Pressure-induced smooth muscle cell depolarization in pulmonary arteries from control and chronically hypoxic rats does not cause myogenic vasoconstriction

Jay S. Naik, Scott Earley, Thomas C. Resta, and Benjimen R. Walker
Vascular Physiology Group, Department of Cell Biology and Physiology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico

Submitted 2 August 2004; accepted in final form 28 September 2004

Naik, Jay S., Scott Earley, Thomas C. Resta, and Benjimen R. Walker. Pressure-induced smooth muscle cell depolarization in pulmonary arteries from control and chronically hypoxic rats does not cause myogenic vasoconstriction. J Appl Physiol 98: 1119–1124, 2005. First published October 22, 2004; doi:10.1152/japplphysiol.00819.2004.—Chronic obstructive pulmonary diseases, as well as prolonged residence at high altitude, can result in generalized airway hypoxia, eliciting an increase in pulmonary vascular resistance. We hypothesized that a portion of the elevated pulmonary vascular resistance following chronic hypoxia (CH) is due to the development of myogenic tone. Isolated, pressurized small pulmonary arteries from control (barometric pressure = 630 Torr) and CH (4 wk, barometric pressure = 380 Torr) rats were loaded with fura 2-AM and perfused with warm (37°C), aerated (21% O2-6% CO2-balance N2) physiological saline solution. Vascular smooth muscle (VSM) intracellular Ca2+ concentration ([Ca2+]i) and diameter responses to increasing intraluminal pressure were determined. Diameter and VSM cell [Ca2+]i responses to KCl were also determined. In a separate set of experiments, VSM cell membrane potential responses to increasing luminal pressure were determined in arteries from control and CH rats. VSM cell membrane potential in arteries from CH animals was depolarized relative to control at each pressure step. VSM cells from both groups exhibited a further depolarization in response to step increases in intraluminal pressure. However, arteries from both control and CH rats distended passively to increasing intraluminal pressure, and VSM cell [Ca2+]i was not affected. KCl elicited a dose-dependent vasoconstriction that was nearly identical between control and CH groups. Whereas KCl administration resulted in a dose-dependent increase in VSM cell [Ca2+]i, in arteries taken from control animals, this stimulus elicited only a slight increase in VSM cell [Ca2+]i, in arteries from CH animals. We conclude that the pulmonary circulation of the rat does not demonstrate pressure-induced vasoconstriction.

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normoxic vasculature since pressures are low. In contrast, upon
the development of pulmonary hypertension secondary to CH
exposure, it is possible that pressures may reach a threshold for
myogenic reactivity not achieved in the nonhypertensive cir-
culation (14). In addition, there is evidence that isolated pul-
monary vascular myocytes are depolarized following CH ex-
posure due to altered ion channel expression (31, 34–37, 39).
However, the existence of stretch-induced VSM depolarization
has not been examined in intact pulmonary arteries from either
control or hypertensive circulations. Because of the similarities
between the pulmonary vasculature of the fetus (i.e., thickened
medial layer and low arterial PO2) and the pulmonary circula-
tion of the adult exposed to CH, we hypothesized that a portion
of the elevated PVR following CH is due to the development
of myogenic reactivity.

METHODS

Animals. All animal protocols employed in this study were re-
viewed and approved by the Institutional Animal Care and Use
Committee of the University of New Mexico, School of Medicine.
Adult male Sprague-Dawley rats (age, 8–10 wk; body wt, 250–350 g;
Harlan Industries) were used for these experiments. CH rats were
exposed to hypobaric hypoxia for 4 wk (barometric pressure 380 Torr,
inspired PO2 ~70 Torr). Control animals were housed in ambient air
conditions (barometric pressure = 630 Torr, inspired PO2 ~122 Torr).
Our laboratory has previously demonstrated that this hypoxic expo-
sure protocol results in the development of pulmonary hypertension
in these animals (33).

Isolated pulmonary artery preparation. Rats were anesthetized
with pentobarbital sodium (50 mg ip). A midsternal incision was made
to expose the heart, and 100 units of heparin were injected directly
into the left ventricle. The left lobe of the lung was excised and placed
in ice-cold physiological saline solution [(PSS) composed of (in mM)
129.8 NaCl, 5.4 KCl, 0.5 NaH2PO4, 0.83 MgSO4, 19 NaHCO3, 1.8
CaCl2, and 5.5 glucose], and it was aerated with a 21% O2/6–8%
C02/73% N2 gas mixture. To separate the effects of stretch on Ems
and vasoconstriction from those of acute hypoxia, all experiments were
performed under normoxic conditions (5, 39). The left lung lobe was
secured in a Silastic-coated petri dish that contained cold aerated PSS.
Small pulmonary arteries (200–350 μm) free of side branches were
dissected away from the adjacent airway and transferred to a vessel
chamber (Living Systems). Arteries were cannulated and pressurized
to 12 Torr with PSS using a servo-controlled peristaltic pump (Living
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using a servo-controlled peristaltic pump (Living Systems). Arteries
were superfused with warmed, aerated PSS following
the loading period to wash out excess dye and to allow hydrolysis of
AM groups by intracellular esterases. Pressure-induced vasoconstric-
tor responses were determined by exposing fura-loaded vessels to a
series of 10-Torr pressure steps beginning at 5 Torr and ending at 45
Torr. Each pressure step was held for 5 min. To determine the passive
diameter at each pressure step, 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 then performed under Ca2+-free conditions.
Separation of the diameters measured in Ca2+-replete and Ca2+-free
PSS is indicative of the degree of myogenic tone at each pressure step.
In a separate set of vessels, vasoconstrictor responses to increasing
concentrations of KCl (15–85 mM) were determined in fura-loaded
pulmonary arteries from both control and CH rats. The internal
diameter (ID) for all studies was continuously monitored using video
microscopy and edge-detection software (IonOptix). Fura-loaded ves-
sels were alternatively excited at 340 and 380 nm, and the respective
510-nm emissions were quantified using a photomultiplier tube and
recorded using IonWizard software (IonOptix). Vessel wall intracel-
larular Ca2+-concentration ([Ca2+]i) was calculated at each pressure
step, and concentration of KCl as the mean F340/F380 from the
background-subtracted 510-nm signal was collected over the last 1 min.

Data analysis. Variances about the means will be quantified by
using standard deviations (SD). Data were analyzed by using two-way
repeated-measures ANOVA. Where significant, main effects oc-
curred in individual groups by intracellular esterases. Pressure-induced vasoconstric-
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RESULTS

Pulmonary VSM cell Ems response to increasing intraluminal pressure.
Changes in pulmonary artery VSM cell Ems in re-
sponse to pressure-induced stretch are presented in Fig. 1. Raw
tracings of Ems recordings made in VSM cells from isolated
pulmonary arteries from control animals pressurized at either
12 or 45 Torr are shown in Fig. 1, A and B, respectively. Pulmonary
VSM cell Ems in arteries from control animals depolarized in response to stretch induced by increasing in-
traluminal pressure from 12 to 45 Torr. These data are sum-
marized in Fig. 1C. Pulmonary VSM cell resting Ems in arteries
from CH animals was depolarized relative to control at each
pressure step. In addition, pulmonary VSM cells in arteries

J Appl Physiol • VOL 98 • MARCH 2005 • www.jap.org

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from control and CH animals depolarized similarly in response to stretch.

**Effect of CH on myogenic responsiveness.** Changes in ID in response to increasing intraluminal pressure are presented in Fig. 2. Increasing intraluminal pressure produced a stepwise increase in ID in arteries from both control (Fig. 2A) and CH (Fig. 2B) rats. The ID determined at each pressure step in Ca$^{2+}$-replete conditions was not significantly different from the passive diameter observed under Ca$^{2+}$-free conditions. Vessel wall [Ca$^{2+}$]$_i$ was not different between arteries taken from control and CH animals. Moreover, [Ca$^{2+}$]$_i$ did not change in response to increasing intraluminal pressure (Fig. 3).

**Effect of CH on KCl-induced vasoconstriction and Ca$^{2+}$.** Changes in ID in response to KCl are presented in Fig. 4. KCl produced a concentration-dependent vasoconstriction that was similar in arteries from control and CH rats (Fig. 4, A and B). KCl-induced increases in vessel wall [Ca$^{2+}$]$_i$ are depicted in Fig. 4, C and D. KCl elicited a dose-dependent increase in vessel wall [Ca$^{2+}$]$_i$ in arteries from control (Fig. 4C) animals. In contrast, the increase in vessel wall [Ca$^{2+}$]$_i$ in response to KCl in arteries from CH (Fig. 4D) animals was attenuated compared with controls.

**DISCUSSION**

The major findings of the present study are as follows: 1) pulmonary VSM cell $E_m$ from control and CH rats depolarized in response to stretch; 2) VSM cell $E_m$ was more depolarized at each intraluminal pressure in arteries from CH animals compared with control; 3) arteries from neither control nor CH rats constricted in response to increases in intraluminal pressure; and 4) KCl-induced vasoconstriction was similar between control and CH groups. However, KCl-mediated increases in VSM cell [Ca$^{2+}$]$_i$ were blunted in arteries from CH rats compared with control. These results suggest that pressure-induced VSM cell depolarization is not a sufficient stimulus to
induce myogenic reactivity in small pulmonary arteries from either control or pulmonary hypertensive rats.

The present study is the first to investigate myogenic vasoconstriction in the pulmonary circulation using pressurized, intact small intrapulmonary arteries (removing possible neural or humoral influences), thus facilitating a direct assessment of the ability of pulmonary VSM cells to respond to stretch. We have shown that, although pulmonary VSM cells depolarize in response to stretch, vessel diameters under Ca\textsuperscript{2+}-replete and Ca\textsuperscript{2+}-free PSS were identical, suggesting no active vasoconstriction (Fig. 2). Because pressure-induced vasoconstriction is dependent on Ca\textsuperscript{2+} influx, our finding that vessel wall Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]) did not increase in response to increasing intraluminal pressure (Fig. 3) is consistent with the absence of myogenic tone. Although there are similarities between the pulmonary circulation of the fetus and that of the CH adult, the results of the present study suggest that the pulmonary arterial circulation does not demonstrate stretch-induced constriction within a physiological pressure range. Previous investigators have shown a myogenic response in the pulmonary circulation of both the fetus and newborn. For example, Belik has demonstrated myogenic tone in the pulmonary circulation of newborn guinea pigs (2) as well as fetal and newborn sheep (3) using arterial rings. In addition, Storme et al. (38) were able to unmask an autoregulatory response to the partial occlusion of the ductus arteriosus in fetal sheep in vivo by inhibiting nitric oxide synthase. However, in these studies, stretch-induced contraction was not present in the adult (2, 3). In addition, Davis et al. (7, 9) transplanted neonatal hamster pulmonary tissue into adult female hamster cheek pouch. These arteries have been shown to constrict to hypoxia and dilate to sodium nitroprusside (9); however, they respond passively to either increases or decreases in transmural pressure (7). Interestingly, renal arteries transplanted in this manner were constricted when transmural pressure was increased and dilated upon a reduction in transmural pressure [i.e., respond actively (15)]. Moreover, PVR has been shown to decrease in response to elevated left atrial pressure, suggesting that they respond passively to stretch (4, 22). To date, only one study has demonstrated a myogenic response in the pulmonary circulation of the adult. Kulik et al. (21) demonstrated active vasoconstriction in response to stretch in small pulmonary arteries of adult cats using an arterial ring preparation. Discrepancies between the findings of Kulik et al. and those of the present study may be due to either species differences or possibly the degree and direction of stretch employed in these different experimental preparations. Because VSM cells wrap circumferentially around arteries, in a pressurized artery preparation, all VSM cells will be exposed to a circumferential force. However, in a ring preparation, the force applied is different depending on the location relative to the point of attachment of the ring to the force transducer. The applied force will be circumferential near the point of attachment, but it will change to a tangential force as one moves away from this point.

We have currently demonstrated that pulmonary VSM cell resting $E_m$ in intact pressurized arteries from CH rats are depolarized compared with VSM cells in arteries from control animals. These results are consistent with the findings of previous studies performed in isolated pulmonary artery myocytes (31, 34–37, 39). For example, Shimoda et al. (36) demonstrated that resting $E_m$ in single pulmonary myocytes isolated from male Wistar rats exposed to 17–21 days of normobaric hypoxia (10% O\textsubscript{2}) were depolarized compared with control. In the systemic circulation, Harder (16) has demonstrated pressure-dependent VSM cell depolarization in intact cat middle cerebral arteries between 10 and 150 mmHg. In this study, $E_m$ at lower intraluminal pressures were similar to those of control pulmonary arteries in the present study (16). In addition, Davis et al. (10) demonstrated that dispersed coronary artery VSM cells depolarized by $\sim$20 mV when cell
length was increased by 25%, demonstrating that mechanosensitivity is an inherent property of VSM cells. Stretch-induced VSM cell depolarization opens voltage-dependent calcium channels (VDCC), resulting in Ca\(^{2+}\) influx and increased [Ca\(^{2+}\)], (30). Blockade of VDCC inhibits the myogenic response (16, 18, 19, 23), illustrating that pressure-induced vasoconstriction results from Ca\(^{2+}\) influx via these channels in systemic arteries. Moreover, the \(E_m\) measured at 45 Torr in the present experiments is near that observed at 40 Torr in mesenteric arteries in an earlier study from our laboratory using identical techniques (11). This observation suggests that the mechanosensitivity of pulmonary VSM may be similar to that of cells from the systemic vasculature. \(E_m\) recorded from unpressurized VSM cells from main pulmonary artery strips from control and CH rats were \(-60\) mV (SD 3) and \(-47\) mV (SD 6), respectively (unpublished observations). These values are similar to those of the present study at 5 Torr, suggesting that there is a threshold for stretch-sensitive ion channel activation in pulmonary artery VSM cells. Although we have demonstrated that pulmonary VSM cells depolarize in response to stretch, VSM cell Ca\(^{2+}\) did not increase. In addition, a pressure-induced decrease in luminal diameter was not observed. Taken together, these results suggest that, under the conditions of the present study, small pulmonary arteries do not exhibit myogenic vasoconstriction. To demonstrate our ability to measure changes in [Ca\(^{2+}\)], in response to depolarizing stimuli in our preparation, we examined KCl-mediated changes in ID and [Ca\(^{2+}\)]. Although vasoconstriction to KCl was similar in arteries taken from control and CH rats, increases in vessel wall [Ca\(^{2+}\)] were blunted in arteries from CH animals compared with control. This blunted [Ca\(^{2+}\)], response to KCl is consistent with the findings of Shimoda et al. (35), demonstrating altered Ca\(^{2+}\) handling following CH. These investigators have shown that the VDCC-mediated increase in VSM cell [Ca\(^{2+}\)] in response to endothelin-1 is blunted in pulmonary myocytes from animals exposed to \(-3\) wk of normobaric hypoxia compared with control. In addition, these authors showed that, while the elevated basal VSM cell [Ca\(^{2+}\)], seen in CH myocytes is dependent on extravascular Ca\(^{2+}\), inhibition of VDCC with nifedipine had no effect on [Ca\(^{2+}\)]. Taken together, these findings and those of the present study support the postulate that Ca\(^{2+}\) influx mechanisms in VSM cells are altered following CH.

Our present findings indicate that KCl-induced vasoconstriction is similar between groups, despite differences in [Ca\(^{2+}\)]. One possible explanation for these findings is that, following CH, there is a switch from a dependence on Ca\(^{2+}\) influx for vasoconstriction to a mechanism that relies on increases in Ca\(^{2+}\) sensitivity. The small GTPase RhoA and its downstream effector Rho kinase (ROK) produce an augmented vasoconstrictor response for a given concentration of intracellular Ca\(^{2+}\) by inhibiting myosin light chain phosphatase. There is recent evidence to suggest that the RhoA/ROK pathway is involved in regulating vascular reactivity in the pulmonary circulation (12, 29). Indeed, KCl-induced increases in perfusion pressure in isolated saline-perfused lungs were nearly eliminated by the ROK inhibitor Y-27632 in lungs from CH rats, with no effect in controls. In this same study, these investigators provided evidence that, although lungs from CH rats have higher basal vascular resistance compared with controls, nifedipine had no effect on perfusion pressure. In contrast, Y-27632 decreased basal perfusion pressure in a concentration-dependent manner in lungs from CH rats but was without effect in controls (29). Moreover, nifedipine has been shown to have a minimal effect on endothelin-1-induced contraction in pulmonary artery rings from CH rats (35). Interestingly, Ledvora et al. (24) have demonstrated in carotid artery strips that phosphorylation of the 20-kDa myosin light chain in response to stretch can still occur in Ca\(^{2+}\)-free PSS. Taken together, these results suggest that, following CH, changes in Ca\(^{2+}\) sensitivity play a greater role in regulating vascular tone than does Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels.

Although we did not observe stretch-induced constriction in small pulmonary arteries (250–300 μm ID), it is possible that this response is restricted to more distal arterioles. Indeed, Davis and colleagues (6, 8) have shown heterogeneity along the vascular tree, with greater myogenic reactivity being observed in more distal segments. In addition, the present experiments were performed under normoxic conditions. Although pulmonary artery VSM cells depolarize in response to stretch under normoxic conditions, it is possible that hypoxia initiates other signaling cascades, which are required for vasoconstriction to occur in response to stretch.

In summary, the present study provides evidence that pulmonary VSM cell \(E_m\) is depolarized following CH compared with controls and that VSM cells from both control and CH animals depolarize in response to stretch. However, arteries from either control or CH animals do not exhibit stretch-induced vasoconstriction. Thus these findings suggest that the pulmonary circulation of the rat does not demonstrate pressure-induced vasoconstriction.

ACKNOWLEDGMENTS

The authors acknowledge Minerva Murphy for technical assistance.

GRANTS

This work was supported by National Institutes of Health Grants HL-58124, HL-63207, RR-16480, and HL-77876.

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