hyperpolarizing factor. We conclude that blunted vasoconstrictor responsiveness after CH is mediated by increased production of CO by the endothelium, which
results in VSM cell hyperpolarization and decreased Ca\(^{2+}\) influx.

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