Fetal cerebrovascular acclimatization responses to high-altitude, long-term hypoxia: a model for prenatal programming of adult disease?

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Longo, Lawrence D., and William J. Pearce. Fetal cerebrovascular acclimatization responses to high-altitude, long-term hypoxia: a model for prenatal programming of adult disease? Am J Physiol Regul Integr Comp Physiol 288: R16–R24, 2005; doi:10.1152/ajpregu.00462.2004.—During the past several decades, many risk factors for cerebrovascular and cardiovascular disease have been identified. More recently, it has been appreciated that inadequate nutrition and/or other intrauterine factors during fetal development may play an important role in the genesis of these conditions. An additional stress factor that may “program” the fetus for disease later in life is chronic hypoxia. In studies originally designed to examine the function of developing cerebral arterial function in response to high-altitude (3,820 m, 12,470 ft) LTH (~110 days). LTH is associated with augmentation or upregulation of presynaptic functions, including responses to perivascular (i.e., sympathetic) nerve stimulation, and structural maturational changes. In contrast, many postsynaptic functions related to the Ca$^{2+}$-dependent contractile pathway tend to be downregulated, whereas elements of the Ca$^{2+}$-independent contraction pathway are upregulated. The results emphasize the role of high-altitude LTH in modulating many aspects of electromechanical and pharmacomechanical coupling in the developing cerebral vasculature. A complicating factor is that the regulation of cerebrovascular tone by Ca$^{2+}$-dependent and Ca$^{2+}$-independent pathways changes significantly as a function of maturational age. In addition to highlighting independent regulation of various elements of the signal transduction cascade, the studies demonstrate the potential for LTH to program the fetus for cerebrovascular and other disease as an adult.
dependent (Type 2) diabetes with low birth weight, the relation of mortality from coronary artery disease to weight at 1 yr of age, and the relation of both newborn ponderal index [weight (g) × 10⁹/crown-heel length (cm)²] and placental-to-fetal weight ratio to hypertension in the adult (see Refs. 6 and 7 for review). During the past decade, numerous studies in experimental animals also have demonstrated a relation between intrapartum stress, particularly that of maternal food deprivation and/or emotional stress, and adult disease (29). Among the major known intrapartum stresses about which the effects on subsequent adult health are largely unknown is fetal hypoxia.

In the present review, we examine the current state of knowledge of fetal cerebrovascular responses to long-term hypoxia (LTH). This is not intended as a review of vascular responses to acute hypoxia or hypoxia in general, nor as a review of the idea of antenatal origins of adult disease. These topics have been reviewed at length elsewhere. Also, as originally formulated, our LTH studies were not designed to test the Barker hypothesis per se, but rather the impact of chronic hypoxia on the developing cardiac, cerebrovasculature, endocrinologic, and other systems. Most of the studies reported here have been in vitro rather than in vivo.

FETAL HYPOXIA

For the developing fetus, the responses to LTH are of particular importance. This is because under normal conditions fetal arterial PO₂ values are low by adult standards. Although cardiac, hemodynamic, and cerebrovascular adjustments to acute hypoxia are well defined, the responses of these mechanisms to LTH are less well known, if at all. This review considers some of those acclimatization responses to high-altitude hypoxia that occur in the fetal cerebrovasculature in a “model” of pregnant sheep that are transported to high altitude (White Mountain Research Station, elevation 3,820 m, 12,470 ft) at 0.21 gestation, where they are kept until study at 0.97 gestation (30 and 140 days, respectively). At this altitude, maternal arterial PO₂ falls to 60 ± 3 Torr compared with a sea-level value of 100 ± 5 Torr and fetal arterial PO₂ decreases from 25 ± 1 to 19 ± 1 Torr (36, 37). Although arterial PCO₂ falls slightly, arterial pH remains unchanged (Table 1 summarizes these physiological variables). Despite this long-term reduction in arterial PO₂ values, there is no reduction in body or organ weights of the near-term fetus, nor is there a major change in the levels of many circulating hormones (27, 34, 35, 47, 48). Thus this appears to represent an example of successful acclimatization.

CEREBROVASCULAR RESPONSES TO LTH

In the fetus, acute hypoxia can increase cerebral blood flow (CBF) severalfold (2, 58). In contrast, LTH is associated with near-normal CBF and O₂ delivery, despite a significant 27% decrease in cardiac output and a 49% decrease in blood flow to the carcass and most other organs (34). Mean arterial blood pressure is increased 17% (from 44 ± 1 to 52 ± 1 mmHg; Ref. 35). These changes imply a modest increase (~17%) in cerebrovascular resistance with a more significant ~40% increase in systemic vascular resistance, (i.e., a decrease in the ratio of cerebrovascular to total peripheral resistance). In contrast, in high-altitude-acclimatized adults, despite changes in respiration and function of many organs, CBF is maintained at relatively normal levels with little change in cerebrovascular resistance (32, 52, 61). These findings raise questions regarding the mechanisms whereby cerebrovascular homeostasis is regulated during chronic hypoxia, and the extent to which the mechanisms that enable redistribution of cardiac output to favor the brain may have a lasting impact into adult life.

COMPOSITION AND STRUCTURE

As noted above, during hypoxic acclimatization in the fetus the maintenance of CBF despite a modest increase in cerebrovascular resistance suggests altered structure and/or composition of the cerebral arteries and their smooth muscle cells (SMC) to favor smaller diameters with increased hydraulic resistance. Alternatively, this may suggest increased perivascular innervation and vasomotor activity in these vessels (see below). Consistent with the first possibility, middle cerebral arteries were characterized by significantly increased arterial wall (medial) thickness and number of layers of vascular SMC in the media. These, with other structural features, were significantly greater for the LTH-acclimatized fetuses, compared with normoxic controls (31). Table 1 summarizes some of these responses for the LTH-acclimatized fetus compared with normoxic controls. Overall, the LTH-induced changes reflected SMC proliferation (medial hyperplasia) and, to a lesser degree, expansion of extracellular matrix (and also augmented perivascular innervation, see below). This greater wall thickness would tend to amplify the effects of vasoconstrictors. Also, in fetal cerebral arteries, LTH significantly increased base soluble protein content, which includes cytosolic and enzymatic but not structural proteins (Ref. 46; Table 1). Depending on the type of proteins expressed, the greater wall thickness could increase resistance to rupture if highly branched collagen was laid down, or the opposite if this were to be replaced with nonbranched collagen. Quite obviously, vascular remodeling with these morphological changes in the LTH vessels could have major effects on cerebrovascular contractility in later life.

ELECTROMECHANICAL COUPLING

Plasma membrane L-type Ca²⁺ channels. In vascular SMC, electromechanical coupling describes the relation between membrane potential and contractile tone intrinsic to excitable smooth muscle. The single most important component of this coupling is the L-type Ca²⁺ channel, which, by virtue of its voltage-dependent conductivity, directly links changes in membrane potential to the rate of Ca²⁺ influx (66). For many vessels, including cerebral arteries (51, 55), calcium entry through L-type Ca²⁺ channels constitutes the main fraction of contractile calcium, owing to sparse or poorly developed sarcoplasmic reticulum. This is particularly true for immature cerebral arteries, which are essentially totally dependent on Ca²⁺ influx through L-type Ca²⁺ channels for contraction (1, 41, 44).

A common approach to assess electromechanical coupling is to monitor the contractions produced by high concentrations of extracellular K⁺. This abolishes the transmembrane K⁺ gradient, depolarizes the cell, and increases Ca²⁺ influx via the L-type Ca²⁺ channels. By this approach, K⁺-induced tensions in LTH-acclimatized fetal cerebral arteries were not significantly different from those of normoxic controls (43, 46). Because L-type Ca²⁺ channel density was significantly greater
<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Hypoxic</th>
<th>%Δ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO2, Torr</td>
<td>25±1</td>
<td>19±1</td>
<td>-24†</td>
<td>34, 35, 36</td>
</tr>
<tr>
<td>[Hbg], g/dl</td>
<td>10±0.7</td>
<td>12.6±0.6</td>
<td>25*</td>
<td>36</td>
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<td>O2 content, ml/dl</td>
<td>7.7±0.5</td>
<td>7.8±0.5</td>
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<tr>
<td>PaCO2, Torr</td>
<td>42±1</td>
<td>38±1</td>
<td>-9*</td>
<td>36</td>
</tr>
<tr>
<td>pH</td>
<td>7.36±0.01</td>
<td>7.37±0.01</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Vessel resting inside luminal diameter, mm</td>
<td>0.94±0.07</td>
<td>1.30±0.04</td>
<td>39*</td>
<td>31</td>
</tr>
<tr>
<td>Media thickness, μm</td>
<td>21±1</td>
<td>30±6</td>
<td>42*</td>
<td>31</td>
</tr>
<tr>
<td>Media cross-sectional area, μm² x 10⁻³</td>
<td>67±7</td>
<td>124±22</td>
<td>85*</td>
<td>31</td>
</tr>
<tr>
<td>Number of layers of SMC in media</td>
<td>5.6±0.2</td>
<td>11.5±2.1</td>
<td>105†</td>
<td>31</td>
</tr>
<tr>
<td>Base-soluble protein, %dry wt</td>
<td>24.5±2.2</td>
<td>30.0±4.1</td>
<td>22*</td>
<td>46</td>
</tr>
<tr>
<td>Water, %wt</td>
<td>80.0±0.8</td>
<td>82.5±1.1</td>
<td>46</td>
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<tr>
<td>K⁺ max tension, g</td>
<td>1.0±0.06</td>
<td>1.1±0.1</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>K⁺ stress, 10⁻⁶ dyn/cm²</td>
<td>0.41±0.05</td>
<td>0.44±0.05</td>
<td>7</td>
<td>46</td>
</tr>
<tr>
<td>L-type Ca²⁺ channel</td>
<td>100±10</td>
<td>100±10</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>%Inhibition tension by nifedipine</td>
<td>7.3±0.1</td>
<td>7.7±0.1</td>
<td>53</td>
<td>40</td>
</tr>
<tr>
<td>pIC50</td>
<td>7.2±0.1</td>
<td>7.6±0.1</td>
<td>68</td>
<td>43</td>
</tr>
<tr>
<td>KATP channel, %inhibition tension by pinacidil</td>
<td>5.2±0.1</td>
<td>4.7±0.1</td>
<td>-10%</td>
<td>43</td>
</tr>
<tr>
<td>KATP channel, %inhibition fluorescence ratio by pinacidil</td>
<td>72±8</td>
<td>90±5</td>
<td>25*</td>
<td>43</td>
</tr>
<tr>
<td>pIC50</td>
<td>4.8±0.1</td>
<td>4.3±0.1</td>
<td>-10%</td>
<td>43</td>
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<tr>
<td>Kc channel, %inhibition tension by NS-1619</td>
<td>100±10</td>
<td>41±5</td>
<td>-59%</td>
<td>43</td>
</tr>
<tr>
<td>pIC50</td>
<td>7.6±0.1</td>
<td>7.5±0.1</td>
<td>43</td>
<td></td>
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<tr>
<td>Kc channel, %inhibition fluorescence ratio by NS-1619</td>
<td>37±6</td>
<td>Unchanged</td>
<td>43</td>
<td></td>
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<tr>
<td>Membrane potential, mV</td>
<td>-26.1±1.4</td>
<td>-42.0±5.2</td>
<td>61†</td>
<td>40</td>
</tr>
<tr>
<td>BK channel current density, pA/pF</td>
<td>57.9±6.6</td>
<td>75.5±4.8</td>
<td>30*</td>
<td>40</td>
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<tr>
<td>P, at 40 mV of dephosphorylation state</td>
<td>0.15±0.04</td>
<td>0.79±0.06</td>
<td>42%</td>
<td>40</td>
</tr>
<tr>
<td>Calcium set point, 10⁻⁶ M</td>
<td>4.7</td>
<td>3.0</td>
<td>-36%</td>
<td>40</td>
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<tr>
<td>Right shift by dephosphorylation state, mV</td>
<td>52±3.1</td>
<td>11±5.6</td>
<td>-79%</td>
<td>40</td>
</tr>
<tr>
<td>V1/2 of dephosphorylated channels, mV</td>
<td>64±4.8</td>
<td>23±6.5</td>
<td>-63%</td>
<td>40</td>
</tr>
<tr>
<td>Endogenous activated kinase shift, mV</td>
<td>32±4.1</td>
<td>30±5.4</td>
<td>-6%</td>
<td>40</td>
</tr>
<tr>
<td>Endogenous PKA shift, mV</td>
<td>30±4.7</td>
<td>23±3.7</td>
<td>-26%</td>
<td>40</td>
</tr>
<tr>
<td>Channel-associated PKG shift, mV</td>
<td>36±0.3</td>
<td>35±0.3</td>
<td>-3%</td>
<td>40</td>
</tr>
<tr>
<td>Amine-induced tension, g (10⁻⁵ M serotonin and 2 x 10⁻⁵ M histamine)</td>
<td>2.0±0.2</td>
<td>1.5±0.2</td>
<td>-26%</td>
<td>46</td>
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<tr>
<td>Amine/K⁺ max, %</td>
<td>85±5</td>
<td>75±5</td>
<td>-12%</td>
<td>46</td>
</tr>
<tr>
<td>NE-induced tension, g</td>
<td>1.4±0.1</td>
<td>1.4±0.1</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>pD₂</td>
<td>6.1±0.1</td>
<td>6.1±0.1</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>NE/K⁺ max, %max</td>
<td>140±12</td>
<td>127±10</td>
<td>-9%</td>
<td>43</td>
</tr>
<tr>
<td>NE-induced fluorescence ratio</td>
<td>0.17±0.03</td>
<td>0.18±0.03</td>
<td>-6%</td>
<td>43</td>
</tr>
<tr>
<td>pD₂</td>
<td>6.5±0.1</td>
<td>6.5±0.1</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>NE/K⁺ max, %max</td>
<td>85±10</td>
<td>82±11</td>
<td>-3%</td>
<td>43</td>
</tr>
<tr>
<td>Slope tension vs. fluorescence ratio</td>
<td>4.1±0.4</td>
<td>3.7±0.6</td>
<td>-10%</td>
<td>43</td>
</tr>
<tr>
<td>α₁-AR density, fmol/mg protein</td>
<td>47±4</td>
<td>11±1</td>
<td>-77%</td>
<td>65</td>
</tr>
<tr>
<td>Ins(1,4,5)P³, basal</td>
<td>40±10</td>
<td>42±7</td>
<td>-6%</td>
<td>65</td>
</tr>
<tr>
<td>Ins(1,4,5)P³, response, %basal</td>
<td>345±27</td>
<td>225±30</td>
<td>-35%</td>
<td>65</td>
</tr>
<tr>
<td>Ins(1,4,5)P³/α₁-AR</td>
<td>5.2±1</td>
<td>11.7±1</td>
<td>125†</td>
<td>65</td>
</tr>
<tr>
<td>Ins(1,4,5)P³-receptor density, fmol/mg protein</td>
<td>115±15</td>
<td>22±3</td>
<td>-80%</td>
<td>71</td>
</tr>
<tr>
<td>α₁-AR density, fmol/mg protein</td>
<td>201±18</td>
<td>122±14</td>
<td>-39%</td>
<td>13, 48</td>
</tr>
<tr>
<td>ERK1/2—total (%control)</td>
<td>1.0</td>
<td>0.9±0.1</td>
<td>-10</td>
<td>69</td>
</tr>
<tr>
<td>—Phosphorylated</td>
<td>1.0</td>
<td>2.5±0.4</td>
<td>150†</td>
<td>69</td>
</tr>
<tr>
<td>MLC20—total (%control)</td>
<td>1.0</td>
<td>5.5±1.0</td>
<td>450†</td>
<td>69</td>
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<tr>
<td>—Phosphorylated</td>
<td>1.0</td>
<td>0.8±0.2</td>
<td>-20%</td>
<td>69</td>
</tr>
<tr>
<td>CPI17—total (%control)</td>
<td>1.0</td>
<td>1.9±0.2</td>
<td>90%</td>
<td>69</td>
</tr>
<tr>
<td>—Phosphorylated</td>
<td>1.0</td>
<td>1.1±0.2</td>
<td>10%</td>
<td>69</td>
</tr>
<tr>
<td>Vessel NE content, 10⁻⁹ g/10⁻³ g tissue</td>
<td>32±7</td>
<td>21±5</td>
<td>-34%</td>
<td>20</td>
</tr>
<tr>
<td>Stimulation-evoked NE release, 10⁻⁹ g/10⁻³ g tissue</td>
<td>820±90</td>
<td>470±80</td>
<td>-43%</td>
<td>20</td>
</tr>
<tr>
<td>Basal NE release, 10⁻⁹ g/10⁻³ g content</td>
<td>4.7±0.5</td>
<td>8.2±0.7</td>
<td>74%</td>
<td>19</td>
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<tr>
<td>Stimulation-evoked fractional NE release after uptake blockade</td>
<td>0.52±0.05</td>
<td>0.69±0.10</td>
<td>33%</td>
<td>19</td>
</tr>
<tr>
<td>Stimulation-induced adrenergic contractions, %Kmax at 8 Hz</td>
<td>0.5±0.1</td>
<td>8±2</td>
<td>&gt;150%</td>
<td>57</td>
</tr>
</tbody>
</table>

Values are means ± SE. %Δ, Percent change; SMC, smooth muscle cell; K⁺ATP, ATP-sensitive K⁺; Kc, calcium-activated K⁺; BK, “big” K⁺; P, open probability; pIC50, negative logarithm of IC₅₀; V₁/₂, current density; NE, norepinephrine; AR, adrenergic receptor; Ins(1,4,5)P₃, inositol 1,4,5-trisphosphate; ERK1/2, ERK 1 and 2; MLC20, 20-kDa myosin regulatory light chain; sGC, soluble guanylate cyclase; eNOS, endothelial nitric oxide synthase. *P < 0.05, †P < 0.01. ‡Because these are logarithmic functions, %Δ is not an accurate or appropriate representation.
in immature than mature cerebral arteries (15), it is possible that chronic hypoxia may depress L-type Ca\(^{2+}\) channel density in fetal arteries, consistent with hypoxia-accelerated maturation (see below). Arguing against this possibility, however, is the finding that LTH had no significant effect on K\(^{-}\)-induced increases in cytosolic Ca\(^{2+}\) in fetal arteries (43). This suggests that L-type Ca\(^{2+}\) channel function may be preserved in LTH-acclimatized fetuses and that hypoxic changes in agonist-induced contractile force are due to alteration of other mechanisms (see below).

**Plasma membrane K\(^{+}\) channels.** As noted above, a primary determinant of SMC tone and contractility is the resting membrane potential, which, in turn, is determined chiefly by plasma membrane K\(^{+}\) channel activity. Thus of particular relevance to this discussion are electrophysiological studies of membrane potential, changes with K\(^{+}\) channel activity, and the role of developmental maturation in this activity. The primary current-carrying, voltage-gated K\(^{+}\) channels in SMC myocytes are the large-conductance calcium-activated K\(^{+}\) (K\(_{Ca}\)) channels and voltage-activated K\(^{+}\) (K\(_{V}\)) channels. These K\(^{+}\) channels largely determine SMC electrical responses to many physiological stimuli, including basal stretch that governs myogenic tone (55). LTH modulates the activity of several types of K\(^{+}\) channels, as suggested by the finding that sensitivity to the ATP-sensitive K\(^{+}\) (K\(_{ATP}\)) channel opener pinacidil decreases significantly in hypoxic fetal arteries compared with controls (43). Similarly, in precontracted arteries from LTH fetuses the ability of the K\(_{Ca}\) channel activators NS-1619 to inhibit tension was significantly decreased compared with normoxic arteries (see Table 1). These findings suggest that either the density of these channels or their ability to be opened by NS-1619 is significantly increased (see below).

In perforated whole cell voltage-clamped preparations of fetal arteries, LTH resulted in increased K\(_{Ca}\) channel currents and outward current density increased approximately 30% (in comparison, current density of the adult myocytes was doubled) (40). In contrast, neither unitary single-channel conductance nor BK\(_{Ca}\) channel expression was altered in fetal myocytes. Also, in response to LTH the increased current density of the BK\(_{Ca}\) channels converted to a calcium set point for fetal myocytes, decreasing an additional 30% (from 4.7 to 3.0 \times 10^{-6} M). This suggests that the increased whole cell current density was a consequence of this lower Ca\(^{2+}\) set point, e.g., higher channel activity (Ref. 40; see Table 1).

An important feature of BK\(_{Ca}\) channels is the role of several enzymes and other factors in channel regulation (55, 60). These include PKA, PKC, and PKG. Other regulatory factors include cellular redox state, reactive O\(_2\) species, NO, carbon monoxide, eicosanoids, and so forth. Excised inside-out patch preparations from LTH fetal myocytes showed similar endogenous activity of both PKA and PKG, each of which are important mediators of BK\(_{Ca}\) phosphorylation and activity. Although BK channel activity is modulated by phosphorylation/dephosphorylation, this of itself could not account for the increased channel activity or decreased Ca\(^{2+}\) set point seen in response to LTH (40). For myocytes from LTH-acclimatized fetuses, the voltage activation curves of the dephosphorylated BK\(_{Ca}\) channels were markedly left-shifted compared with normoxic controls. Nonetheless, there was no significant difference in levels of endogenous PKA activity nor in either the amount of channel-associated PKA or the response to exogenously added catalytic subunit of PKA (PKAc). Likewise in these myocytes, endogenously activated PKG activity, amount of channel-associated PKG, and response to exogenous PKG were similar (40).

In summary, these results support the idea that in response to LTH fetal myocyte BK\(_{Ca}\) channel activity hyperpolarizes the cell at a lower [Ca\(^{2+}\)], thus contributing to decreased vascular tone. Nonetheless, these intrinsic differences appear independent of phosphorylation state and may be a consequence of channel \(\alpha\)-subunit splice variants, differences in association of \(\beta\)-subunits, or other factors. Quite obviously, the significant LTH-associated changes noted above for membrane potential, BK\(_{Ca}\) channel activity, and phosphorylation state have profound implications for the adult.

**PHARMACOMECHANICAL COUPLING**

**Calcium-dependent responses: adrenergic mediated.** In contrast to electromechanical coupling, which mainly governs the relations among membrane potential, calcium influx, and cytosolic [Ca\(^{2+}\)], pharmacomechanical coupling involves the influences of membrane receptor activation on second messenger signaling, contractile protein phosphorylation state, and thereby changes in contractile tone. This coupling, in turn, is highly specialized for each of the many receptor types and second messenger systems present in SMC and, in general, operates via both Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent mechanisms. One pharmacomechanical pathway modulated by LTH is that activated by the binding of the sympathetic neurotransmitter norepinephrine (NE) to adrenergic receptors.
As indicated by NE-induced contractility studies, although in the adult LTH attenuated NE-induced contraction such an effect was not seen in the fetus (43). Much of this effect appears attributable to hypoxic downregulation of adrenergic receptor density. In LTH-acclimatized fetal cerebral arteries, densities for $\alpha_1$- and $\alpha_2$-adrenergic receptors (AR) were decreased by 77% and 39%, respectively (Refs. 48, 65; Table 1). Because the magnitudes of the decreases in $\alpha_1$-AR density were much greater than the corresponding decreases in contractility, these results suggest the presence of a substantial receptor reserve (71) for $\alpha_1$-AR that is ablated by LTH. In the context of these studies, receptor reserve is defined as the number of receptors present in excess of the number required to obtain a maximal contractile response. Consistent with this concept of $\alpha$-AR reserve, NE-induced inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] mobilization following $\alpha_1$-AR activation was decreased 35% in cerebral arteries from LTH fetuses (65). Given that the ratio of the NE-induced Ins(1,4,5)P$_3$ signal to $\alpha_1$-AR density was increased by LTH in these vessels, the results also support the idea of considerable receptor reserve. Alternatively, LTH might also either enhance rates of Ins(1,4,5)P$_3$ synthesis or depress rates of Ins(1,4,5)P$_3$ turnover through mechanisms that have yet to be studied in cerebral arteries. Although LTH was without effect on basal Ins(1,4,5)P$_3$ levels in fetal arteries (65), their Ins(1,4,5)P$_3$ receptor density was decreased 80% (71). Despite these changes, NE-induced increases in cytosolic [Ca$^{2+}$] were unaltered in fetal arteries (although this was decreased 21% in the adult vessels). Nonetheless, it is clear that LTH affects multiple components of the $\alpha$-adrenergic signal transduction pathway.

Serotonin-mediated responses. Another important pharmacomechanical coupling pathway in cranial arteries is that activated by the binding of 5-HT to 5-HT$\alpha_2$ receptors. In ovine cranial arteries the 5-HT$\alpha_2$ subtype is most common, and LTH had no significant effect on this subtype expression in this model (64). Nonetheless, LTH appears to modulate the signaling pathway initiated by the 5-HT$\alpha_2$ receptor in a manner distinct from that observed for the $\alpha$-adrenergic pathway. Importantly, despite a hypoxia-induced decrease in 5-HT$\alpha_2$ receptor density of 49% in the fetal carotid arteries, LTH did not depress 5-HT-induced contractions in the middle cerebral artery. Correspondingly, the size of the 5-HT-induced Ins(1,4,5)P$_3$ signal was decreased 52% in response to LTH (64). Also, in skinned fiber preparations, LTH had no effect on myofilament Ca$^{2+}$ sensitivity in the fetal vessels. Thus, as for the $\alpha$-adrenergic system, the finding that LTH had little effect on 5-HT-induced contractility, despite large decreases in receptor density and Ins(1,4,5)P$_3$ signal, suggests that LTH also reduces receptor reserve for serotoninergic receptors. As for the $\alpha$-adrenergic pathway, it is clear that hypoxia affects multiple components of the serotoninergic pathway, but the mechanisms through which these influences are mediated remain unexplored. The serotoninergic pathway plays a critical role in hemostasis and appears to be involved in the development of vasogenic headache as well as cerebral vasospasm after intracranial hemorrhage (63). In this regard it may help to couple perfusion and metabolism via serotoninergic perivascular innervation (16).

This complex and age-dependent pattern of hypoxic effects reveals that for both the $\alpha$-adrenergic and serotoninergic pathways pharmacomechanical coupling is closely regulated by multiple physiological mechanisms. The mechanisms by which LTH modulates myocyte signaling, by changes in gene expression or in efficiency of message translation or through post-translational modifications or turnover of key signaling proteins, remain undetermined. The possible long-term sequelae of such changes are of obvious importance. Such investigations of these promising targets are clearly warranted, given that these pathways are critically important for cardiovascular regulation during embryonic and fetal development (23, 53) and have lasting impact on adult cerebrovascular homeostasis.

Calcium-independent responses. In contrast to the Ca$^{2+}$-dependent pathways described above, accumulating evidence suggests that Ca$^{2+}$-independent pathways also are important in modulating vascular tone. Of particular importance in this regard is the MAPK cascade and its substrates, ERK1 and ERK2 (ERK1/2) of 44 kDa (p44) and 42 kDa (p42), respectively (14, 17). The relationship of ERK1/2 activation to nuclear transcriptional events (22, 24) is well established (21, 62). However, relatively less is known about the role of ERKs in SMC contraction/relaxation. Although in normoxic fetal cerebral arteries the levels of phosphorylated (i.e., activated) ERK1/2 were significantly less than those of adults (68), in response to LTH the levels of phosphorylated ERK1/2 increased significantly (69). In addition, under these conditions the levels of phosphorylated 20-kDa myosin regulatory light chain and several associated proteins also increased significantly (Ref. 69; Table 1).

The alterations in many elements of signal transduction raise important questions in regard to LTH-related changes in myofilament Ca$^{2+}$ sensitivity. In normoxic control fetuses, myofilament Ca$^{2+}$ sensitivity is somewhat decreased in intact middle cerebral arteries compared with the adult (26, 41, 43). However, it appears to be greater in $\beta$-escin-permeabilized fetal cerebral arteries in relation to the adult (1). Nonetheless, despite the accelerated accelerated maturational effect of hypoxic acclimatization (see below), this probably does not involve modulation of myofilament Ca$^{2+}$ sensitivity.

Perivascular innervation. An additional important influence on cerebrovascular resistance is the release of vasoactive neurohormones from perivascular nerves. Cranial arteries receive an abundant perivascular innervation that includes adrenergic, cholinergic, and peptidergic components (49). In studies of electrical stimulation-induced NE release in fetal middle cerebral arteries, LTH was associated with a significant 30–40% decrease in vessel NE content and stimulation-evoked NE release and a 75% increase in basal NE release (Ref. 20; Table 1). Because inhibition of NO synthesis with N$^\omega$-nitro-l-arginine methyl ester (L-NAME; 10–5 M) significantly depressed electrical stimulation-induced NE release in the normoxic fetal middle cerebral artery, basal NO release appears to facilitate NE release. Of interest, this effect was abolished in arteries from LTH-acclimatized animals (20, 50). This may have resulted from the significant reduction of the relative abundance of neuronal NO synthase in the LTH arteries (50). In addition to this inhibitory effect on NO-mediated facilitation of NE release, LTH also may attenuate presynaptic inhibition of electrical stimulation-induced NE release. Blockade of prejunctional $\alpha_2$-adrenoceptors with idazoxan increased electrical stimulation-induced NE release by blocking presynaptic inhibition. The magnitude of this increase was attenuated by LTH, indicating that the ability of the presynaptic $\alpha_2$-adreno-
ceptor pathway to inhibit NE release was modulated by hypoxia (19). In contrast, LTH had no effect on NE overflow when both neuronal and extraneuronal reuptake were blocked (19). This suggests a negligible effect of hypoxia on synaptic reuptake mechanisms. Thus LTH attenuated both NO-mediated facilitation of NE release as well as $\alpha_2$-adrenoceptor-mediated inhibition of NE release. One consequence of these effects is that nerve stimulation-induced adrenergic contractions were enhanced to a considerable degree in fetal cerebral arteries (Ref. 57; Table 1). This LTH effect cannot be explained by the effects of hypoxia on fetal artery NE release. Rather, this suggests that hypoxia may accelerate maturation of the neuromuscular junction, perhaps by decreasing synaptic cleft width or in association with hypertrophy of the nerves. This idea would fit with the morphometric findings of greatly enlarged hyperplastic perivascular nerves (31). Again, this accelerated maturation may have profound implications for the cardiovascular system of the adult.

In regard to the effects of chronic hypoxia on perivascular peptidergic influences, the only transmitter studied to date is neuropeptide Y (NPY), which typically is coreleased with NE. Thus the effects of hypoxia on NPY release are of particular interest. Furthermore, the cerebral arteries of fetal rabbits subjected to intermittent or prolonged hypoxia have considerably enhanced NPY release (Table 1). Because the magnitude of this effect was modest, it is clear that the minimally altered basal cerebrovascular resistance characteristic of hypoxic acclimatization must involve other mechanisms. Recent ongoing studies have suggested that in fetal vessels LTH is associated with decreased abundance and activity of both endothelial NO synthase and soluble guanylate cyclase (Table 1). These changes do not appear to reduce basal cerebrovascular resistance significantly but may have important and lasting effects on cerebral vasoreactivity (C. R. White, J. M. Williams, and W. J. Pearce, unpublished observations).

**Perspectives**

Oxygen being essential for aerobic metabolism and life, high-altitude hypoxemia and cellular hypoxia pose significant challenges for survival. Fortunately, cardiovascular, hematologic, endocrinologic, and other acclimatization responses to chronic hypoxia mitigate that risk and help to preserve oxygen homeostasis at the organismal/cellular level. Thus high-altitude hypoxia has proven to be a useful model to explore the physiological mechanisms of both short- and long-term acclimatization, as well as the genetic adaptations that occur over multiple generations. Particularly because of its potent influence on function of the central nervous system, and also that of the cardiovascular and other organ systems, the pathophysiology of cerebral hypoxia, the regulation of cerebral blood flow at altitude, and the mechanisms involved in the development of, and responses to, cerebral edema and ischemia are of more than passing interest.

Under normal circumstances the developing fetus has an arterial PO$_2$ simulating “Mount Everest in utero” (25, 45), with severe hypoxia of more than momentary duration posing a particular peril. In turn, the mechanisms whereby the fetus of the mother at high altitude “acclimatizes” are of importance. Thus the basic cellular mechanisms of cerebrovascular signal transduction (electromechanical and pharmacomechanical coupling) and their role in the pathogenesis of dysregulation of CBF in the premature fetus and/or those near-term fetuses subjected to intermittent or prolonged hypoxia have considerable clinical implications.

A critical aspect of the studies reported here is the complexity of the problem. That different elements of the cerebral artery agonist-mediated presynaptic and postsynaptic contraction and relaxation mechanisms (including K$^+$ and Ca$^{2+}$ channel function, adrenergic- and serotoninergic-mediated responses, Ca$^{2+}$-dependent and -independent mechanisms) should be independently regulated and show differing responses to LTH should come as no surprise. Similarly, the findings that these responses to LTH in the fetal arteries differ significantly from those of the adult only strengthen the view that physiological stresses differ in immature as opposed to more mature animals, as do the homeostatic responses to these stresses. Whereas hypoxia is perhaps the most important primary stimulus at high altitude, clearly multiple secondary responses are of critical importance to the process of acclimatization. These include levels of circulating catecholamines, 5-HT, cortisol, other stress-related hormones, and growth factors and related cellular responses. Thus, overall in the cerebral vasculature, multiple mechanisms are recruited in response to chronic hypoxia both to promote relaxation and to attenuate...
contraction. Within this general framework, however, are multiple independent effects that are highly heterologous and unique for each signal transduction pathway.

For fetal cerebral arteries, the general character of many of the acclimatization responses is similar to that of the adult, with the trend that the magnitudes of hypoxic changes are smaller for contractility and larger for changes in receptor density and some other individual components. From a teleological point of view, one might argue that the studies reported here, such as the electrophysiological studies of K⁺ channel activity, serve to optimize CBF and thus O₂ delivery in the face of decreased O₂ levels. LTH appears to accelerate maturation of many of the fetal cerebrovascular mechanisms and serves to illustrate the concept of “developmental plasticity.” Whereas at high altitude changes in the fetal environment are buffered by maternal homeostatic mechanisms (for instance, a relatively small decrease in P₀₂ with no significant decrease in O₂ content or pH), it is also clear that the fetus constitutes a stress for the mother at altitude. From this perspective, it would enhance the chances for survival for both mother and fetus if the fetus were to mature more quickly. Although this is an attractive hypothesis, it remains highly speculative and in need of definitive experimental verification.

Several caveats are appropriate in terms of the present studies. Because fetal weights were not significantly decreased in the high-altitude animals (34), this probably does not constitute an appropriate “model” for intrauterine growth restriction but rather one of successful acclimatization. Also, many of the subcellular responses to LTH in the fetal cerebrovasculature differed significantly from those of the adult (data not discussed). This may have resulted from the degree of both absolute and relative hypoxia differing in the two age groups. Thus some of the changes observed probably reflect more than maturational differences. An additional consideration is that of the heterogeneity of vascular responses, not only within the several segments of a given vessel, but also in different vascular beds. Although the present studies apply to cerebral arteries, the extent to which such changes occur in cardiac, pulmonary, renal, or other vessels remains to be determined. In addition, we have few data on the extent to which the in vivo changes reported here are reflected in the in vivo regulation of CBF. Finally, and of vital importance, findings of the present studies demand long-term follow-up to ascertain the extent to which these or other alterations persist into adult life. Although we have initiated such studies on cardiovascular sequelae in the adult, these are ongoing and, as yet, not ready to report.

A critical element of the hypothesis of the fetal origins of adult cerebrovascular, cardiac, and other disease is that a given genotype can allow different phenotypes, depending on environmental conditions. One would postulate that such developmental plasticity, as observed in stress-induced alterations in the structure and function of these tissues, persists into adult life with potentially important consequences. Thus the structural and functional changes described in the present series of studies may have great relevance to the issue of prenatal programming. (As an aside, the extent to which such changes lay the groundwork for conditions such as acute mountain sickness and high-altitude cerebral edema in the adult also remains to be determined.)

A number of other issues remain. Of fundamental importance is the biological basis of both acclimatization responses per se and their role in programming. The mechanism(s) by which vascular endothelial cells and SMC sense hypoxia and identification of the signals remains a critical unknown. Related issues include the extent to which small artery function differs from that of larger vessels and the role of vascular innervation and growth factors in these responses to LTH. Many of these questions have been pursued for decades without elucidation. These and other factors may have profound implications for fetal programming of adult disease. Recent advances are promising, however, in that they point out the direction for the focus of more cellular and molecular investigations. The rapidly growing diversity and power of these new tools offer unprecedented opportunity and great promise for furthering our understanding of chronic hypoxia and the mechanisms by which it modulates cerebrovascular regulation. Hopefully, a new generation of studies of hypoxic-induced gene regulation and posttranscriptional modifications will yield key clues for which this field has so long been searching.

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