IN RESPONSE TO HYPOXIA, cerebral vascular contractile and relaxation responses are markedly altered. In general, acute hypoxia is associated with vasodilation of systemic blood vessels, with increased blood flow and oxygen delivery. (In contrast, in the pulmonary vascular bed, hypoxia-induced vasoconstriction serves to direct blood away from poorly oxygenated alveoli.) In long-term hypoxia (LTH), organ blood flows may be increased, decreased, or little changed from normoxic control values depending on the species, organ, degree of hypoxia, and other factors. On going to high altitude, cerebral blood flow initially increases and then with acclimatization returns to near normal values (8, 32).

Studies of acute hypoxia-mediated vascular smooth muscle (VSM) responses suggest these to be mediated, in part, by K+ channel activation, which, in turn, plays a major role in modulating vessel contractility (1, 27). Although we and others have described a number of effects of LTH on cerebral vascular reactivity (16, 17, 34, 38), less well defined are the relative roles of K+ channels in these responses. In vascular smooth muscle, K+ channel activation plays a major role in modulating membrane potential, voltage-gated L-type Ca2+ channel activity, intracellular Ca2+ concentration ([Ca2+]i), and myogenic tone (22). Four types of K+ channels have been identified in cerebral and other arteries: ATP sensitive (KATP), Ca2+-activated (KCa), voltage dependent (Kv), and inward rectifier (KIR). Recently, we reported on some aspects of the role of these several channels in modulating the NE-induced [Ca2+]i, and contractile responses in normoxic adult and fetal cerebral vessels (14). LTH-mediated cerebrovascular responses are less well defined, and few studies have examined the relative roles of K+ channels in association with chronic hypoxia.

In the present studies, we tested the hypothesis that, in part, the LTH-associated depressed or otherwise altered tension responses seen in cerebral arteries were secondary to altered plasma membrane K+ channel function. We also tested the hypothesis that in the fetus the responses of cerebral K+ channels to LTH differed from those responses in the adult.

METHODS

Tissue preparation. We obtained main branch middle cerebral arteries (MCA) from 29 near-term fetuses (~140 days gestation) and 30 nonpregnant adult sheep (age 18–24 mo) that had been maintained near sea level [300 m; arterial PO2 (PaO2) = 95 ± 5 and 25 ± 1 Torr for ewe and fetus, respectively]. We also obtained MCA from 28 fetuses and 30 adult ewes that had been acclimatized to high altitude (3,820 m, 12,470 ft, PaO2 = 60 ± 5 and 19 ± 3 Torr for ewe and fetus, respectively; Barcroft Laboratory, White Mountain Research Station, Bishop, CA) for 110+ days. (Note: to optimize animal use, other tissues from these animals were shared with other investigators in our research group.) Both nonpregnant and pregnant ewes were obtained from Nebeker Ranch (La-
caster, CA), as previously described (16, 19). The animals from high altitude were transported to our laboratory just before the studies. Shortly after arrival, we placed a tracheal catheter in the ewe, through which N2 flowed at a rate to maintain PaO2 at ~60 Torr (9), and this was maintained until the time of the experimental study. All hypoxicem fetuses were singletons, and when normoxic twin fetuses were obtained, we used only one fetus of the pair. The ewes were anesthetized and euthanized with 100 mg/kg intravenous pentobarbital sodium. We removed the fetal or adult brain, placed it in iced saline, and dissected out and cleaned the cerebral arteries. Our laboratory has shown that this method of death has no significant effect on vessel reactivity compared with use of other anesthetic agents (18). To avoid the complication of endothelium-mediated effects, we removed the endothelium by carefully inserting a small wire three times (18). To confirm endothelium removal, we contracted the vessel with 10−5 M 5-hydroxytryptamine and at the plateau added 10−6 M ADP. Vessels that relaxed >20% after this treatment were rejected for further study. Cerebral arteries [diameter of 450 ± 25 μm for adult and 350 ± 25 μm for fetus (15), with no difference in sea level vs. high altitude] were used within a few minutes of being obtained for simultaneous measurements of tension and [Ca2+]i (15). Unless otherwise noted, all chemical compounds were purchased from Sigma Chemical (St. Louis, MO).

Contractility and intracellular Ca2+ measurements. We cut the middle cerebral arteries into rings 2 mm in length and mounted them on two tungsten wires (0.13 mm diameter; A-M Systems, Carlsborg, WA). We attached one wire to an isometric force transducer (Kent Scientific, Litchfield, CT) and the other to a post attached to a micrometer that was used to vary resting tension in a 5-ml tissue bath mounted on Jasco CAF-110 intracellular Ca2+ analyzer (Jasco, Easton, MD). We then measured vascular tension, as previously described (13, 15). This, with vessel inside diameter measurements in combination with measurements of vessel wall thickness, length, and potassium-induced force, enabled calculation of force per unit cross-sectional area, as previously described. MCA rings were equilibrated under 0.3-g tension at 25°C for 40 min before being loaded with the acetoxyethyl ester of fura 2 (fura 2-AM; Molecular Probes, Eugene, OR), a fluorescent Ca2+ indicator that is a measure of mean cytoplasmic [Ca2+]i (7). Fura 2 fluorescence and force were measured simultaneously at 38°C, as previously described (15). As we have noted, although some investigators may prefer the transformation of fluorescence to [Ca2+]i, in tissues such as cerebral arteries the presentation of the ratio is less ambiguous (15). During all contractility experiments, we continuously digitized, normalized, and recorded contractile tensions and the fluorescence ratio (F340/380) using an on-line computer. For all vessels, we evaluated the contractile response for tension and fluorescence ratio by measuring the maximum peak height and expressing it as percent of maximal tension achieved to 120 mM K+ (Kmax, a measure of “efficacy”) and calculated pD2 (the negative logarithm of the 50% effective concentration, or half-maximal concentration for norepinephrine (NE), and an index of tissue “sensitivity” or “potency”) (15).

Relative roles of the several K+ channels with hypoxia. To determine the potential role of the several types of K+ channels in modulating NE-induced changes in [Ca2+]i, and vascular tension in the two oxygenation states, we quantified these variables in the presence of selective K+ channel activators or blockers. For all studies, after initial K+ (120 mM) depolarization to determine Kmax, we performed NE dose-response curves to establish the maximum contraction, percent Kmax, and pD2.

To examine the role of activation of KATP channels on [Ca2+]i and tension, we first stimulated the vessel with 10−5 M NE. Then, on the plateau of the response curve, we added increasing doses of pinacidil (10−7 to 10−3 M) to stimulate K+ efflux, hyperpolarize the vessel, and cause relaxation. From these data, we plotted the percent relaxation (or percent inhibition) as a function of agonist dose. In a separate study, we gave 10−5 M pinacidil and in 15 min performed a NE dose-response study (10−9 to 10−4 M); from these data we plotted the shift in the NE dose-response curve. To examine the effect of KATP channel inhibition, we quantified NE-induced change in [Ca2+]i, and tension after administration of the KATP channel blocker glibenclamide (3 × 10−7 M). In a separate study, we first gave 3 × 10−7 M NE to achieve a 30% maximum response and then administered glibenclamide in increasing doses (10−7 to 3 × 10−5 M) to increase tension and [Ca2+]i. In addition, to examine the effect of glibenclamide alone, we administered 10−7 to 3 × 10−5 M of that compound (14).

To examine the role of Kca channels on NE-induced [Ca2+]i, and vascular tension, we quantified these after administration of 10−5 M NE. Then, on the plateau of the response, we administered increasing doses of the Kca channel opener NS-1619 (10−9 to 10−6 M) to stimulate K+ efflux. In a separate study, we first gave NS-1619 (10−7 M) and in 15 min determined the NE dose response (10−9 to 10−4 M). To examine the role of inhibition of the Kca channel on [Ca2+]i, and tension, we performed NE dose-response curves in the presence of the channel blocker (15 min) iberiotoxin (10−7 M). In a separate study, we gave 3 × 10−7 M NE to achieve ~30% maximum response and then gave increasing doses of iberiotoxin (10−9 to 10−6 M). In addition, to examine the effect of iberiotoxin alone, we administered 10−9 to 10−6 M of that compound (14).

To examine the potential role of inhibition of KIR channels on Ca2+ channel activity in fetal and adult cerebral arteries, we measured NE-induced [Ca2+]i, and tension after administration of 4-aminopyridine (4-AP; 10−4 M). Alternatively, we administered 3 × 10−7 M NE and then gave increasing doses of 4-AP (10−5 to 10−3 M). Finally, to determine the possible role of KIR channels in modulating Ca2+ channel activity, we measured NE-induced [Ca2+]i, and tension after addition of KCl (1.5 × 10−2 M) to activate the channel or BaCl2 (10−5 M) to block it. In addition, we administered 3 × 10−7 M NE to achieve ~30% maximum response and then gave increasing doses of BaCl2 (14). In all studies noted above, to avoid the complication of previous agonists and/or antagonists affecting the response to a subsequent compound, only a single agonist or antagonist was used.

Statistical analysis. All values were calculated as means ± SE. In all cases, n refers to the number of vessel segments (which corresponds to the number of animals) studied. The n values for the different experiments are given in Table 1. Because of the nature of these studies, several statistical tests were used to test for significant differences. For testing differences between two groups, we used a simple unpaired Student’s t-test. For multiple comparisons, one-way and two-way ANOVA (age, oxygen status) coupled with Duncan’s multiple-range test was used. When appropriate, we used ANOVA with repeated measures. A P value of <0.05 was considered significant.

RESULTS

Contractile and [Ca2+]i responses to K+ and NE. Figure 1 shows the NE-induced responses of vascular tension and the fura 2 fluorescence ratio (F340/380), a
Table 1. Peak responses of vascular tension and fluorescence ratio

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Fetus</th>
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<tbody>
<tr>
<td></td>
<td>Delta Maximum Tension, g</td>
<td>Delta Maximum Tension, g</td>
</tr>
<tr>
<td>Kmax (120 mM)</td>
<td>1.6 ± 0.1(15)</td>
<td>0.15 ± 0.02</td>
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<tr>
<td>NE (10−6 M)</td>
<td>1.7 ± 0.1(12)</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>10−5 M NE as</td>
<td></td>
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<tr>
<td>% Kmax</td>
<td>106 ± 10</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>pD2</td>
<td>6.1 ± 0.1</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Pinacidil inhibition dose response (% inhibition)</td>
<td>100 ± 10% (4)</td>
<td>45.5%</td>
</tr>
<tr>
<td>pIC50</td>
<td>5.0 ± 0.1</td>
<td>4.7 ± 0.1</td>
</tr>
<tr>
<td>10−5 M Prn + NE</td>
<td>59 ± 8% (3)</td>
<td>89 ± 10%</td>
</tr>
<tr>
<td>pD2</td>
<td>6.0 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>3 × 10−7 M Glib + NE</td>
<td>76 ± 9% (3)</td>
<td>57 ± 8%</td>
</tr>
<tr>
<td>pD2</td>
<td>5.9 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>NS-1619 inhibition dose response (% inhibition)</td>
<td>42 ± 9% (4)</td>
<td>0</td>
</tr>
<tr>
<td>pIC50</td>
<td>7.6 ± 0.1</td>
<td>7.8 ± 0.1</td>
</tr>
<tr>
<td>10−7 M NS-1619 + NE</td>
<td>93 ± 9% (3)</td>
<td>89 ± 10%</td>
</tr>
<tr>
<td>pD2</td>
<td>6.3 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>10−9–10−6 M IbTx dose response</td>
<td>100 ± 12% (3)</td>
<td>88 ± 10%</td>
</tr>
<tr>
<td>pD2</td>
<td>7.1 ± 0.1</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>10−7 M IbTx + NE</td>
<td>92 ± 7% (4)</td>
<td>86 ± 9%</td>
</tr>
<tr>
<td>pD2</td>
<td>6.1 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>10−4 M 4-AP + NE</td>
<td>75 ± 5% (3)</td>
<td>99 ± 11%</td>
</tr>
<tr>
<td>pD2</td>
<td>5.9 ± 0.1</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>10−5 M BaCl2 + NE</td>
<td>100 ± 12% (3)</td>
<td>71 ± 8%</td>
</tr>
<tr>
<td>pD2</td>
<td>6.4 ± 0.1</td>
<td>6.3 ± 0.1</td>
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</table>

Values are means ± SE expressed in absolute terms as % maximal tension achieved to 120 mM K+ (Kmax; see METHODS for details) or as % norepinephrine (NE) maximum. Tension and intracellular Ca²⁺ concentration ([Ca²⁺]i) were measured simultaneously; thus the n values (i.e., those given with tension) were the same. Δ, Change in maximum tension and fluorescence ratio (F540/380) from baseline; Pin, pinacidil; 4-AP, 4-aminopyridine; Glib, glibenclamide; IbTx, iberiotoxin; pD2, negative logarithm of the 50% effective concentration, or half-maximal concentration for NE. Nos. in parentheses, no. of individual experiments for each protocol for adult and fetus. *P < 0.05, †P < 0.01 compared with control 10−5 M NE. See text for exact drug doses and timing.

For normoxic control adult MCA, Fig. 2A shows NE-induced tension (g) as a function of fluorescence ratio (F540/380), slope = 7.3 ± 0.6. For the LTH adult vessels, Fig. 2B shows NE-induced tension as a function of fluorescence ratio, slope = 15.5 ± 1.3 (see Table 1). Also, as seen in Fig. 2, C and D, for fetal MCA the relations of tension (g) to fluorescence ratio in response to NE stimulation were not significantly different in the two oxygen exposure groups (slopes = 4.1 ± 0.4 and 3.7 ± 0.6, respectively).

Role of KATP in vascular [Ca²⁺]i and tension. To examine the role of ATP-sensitive K⁺ channel activation on adult and fetal MCA tension and fluorescence ratio, we first contracted the vessels with 10−5 M NE and then on the plateau of the contractile response administered pinacidil in increasing half-log dose increments (10−7 to 10−3 M). As shown in Fig. 3A for adult MCA, at concentrations above 10−6 M, pinacidil inhibited tension with little difference between normoxic

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and LTH vessels [see Table 1 for half-maximal inhibitory concentration (pIC_{50}) values]. In contrast, whereas the fluorescence ratio showed essentially no change in normoxic adult vessels (14), long-term hypoxic vessels displayed ~40% inhibition (Fig. 3B). As shown in Fig. 3C, fetal cerebral vessels from the LTH animals were much less sensitive to increasing pinacidil concentrations (see Table 1 for pIC_{50} values). In addition, hypoxic fetal vessels also showed less sensitivity to pinacidil-induced inhibition of [Ca^{2+}]_{i} (Fig. 3D, Table 1).

Fig. 1. Norepinephrine (NE) dose-response relationships for adult and fetal main branch middle cerebral arteries (MCA) under normoxic control conditions and in response to long-term hypoxia. Arterial segments were first contracted with 120 mM K^+ to obtain peak tension. After washing and reequilibration to baseline tension, we induced subsequent contractions using cumulative doses of NE added in half-log increments (see METHODS for details). Points shown are means ± SE. A: vascular tensions (g) for normoxic (○) and long-term hypoxic (□) adult MCA. B: fluorescence ratios (F_{340/380}) for normoxic and hypoxic adult MCA. C: vascular tensions (g) for normoxic and long-term hypoxic fetal MCA. D: F_{340/380} for normoxic and hypoxic fetal MCA.

Fig. 2. Long-term hypoxia is associated with an increase in Ca^{2+} sensitivity in adult, but not fetal, cerebral arteries. A: NE-induced vascular tension (g) as a function of F_{340/380} for normoxic control adult MCA. Slope = 7.3 ± 0.6. B: NE-induced tension (g) as a function of F_{340/380} for long-term hypoxic adult MCA. Slope = 15.5 ± 1.3 (P < 0.01 different from normoxic control). C: NE-induced vascular tension (g) as a function of F_{340/380} for normoxic control fetal MCA. Slope = 4.1 ± 0.4. D: NE-induced tension (g) as a function of F_{340/380} for long-term hypoxic fetal MCA. Slope = 3.7 ± 0.6.
To examine further the role of hypoxia in altering K\textsubscript{ATP} channel function in adult MCA, in another set of vessels we quantified vascular tension and fluorescence ratio in response to increasing doses of NE (10\textsuperscript{-9} to 10\textsuperscript{-4} M) alone or 15 min after administration of 10\textsuperscript{-5} M pinacidil. Normoxic adult MCA showed typical increases in vascular tension and [Ca\textsuperscript{2+}]\textsubscript{i} in response to NE, as described above. As we have reported, after K\textsubscript{ATP} channel activation by pinacidil the maximum NE-induced contractile response in normoxic adult MCA was attenuated to 59 ± 8%, whereas neither the fluorescence ratio nor the pD\textsubscript{2} values were significantly different from control (14). Also, in adult cerebral arteries from animals acclimatized to LTH, in the presence of 10\textsuperscript{-5} M pinacidil, the maximal NE-induced tension and fluorescence ratios, as well as pD\textsubscript{2} values, were not significantly different from normoxic controls (see Table 1 for n values and % change). In LTH fetal MCA in response to NE alone or to NE after administration of 10\textsuperscript{-5} M pinacidil, the values for neither NE-induced tension nor [Ca\textsuperscript{2+}]\textsubscript{i} were significantly different from those of normoxic controls (Table 1).

To examine further the effect of LTH and K\textsubscript{ATP} channel inhibition on NE-induced [Ca\textsuperscript{2+}]\textsubscript{i} and tension, we first administered 3 × 10\textsuperscript{-7} M glibenclamide and then in 15 min determined the NE concentration-response curve relations. As we have reported for normoxic adult MCA (14), glibenclamide had a modest effect in lowering maximum tension and fluorescence ratio 20–30% (Table 1). In contrast, in vessels from LTH adult animals, aside from decreased maximal tension and fluorescence ratios in response to NE alone, in the presence of 3 × 10\textsuperscript{-7} M glibenclamide the NE-induced responses were not significantly different from those responses with NE alone (Table 1). In a manner similar to the adult, in the hypoxic fetal MCA, the NE-induced responses in the presence of 3 × 10\textsuperscript{-7} M glibenclamide were not significantly different from those of normoxic control vessels (Table 1).

Role of K\textsubscript{Ca} in vascular [Ca\textsuperscript{2+}], and tension. Figure 4 shows the effect of the K\textsubscript{Ca} channel opener NS-1619 (10\textsuperscript{-9} to 10\textsuperscript{-6} M) on hypoxic and normoxic precontracted adult and fetal MCA. In the adult LTH vessel, tension was only modestly inhibited at NS-1619 concentrations above 3 × 10\textsuperscript{-8} M, 27 ± 8% at 10\textsuperscript{-8} M, significantly less than the 42 ± 9% inhibition seen in the normoxic vessel (14) (Fig. 4A; Table 1). In contrast, in hypoxic adult artery the fluorescence ratio did not change significantly in response to NS-1619 (Fig. 4B). In comparison, Fig. 4C shows the effect of K\textsubscript{Ca} channel activation (at concentrations above 3 × 10\textsuperscript{-8} M NS-1619) in inhibiting vascular tension only 41 ± 5% at 10\textsuperscript{-8} M in the hypoxic fetal MCA. This significantly smaller inhibition contrasts with essentially 100% inhibition seen in the normoxic fetal vessel (14). As shown in Fig. 4D, the fluorescence ratio was essentially unchanged in the hypoxic fetal MCA in response to NS-1619 (Table 1).

To evaluate further K\textsubscript{Ca} channel activation in hypoxic adult MCA, we administered 10\textsuperscript{-7} M NS-1619 and then in 15 min performed a NE dose-response curve. Under these conditions, NE-induced tension and fluorescence ratio of long-term hypoxic adult MCA were minimally affected and did not differ significantly from the responses seen in normoxic vessels (14) (Table 1). Again, in hypoxic fetal MCA, NE dose responses
after administration of 10^{-7} M NS-1619 were not significantly different from those responses reported in the normoxic vessel (14) (Table 1).

To examine the effect of K_{Ca} channel inhibition in hypoxic adult and fetal cerebral arteries, we administered iberiotoxin in half-log doses (10^{-9} to 10^{-6} M). In hypoxic adult MCA at 3 \times 10^{-8} M and above, iberiotoxin resulted in significant increase of both tension and [Ca^{2+}], with no significant difference from those responses seen in the normoxic group (Table 1). In a similar manner in the hypoxic fetal artery, at 3 \times 10^{-8} M iberiotoxin and above, tension increased significantly, with no significant difference in the response from that seen in the normoxic vessels (14). In contrast, in the hypoxic fetal vessel [Ca^{2+}], increased much less in response to K_{Ca} channel inhibition than in the normoxic vessel (Table 1). In another study, we first administered the L-type Ca^{2+} channel blocker nifedipine (10^{-6} M) and then in 15 min determined the iberiotoxin dose-response relations (n = 3). Under these circumstances, neither adult nor fetal MCA showed a significant increase in tension or [Ca^{2+}].

In further studies on the effect of K_{Ca} channel inhibition on tension and fluorescence ratio in LTH adult MCA, when the NE dose-response was performed 15 min after administration of 10^{-7} M iberiotoxin, neither tension nor fluorescence ratio was significantly altered compared with NE alone (Table 1). In the LTH fetal vessels, in contrast, iberiotoxin pretreatment resulted in a 20% decrease in NE-induced maximum tension (a change not seen in the normoxic vessel), but this was not statistically significant. Also there was no significant change in [Ca^{2+}], (Table 1).

**Role of K_{IR} in [Ca^{2+}], and tension.** We also examined the role of inhibition of K_{IR} channels on tension and fluorescence ratio of hypoxic adult cerebral arteries. As in the other studies, we measured these variables after the administration of 10^{-4} M 4-AP followed in 15 min by a NE dose response. In LTH adult MCA, 4-AP pretreatment was associated with 30–40% decreases in NE-induced tension and [Ca^{2+}], which were only slightly greater inhibition than that of normoxic controls (14) (Table 1). For both LTH and normoxic fetal MCA, administration of 10^{-4} M 4-AP had no significant effect on either the NE dose-response of tension or fluorescence ratio (Table 1).

**Role of K_{IR} in [Ca^{2+}], and tension.** To explore the possible effect of hypoxia in modulating K_{IR} channel activity, we administered 10^{-5} M BaCl_{2} to inhibit these channels, after which we performed a NE dose response. As we have reported (14), in normoxic adult MCA maximum vascular tension was not significantly altered (Fig. 5A), despite a modest decrease in fluorescence ratio (Fig. 5B). In contrast, in vessels from ewes subjected to LTH, BaCl_{2} resulted in significant 50% reduction of maximum NE-induced tension (Fig. 5C) and fluorescence ratio (Fig. 5D) (Table 1). In the hypoxic fetal MCA, 10^{-5} M BaCl_{2} significantly inhibited both the NE-induced tension and fluorescence ratio, but these responses were not significantly different from those seen in normoxic fetal vessels (Table 1).

**DISCUSSION**

Cerebral arteries, like most VSM, are exquisitely sensitive to K^{+} channel activation in modulating mem-

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**Fig. 4.** Long-term hypoxia is associated with decreased inhibition of tension by the K_{Ca} channel activator NS-1619 in fetal cerebral arteries. Points shown are means ± SE. A: percent inhibition of NE-induced tension in normoxic (●) and hypoxic (○) adult main branch MCA in response to NS-1619 (n = 4 each). Vessels were first contracted with 10^{-5} M NE, and then at the plateau of contraction the channel agonist was given in half-log increments (see METHODS for details). B: lack of effect of NS-1619 in inhibiting NE-induced F_{405/380} response in either normoxic or hypoxic adult MCA. C: percent inhibition of NE-induced tension in normoxic and hypoxic fetal main branch MCA in response to NS-1619 (n = 4 each). Vessels were first contracted with 10^{-5} M NE, and then at the plateau of contraction the channel agonist was given in half-log increments (see METHODS for details). Points shown are means ± SE. D: relative lack of effect of NS-1619 in inhibiting NE-induced F_{405/380} response in either normoxic or hypoxic fetal MCA.
brane potential and voltage-gated L-type Ca$^{2+}$ channel activity. The present studies offer several important observations on the K$^+$ channel responses of adult and fetal cerebral arteries exposed to long-term, high-altitude hypoxia. First, in response to K$_{Ca}$ channel activation by NS-1619, LTH fetal MCA showed significantly less inhibition of NE-induced tension and [Ca$^{2+}$]$_i$ than normoxic controls (Fig. 4, A and B). Second, in response to pinacidil-induced K$_{ATP}$ channel activation, LTH fetal MCA showed significantly decreased sensitivity (e.g., a lower pIC$_{50}$ value) of both tension and [Ca$^{2+}$]$_i$, compared with normoxic controls (Fig. 3, C and D). In contrast, in hypoxic adult MCA the effect of activation of K$_{ATP}$ channel on tension was little different from that of normoxic controls, whereas the significantly greater inhibition of [Ca$^{2+}$]$_i$ again suggests somewhat greater Ca$^{2+}$ sensitivity (Fig. 3, A and B). Third, in adult MCA, hypoxia was associated with significant inhibition by BaCl$_2$ of NE-induced tension and to a lesser extent [Ca$^{2+}$]$_i$, again demonstrating the greater sensitivity to Ca$^{2+}$ under conditions of chronic hypoxia (Fig. 5). In hypoxic fetal MCA, the BaCl$_2$-induced inhibition was not significantly different from that observed in the normoxic vessels. Fourth, notable in these studies was the fact that both the adult and fetal hypoxic cerebral arteries showed little alteration in NE-induced tension or [Ca$^{2+}$]$_i$ responses after activation or blockade of K$_{ATP}$, K$_{Ca}$, or K$_v$ channels compared with normoxic controls. This suggests that, in terms of overall regulation of cerebral artery responses to LTH, K$^+$ channel activity and its linkage to myogenic tone is probably of greater importance than adrenergic-mediated mechanisms per se.

Fifth, in adult, but not fetal, MCA, LTH was associated with a decrease in both maximal NE-induced tension and fluorescence ratio (e.g., decreased efficacy) (Fig. 1). Because this decrease in tension was less than the decrease in [Ca$^{2+}$]$_i$, LTH was associated with a significant increase in Ca$^{2+}$ sensitivity in adult vessels (Fig. 2). Sixth, overall in response to LTH, adult cerebral arteries demonstrated increased Ca$^{2+}$ sensitivity (Figs. 2, A and B; 3, A and B; 5, C and D). In contrast, LTH fetal cerebral arteries showed relatively little change in Ca$^{2+}$ sensitivity (Fig. 2, C and D), with only a slight decrease when K$_{Ca}$ channels were activated (Fig. 4, C and D). That is, in fetal MCA, exposure to LTH significantly decreased the inhibition of vascular tone produced by activation of K$_{Ca}$, but not K$_{ATP}$ channels. This was associated with a tendency for the fetal cerebral arteries to function in a manner similar to more “mature” adult vessels and was particularly evident in terms of the effect of the K$_{Ca}$ channel opener NS-1619 (Fig. 4, C and D; Table 1). In addition, the differing patterns of response to activation of K$_{ATP}$ and K$_{Ca}$ channels suggests differential regulation of these channels by LTH. Overall, the present studies support the hypothesis that LTH is associated with significantly altered K$_{ATP}$ and K$_{Ca}$ channel function in cerebral arteries, especially in those of the fetus.

**Developmental differences in contractile responses in long-term hypoxemia.** Previously, we and others have reported significant differences in cerebral artery signal transduction mechanisms with development from fetus to adult (13, 15, 19, 26, 38), and this includes the function of K$_{ATP}$ and K$_{Ca}$ channels (14).
Acclimatization to LTH has significant effects on cerebral vascular tone. In previous studies, our laboratory showed that, in high-altitude-acclimatized sheep, neither adult nor fetal MCA showed significant change in $K^+$-induced depolarization either in absolute terms (g tension) or as $K^+$-induced stress ($10^5$ dyn/cm$^2$) (16), an observation confirmed in the present study. We previously reported decreased NE-induced contraction in LTH adult MCA (17), but our suggestion that this also was the case for the fetus was not confirmed in the present study. As we have also reported, cerebral arteries of LTH fetal and adult animals demonstrated significant decreases in $\alpha_1$-adrenergic-receptor density and inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] responses (34), as well as in Ins(1,4,5)P$_3$-receptor density (38). One might argue that the LTH-mediated decrease in cerebral artery contractility is a defense mechanism to protect the brain against excessive $\alpha$-adrenergic activity; but this, of course, is unknown. As is well established, the fetal and adult cerebral vasculature dilates in response to acute hypoxia, despite a large increase in circulating catecholamines (this is not surprising, however, given that catecholamines do not cross the blood-brain barrier). Thus hypoxia-mediated activation of cerebrovascular adrenergic receptors must be secondary to neurally released NE.

Hypoxic modulation of $K^+$ channel function in cerebral arteries. In previous studies, our laboratory has reported for cerebral arteries of both adult and fetus that LTH is associated with a significant decrease or downregulation of $\alpha_1$-adrenergic-receptor density and NE-induced Ins(1,4,5)P$_3$ responses (34), Ins(1,4,5)P$_3$-receptor density (38), and other signal transduction components (16, 17). Under normoxic conditions, vessels in the cerebral microcirculation exist in a partially contracted state and constrict further or dilate depending on the tissue requirements for blood and/or O$_2$. This tone is an important determinant of vascular resistance and blood pressure and to a great extent is regulated by the VSM membrane potential, which, in turn, is regulated by the plasma membrane $K^+$ channels (22). Little is known of the regulation of these channels in chronic hypoxia, however. As noted earlier, the main branch MCA employed in the present studies is not the major site of vascular resistance; however, because the $K^+$ channels in the smaller arterioles cannot be examined in the manner of the present studies, we trust that the insights gained from these data may also apply to the smaller vessels. Although the four distinct types of $K^+$ channels are fairly well defined structurally, and selective pharmacological blockers exist for each channel type, selective activators have been described for only the $K_{ATP}$ and $K_{Ca}$ channels.

Role of $K_{ATP}$ channels. The sensitivity of these channels to changes in cellular metabolic state (opening in response to a decrease in intracellular ATP concentration) suggests that they would be activated by hypoxia. Although some studies suggest that acute moderate hypoxia may not result in significant ATP decrease, the extent to which this changes with chronic hypoxia is unknown. In rat cerebral arteries, the $K_{ATP}$ channel inhibitor glibenclamide reduced hypoxia-induced vasodilation (33). In the present study, in hypoxic adult MCA, the $K_{ATP}$ channel opener pinacidil inhibited NE-induced elevation in $[Ca^{2+}]_i$, compared with its minimal effect in the normoxic vessel (Fig. 3B). The mechanistic basis of this hypoxia-associated increase in $Ca^{2+}$ sensitivity is not clear. LTH-associated changes in RhoA-Rho kinase, or other enzymes, by increasing phosphorylation of myosin light chain phosphatase, may increase $Ca^{2+}$ sensitivity; however, this remains to be determined. In hypoxic fetal MCA, the reduction in pIC$_{50}$ values of pinacidil-induced inhibition of both tension and $[Ca^{2+}]_i$ of about one-half log (i.e., a threefold change) (Fig. 3, C and D) may be related to a similar, or other, mechanism. Again in hypoxic fetal MCA, administration of pinacidil lowered maximum NE-induced tension and $[Ca^{2+}]_i$ (Table 1). As an aside, the pinacidil concentrations required for half-maximal inhibition of $\sim 10^{-5}$ M is an order of magnitude higher than that observed in anesthetized cat pial arteries in vivo (12). This raises the issue of relative insensitivity of these in vitro endothelium-denuded vessel rings, compared with vessels in vivo.

Several endogenous substances produce hyperpolarization and relaxation of cerebral arteries, which may be mediated by activation of $K_{ATP}$ channels (6). For instance, the vasodilators adenosine and calcitonin gene-related peptide increased glibenclamide-sensitive currents, whereas the vasoconstrictors serotonin and histamine inhibited pinacidil-induced $K^+$ currents (10, 22). We do not know the extent to which these were factors in the present studies. Also, in isolated VSM cells, hypoxia can activate $K_{ATP}$ channels (5, 37). Whether this occurs by a reduction in mitochondrial ATP generation or by an O$_2$ sensor as part of, or coupled to, the channel is unknown (29).

Role of $K_{Ca}$ channels. Although large-conductance $K^+$ channels activated by membrane depolarization and an increase in $[Ca^{2+}]_i$, are an important feature of essentially all smooth muscle cells (21), the physiological regulation of $K_{Ca}$ channel activity is largely unknown (22). Hypoxia activates $K_{Ca}$ channels in pulmonary artery (35) and portal vein (20) VSM cells, and such activation is associated with $Ca^{2+}$ release from intracellular stores. With development from fetus to adult, ovine pulmonary arteries demonstrate a maturation change from $K_{Ca}$ to $K_{Ca}$ channels (30), but whether such change occurs in other vessels is unknown.

A critical observation of the present study is that, in fetal cerebral arteries under conditions of chronic hypoxia, the $K_{Ca}$ channel activator NS-1619 inhibited vascular tension much less than in the normoxic vessel (Fig. 4). This was not accompanied by significant inhibition of $[Ca^{2+}]_i$, suggesting decreased $Ca^{2+}$ sensitivity. Nevertheless, in the hypoxic fetal vessel, $K_{Ca}$ channel activation was not associated with such a significant inhibition of NE-induced tension (or fluorescence ratio; Table 1). These results suggest that hypoxia results in “maturation” of fetal arteries so that they function more like those of the adult.
in hypoxic fetal MCA, the KCa channel inhibitor iberiotoxin resulted in significantly less increase in \([\text{Ca}^{2+}]_i\). This is in marked contrast to the lack of such effect by the \(\text{K}_{\text{ATP}}\) channel blocker glibenclamide. In addition, in the hypoxic fetal (but not adult) artery, preadministration of iberiotoxin inhibited NE-induced tension (Table 1). Recently, Schubert and Nelson (31) reviewed the role of several protein kinases in modulating VSM KCa channel function. The extent to which these or other mechanisms(s) may play a role in the effects of LTH on fetal and adult KCa channels is unknown.

**Role of \(K_v\) channels.** These channels (also called delayed-rectifier channels) open to allow \(K^+\) efflux and membrane repolarization when the membrane is depolarized (22). In pulmonary artery VSM cells, \(K_v\) channels can be regulated by cellular \(O_2\) tension (1, 2, 27), and hypoxia-induced inhibition of \(K_v\) channels is an important mechanism of pulmonary hypoxic vasorelaxation (23, 36). A novel delayed-rectifier \(K_v\) channel in pulmonary arterial smooth muscle cells, activated by ATP, may be the channel inhibited by hypoxia (24, 25). Nonetheless, in the present study LTH showed little influence on 4-AP modulation of NE-induced contraction (Table 1).

**Role of \(K_{IR}\) channels.** In contrast to \(K_{Ca}\) and \(K_v\) channels, which are activated by membrane depolarization, the \(K_{IR}\) channels are activated by membrane hyperpolarization and may play a role in maintaining resting membrane potential, although this is poorly understood (6, 22). As noted, the effect of BaCl2 in both LTH and normoxic fetal MCA may have been secondary to Ba\(^{2+}\) blockade of \(L\)-type Ca\(^{2+}\) channels rather than to inhibition of \(K_{IR}\) channels per se (Table 1), and the mechanisms of the significant inhibition of NE-induced contraction by BaCl2 in hypoxic adult vessels (Fig. 5C) are unclear.

**Perspectives.** The role of cerebral artery plasma membrane K+ channels, and their sensitivity to various agents, may differ greatly as a function of species and developmental age. Here we show that particularly the \(K_{Ca}\) and \(K_{ATP}\) channels are affected by LTH. By quantifying simultaneously \([\text{Ca}^{2+}]_i\) and tension, the present studies are the first to demonstrate in fetal and adult cerebral arteries the effect of high-altitude \(O_2\) tension on the function of several \(K^+\) channels. The dependence of fetal cerebral arteries on extracellular Ca\(^{2+}\) (15) is associated with these vessels being exquisitely sensitive to \(K_{ATP}\) and \(K_{Ca}\) channel activation, compared with adult, and these channels play an important, albeit differing, role in the regulation of vascular tone in both fetal and adult cerebral arteries (14). As evidenced in the present report, in adult cerebral arteries LTH significantly decreased NE-induced contractility (Fig. 1) and increased \([\text{Ca}^{2+}]_i\) sensitivity (Fig. 2). In addition, although hypoxemic adult cerebral arteries were only moderately affected by the \(K_{ATP}\) channel opener pinacidil or the \(K_{Ca}\) channel opener NS-1619, their sensitivity to Ca\(^{2+}\) was significantly increased. In contrast, LTH fetal cerebral arteries showed significantly decreased inhibition of NE-induced contraction by the \(K_{Ca}\) channel opener NS-1619 and to a lesser extent by the \(K_{ATP}\) channel opener pinacidil. The differing pattern of these responses strongly supports the idea of differential regulation of these channels by hypoxia. In some respects (e.g., less inhibition by NS-1619), LTH was associated with the fetal cerebral arteries functioning more like mature adult than fetal normoxic vessels. In both adult and fetal vessels, LTH appeared to affect \(K_{ATP}\) and \(K_{Ca}\) function per se, without significantly altering the coupling of these channels to adrenergic-mediated contraction. Because cerebral artery vascular tone appears to be determined primarily by myogenic regulation, and because both \(K_{ATP}\) and \(K_{Ca}\) channels play a key role in regulating this tone (21, 22, 28), the present findings that chronic hypoxia suppressed or otherwise altered \(K_{ATP}\) and \(K_{Ca}\) channel activities in a developmentally age-dependent manner supports the hypotheses that stimulated the study. This may have significant implications in understanding the regulation of cerebral blood flow in both the chronically hypoxic fetus and adult. For instance, in the hypoxic fetus the cerebral arteries may be near-maximally dilated so that \(K^+\) channel activation with hyperpolarization can result in little further inhibition of tone. Obviously, many questions remain as to the mechanisms by which LTH alters \(K^+\) channel activity and Ca\(^{2+}\) sensitivity.

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