J Appl Physiol 92: 1859–1864, 2002;
10.1152/japplphysiol.00797.2001.

Chronic hypoxia increases MCA contractile response to U-46619 by reducing NO production and/or activity

A. HUGO SILLAU,[1,2] ROBERT E. McCULLOUGH,[1] REBECCA DYCKES,[1]
MARGUERITTE M. WHITE,[1] AND LORNA G. MOORE[1,3]
[1]*Women's Health Research Center, University of Colorado Health Sciences Center, and* [3]*Department of Anthropology, University of Colorado at Denver, Denver, Colorado 80262; and* [2]*Department of Physiology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico 00936*

Received 30 July 2001; accepted in final form 17 December 2001

Sillau, A. Hugo, Robert E. McCullough, Rebecca Dyckes, Margueritte M. White, and Lorna G. Moore. Chronic hypoxia increases MCA contractile response to U-46619 by reducing NO production and/or activity. *J Appl Physiol* 92: 1859–1864, 2002; 10.1152/japplphysiol.00797.2001.—Chronic hypoxia alters contractile sensitivity of isolated arteries to α-adrenergic stimulation and other agonists. However, most studies have been performed in thoracic aortas or other large vessels making little contribution to vascular resistance in their respective circulations. To determine the effect of chronic hypoxia on the vasoconstrictor response in a small, resistance-sized vessel, we studied second and third generation middle cerebral arteries (MCA; ~75-μm internal diameter before mounting). MCA were isolated from normoxic (inspired oxygen = 125 Torr) and hypoxic (8 wk at 3,960 m; inspired oxygen = 90 Torr) guinea pigs, and their vasoconstrictor responses were determined to the thromboxane mimetic U-46619 by using dual-pipette video microscopy. Arteries from hypoxic animals had greater contractile sensitivity to U-46619 compared with those of the normoxic animals ($-\log EC_{50} = 7.86 \pm 0.11$ vs. 7.62 ± 0.06, respectively, $P < 0.05$). Addition of the nitric oxide (NO) inhibitor nitro-L-arginine (200 μM) to the vessel bath eliminated the differences in contractile sensitivity between the MCA from the normoxic and chronically hypoxic groups. Supplementation with L-arginine in the drinking water sufficient to raise plasma L-arginine levels 41% reduced MCA contractile sensitivity to U-46619 in the normoxic group ($-\log EC_{50} = 7.22 \pm 0.31$, $P < 0.05$ compared with the nonsupplemented normoxic group) but not in the chronically hypoxic group. These results show that chronic hypoxia increases the sensitivity of the MCA to the vasoconstrictor U-46619, likely because of a reduction in NO production and/or activity.

vascular reactivity; nitric oxide; thromboxane; L-arginine; cerebral circulation; middle cerebral arteries

BRAIN BLOOD FLOW IS RIGOROUSLY DEFENDED under conditions of acute as well as chronic hypoxia. Cerebral vasoconstrictor and vasodilator responses are important mechanisms by which brain blood flow is maintained. A number of studies have shown that chronic hypoxia profoundly influences vascular control, altering both vasoconstrictor as well as vasodilator responses in isolated cerebral vessels (2, 16). Such effects may be important for the fetus in relation to the occurrence of cerebral vascular injury during the neonatal transition. They also may be important in the adult in whom disorders involving the cerebral circulation occur under conditions of acute and chronic hypoxia. A rare but often fatal complication after acute hypoxia is high-altitude cerebral edema in which sustained vasodilatation combined with probable endothelial dysfunction disrupts the blood-brain barrier and causes leakage (11). Chronic mountain sickness can develop after years of high-altitude residence (32) and is characterized by profound hypoxia, particularly during sleep, excessive polycythemia, and possible loss of cerebral blood flow autoregulation (28).

We hypothesized that chronic hypoxia augmented contractile sensitivity to the thromboxane mimetic U-46619 in isolated cerebral vessels as the result of reduced nitric oxide (NO) production and/or activity. We studied the middle cerebral artery (MCA) as a vessel making a substantial contribution to cerebral vascular resistance that could be readily isolated and was amenable to being studied by video microscopy, a technique for measuring actual contractile response rather than the increase in isometric tension. U-46619 was chosen as a physiologically relevant contractile agonist, given that thromboxane is present in the cerebral circulation under circumstances of hemolysis (33) and has been reported to be increased during hypoxia (3). Study results indicated that chronic hypoxia increased the contractile sensitivity to U-46619 as hypothesized. Administration of the nonspecific NO synthase (NOS) inhibitor nitro-L-arginine (NLA) eliminated the difference in contractile sensitivity between the vessels from the normoxic and chronically hypoxic animals, suggesting that reduction in NO production and/or activity was responsible for the increased contractile sensitivity observed. Because cofactors for NOS are not saturating in the cerebral circulation (25) and dietary supplementation with L-arginine has been

Address for reprint requests and other correspondence: L. G. Moore, Box B133, WHRC, Univ. of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262. (E-mail: lorna.g.moore@uchsc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

shown to at least partially reverse hypoxia-associated increases in contractile responsiveness in other circulations (13, 21), we reasoned that dietary supplementation with L-arginine might restore NO production and/or activity and thereby reduce contractile sensitivity in vessels from chronically hypoxic animals to normoxic values. Results indicated that this did not occur, suggesting that other factors served to reduce NO production and augment MCA contractility under conditions of chronic hypoxia.

METHODS

Animals. Animals for these experiments were 10 guinea pigs kept at the laboratory altitude of 1,600 m (normoxia, inspired O_2 = 125 Torr) and 11 animals who were exposed to a simulated altitude of 3,960 m in a hypobaric chamber (hypoxic, inspired O_2 = 90 Torr) for 8 wk. Guinea pigs were chosen as the experimental animal because their small size permits them being housed in our hypobaric chamber and in relation to the goals of a larger study concerned with the effects of chronic hypoxia on vascular responses to pregnancy. At the time of study, body weight was similar in the two groups (724 ± 117 and 797 ± 95 g, respectively, P = not significant). Five animals in each altitude group were supplemented with L-arginine in the drinking water (22.5 g/l) for 8 wk before study. In a separate group of six normoxic animals, this dosage of L-arginine raised serum L-arginine levels 41 ± 7% (from 132 to 177 nM/ml, $P < 0.05$) as measured by use of gas chromatography/mass spectrometry (19).

Isolated vessel preparation. The brain was removed under chloralose-urethane anesthesia (200 mg/kg), and MCA were carefully dissected to avoid mechanical damage. These were second- to third-generation, ~75-μm (before mounting) vessels off the circle of Willis that were devoid of collaterals. Both ends of the vessel were cannulated by using a dual-pipette system described previously (5, 6) and visualized by use of an inverted binocular light microscope (Olympus model CK, Tokyo, Japan) with signals being recorded by video camera (model JE 7442, Javelin Electronics, Los Angeles, CA) and displayed on a monochrome monitor (model BWM 12A, Javelin Electronics, Torrance, CA). Vessels were mounted by inserting an inner pipette into each end of the vessel and, in turn, advancing the inner pipette into an outer, holding pipette connected to the microscope stage by using four-axis micromanipulators (Spiderwort Design, Colorado Springs, CO). The mounted vessel assembly was submerged in a 2-ml perfusion bath maintained at 37°C by a thermal control circuit (model 16060, Love Controls, Wheeling, IL). The vessels were perfused externally with a physiological salt solution maintained at pH 7.4 ± 0.04, and the vessel lumen was perfused with the same solution with the addition of 1% bovine albumin (A-2934, Sigma Chemical, St, Louis, MO). Vessels were studied under conditions of no flow through the vessel lumen.

Protocol. The mounted vessels were pressurized to 10 Torr and then to 20 Torr, which we previously determined to be that which yielded the greatest contraction to 40 mM KCl. (Guinea pig arterial blood pressure is ~55 Torr.) After a 60-min equilibration period, vessels were contracted with a submaximal dose of KCl (40 mM), rinsed, and allowed to return to their initial diameter. These inner diameters were similar for the normoxic and hypoxic animals, averaging 271 ± 13 μm. Vessels were considered viable if a test dose of U-46619 (3×10^{-7} M) reduced inner diameter at least 25% from the initial resting value, and acetylcholine (3×10^{-5} M) reversed at least 50% of this U-46619-induced contraction.

The dose response to the thromboxane mimetic U-46619 (1×10^{-10} to 3×10^{-6} M or 0.1 nM to 3 μM) was then examined. In preliminary experiments, lower concentrations of U-46619 had no effect and higher dosages did not produce further contraction. Vessels were treated with NLA (200 μM), an inhibitor of NO production, and the contractile responses to U-46619 were repeated. In separate studies in normoxic or chronically hypoxic animals (n = 4 each group), there were no differences in maximal contraction, contractile sensitivity, or Hill coefficient between two successive dose-response curves.

Data analysis. Data are reported as means ± SE. Changes in inner diameter in response to chemicals added to the bath were considered to represent the contractile response. Contractile responses to U-46619 were recorded as absolute values, and the percent of maximal contraction was calculated at each dose. Maximum contractile response was calculated for each vessel as the difference between the initial resting diameter and the minimal diameter observed (C_{max}). The percent maximal contraction at each dose of U-46619 was plotted, and nonlinear regression analysis was used to calculate the best fitting contraction-response curve, the negative log of the half-maximal contractile response or EC_{50} (pD_2), and the slope of the sigmoidal portion of the dose-response curve (Hill coefficient). C_{max}, pD_2, and Hill coefficients were compared among groups by using paired or unpaired t-tests with Student-Newman-Keuls multiple comparisons as appropriate. Comparisons were considered significant when $P < 0.05$.

RESULTS

Response to U-46619 in vessels from normoxic and chronically hypoxic animals. The MCA from the normoxic as well as the chronically hypoxic animals contracted in response to U-46619 (Fig. 1, Table 1). Chronic hypoxia increased contractile sensitivity to the thromboxane mimetic U-46619, shifting the dose response curve to the left (Fig. 1*A*) and raising the pD_2 (Table 1). Neither the C_{max} nor the Hill coefficient differed in the hypoxic vs. normoxic groups (Table 1). The contractile response to 40 mM KCl was also similar, with the vessels decreasing in inner diameter by 15 ± 6 and 19 ± 6% in the normoxic and chronically hypoxic groups, respectively.

Effect of NO synthesis inhibition on the contractile response to U-46619. Treatment with the NO synthesis inhibitor NLA eliminated the differences in MCA contractile sensitivity between the normoxic and chronically hypoxic groups (Fig. 1*B*). Although NLA administration increased contractile sensitivity in both groups (Table 1), the magnitude of change in pD_2 was considerably greater in the normoxic than the chronically hypoxic group (EC_{50} values = 24.9 ± 2.9 nM before vs. 5.9 ± 0.6 nM after NLA in the normoxic group; 15.4 ± 2.4 nM before vs. 9.0 ± 2.2 nM after NLA in the hypoxic group). Maximum contraction and the Hill coefficients were unaffected by NLA administration. Together with the greater effect of NO inhibition in the normoxic than the hypoxic vessels (Table 1), this supported the likelihood that reduced NO production

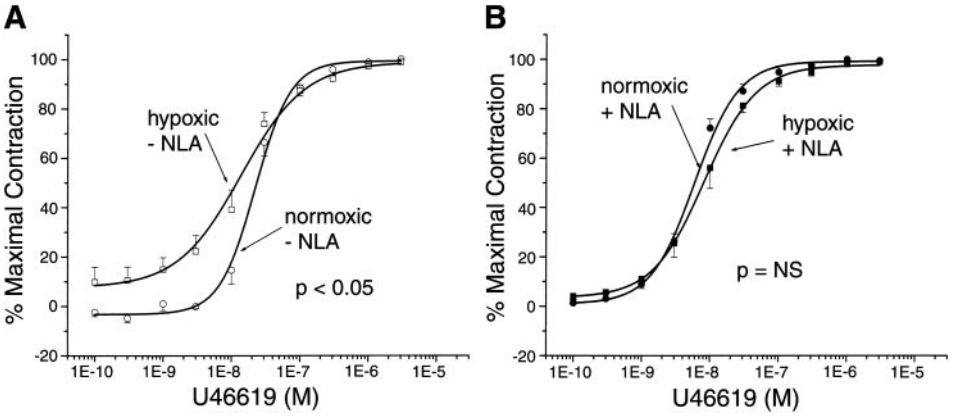

Fig. 1. Middle cerebral arteries (MCA) from chronically hypoxic guinea pigs had greater contractile sensitivity to U-46619 in the absence (−) of nitric oxide (NO) inhibition by nitro-L-arginine (NLA) (*A*) but not after NO inhibition (*B*). Values are means ± SE, and sample sizes are 5–6 animals in each group. *P* values refer to comparison of curve position as judged by the negative log of the EC_{50} (pD_2). NS, not significant.

was responsible for the greater contractile response of the chronically hypoxic vs. normoxic group.

Effect of L-arginine supplementation on the contractile response to U-46619. In normoxic animals, L-arginine supplementation shifted the dose response curves to the right (Fig. 2*A*, Table 1), suggesting that substrate supplementation raised NO production to thereby decrease contractile sensitivity. No difference was observed in maximum contraction to U-46619 or the Hill coefficient (Table 1). The contractile response to 40 mM KCl was diminished in the vessels from the L-arginine-supplemented animals compared with those from the nonsupplemented group (3 ± 2 vs. 15 ± 6%, respectively, $P < 0.05$).

In chronically hypoxic animals, L-arginine supplementation did not change the pD_2 (Fig. 2*B*, Table 1). The contractile response to 40 mM KCl was lower in the vessels from the chronically hypoxic L-arginine-supplemented animals vs. the nonsupplemented group (0 ± 2 vs. 19 ± 6%, respectively, $P < 0.05$).

In normoxic L-arginine-supplemented animals, NLA administration to the vessel bath markedly raised maximum contraction and contractile sensitivity (Table 1, Fig. 3*A*). NLA administration to vessels from chronically hypoxic animals also raised contractile sensitivity, but the effect was less marked than in normoxic animals (Fig. 3*B*). NLA administration did not change the Hill coefficient in either group (Table 1).

Table 1. *Dose-response characteristics of middle cerebral arteries from normoxic or chronically hypoxic guinea pigs treated with the thromboxane mimetic U-46619*

	Normoxia		Chronic Hypoxia	
	Without NLA	With NLA	Without NLA	With NLA
Without L-arginine supplementation				
pD_2	7.62 ± 0.06	8.24 ± 0.06†	7.86 ± 0.11*	8.11 ± 0.10†
C_{max}, μm	113 ± 26	130 ± 21	117 ± 23	152 ± 25
Hill coefficient	2.02 ± 0.41	1.57 ± 0.17	1.64 ± 0.45	1.40 ± 0.12
With L-arginine supplementation				
pD_2	7.22 ± 0.31‡	8.36 ± 0.25†	7.69 ± 0.34	8.26 ± 0.53†
C_{max}, μm	92 ± 28	206 ± 13‡	138 ± 27	169 ± 27
Hill coefficient	1.22 ± 0.16	1.19 ± 0.12	1.82 ± 0.32	2.57 ± 0.79

Values are means ± SE for sample sizes of 5–6 animals. Maximum contraction (C_{max}) is measured directly. Contractile sensitivity or the pD_2 and the Hill coefficient are calculated as the negative log of the U-46619 concentration producing 50% of the maximum contraction (−log mol/l) and the sigmoidal slope of the best-fitting nonlinear regression curve, respectively. *$P < 0.05$ for comparison of normoxia vs. chronic hypoxia; †$P < 0.05$ for comparison of without vs. with NLA; ‡$P < 0.05$ for comparison of without L-arginine vs. with L-arginine supplementation

DISCUSSION

In this study, we found that chronic hypoxia raised MCA contractile sensitivity to the thromboxane A_2 mimetic U-46619. This increase appeared to be due to a diminution in NO production and/or activity as demonstrated by the similarity in contractile sensitivity between the normoxic and chronically hypoxic groups after pharmacological inhibition of NOS. The apparent reduction of NO production and/or activity was not due to reduced levels of the NOS substrate L-arginine because dietary supplementation with L-arginine reduced contractile sensitivity only in vessels from normoxic animals.

The validity of our study findings was dependent on considerations regarding choice of vessel, study design, technique, agonist, and means for evaluating the effects of NO production and/or activity. Although second to third generation ~75 μm MCA are not end arterioles and thus the site of greatest cerebral vascular resistance, they make a substantial contribution to vascular resistance and hence can be considered as resistance vessels (23). Furthermore, the MCA has the advantage of being comparatively free of adjacent tissue and hence easily isolated. Our study design entailed examining MCA from guinea pigs that had been at the laboratory altitude (1,600 m) or simulated high altitude (3,960 m) for 8 wk as part of a larger study concerned with the effects of chronic hypoxia and pregnancy on the cerebral vascular vasoconstrictor and vasodilator responses (22). This duration of high-altitude exposure was chosen to encompass nearly the whole of guinea pig gestation (term = 9 wk). The guinea pig was selected because it, like humans, demonstrates fetal

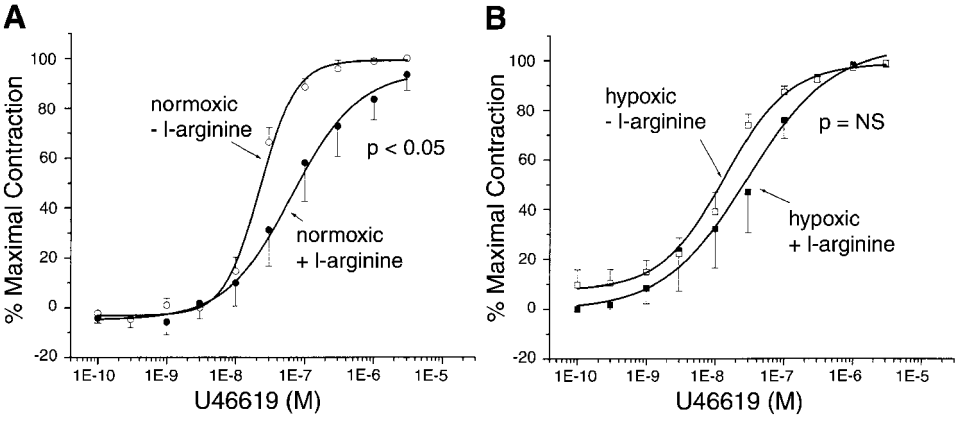

Fig. 2. Chronic L-arginine supplementation for 8 wk decreased contractile sensitivity in MCA from normoxic guinea pigs (*A*) but not in chronically hypoxic animals (*B*). Values are means ± SE, and sample sizes are 5–6 animals in each group. *P* values refer to comparison of curve position as judged by the pD_2.

growth retardation at high altitude and its small size permits placing it in a hypobaric chamber for simulating high altitude. We used video microscopy (sometimes termed pressure myography) rather than vessel rings (isometric myography) to avoid the possibility of endothelial damage in these small vessels and because it enables measurement of the actual contractile response rather than an increase in isometric tension as afforded with vessel ring preparations (4). We chose the thromboxane mimetic U-46619 as the contractile agonist because thromboxane is found in the cerebral circulation under conditions of hypoxia (3) and, in our hands, U-46619 was a consistent contractile agonist. Alterations in contractile response were analyzed by using well-accepted measures, namely, curve position or pD_2, C_{max}, and the slope of the dose-response curve (Hill coefficient). We administered NLA in standard dosages previously shown to inhibit NO production by endothelial or type III NOS as well as the other NOS isoforms (30). In support of its efficacy in our study preparation was the ability of NLA to reverse the rightward shift in the contractile dose-response curve observed with L-arginine supplementation. Thus we concluded that our study findings indicated that chronic hypoxia raised MCA contractile sensitivity to U-46619 and that this was due, at least in part, to reduced NO production and/or activity.

Circulating substances do not normally reach cerebral tissue when the blood-brain barrier is intact. Endogenous norepinephrine released by stimulation of arterial nerves at physiological frequencies causes vasoconstriction in isolated sheep cerebral vessels (17) but not, in our experience, when exogenously applied to the guinea pig MCA. Thromboxane is released locally under conditions of hemolysis and is a potent contractile substance both in vivo and in vitro. Furthermore, it acts synergistically with endothelin-1, serotonin, and other products of hemolysis to exert an even more potent contractile effect (12, 33). Our results were consistent with these previous studies, showing that exogenous administration U-46619 caused brisk and marked vasoconstriction.

Chronic hypoxia alters the contractile response of isolated cerebral vessels. In isolated MCA from adult sheep, chronic hypoxia increased the contractile response to high-dose KCl (135 mM) and serotonin as well as histamine when normalized by the KCl response (16). However, chronic hypoxia decreased the contractile response to norepinephrine in adult middle cerebral and basilar arteries (18). In an elegant series of studies, this decreased contractile responsiveness to norepinephrine has been shown to be due to downregulation at several points in the signal transduction cascade (18, 29), perhaps as the result of prolonged sympathetic stimulation seen at high altitude (26). The factors responsible for the increased contractile responsiveness to serotonin and histamine have not been identified. Nor has the effect of chronic hypoxia on the

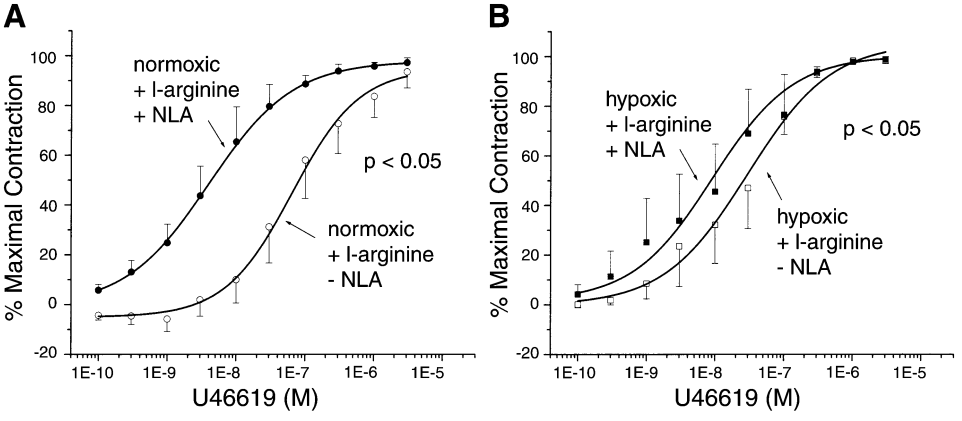

Fig. 3. NO synthesis inhibitor NLA markedly raised contractile sensitivity to U-46619 in MCA from L-arginine-supplemented normoxic animals (*A*) and, albeit to a lesser extent, in MCA from L-arginine-supplemented, chronically hypoxic animals (*B*). Values are means ± SE, and sample sizes are 5–6 animals in each group. *P* value refers to comparison of curve position as judged by the pD_2.

contractile response to U-46619 been previously studied in cerebral vessels. Chronic hypoxia reduced the contractile response to U-46619 in isolated fetal sheep coronary arteries as the result of an increased NO contribution (10). Interestingly, an extensive series of studies has shown that the effects of chronic hypoxia on the contractile response of isolated fetal cerebral vessels are opposite to those seen in adult vessels (17, 24). Thus our results showing an increased contractile response to U-46619 are consistent with the increased responsiveness to serotonin and histamine in adult vessels. And if we may speculate, our results are also consistent with the decreased contractile response to U-46619 in isolated fetal sheep coronary arteries given the pattern for chronic hypoxia to have opposite effects on contractile responsiveness in fetal vs. adult vessels.

NO is an important modulator of cerebral vascular tone in vivo and in vitro (2, 7, 15, 31). Consistent with these reports, we found that inhibition of NO production with NLA increased MCA contractile sensitivity to U-46619, implying that basal release of NO opposed U-46619-induced vasoconstriction. This was true in vessels from both normoxic and chronically hypoxic guinea pigs, but the effect of NLA was greater in MCA from normoxic vs. hypoxic animals. Because, by design, only vessels that vasodilated in response to acetylcholine were used and vessels were studied by using video microscopy in which there is less possibility of partial loss of endothelial function than with isometric myography (because no object is introduced into the vessel lumen), it was unlikely that differences between groups in contractile sensitivity were due to differing amounts of endothelial damage. Because the contractile sensitivity to U-46619 was the same in the MCA from chronically hypoxic and normoxic animals after NOS inhibition, we concluded that chronic hypoxia reduced basal NO production and/or activity during U-46619-induced contraction.

Chronic hypoxia is known to influence NO modulation of contractile response. A decrease in NO production or activity as measured by exhaled or plasma NOx (8, 9) or cGMP generation (27) has been observed under chronic hypoxia. This could be due to decreased NOS protein levels or activity, reduced availability or uptake of its substrates L-arginine and oxygen, increased NO degradation, or reduced cGMP production. In the brain, long-term hypoxia has been reported to reduce brain NOS activity, endothelial NOS (eNOS) protein, and mRNA (1). The effects of chronic hypoxia on expression of eNOS protein and mRNA in other tissues are variable, being reported as both increased and decreased (9, 14, 20). Reduced oxygen tension itself may limit NO production, given that oxygen is a substrate for NOS (14). Supporting a possible limitation by L-arginine, L-arginine supplementation raised NO production (8) and decreased hypoxic pulmonary vasoconstriction and pulmonary vascular remodeling in chronically hypoxic rats (21). Effects of L-arginine supplementation are not limited to the pulmonary circulation, as shown by increased renal blood flow after systemic L-arginine infusion (13). Chronic hypoxia has been shown to impair L-arginine uptake (8), suggesting that this may be one mechanism by which chronic hypoxia might limit NO production. Because NOS substrate levels are not saturating in cerebral tissue (25), we reasoned that L-arginine supplementation might reduce MCA contractile sensitivity to U-46619 in hypoxic animals to levels observed in vessels from normoxic animals. Consistent with this, L-arginine supplementation decreased contractile sensitivity and potentiated the effect of NOS inhibition, but the effect was less, not greater, in the hypoxic vessels. This suggested that some factor other than decreased L-arginine availability was involved. Furthermore, because the isolated vessels were studied under normoxic conditions, it appeared unlikely that reduced levels of oxygen were responsible. This implies that reduced levels of eNOS protein and/or message are involved. Studies to evaluate the effect of chronic hypoxia on NO production and/or activity under conditions such as acetylcholine administration or increased shear stress are warranted to determine whether stimulated NO production is similarly reduced.

In summary, MCA contractile sensitivity to U-46619 was augmented under conditions of chronic hypoxia. That this was due, at least in part, to decreased NO production and/or activity was supported by the similarity in contractile sensitivity between the chronically hypoxic and normoxic vessels after pharmacological inhibition of NO production. The cause of the greater contractile sensitivity to U-46619 in MCA from chronically hypoxic animals was not the result of reduced levels of its L-arginine substrate. The possibilities that decreased NOS protein levels or activity, increased NO degradation, or reduced cGMP production were responsible await to be tested in future studies. Such studies are of importance, given that endothelial or NO-related mechanisms may contribute to the cerebral complications that have been described under conditions of acute and chronic hypoxia.

The help of Drs. Fennessy and Pike in the Clinical Mass Spectrometry Resource at the University of Colorado Health Sciences Center is gratefully appreciated. We also thank Linda Min for helping with the preparation of the figures.

The conduct of these studies was aided by grant support from National Institutes of Health Grants HL-60131, HL-14985, DK-48520, and HD-08315.

REFERENCES

1. **Aguan K, Murotsuki J, Gagnon R, Thompson LP, and Weiner CP.** Effect of chronic hypoxemia on the regulation of nitric-oxide synthase in the fetal sheep brain. *Brain Res Dev Brain Res* 111: 271–277, 1998.
2. **Brian JE, Faraci FM, and Heistad DD.** Recent insights into the regulation of cerebral circulation. *Clin Exp Pharmacol Physiol* 23: 449–457, 1996.
3. **Coker SJ, Marshall RJ, Paratt JR, and Zeitlin IJ.** Does the local myocardial release of prostaglandin E_2 or $F_{2\alpha}$ contribute to the early consequences of myocardial ischemia? *J Cell Cardiol Mol* 13: 425–434, 1981.
4. **Davis MJ and Hill MA.** Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev* 79: 387–423, 1999.
5. **Duling BR, Gore RW, Dacey RG Jr, and Damon DN.** Methods for isolation, cannulation, and in vitro study of single mi-

crovessels. *Am J Physiol Heart Circ Physiol* 241: H108–H116, 1981.
6. **Duling BR and Rivers RJ.** Isolation, cannulation and perfusion of microvessels. In: *Microcirculatory Technology*, edited by Baker CH and Nastuk WL. New York: Academic, 1986, p. 265–280.
7. **Faraci FM.** Role of endothelium-derived relaxing factor in cerebral circulation: large arteries vs. microcirculation. *Am J Physiol Heart Circ Physiol* 261: H1038–H1042, 1991.
8. **Fike CD, Kaplowitz MR, Rehorst-Paea LA, and Nelin LD.** L-Arginine increases nitric oxide production in isolated lungs of chronically hypoxic newborn pigs. *J Appl Physiol* 88: 1797–1803, 2000.
9. **Fike CD, Kaplowitz MR, Thomas CJ, and Nelin LD.** Chronic hypoxia decreases nitric oxide production and endothelial nitric oxide synthase in newborn pig lungs. *Am J Physiol Lung Cell Mol Physiol* 274: L517–L526, 1998.
10. **Garcia FC, Stiffel VM, and Gilbert RD.** Effects of long-term high-altitude hypoxia on isolated fetal ovine coronary arteries. *J Soc Gynecol Investig* 7: 211–217, 2000.
11. **Hackett PH.** High altitude cerebral edema and acute mountain sickness: a pathophysiology update. *Adv Exp Med Biol* 474: 23–45, 1999.
12. **Hempelmann RG, Pradel RH, Barth HL, Mehdorn HM, and Ziegler A.** Interaction between vasoconstrictors in isolated human cerebral arteries. *Acta Neurochir (Wien)* 139: 574–581, 1997.
13. **Howes TQ, Keilty SE, Maskrey VL, Deane CR, Baudouin SV, and Moxham J.** Effect of L-arginine on renal blood flow in normal subjects and patients with hypoxic chronic obstructive pulmonary disease. *Thorax* 51: 516–519, 1996.
14. **Le Cras TD and McMurtry IF.** Nitric oxide production in the hypoxic lung. *Am J Physiol Lung Cell Mol Physiol* 280: L575–L582, 2001.
15. **Librizzi L, Folco G, and de Curtis M.** Nitric oxide synthase inhibitors unmask acetylcholine-mediated constriction of cerebral vessels in the in vitro guinea-pig brain. *Neuroscience* 101: 283–287, 2000.
16. **Longo LD, Hull AD, Long DM, and Pearce WJ.** Cerebrovascular adaptations to high-altitude hypoxemia in fetal and adult sheep. *Am J Physiol Regulatory Integrative Comp Physiol* 264: R65–R72, 1993.
17. **Longo LD and Pearce WJ.** High altitude, hypoxemic-induced responses in adult and fetal cerebral blood vessels. *Acta Andina* 6: 152–160, 1997.
18. **Longo LD and Pearce WJ.** High altitude, hypoxic-induced modulation of noradrenergic-mediated responses in fetal and adult cerebral arteries. *Comp Biochem Physiol A Physiol* 119: 683–694, 1998.
19. **Loy GL, Quick AN Jr, Teng CC, Hay WW Jr, and Fennessey PV.** Versatile stable isotope technique for the measurement of amino acids and keto acids: comparison with radioactive isotope and its use in measuring in vivo disposal rates. *Anal Biochem* 185: 1–9, 1990.
20. **McQuillan LP, Leung GK, Marsden PA, Kostyk SK, and Kourembanas S.** Hypoxia inhibits expression of eNOS via transcriptional and posttranslational mechanisms. *Am J Physiol Heart Circ Physiol* 267: H1921–H1927, 1994.
21. **Mitani Y, Maruyama K, and Sakurai M.** Prolonged administration of L-arginine ameliorates chronic pulmonary hypertension and pulmonary vascular remodeling in rats. *Circulation* 96: 689–697, 1997.
22. **Moore LG, Dyckes R, McCullough RE, and White MM.** Chronic hypoxia prevents pregnancy-associated increase in cerebral middle connecting artery (MCA) vasodilator response to acetylcholine (ACH) (Abstract). *J Soc Gynecol Investig* 5: 146A, 1998.
23. **Mulvany MJ and Halpern W.** Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ Res* 41: 19–26, 1977.
24. **Pearce WJ.** Cerebrovascular development at altitude. In: *Hypoxia and the Brain*, edited by Sutton JR, Houston C, and Coates G. Burlington, VT: Queen City, 1995, p. 125–141.
25. **Pearce WJ, Tone B, and Ashwal S.** Maturation alters cerebral NOS kinetics in the spontaneously hypertensive rat. *Am J Physiol Regulatory Integrative Comp Physiol* 273: R1367–R1373, 1997.
26. **Reeves JT, Moore LG, Wolfel EE, Mazzeo RS, Cymerman A, and Young AJ.** Activation of the sympatho-adrenal system at high altitude. In: *High Altitude Medicine*, edited by Ueda G. Matsumoto, Japan: Shinshu University Press, 1992, p. 10–23.
27. **Shaul PW, Wells LB, and Horning KM.** Acute and prolonged hypoxia attenuate endothelial nitric oxide production in rat pulmonary arteries by different mechanisms. *J Cardiovasc Pharmacol* 22: 819–827, 1993.
28. **Sun SF, Oliver-Pickett C, Droma T, Micco AJ, Zamudio S, Zhuang J, McCullough RG, Cymerman A, Ping Y, and Moore LG.** Breathing and brain blood flow during sleep in patients with chronic mountain sickness. *J Appl Physiol* 81: 611–618, 1996.
29. **Ueno N, Zhao Y, Zhang L, and Longo LD.** High altitude-induced changes in α_1-adrenergic receptors and Ins(1,4,5)P_3 responses in cerebral arteries. *Am J Physiol Regulatory Integrative Comp Physiol* 272: R669–R674, 1997.
30. **Vargas HM, Cuevas JM, Ignarro LJ, and Chaudhuri G.** Comparison of the inhibitory potencies of NG-methyl-, NG-nitro- and NG-amino-L-arginine on EDRF function in the rat: evidence for continuous basal EDRF release. *J Pharmacol Exp Ther* 257: 1208–1215, 1991.
31. **Wallis SJ and Martin W.** Conditions permitting suppression of stretch-induced and vasoconstrictor tone by basal nitric oxide activity in porcine cerebral artery. *Br J Pharmacol* 130: 567–574, 2000.
32. **Winslow RM and Monge CC.** *Hypoxia, Polycythemia, and Chronic Mountain Sickness*. Baltimore, MD: Johns Hopkins University Press, 1987.
33. **Yakubu MA and Leffler CW.** Enhanced pial arteriolar sensitivity to bioactive agents following exposure to endothelin-1. *Life Sci* 66: 307–316, 2000.
34. **Zhou L, Zhao Y, Nijland R, Zhang L, and Longo LD.** Ins(1,4,5)P_3 receptors in cerebral arteries: change with development and high-altitude hypoxia. *Am J Physiol Regulatory Integrative Comp Physiol* 272: R1954–R1959, 1997.