



# HHS Public Access

Author manuscript

*Semin Pediatr Neurol.* Author manuscript; available in PMC 2019 December 01.

Published in final edited form as:

*Semin Pediatr Neurol.* 2018 December ; 28: 17–28. doi:10.1016/j.spen.2018.05.003.

## Fetal Cerebrovascular Maturation: Effects of Hypoxia

**William J. Pearce, Ph.D.**

Professor of Physiology & Associate Director Center for Perinatal Biology, Loma Linda University School of Medicine

### Abstract

The human cerebral vasculature originates in the fourth week of gestation and continues to expand and diversify well into the first few years of postnatal life. A key feature of this growth is smooth muscle differentiation, whereby smooth muscle cells within cerebral arteries transform from migratory to proliferative to synthetic and finally to contractile phenotypes. These phenotypic transformations can be reversed by pathophysiological perturbations such as hypoxia, which causes loss of contractile capacity in immature cerebral arteries. In turn, loss of contractility affects all whole-brain cerebrovascular responses, including those involved in flow-metabolism coupling, vasodilatory responses to acute hypoxia and hypercapnia, cerebral autoregulation, and reactivity to activation of perivascular nerves. Future strategies to minimize cerebral injury following hypoxia-ischemic insults in the immature brain might benefit by targeting treatments to preserve and promote contractile differentiation in the fetal cerebrovasculature. This could potentially be achieved through inhibition of RTK mediated growth factors, such as VEGF and PDGR, which are mobilized by hypoxic and ischemic injury and which facilitate contractile dedifferentiation. Interruption of the effects of other vascular mitogens, such as endothelin and angiotensin-II, and even some miRNA species, also could be beneficial. Future experimental work that addresses these possibilities offers promise to improve current clinical management of neonates who have suffered and survived hypoxic, ischemic, asphyxic, or inflammatory cerebrovascular insults.

### Introduction

Recent innovations in high resolution imaging and genomic analysis have dramatically expanded studies of vascular biology, and these new approaches are now beginning to find applications in the study of the dynamic relations between structure and function in the immature cerebral circulation. This review summarizes some of these advances, with emphasis on the functional competence of the term fetal cerebral circulation, how it is affected by an adverse intrauterine environment, and possible new strategies to ameliorate fetal and neonatal cerebrovascular compromise.

**Mailing Address:** William J. Pearce, Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, California, 92350; wpearce@llu.edu, Phone: 909-558-4325.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## Origins of the Fetal Cerebrovasculature

The cerebral vasculature originates at about 24 days of gestation, and grows from an initial leptomenigeal plexus into identifiable vascular structures by 28 days<sup>1</sup>. These early vessels continuously grow and differentiate in waves that spread radially from the base of the brain towards the midbrain convexity. Between 6 and 7 weeks of gestation, portions of the dorsal aorta, first aortic arch, and third aortic arch merge to form the internal carotid artery. From this point onward, the cerebrovascular bed arborizes rapidly to facilitate perfusion of the developing cortex<sup>2</sup>. Human cerebral arteries continue to grow and diversify well into the third postnatal month at which time vessel density begins to stabilize<sup>3</sup>. In contrast, capillary density levels off near term in telencephalic white matter but continues to increase in cortical gray matter throughout the first three or four years of postnatal life<sup>4</sup>. This regional and temporal heterogeneity emphasizes the highly dynamic character of cerebral vasculogenesis, which in turn helps explain why immature cerebral vessels are so vulnerable to metabolic and mechanical injury. Importantly, throughout life the entire cerebral vasculature continues to undergo remodeling and angiogenesis, and can dedifferentiate in response to injury or stress even in the adult brain.

One of the most common and important pathophysiological stresses on the developing cerebrovasculature is hypoxia. When oxygen delivery cannot sustain local increases in metabolic activity, tissue hypoxia results. In turn, hypoxia stimulates multiple cell types within the brain to produce and release HIF-1 $\alpha$  (Hypoxia Inducible Factor), which is a transcription factor that can translocate to the cell nucleus. Within the nucleus, HIF-1 $\alpha$  can combine with its coactivator, HIF-1 $\beta$ , and bind the promoter regions of numerous genes that help mediate cellular responses to hypoxia. These include genes for Vascular Endothelial Growth Factor and Erythropoietin, as well as for glycolytic enzymes and proteins regulating mitochondrial function<sup>5,6</sup>. In combination with other angiogenic factors such as angiopoietin<sup>7</sup>, transforming growth factor- $\alpha$ <sup>8</sup>, and Wnts<sup>9</sup>, these factors govern the formation and differentiation of new cerebral vessels. This diversity of factors that regulate angiogenesis enables a highly robust and redundant regulation that assures close matching between vascular architecture and local metabolic activity. Such a system is essential to support the high rates of growth and expansion characteristic of the immature brain.

## Smooth Muscle Differentiation

In contrast to cardiac myocytes, which undergo terminal differentiation, smooth muscle does not<sup>10</sup>. Instead, smooth muscle, and in particular vascular smooth muscle, exists in a continuum of different phenotypes that include broad variations in the capacity for migration, proliferation, secretion and contraction<sup>10,11</sup>. In addition, these phenotypes can transform into one another under the influence of numerous growth factors, including Vascular Endothelial Growth Factors, Platelet-Derived Growth Factor, Fibroblast Growth Factors, amines such as norepinephrine and serotonin, peptides such as angiotensin and endothelin, and many others<sup>10</sup>. Correspondingly, the receptors for these vascular growth factors include both Receptor Tyrosine Kinases and G-Protein Coupled Receptors that convey information and signals from outside the smooth muscle cell into the vascular smooth muscle cytoplasm, and in many cases, on into the nucleus. Indeed, many of these

Author Manuscript

cytosolic signaling pathways involve selective phosphorylation and nuclear translocation of key vasoregulatory transcription factors such as myocardin, serum response factor, ternary complex factor, and Elk-1<sup>12</sup>. Another critically important influence on smooth muscle differentiation is “excitation-transcription coupling,” as first articulated by Wamhoff<sup>13</sup>. This idea couples cyclical changes in cytosolic calcium concentration, as occur during smooth muscle contraction and activation, with changes in gene expression through the actions of calcium-dependent kinases and phosphatases that target transcription factors. As such, these mechanisms help smooth muscle phenotype adapt to its history of activation by physiological stimuli such as changes in vasoactive agonist concentrations, sheer stress, and pressure-induced stretch. Together, these diverse influences constitute a near continuous barrage of factors that work together to govern the phenotype, structural, and functional characteristics of all cerebrovascular smooth muscle.

Author Manuscript

Owing to the diversity of factors that govern cerebrovascular smooth muscle phenotype, the dynamic and variable nature of their influences, and regional differences in their production, clearance, and/or activity, smooth muscle phenotype in immature cerebral arteries is highly heterogeneous. This is important when comparing cerebral arteries from different brain regions, but even within the same artery, adjacent smooth muscle cells may be of different phenotypes such that the artery wall includes cells in proliferative, migratory, synthetic, and contractile phenotypes at any given time. A key marker for smooth muscle phenotype in immature vascular smooth muscle is the myosin heavy chain isoform it expresses. At the beginning of smooth muscle differentiation, the cells initially express non-muscle myosin heavy chain. As differentiation proceeds, this non-muscle myosin is gradually replaced with smooth muscle myosin heavy chain of the S1 type<sup>14</sup>. As differentiation continues further, the cells begin to express the S2 isoform of smooth muscle myosin heavy chain, which is approximately 34 amino acids shorter than the S1 isoform and is required for maximal contractile efficiency. At full contractile differentiation, the S1 and S2 isoforms of myosin heavy chain are approximately equal in abundance, and these complex together to form the myosin hexamers that mediate contraction<sup>14,15</sup>. Similarly, many other contractile proteins also exhibit graded expression during differentiation, including smoothelin, calponin, caldesmon, and many others, each on an independent time course<sup>10,11</sup>. In aggregate, these changing patterns of expression and intracellular organization reveal that smooth muscle phenotype is a continuum of many overlapping characteristics that together define not just the state of differentiation at any moment in time, but also smooth muscle structure and function. In general, smooth muscle cells in fetal cerebral arteries exhibit a smaller proportion of fully contractile cells than found in adult arteries, which helps explain their reduced overall contractility, and differential reactivity to pathophysiological stimuli such as infection, inflammation, ischemia, and hypoxia<sup>15–17</sup>.

## Effects of Hypoxia on Cerebrovascular Structure and Function

Author Manuscript

One of the most common and important pathophysiological factors influencing cerebrovascular development is hypoxia<sup>18,19</sup>. At the level of the microcirculation, hypoxia acts through HIF-1a to promote angiogenesis, increase capillary density, and thereby reduce inter-capillary distances over which oxygen must diffuse to reach every cell within the brain parenchyma<sup>20–28</sup>. Given that hypoxia is a prominent aspect of ischemia, cerebral ischemia

also promotes many of the same microcirculatory responses<sup>29</sup>. In cerebral arteries upstream of the microcirculation, hypoxia causes significant changes in composition, including increases in wall thickness and protein content in a manner that is graded with the severity and duration of hypoxia<sup>18,30–32</sup>. Age is also a critical factor in remodeling responses to hypoxia<sup>33–35</sup> due in large part to developmental and maturational differences in the populations of cell surface receptors on the smooth muscle cells for both RTK-dependent and GPCR dependent vasotrophic factors such as VEGF<sup>15</sup> and endothelin-1<sup>19,36</sup>. For VEGF, receptor levels for both the FLT and FLK types are expressed at lower levels in fetal than adult arteries, and hypoxia dramatically increases both receptor types in both age groups<sup>37,38</sup>. Hypoxia also increases expression of endothelin-A receptors in fetal cerebral arteries<sup>36</sup>.

Commensurate with hypoxic changes in composition, cerebrovascular function is also modulated by hypoxia. Long-term hypoxia depresses contractile capacity through depressed expression of GPCR receptors that stimulate contraction<sup>30,39</sup>. Chronic hypoxia also can depress cerebrovascular IP<sub>3</sub> synthesis and IP<sub>3</sub> receptor density<sup>40</sup>. At the level of the contractile proteins, chronic hypoxia attenuates calcium-dependent myosin light chain phosphorylation but simultaneously enhances the ability of phosphorylated myosin to generate contractile force. The net result of these opposing effects is that myofilament calcium sensitivity is increased by chronic hypoxia in fetal cerebral arteries<sup>34</sup>. Chronic hypoxia also increases the agonist binding affinity of serotonergic receptors<sup>41</sup> and decreases the ability of calcium-sensitive and ATP-sensitive potassium channels to mediate vasorelaxation<sup>42,43</sup>. The integrated result of these effects is that reactivity to both contractile and relaxant stimuli is attenuated by chronic hypoxia in fetal cerebral arteries. As a result, fetal cerebral arteries respond more slowly and less forcefully to vasoactive stimuli. An obvious consequence of this pattern is that under conditions of hypoxia, fetal cerebral arteries are less able to match cerebral perfusion to local metabolic activity, constrict more slowly in response to rising arterial pressure, and overall are less efficient at maintaining cerebrovascular homeostasis.

Chronic hypoxia also influences the density and efficacy of the perivascular innervation of cerebral arteries. In perivascular nitridergic nerves, chronic hypoxia reduces the content of neuronal nitric oxide synthase and the ability of these nerves to release nitric oxide<sup>44</sup>. Given that NO released from nitridergic nerves can enhance norepinephrine release from nearby adrenergic nerve endings, the effects of chronic hypoxia on nitridergic nerves secondarily decrease the release of norepinephrine from perivascular adrenergic nerves<sup>35,45</sup>. In opposite fashion, chronic hypoxia upregulates the ability of perivascular adrenergic nerves to activate adrenergic receptors on fetal cerebral arteries<sup>32,46,47</sup>. These increases in adrenergic activation potentially influence phenotypic differentiation in fetal vascular smooth muscle, which in turn contribute to increased contractile differentiation in hypoxic fetal cerebral arteries<sup>48</sup>.

Just as gestational hypoxia can dramatically alter the pattern and timing of cerebrovascular development, it also powerfully influences neuronal and glial development and many of these effects have important secondary effects on cerebrovascular development. Antenatal maternal hypoxia can alter the expression of numerous genes involved in neuronal growth

and death, and as such hypoxia can precipitate neurological disorders in the neonate if it acts during key developmental time periods<sup>49–51</sup>. For example, hypoxia can act through epigenetic mechanisms to alter the renin-angiotensin system in both extracerebral<sup>52,53</sup> and cerebral tissues<sup>54–58</sup>. Owing to the potent influences of angiotensin on smooth muscle differentiation and phenotype<sup>59</sup>, it follows that hypoxic modulation of neuronal and glial angiotensin pathways will also influence local cerebrovascular development. Chronic hypoxia modulates the hypothalamo-pituitary-adrenal (HPA) axis, and alters the influence of glucocorticoids on fetal cerebrovascular maturation<sup>60,61</sup>; changes in circulating concentrations of glucocorticoids can program fetal cerebral arteries, with long-term consequences for cerebrovascular structure and function, including depression of myogenic reactivity and regulation of cytosolic calcium within cerebrovascular smooth muscle<sup>62</sup>. These myriad effects of hypoxia vary with the intensity and duration of hypoxia, and are heavily dependent upon developmental age and brain region. Although this heterogeneity can greatly complicate understanding of how a specific insult will affect the immature brain and its vascular supply, it also emphasizes that growth and development of the fetal brain as a whole is highly integrated across multiple cell types, is robust due to redundancies in the mechanisms regulating growth, and that together these systems normally enable highly coordinated patterns of functional maturation.

### Cerebrovascular Endothelium and the Blood-Brain Barrier

As in all vascular beds, the endothelium of fetal cerebral arteries serves four main functions important for: 1) angiogenesis; 2) hemostasis; 3) blood brain barrier function; and 4) vascular tone. The first of these to mature is the ability of the endothelium to initiate capillary angiogenesis through the release of vascular growth factors such as Platelet-Derive Growth Factors, Fibroblast Growth Factors, Thrombospondin, and Insulin-Like Growth Factors, all of which promote angiogenesis<sup>63</sup>. Conversely, the endothelium can release angiostatic factors that inhibit angiogenesis, including nitric oxide, prostacyclin, TGF- $\beta$ , heparin, and heparin sulfate. As a whole, this diversity of factors enables the immature endothelium of fetal cerebral arteries to closely control the high rates of vascular growth and expansion needed to support the rapid growth of the brain during late fetal and early neonatal life.

The hemostatic functions of the cerebrovascular endothelium become functionally competent in the 3<sup>rd</sup> trimester, at which time intact endothelial cells begin to continuously release nitric oxide and prostacyclin, which in turn inhibits initiation of hemostasis.<sup>64</sup>. Healthy endothelial cells also contain multiple pro-coagulant factors, including tissue factor (thromboplastin), von Willebrand factor, factor V, factor X, and platelet activating factor. Endothelial injury causes the release of these factors and initiation of hemostasis. Throughout late fetal development and early postnatal maturation, endothelial content and the ability to release these factors increases steadily<sup>65</sup>, as does production and release of nitric oxide<sup>66–68</sup>.

A critically important and unique characteristic of cerebrovascular endothelium is its ability to efficiently facilitate or restrict the movement of substances between circulating blood and the brain interstitial space<sup>69</sup>. This endothelial blood-brain-barrier function is due in large

part to tight junctions that bind adjacent endothelial cells to one another. Cerebrovascular endothelium also expresses the integral membrane protein, occludin, which spans intracellular gaps and further binds adjacent endothelial cells together<sup>70</sup>. In addition, cerebrovascular endothelium also expresses claudins, a family of cell-cell adhesion molecules that increase electrical and hydraulic resistance across the cerebrovascular endothelial layer<sup>71,72</sup>. The expression and localization of all of these components of the blood-brain-barrier continues throughout fetal life, and attain full functional maturity, as characterized by low hydraulic permeability, not until well after birth<sup>73–75</sup>.

A major limit on the rate at which the immature blood-brain-barrier tightens to reduce its overall permeability is the rapid pace of angiogenesis in the fetal brain. In newly formed capillaries, tight junctions, occludins, and claudins appear gradually over several days or weeks and during this initial maturation period, initial permeability is high as is electrical conductivity. The astrocytes that envelop cerebral capillaries are not fully mature at birth<sup>76,77</sup>.

Similarly, subendothelial pericytes, which help tighten and maintain the blood-brain-barrier, also are more sparse in fetal than in adult cerebral arteries<sup>72</sup>. Together, these characteristics render the immature blood-brain barrier less efficient and more vulnerable to stress and injury than the fully mature blood-brain barrier that begins to appear only in early postnatal life<sup>78</sup>.

Another important role of the cerebrovascular endothelium is the regulation of local vascular tone through the release of vasoactive factors, one of the most important of which is nitric oxide<sup>79</sup>. The short half-life of nitric oxide restricts its influence to nearby smooth muscle, in which it activates soluble guanylate cyclase and promotes vasodilatation<sup>80</sup>. The physiological release of nitric oxide is stimulated by shear stress, but this mechanism is attenuated in fetal compared to adult cerebral arteries<sup>66–68</sup>. Even so, this effect of shear stress, in turn, implies that any influence that increases blood viscosity (polycythemia, hyperproteinemia, dehydration, etc.) or blood flow velocity, will correspondingly enhance nitric oxide release from a healthy cerebrovascular endothelium. The fetal cerebrovascular endothelium also releases the vasodilator prostacyclin<sup>81</sup>, but through pathways that are fully developed in fetal cerebrovascular endothelium<sup>82</sup>. Fetal cerebrovascular endothelium also can release a broad variety of other vasoactive molecules, including the vasodilator Endothelium-Derived Hyperpolarizing Factor<sup>83</sup>, and vasoconstrictors such as endothelin, thromboxane A<sub>2</sub>, and superoxide<sup>84</sup>. Overall, however, the structural and functional immaturity of the fetal cerebrovascular endothelium generally attenuates its influence on vascular tone in fetal, compared to adult, cerebral arteries.

Hypoxia of even short duration can modulate multiple aspects of endothelial function in cerebral arteries, including dysregulation of initial vessel formation and architecture (vasculogenesis) secondary to increased VEGF activity. Thus, sustained in utero hypoxia during human gestation can increase fetal blood-brain permeability<sup>85</sup> and attenuate endothelium-dependent vasodilatation<sup>30</sup> through mechanisms that appear to be conserved across many vertebrate species, including birds<sup>86</sup>. The depressive effects of chronic hypoxia on endothelium-dependent vasodilatation involve depression of transcription of the eNOS

gene, and inhibition of synthesis of functional eNOS protein<sup>87</sup>. In parallel, hypoxia also inhibits expression of soluble guanylate cyclase in cerebrovascular smooth muscle, and thus reduces the ability of endothelial NO to stimulate cGMP synthesis and promote vasodilatation<sup>88</sup>. In contrast to chronic hypoxia, acute hypoxia can enhance the release of endothelium-dependent relaxant factors such as nitric oxide<sup>89</sup>, although the direct contribution of prostanoids in this effect appears to be minimal<sup>90</sup>. The effects of hypoxia, either acute or chronic, on the release of vasoactive molecules other than nitric oxide or prostanoids from the fetal cerebrovascular endothelium remain largely unexplored.

## Fetal and Neonatal Whole Brain Cerebrovascular Reactivity

A main theme of the fetal and neonatal brain is heterogeneity: structural, functional, and regional. Cerebral arteries of different size also exhibit different characteristics, variations in the size and phenotype of smooth muscle cells, extent and type of innervation at the adventitial-medial junction, and patterns of receptor and ion-channel expression, etc. A cerebral artery with a diameter of 150  $\mu\text{m}$  from the brainstem also has different characteristics than a similarly-sized artery from the cortex or pial circulation; local factors heavily influence vascular differentiation. Developmental age further accentuates this heterogeneity such that cerebral arteries more proximal to the heart appear to mature more quickly than those distal to the heart. Another important type of cerebrovascular heterogeneity is chronological heterogeneity, which reflects the recent history of activation of smooth muscle by local vasoactive influences. These influences typically change patterns of phosphorylation of contractile proteins, membrane receptors, and other determinants of contractility, such that vasoreactivity to any given stimulus depends heavily upon the recent past. Such diversity in heterogeneity related to artery size, age, region of origin, and history of activation, complicates appreciation of global patterns of regulation, but is the very feature that enables the immature brain to maintain cerebrovascular homeostasis despite constantly changing and highly variable conditions.

## Flow-Metabolism Coupling

At its most fundamental level, cerebrovascular homeostasis requires a tight coupling between local cerebral metabolic rate and local perfusion. From this perspective, local metabolic rate is the primary variable driving changes in local cerebral perfusion, regardless of all other aspects of cerebrovascular heterogeneity. Given that brain metabolism is uniquely reliant on the catabolism of glucose, the coupling between blood and cerebral metabolism typically involves simultaneous coupling between the rates of glucose oxidation, oxygen consumption, and local cerebral perfusion<sup>91</sup>. Compared to adult values, human neonatal cerebral blood flow and metabolic rate are both low at birth but increase gradually through the first few years of postnatal life and reach a maximum at about age six, after which rates of cerebral blood flow and metabolism begin a gradual decline that continues throughout juvenile, adolescent, and adult life<sup>92</sup>. At all ages, however, the rates of glucose catabolism, oxygen consumption, and cerebral perfusion are tightly coupled in virtually all mammalian species, including humans<sup>93-95</sup>.

Decades of intensive research have revealed that coupling between cerebral metabolism and perfusion is mediated by a broad variety of factors, one of the most important of which is adenosine<sup>96</sup>. As the oxygen supply-demand ratio begins to fall, ATP becomes progressively converted to ADP, AMP and then adenosine, which is released from all cell types in the brain parenchyma<sup>91</sup>. The metabolic pathways that mediate this coupling between the oxygen supply-demand ratio and adenosine release are fully functional in the fetal brain, and as the overall rate of cerebral metabolism increases throughout late fetal and early postnatal life, so too does the brain interstitial concentration of adenosine<sup>97</sup>. The adenosine A2a receptor subtype that promotes vasodilatation when bound to adenosine, is expressed on cerebrovascular smooth muscle cells early in fetal brain development and the presence of these receptors in cerebral arteries persists throughout adult life<sup>98</sup>. In turn, A2a receptors enable feedback regulation that ultimately couples increased extracellular adenosine concentrations to increased cerebral perfusion and increased oxygen supply in the immature brain<sup>99–101</sup>. This coupling is critically important during postnatal life as fetal hemoglobin is gradually replaced with adult hemoglobin, which in turn increases the mass of oxygen that can be extracted from each unit of hemoglobin<sup>101,102</sup>. The adenosine pathway is also a critical mediator of hypoxic vasodilatation in cerebral arteries of all ages, which implies that any pathophysiological disturbance that interferes with the release of adenosine, or its binding to vascular A2a receptors, will interrupt hypoxic flow-metabolism coupling<sup>103</sup>.

### Hypercapnic Vasodilatation

Carbon dioxide is a universally recognized cerebral vasodilator that can increase cerebral blood flow in many different species of all ages, from fetus to adult<sup>93,104</sup>. Importantly, the magnitude of the vasodilator response to hypercapnia gradually increases during postnatal life<sup>105–107</sup>, which has been attributed to parallel increases in reactivity to reduced pH as cerebral arteries mature<sup>108</sup>. Age-related decreases in hematocrit have also been suggested to contribute to age-related increases in cerebral CO<sub>2</sub> reactivity<sup>109</sup>. Hypercapnia also appears to stimulate the synthesis and release of vasodilator prostaglandins<sup>110–112</sup>, although their cellular origin remains uncertain and may include astrocytes<sup>113</sup> and cerebrovascular endothelial cells<sup>114</sup> as well as other cell types of the cerebral parenchyma. Other mechanisms may also contribute to hypercapnic vasodilatation, including release of nitric oxide from either neurons or endothelial cells<sup>115,116</sup>, and activation of ATP-sensitive and calcium-sensitive potassium channels<sup>117,118</sup>. Conversely, hypercapnic vasodilatation in the immature cerebral circulation appears not to depend upon changes in cerebral metabolic rate<sup>106,119,120</sup> or activation of perivascular nerves<sup>121</sup>. Without doubt, multiple mechanisms mediate the cerebrovascular response to hypercapnia, which appears due in large part to the ability of carbon dioxide to readily diffuse into all cell types of the brain, where it can differentially disturb intracellular acid-base balance.

### Acute Hypoxic Vasodilatation

Given that the human cerebral circulation is largely inaccessible to investigation, particularly in the fetus, most understanding of hypoxic cerebral vasodilatation in the immature brain has originated from studies in animals<sup>122</sup>.



Across all mammalian species, acute or short-term hypoxia produces a marked cerebral vasodilatation in all age groups including fetuses and neonates<sup>97,108,123,124</sup>. Investigations of these responses in premature human neonates have largely corroborated the results from animal models<sup>125</sup>. As might be expected, the areas of the brain that exhibit the highest metabolic rates also demonstrate the strongest immediate responses to hypoxia<sup>75,126–129</sup>. The magnitude of the cerebrovascular response to hypoxia is also graded with the severity of hypoxia, with the important result that hypoxic increases in cerebral blood flow at least initially maintain the oxygen supply-demand ratio and the oxygen extraction fraction over a broad range of blood oxygen tension values<sup>130</sup>. If hypoxia becomes more severe and arterial oxygen tensions fall below about 50 mm Hg, rates of cerebral perfusion can become maximal, in which case the oxygen extraction fraction begins to increase. Once both cerebral perfusion and oxygen extraction are maximal, sustained or more severe hypoxia will decrease cerebral oxygen consumption, increase rates of anaerobic glycolysis, and ultimately cause lactic acidosis<sup>131</sup>. In essence, this sequence of events is an extrapolation of the adenosine-dependent mechanisms that govern physiological maintenance of the oxygen supply-demand ratio and are fully functional in neonates of most vertebrate species<sup>97,123,132</sup>. Aside from adenosine, acute hypoxia promotes release of many other vasoactive factors, including vasodilator prostaglandins<sup>133</sup> and endothelial factors<sup>89</sup>. Hypoxia also has potent direct effects on cerebrovascular smooth muscle that attenuate the influx and mobilization of calcium essential for contraction<sup>134</sup>. Again, the relative importance of each of these mechanisms varies significantly amongst arteries of different size, from different regions, and at different ages. Even so, this pronounced redundancy of mechanisms constitutes a strong resistance to hypoxic injury and fails to maintain flow-metabolism coupling only under extreme conditions.

### Autoregulation

The efficiency of baroreceptor-mediated regulation of blood pressure improves with fetal weight and age<sup>135</sup>, which explains why slow oscillations in blood pressure are more common in fetuses and neonates than adults. These dynamic swings in blood pressure require that cerebral autoregulatory mechanisms, which help maintain flow-metabolism coupling despite changes in blood pressure, are intact in the fetal and neonatal brain. When assessed using indirect methods, cerebral autoregulation in human fetuses appears in the third trimester, perhaps as early as 23 weeks, and improves steadily thereafter<sup>93,136</sup>. Strictly defined, however, cerebral autoregulation maintains a relatively constant rate of cerebral blood flow in the face of changes in cerebral perfusion pressure<sup>137</sup>. Cerebral perfusion pressure, in turn, is calculated as the difference between mean arterial pressure and the greater of either intracranial pressure or cerebral venous pressure. This is an important distinction, because intracranial hypertension directly compresses the cerebral veins, obstructs venous outflow, and decreases cerebral perfusion pressure<sup>138,139</sup>.

In the neonates of most species, including humans, autoregulatory mechanisms promote a progressive vasodilatation as perfusion pressure falls to about 30 to 40 mmHg, which is the lower limit of autoregulation<sup>75,108,140,141</sup>. At perfusion pressures below the lower limit of autoregulation, cerebral blood flow falls in direct proportion to perfusion pressure.

Just as blood pressure gradually rises with developmental age, so too does the upper limit of autoregulation, which typically falls between 70 and 100 mm Hg in neonates. Under some circumstances, however, infant blood pressure oscillations can drive blood pressure above the upper limit of cerebral autoregulation, which increases the risk of vessel injury and rupture with a subsequent intracranial bleed<sup>141–143</sup>. This upper limit extends as high as 140 to 150 mm Hg in adults<sup>137</sup>, due in part to reflex sympathetic vasoconstriction<sup>144</sup>; this mechanism is not fully functional in immature cerebral arteries<sup>145</sup>.

Despite decades of intensive investigation, the cellular mechanisms that mediate cerebral autoregulation remain uncertain, and appear to involve a blend of myogenic, metabolic, and also neurogenic mechanisms<sup>137,146</sup>. As the name implies, myogenic reactivity originates within the smooth muscle itself as a direct response to stretch, which mobilizes intracellular calcium<sup>147,148</sup>. Numerous mechanisms have been suggested to help mediate this response, including the activation of cell-surface integrins<sup>149</sup> or stretch activation of TRP calcium channels<sup>150</sup>. In immature cerebral arteries, most of these cellular mechanisms are not yet fully developed, due in part to the relatively low fraction of smooth muscle cells that have undergone complete contractile differentiation. Correspondingly, any perturbation that promotes contractile dedifferentiation will also attenuate autoregulatory capacity, which helps explain why autoregulation is so vulnerable to injury in immature cerebral arteries.

Mechanisms that couple flow to metabolism reinforce myogenic mechanisms of neonatal cerebral autoregulation. Indeed, cerebral autoregulation can be conceived as a family of blood flow-perfusion pressure curves, one for each level of cerebral metabolic rate. In support of this view, decreases in cerebral perfusion of the neonatal brain increase interstitial concentrations of adenosine<sup>151,152</sup>, as well as prostanoids<sup>153,154</sup>, opioids<sup>155</sup>, and other vasodilator molecules. To an extent limited by the functional maturation of the perivascular innervation, sympathetic adrenergic mechanisms also can contribute to cerebral autoregulation, and extend the upper limit in neonates<sup>156,157</sup>, as in adults<sup>158</sup>. Given that cerebral autoregulation depends on mechanisms that are not fully mature in fetal and neonatal cerebral arteries, such as the extent of contractile differentiation of cerebrovascular smooth muscle and the functional efficiency of the perivascular innervation, it follows that neonatal cerebral autoregulation is somewhat fragile and highly vulnerable to many insults<sup>159</sup> including acidosis<sup>160</sup>, sustained or severe hypoxia<sup>161</sup>, asphyxia<sup>162</sup>, cerebral ischemia<sup>153</sup>, and intracranial hemorrhage<sup>163</sup>.

### Neurovascular Mechanisms

Fully mature cerebral arteries are innervated by a rich plexus of nerves that terminate near the adventitial-medial junction within the arterial wall<sup>164</sup>. This perivascular plexus includes contributions from adrenergic, cholinergic, and peptidergic nerves, all of which originate during a late embryonic outgrowth phase followed by a synthesis phase during which the pathways essential for neurotransmitter synthesis and storage are developed. Lastly, the synaptic varicosities of these nerves differentiate and become functional<sup>165</sup>. Typically, fetal arterial smooth muscle cells express post-synaptic receptors well before the nerves become functional with the result that reactivity to autonomic neurotransmitters is often greater in fetal than adult arteries<sup>166</sup>, due, in part, to the absence of presynaptic neuronal reuptake<sup>145</sup>.

As for many other aspects of fetal cerebrovascular development, the pace of maturation of the perivascular innervation is highly heterogeneous and depends on artery size, nerve type, region, and developmental age.

Adrenergic nerves are the most widely studied of all nerve types that make up the cerebrovascular perivascular innervation. Sympathetic fibers first develop in arteries supplying rostral brain regions and begin to establish functional varicosities between about 19 and 23 weeks of gestation in the human<sup>164</sup>. By term, exogenously applied norepinephrine can activate  $\alpha 1$  adrenergic receptors in small cerebral arteries<sup>167</sup> and  $\alpha 2$  adrenergic receptors in pial arterioles<sup>168</sup>, which elicits contraction<sup>168–170</sup>. Noradrenergic activation can also stimulate the synthesis and release of the vasodilator prostaglandin PGE<sub>2</sub>, which can attenuate vasoconstrictor responses to norepinephrine<sup>168</sup>. Electrical activation of perivascular sympathetic nerves can constrict cerebral arteries and decrease cerebral perfusion up to 25%, depending upon brain region and developmental age<sup>171,172</sup>. Together, these findings emphasize the regulatory importance of noradrenergic mechanisms in immature cerebral arteries<sup>170</sup>.

In parallel with adrenergic nerves, cholinergic nerves also begin to appear in the perivascular innervation somewhere between 19 and 23 weeks of gestation in human cerebral arteries, and become functional at term<sup>164</sup>. In contrast to adult cerebral arteries, fetal and neonatal cerebral arteries dilate in response to low concentrations of acetylcholine<sup>173</sup>, but contract in response to high concentrations<sup>166</sup>. Given that indomethacin can markedly attenuate acetylcholine-induced contractions in immature cerebral arteries, these contractions appear dependent upon the synthesis and release of vasoconstrictor prostaglandins such as thromboxane A<sub>2</sub> or PGF<sub>2</sub> $\alpha$ <sup>174</sup>. In turn, these findings suggest that the vasoactive effects of indomethacin, or any other cyclooxygenase inhibitor, when these drugs are administered to neonates.

Nerves that contain and release peptide neurotransmitters are the third component of the perivascular innervation of cerebral arteries<sup>175</sup>. Among this class of perivascular nerves, those that release neuropeptide-Y or vasoactive intestinal polypeptide are most common in human fetal cerebral arteries, particularly in arteries of the Circle of Willis and pial circulation<sup>176</sup>. The fetal perivascular innervation also includes sensory fibers that originate from the trigeminal ganglion and can release vasoactive intestinal polypeptide, calcitonin gene-related peptide, substance-P, somatostatin, and cholecystokinin<sup>177</sup>. Whereas the functions of the vasodilator peptides have been extensively studied in the adult cerebral circulation due to their possible involvement in migraine<sup>178</sup>, almost nothing is known of the functions of these neurotransmitters in the fetal cerebral circulation. In turn, studies of the involvement of perivascular peptides in the fetal cerebral circulation seem warranted, particularly in light of findings that these peptides contribute significantly to adult cerebrovascular responses to ischemia and intracranial hemorrhage<sup>175,179–181</sup>.

## Future Directions

One of the most common pathophysiological challenges for a developing fetus is in utero hypoxia, particularly if hypoxia persists over the final days or weeks before birth. In fetal

cerebral arteries, chronic hypoxia increases water content, total protein content, and wall thickness<sup>30</sup>. Accompanying these significant changes in structure and composition are depressed overall contractility and depressed endothelial vasodilator capacity<sup>30,31</sup> that is attributable both to a decreased capacity for endothelial NO synthesis, reduced soluble guanylate cyclase activity within cerebrovascular smooth muscle, and an attenuated ability of PKG to act on BK channels<sup>43,182,183</sup>. Chronic hypoxia also modulates the abundances, organization and function of contractile proteins, due partially to the effects of VEGF, resulting in a less efficient contractile apparatus<sup>15,34,184</sup>. Conversely, hypoxia also promotes the growth and expansion of the perivascular adrenergic innervation, which helps maintain contractile differentiation of cerebrovascular smooth muscle<sup>48,185</sup>. However, in aggregate these hypoxic changes yield a cerebrovasculature that is generally less reactive and slower to respond to changes in blood pressure, blood gases and other contractile stimuli than typical of a healthy normoxic fetus<sup>36,41,161</sup>. What then, is the best strategy to manage an infant that has suffered but survived in utero or perinatal hypoxia?

Fortunately, the fetal and neonatal cerebrovasculature is endowed with a broad variety of cellular mechanisms that enable compensation for stress and injury. When injury is severe but does not cause extensive apoptosis or death of local arterial smooth muscle, this compensation can cause the phenotypic dedifferentiation of cerebrovascular smooth muscle resulting in loss of contractile capacity. This is an important factor contributing to the long-recognized pattern of “pressure passive” cerebral blood flow following severe injury in the neonate<sup>186</sup>. In turn, this pattern of compensatory dedifferentiation implies that a “pressure passive” cerebral circulation does not always indicate irreversible loss of neonatal cerebrovascular function. The challenge then is to know how to rescue injured cerebrovascular smooth muscle and restore its contractile function and capacity for flow-metabolism coupling. Assuming that some assessment of cerebrovascular reactivity, perhaps using perfusion-weighted MRI techniques<sup>187</sup>, reveals persistence of at least some vascular reactivity to perturbations such as mild hypocapnia or hypercapnia, the strategy would be to promote contractile differentiation in the surviving cerebrovascular smooth muscle.

One possible approach to stimulating contractile differentiation might be to administer nitric oxide, which is a standard clinical therapy<sup>188</sup>. Nitric oxide stimulates the synthesis of cGMP, which in turn activates Protein Kinase G and promotes contractile differentiation<sup>189</sup>. Another strategy may be to antagonize VEGF, whose synthesis and release are directly stimulated by hypoxia<sup>190</sup>. VEGF, in turn, promotes not only angiogenesis, but also contractile dedifferentiation in arterial smooth muscle<sup>15</sup>. A molecule with about 60% homology to VEGF is PDGF, which can also promote contractile dedifferentiation<sup>191,192</sup>. Correspondingly, antagonism of the PDGF receptor following intracerebral hemorrhage has proven to help preserve contractile differentiation and reduce overall cerebral injury<sup>193</sup>. A variety of G-Protein Coupled Receptors, including those activated by endothelin<sup>36</sup> and angiotensin II<sup>59</sup>, also potently influence contractile differentiation, although the effects of inhibitors of these peptides have yet to be investigated in the context of hypoxic-ischemic injury in the immature brain. Finally, recent discoveries that multiple miRNA molecules, including miR-1<sup>194</sup>, miR-29c<sup>195</sup>, miR-145<sup>196</sup>, and potentially many others<sup>197</sup> can significantly influence smooth muscle phenotype. Future therapies may be possible through selective administration of these molecules, or their antagonists, to preserve or promote

contractile differentiation in the immature cerebrovasculature. This therapeutic potential is currently motivating intensive interest in the vascular actions of these molecules. Meanwhile, in the immediate future it seems reasonable to focus on the potential therapeutic benefit of treatments already approved for human use, including nitric oxide administration and antagonism of VEGF and PDGF receptors.

## Acknowledgments

### Grant Support:

Supported by USPHS grants P01-NS082184, P01-HD083132, P01-HD31226, and R01-NS076945

## Literature Cited

1. Gilles, FH, Leviton, A, Dooling, EC. The Developing Human Brain: Growth and Epidemiologic Neuropathology. Littleton, MA: John Wright; 1983.
2. Rhodes AJ, Hyde JB. POSTNATAL GROWTH OF ARTERIOLES IN THE HUMAN CEREBRAL CORTEX. *Growth*. 29:173–182.1965; [PubMed: 14324920]
3. Harnarine-Singh D, Hyde JB. Post-natal growth of the arterial net in the human cerebral pia mater. *Nature*. 225(5227):86–87.1970; [PubMed: 5410210]
4. Otto KB, Lierse W. The capillaries of various parts of the human brain in the fetal period and during the first years of life. *Acta Anat (Basel)*. 77(1):25–36.1970; [PubMed: 5533982]
5. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol*. 12(12):5447–5454.1992; [PubMed: 1448077]
6. Ham PB 3rd, Raju R. Mitochondrial function in hypoxic ischemic injury and influence of aging. *Prog Neurobiol*. 157:92–116.2017; [PubMed: 27321753]
7. Suri C, Jones PF, Patan S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 87(7):1171–1180.1996; [PubMed: 8980224]
8. Yun C, Mendelson J, Blake T, Mishra L, Mishra B. TGF-beta signaling in neuronal stem cells. *Disease markers*. 24(4–5):251–255.2008; [PubMed: 18525119]
9. Stenman JM, Rajagopal J, Carroll TJ, Ishibashi M, McMahon J, McMahon AP. Canonical Wnt signaling regulates organ-specific assembly and differentiation of CNS vasculature. *Science*. 322(5905):1247–1250.2008; [PubMed: 19023080]
10. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev*. 84(3):767–801.2004; [PubMed: 15269336]
11. Rensen SS, Doevendans PA, van Eys GJ. Regulation and characteristics of vascular smooth muscle cell phenotypic diversity. *Neth Heart J*. 15(3):100–108.2007; [PubMed: 17612668]
12. Parmacek MS. Myocardin-related transcription factors: critical coactivators regulating cardiovascular development and adaptation. *Circ Res*. 100(5):633–644.2007; [PubMed: 17363709]
13. Wamhoff BR, Bowles DK, Owens GK. Excitation-transcription coupling in arterial smooth muscle. *Circ Res*. 98(7):868–878.2006; [PubMed: 16614312]
14. Eddinger TJ, Meer DP. Myosin II isoforms in smooth muscle: heterogeneity and function. *Am J Physiol Cell Physiol*. 293(2):C493–508.2007; [PubMed: 17475667]
15. Hubbell MC, Semotiuk AJ, Thorpe RB, et al. Chronic hypoxia and VEGF differentially modulate abundance and organization of myosin heavy chain isoforms in fetal and adult ovine arteries. *Am J Physiol Cell Physiol*. 303(10):C1090–1103.2012; [PubMed: 22992677]
16. Gomis P, Kacem K, Sercombe C, Seylaz J, Sercombe R. Confocal microscopic evidence of decreased alpha-actin expression within rabbit cerebral artery smooth muscle cells after subarachnoid haemorrhage. *Histochem J*. 32(11):673–678.2000; [PubMed: 11272807]

17. Hellstrand P, Albinsson S. Stretch-dependent growth and differentiation in vascular smooth muscle: role of the actin cytoskeleton. *Can J Physiol Pharmacol.* 83(10):869–875.2005; [PubMed: 1633359]
18. Pearce W. Hypoxic regulation of the fetal cerebral circulation. *Journal of applied physiology* (Bethesda, Md : 1985). 100(2):731–738.2006;
19. Silpanisong J, Pearce WJ. Vasotrophic regulation of age-dependent hypoxic cerebrovascular remodeling. *Curr Vasc Pharmacol.* 11(5):544–563.2013; [PubMed: 24063376]
20. Xu K, Lamanna JC. Chronic hypoxia and the cerebral circulation. *J Appl Physiol.* 100(2):725–730.2006; [PubMed: 16421279]
21. Patt S, Sampaolo S, Theallier-Janko A, Tschairkin I, Cervos-Navarro J. Cerebral angiogenesis triggered by severe chronic hypoxia displays regional differences. *J Cereb Blood Flow Metab.* 17(7):801–806.1997; [PubMed: 9270497]
22. Boero J, Ascher J, Arregui A, Rovainen C, Woolsey T. Increased brain capillaries in chronic hypoxia. *J Appl Physiol.* 86(4):1211–1219.1999; [PubMed: 10194205]
23. Milner R, Hung S, Erokwu B, Dore-Duffy P, LaManna JC, del Zoppo GJ. Increased expression of fibronectin and the alpha 5 beta 1 integrin in angiogenic cerebral blood vessels of mice subject to hypobaric hypoxia. *Mol Cell Neurosci.* 38(1):43–52.2008; [PubMed: 18343155]
24. LaManna JC, Chavez JC, Pichiule P. Structural and functional adaptation to hypoxia in the rat brain. *J Exp Biol.* 207(Pt 18):3163–3169.2004; [PubMed: 15299038]
25. LaManna JC, Vendel LM, Farrell RM. Brain adaptation to chronic hypobaric hypoxia in rats. *Journal of applied physiology.* 72(6):2238–2243.1992; [PubMed: 1629078]
26. Li L, Welser JV, Milner R. Absence of the alpha v beta 3 integrin dictates the time-course of angiogenesis in the hypoxic central nervous system: accelerated endothelial proliferation correlates with compensatory increases in alpha 5 beta 1 integrin expression. *J Cereb Blood Flow Metab.* 30(5):1031–1043.2010; [PubMed: 20087368]
27. Kanaan A, Farahani R, Douglas RM, Lamanna JC, Haddad GG. Effect of chronic continuous or intermittent hypoxia and reoxygenation on cerebral capillary density and myelination. *American journal of physiology Regulatory, integrative and comparative physiology.* 290(4):R1105–1114.2006;
28. Ment LR, Stewart WB, Fronc R, et al. Vascular endothelial growth factor mediates reactive angiogenesis in the postnatal developing brain. *Brain Res Dev Brain Res.* 100(1):52–61.1997; [PubMed: 9174246]
29. Coulson RJ, Chesler NC, Vitullo L, Cipolla MJ. Effects of ischemia and myogenic activity on active and passive mechanical properties of rat cerebral arteries. *Am J Physiol Heart Circ Physiol.* 283(6):H2268–2275.2002; [PubMed: 12388247]
30. Longo LD, Hull AD, Long DM, Pearce WJ. Cerebrovascular adaptations to high-altitude hypoxemia in fetal and adult sheep. *Am J Physiol.* 264(1 Pt 2):R65–72.1993; [PubMed: 8430888]
31. Williams JM, Pearce WJ. Age-dependent modulation of endothelium-dependent vasodilatation by chronic hypoxia in ovine cranial arteries. *J Appl Physiol.* 100(1):225–232.2006; [PubMed: 16179402]
32. Longo LD, Pearce WJ. 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(1):R16–24.2005; [PubMed: 15590993]
33. Butler SM, Abrassart JM, Hubbell MC, et al. Contributions of VEGF to age-dependent transmural gradients in contractile protein expression in ovine carotid arteries. *Am J Physiol Cell Physiol.* 301(3):C653–666.2011; [PubMed: 21653901]
34. Nauli SM, Williams JM, Gerthoffer WT, Pearce WJ. Chronic hypoxia modulates relations among calcium, myosin light chain phosphorylation, and force differently in fetal and adult ovine basilar arteries. *J Appl Physiol.* 99(1):120–127.2005; [PubMed: 16036903]
35. Buchholz J, Duckles SP. Chronic hypoxia alters prejunctional alpha(2)-receptor function in vascular adrenergic nerves of adult and fetal sheep. *Am J Physiol Regul Integr Comp Physiol.* 281(3):R926–934.2001; [PubMed: 11507010]
36. Silpanisong J, Kim D, Williams JM, Adeoye O, Thorpe R, Pearce WJ. Chronic Hypoxia Alters Fetal Cerebrovascular Responses to Endothelin1. *Am J Physiol Cell Physiol.* 2017

37. Adeoye O, Bouthors V, Hubbell MC, Williams JM, Pearce WJ. VEGF Receptors Mediate Hypoxic Remodeling of Adult Ovine Carotid Arteries. *Journal of applied physiology* (Bethesda, Md : 1985). 2014
38. Adeoye OO, Butler SM, Hubbell MC, Semotiuk A, Williams JM, Pearce WJ. Contribution of increased VEGF receptors to hypoxic changes in fetal ovine carotid artery contractile proteins. *Am J Physiol Cell Physiol*. 304(7):C656–665.2013; [PubMed: 23325408]
39. Ueno N, Zhao Y, Zhang L, Longo LD. High altitude-induced changes in alpha1-adrenergic receptors and Ins(1,4,5)P3 responses in cerebral arteries. *Am J Physiol*. 272(2 Pt 2):R669–674.1997; [PubMed: 9124493]
40. Zhou L, Zhao Y, Nijland R, Zhang L, Longo LD. Ins(1,4,5)P3 receptors in cerebral arteries: changes with development and high-altitude hypoxia. *Am J Physiol*. 272(6 Pt 2):R1954–1959.1997; [PubMed: 9227613]
41. Teng GQ, Williams J, Zhang L, Purdy R, Pearce WJ. Effects of maturation, artery size, and chronic hypoxia on 5-HT receptor type in ovine cranial arteries. *Am J Physiol*. 275(3 Pt 2):R742–753.1998; [PubMed: 9728071]
42. Long W, Zhang L, Longo LD. Fetal and adult cerebral artery K(ATP) and K(Ca) channel responses to long-term hypoxia. *J Appl Physiol*. 92(4):1692–1701.2002; [PubMed: 11896039]
43. Thorpe RB, Hubbell MC, Silpanisong J, Williams JM, Pearce WJ. Chronic Hypoxia Attenuates the Vasodilator Efficacy of Protein Kinase G in Fetal and Adult Ovine Cerebral Arteries. *Am J Physiol Heart Circ Physiol*. 2017
44. Mbaku EM, Zhang L, Pearce WJ, Duckles SP, Buchholz J. Chronic hypoxia alters the function of NOS nerves in cerebral arteries of near-term fetal and adult sheep. *Journal of applied physiology*. 94(2):724–732.2003; [PubMed: 12433849]
45. Buchholz J, Edwards-Teunissen K, Duckles SP. Impact of development and chronic hypoxia on NE release from adrenergic nerves in sheep arteries. *Am J Physiol*. 276(3 Pt 2):R799–808.1999; [PubMed: 10070141]
46. Longo LD, Pearce WJ. High altitude, hypoxic-induced modulation of noradrenergic-mediated responses in fetal and adult cerebral arteries. *Comp Biochem Physiol A Mol Integr Physiol*. 119(3):683–694.1998; [PubMed: 9683407]
47. Gilbert RD, Pearce WJ, Longo LD. Fetal cardiac and cerebrovascular acclimatization responses to high altitude, long-term hypoxia. *High Alt Med Biol*. 4(2):203–213.2003; [PubMed: 12855052]
48. Adeoye OO, Silpanisong J, Williams JM, Pearce WJ. Role of the sympathetic autonomic nervous system in hypoxic remodeling of the fetal cerebral vasculature. *J Cardiovasc Pharmacol*. 65(4):308–316.2015; [PubMed: 25853949]
49. Lewis DA, Cruz D, Eggen S, Erickson S. Postnatal development of prefrontal inhibitory circuits and the pathophysiology of cognitive dysfunction in schizophrenia. *Ann N Y Acad Sci*. 1021:64–76.2004; [PubMed: 15251876]
50. Laviola G, Adriani W, Rea M, Aloe L, Alleve E. Social withdrawal, neophobia, and stereotyped behavior in developing rats exposed to neonatal asphyxia. *Psychopharmacology (Berl)*. 175(2):196–205.2004; [PubMed: 14985924]
51. Blumberg HP, Kaufman J, Martin A, Charney DS, Krystal JH, Peterson BS. Significance of adolescent neurodevelopment for the neural circuitry of bipolar disorder. *Ann N Y Acad Sci*. 1021:376–383.2004; [PubMed: 15251913]
52. Goyal R, Leitzke A, Goyal D, Gheorghe CP, Longo LD. Antenatal maternal hypoxic stress: adaptations in fetal lung Renin-Angiotensin system. *Reprod Sci*. 18(2):180–189.2011; [PubMed: 20978179]
53. Goyal R, Lister R, Leitzke A, Goyal D, Gheorghe CP, Longo LD. Antenatal maternal hypoxic stress: adaptations of the placental renin-angiotensin system in the mouse. *Placenta*. 32(2):134–139.2011; [PubMed: 21130492]
54. Goyal R, Goyal D, Leitzke A, Gheorghe CP, Longo LD. Brain renin-angiotensin system: fetal epigenetic programming by maternal protein restriction during pregnancy. *Reprod Sci*. 17(3):227–238.2010; [PubMed: 19923380]
55. Lavoie JL, Sigmund CD. Minireview: overview of the renin-angiotensin system—an endocrine and paracrine system. *Endocrinology*. 144(6):2179–2183.2003; [PubMed: 12746271]

56. Lenkei Z, Palkovits M, Corvol P, Llorens-Cortes C. Expression of angiotensin type-1 (AT1) and type-2 (AT2) receptor mRNAs in the adult rat brain: a functional neuroanatomical review. *Frontiers in neuroendocrinology*. 18(4):383–439.1997; [PubMed: 9344632]
57. McKinley MJ, Albiston AL, Allen AM, et al. The brain renin-angiotensin system: location and physiological roles. *Int J Biochem Cell Biol*. 35(6):901–918.2003; [PubMed: 12676175]
58. Nguyen G, Delarue F, Burckle C, Bouzahir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest*. 109(11):1417–1427.2002; [PubMed: 12045255]
59. Montezano AC, Nguyen Dinh Cat A, Rios FJ, Touyz RM. Angiotensin II and Vascular Injury. *Curr Hypertens Rep*. 16(6):431.2014; [PubMed: 24760441]
60. Myers DA, Ducsay CA. Adrenocortical and adipose responses to high-altitude-induced, long-term hypoxia in the ovine fetus. *J Pregnancy*. 2012:681306.2012; [PubMed: 22666594]
61. Newby EA, Myers DA, Ducsay CA. Fetal endocrine and metabolic adaptations to hypoxia: the role of the hypothalamic-pituitary-adrenal axis. *Am J Physiol Endocrinol Metab*. 309(5):E429–439.2015; [PubMed: 26173460]
62. Durrant LM, Khorram O, Buchholz JN, Pearce WJ. Maternal food restriction modulates cerebrovascular structure and contractility in adult rat offspring: effects of metyrapone. *Am J Physiol Regul Integr Comp Physiol*. 306(6):R401–410.2014; [PubMed: 24477541]
63. Inagami T, Naruse M, Hoover R. Endothelium as an endocrine organ. *Annu Rev Physiol*. 57:171–189.1995; [PubMed: 7778863]
64. Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. *The American journal of pediatric hematology/oncology*. 12(1):95–104.1990; [PubMed: 2178462]
65. Bleyer WA, Hakami N, Shepard TH. The development of hemostasis in the human fetus and newborn infant. *J Pediatr*. 79(5):838–853.1971; [PubMed: 4940408]
66. White CR, Hamade MW, Siami K, et al. Maturation enhances fluid shear-induced activation of eNOS in perfused ovine carotid arteries. *Am J Physiol Heart Circ Physiol*. 289(5):H2220–2227.2005; [PubMed: 15923310]
67. Liang Y, Fang M, Li J, Yew DT. Immunohistochemical localization of endothelial isoform (eNOS) in human cerebral arteries and the aorta. *Int J Neurosci*. 116(12):1403–1417.2006; [PubMed: 17145676]
68. Williams JM, Hull AD, Pearce WJ. Maturation modulation of endothelium-dependent vasodilatation in ovine cerebral arteries. *Am J Physiol Regul Integr Comp Physiol*. 288(1):R149–157.2005; [PubMed: 15358604]
69. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis*. 37(1):13–25.2010; [PubMed: 19664713]
70. Hirase T, Staddon JM, Saitou M, et al. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci*. 110(Pt 14):1603–1613.1997; [PubMed: 9247194]
71. Haseloff RF, Dithmer S, Winkler L, Wolburg H, Blasig IE. Transmembrane proteins of the tight junctions at the blood-brain barrier: structural and functional aspects. *Semin Cell Dev Biol*. 38:16–25.2015; [PubMed: 25433243]
72. Lee HS, Han J, Bai HJ, Kim KW. Brain angiogenesis in developmental and pathological processes: regulation, molecular and cellular communication at the neurovascular interface. *FEBS J*. 276(17):4622–4635.2009; [PubMed: 19664072]
73. Risau W, Wolburg H. Development of the blood-brain barrier. *Trends Neurosci*. 13(5):174–178.1990; [PubMed: 1693235]
74. Stewart PA, Hayakawa EM. Interendothelial junctional changes underlie the developmental ‘tightening’ of the blood-brain barrier. *Brain Res*. 429(2):271–281.1987; [PubMed: 3567665]
75. Buckley NM. Maturation of circulatory system in three mammalian models of human development. *Comp Biochem Physiol A Comp Physiol*. 83(1):1–7.1986; [PubMed: 2868826]
76. Garcia CM, Darland DC, Massingham LJ, D’Amore PA. Endothelial cell-astrocyte interactions and TGF beta are required for induction of blood-neural barrier properties. *Brain Res Dev Brain Res*. 152(1):25–38.2004; [PubMed: 15283992]



77. Molofsky AV, Deneen B. Astrocyte development: A Guide for the Perplexed. *Glia*. 63(8):1320–1329.2015; [PubMed: 25963996]
78. Haddad-Tovoli R, Dragano NRV, Ramalho AFS, Velloso LA. Development and Function of the Blood-Brain Barrier in the Context of Metabolic Control. *Frontiers in neuroscience*. 11:224.2017; [PubMed: 28484368]
79. Feletou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol*. 291(3):H985–1002.2006; [PubMed: 16632549]
80. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev*. 43(2):109–142.1991; [PubMed: 1852778]
81. Radomski MW, Palmer RM, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol*. 92(3):639–646.1987; [PubMed: 3322462]
82. Parfenova H, Levine V, Gunther WM, Pourcyrus M, Leffler CW. COX-1 and COX-2 contributions to basal and IL-1 beta-stimulated prostanoid synthesis in human neonatal cerebral microvascular endothelial cells. *Pediatric research*. 52(3):342–348.2002; [PubMed: 12193665]
83. Garland CJ, Hiley CR, Dora KA. EDHF: spreading the influence of the endothelium. *Br J Pharmacol*. 164(3):839–852.2011; [PubMed: 21133895]
84. Vanhoutte PM, Luscher TF, Graser T. Endothelium-dependent contractions. *Blood Vessels*. 28(1–3):74–83.1991; [PubMed: 1900447]
85. Savel'eva GM, Chekhonin VP, Pavlova TA, et al. An immunochemical analysis of the function of the hemato-encephalic barrier in acute fetal hypoxia and asphyxia neonatorum. *Akush Ginekol (Mosk)*. (2):43–46.1991;
86. Ruijtenbeek K, Kessels LC, De Mey JG, Blanco CE. Chronic moderate hypoxia and protein malnutrition both induce growth retardation, but have distinct effects on arterial endothelium-dependent reactivity in the chicken embryo. *Pediatric research*. 53(4):573–579.2003; [PubMed: 12612217]
87. Aguan K, Murotsuki J, Gagnon R, Thompson LP, Weiner CP. Effect of chronic hypoxemia on the regulation of nitric-oxide synthase in the fetal sheep brain. *Brain research Developmental brain research*. 111(2):271–277.1998; [PubMed: 9838160]
88. Pearce WJ, Williams JM, White CR, Lincoln TM. Effects of chronic hypoxia on soluble guanylate cyclase activity in fetal and adult ovine cerebral arteries. *Journal of applied physiology*. 107(1):192–199.2009; [PubMed: 19407253]
89. Pearce WJ, Ashwal S, Cuevas J. Direct effects of graded hypoxia on intact and denuded rabbit cranial arteries. *Am J Physiol*. 257(3 Pt 2):H824–833.1989; [PubMed: 2675633]
90. Nishida N, Blood AB, Hunter CJ, et al. Role of prostanoids in the regulation of cerebral blood flow during normoxia and hypoxia in the fetal sheep. *Pediatric research*. 60(5):524–529.2006; [PubMed: 16988195]
91. Kuschinsky W. Coupling of function, metabolism, and blood flow in the brain. *Neurosurg Rev*. 14(3):163–168.1991; [PubMed: 1944930]
92. Kennedy C, Grave GD, Jehle JW, Sokoloff L. Blood flow to white matter during maturation of the brain. *Neurology*. 20(6):613–618.1970; [PubMed: 5463613]
93. Pryds O, Andersen GE, Friis-Hansen B. Cerebral blood flow reactivity in spontaneously breathing, preterm infants shortly after birth. *Acta Paediatr Scand*. 79(4):391–396.1990; [PubMed: 2112295]
94. Ramaekers VT, Casaer P. Defective regulation of cerebral oxygen transport after severe birth asphyxia. *Developmental Medicine & Child Neurology*. 32(1):56–62.1990; [PubMed: 2404811]
95. Walter B, Bauer R, Kuhnen G, Fritz H, Zwiener U. Coupling of cerebral blood flow and oxygen metabolism in infant pigs during selective brain hypothermia. *J Cereb Blood Flow Metab*. 20(8):1215–1224.2000; [PubMed: 10950382]
96. Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci*. 24:31–55.2001; [PubMed: 11283304]
97. Aranda JV, Beharry K, Laudignon N, Sasyniuk BI. Ontogeny of adenosine production and degradation and its implications in neonatal cerebral blood flow regulation. *Dev Pharmacol Ther*. 13(2–4):96–103.1989; [PubMed: 2693008]

98. Weaver DR. A2a adenosine receptor gene expression in developing rat brain. *Brain Res Mol Brain Res.* 20(4):313–327.1993; [PubMed: 8114618]
99. Jones MD Jr, Traystman RJ, Simmons MA, Molteni RA. Effects of changes in arterial O<sub>2</sub> content on cerebral blood flow in the lamb. *Am J Physiol.* 240(2):H209–215.1981; [PubMed: 7468816]
100. Jones MD Jr, Rosenberg AA, Simmons MA, Molteni RA, Koehler RC, Traystman RJ. Oxygen delivery to the brain before and after birth. *Science.* 216(4543):324–325.1982; [PubMed: 6801768]
101. Koehler RC, Traystman RJ, Rosenberg AA, Hudak ML, Jones MD Jr. Role of O<sub>2</sub>-hemoglobin affinity on cerebrovascular response to carbon monoxide hypoxia. *Am J Physiol.* 245(6):H1019–1023.1983; [PubMed: 6660302]
102. Rosenberg AA, Harris AP, Koehler RC, Hudak ML, Traystman RJ, Jones MD Jr. Role of O<sub>2</sub>-hemoglobin affinity in the regulation of cerebral blood flow in fetal sheep. *Am J Physiol.* 251(1 Pt 2):H56–62.1986; [PubMed: 2425642]
103. O'Regan M. Adenosine and the regulation of cerebral blood flow. *Neurol Res.* 27(2):175–181.2005; [PubMed: 15829181]
104. Wyatt JS, Edwards AD, Cope M, et al. Response of cerebral blood volume to changes in arterial carbon dioxide tension in preterm and term infants. *Pediatric research.* 29(6):553–557.1991; [PubMed: 1907730]
105. Hernandez MJ, Brennan RW, Vannucci RC, Bowman GS. Cerebral blood flow and oxygen consumption in the newborn dog. *Am J Physiol.* 234(5):R209–215.1978; [PubMed: 645940]
106. Gregoire NM, Gjedde A, Plum F, Duffy TE. Cerebral blood flow and cerebral metabolic rates for oxygen, glucose, and ketone bodies in newborn dogs. *J Neurochem.* 30(1):63–69.1978; [PubMed: 340616]
107. Pryds O, Greisen G, Lou H, Friis-Hansen B. Heterogeneity of cerebral vasoreactivity in preterm infants supported by mechanical ventilation. *J Pediatr.* 115(4):638–645.1989; [PubMed: 2507767]
108. Mann LI. Fetal brain metabolism and function. *Clin Obstet Gynecol.* 13(3):638–651.1970; [PubMed: 5493916]
109. Raju TN, Kim SY. The effect of hematocrit alterations on cerebral vascular CO<sub>2</sub> reactivity in newborn baboons. *Pediatric research.* 29(4 Pt 1):385–390.1991; [PubMed: 1906596]
110. Leffler CW, Busija DW. Prostanoids in cortical subarachnoid cerebrospinal fluid and pial arterial diameter in newborn pigs. *Circ Res.* 57(5):689–694.1985; [PubMed: 4053302]
111. DeGiulio PA, Roth RA, Mishra OP, Delivoria-Papadopoulos M, Wagerle LC. Effect of indomethacin on the regulation of cerebral blood flow during respiratory alkalosis in newborn piglets. *Pediatric research.* 26(6):593–597.1989; [PubMed: 2513548]
112. Leffler CW, Mirro R, Thompson C, et al. Activated oxygen species do not mediate hypercapnia-induced cerebral vasodilation in newborn pigs. *American Journal of Physiology.* 261(2 Pt 2):H335–H342.1991; [PubMed: 1877661]
113. Howarth C, Sutherland B, Choi HB, et al. A Critical Role for Astrocytes in Hypercapnic Vasodilation in Brain. *J Neurosci.* 37(9):2403–2414.2017; [PubMed: 28137973]
114. Kovacs K, Komjati K, Marton T, Skopal J, Sandor P, Nagy Z. Hypercapnia stimulates prostaglandin E<sub>2</sub> but not prostaglandin I<sub>2</sub> release in endothelial cells cultured from microvessels of human fetal brain. *Brain Res Bull.* 54(4):387–390.2001; [PubMed: 11306189]
115. Iadecola C, Zhang F, Xu X. SIN-1 reverses attenuation of hypercapnic cerebrovasodilation by nitric oxide synthase inhibitors. *Am J Physiol.* 267(1 Pt 2):R228–235.1994; [PubMed: 7519410]
116. Wang Q, Pelligrino DA, Koenig HM, Albrecht RF. The role of endothelium and nitric oxide in rat pial arteriolar dilatory responses to CO<sub>2</sub> in vivo. *J Cereb Blood Flow Metab.* 14(6):944–951.1994; [PubMed: 7929657]
117. Lindauer U, Vogt J, Schuh-Hofer S, Dreier JP, Dirnagl U. Cerebrovascular vasodilation to extraluminal acidosis occurs via combined activation of ATP-sensitive and Ca<sup>2+</sup>-activated potassium channels. *J Cereb Blood Flow Metab.* 23(10):1227–1238.2003; [PubMed: 14526233]
118. Wang X, Wu J, Li L, Chen F, Wang R, Jiang C. Hypercapnic acidosis activates KATP channels in vascular smooth muscles. *Circ Res.* 92(11):1225–1232.2003; [PubMed: 12738754]

119. Rosenberg AA, Jones MD Jr, Traystman RJ, Simmons MA, Molteni RA. Response of cerebral blood flow to changes in PCO<sub>2</sub> in fetal, newborn, and adult sheep. *Am J Physiol.* 242(5):H862–866.1982; [PubMed: 6805337]
120. Rosenberg AA. Response of the cerebral circulation to profound hypocarbia in neonatal lambs. *Stroke.* 19(11):1365–1370.1988; [PubMed: 3142110]
121. Massik J, Jones MD Jr, Miyabe M, et al. Hypercapnia and response of cerebral blood flow to hypoxia in newborn lambs. *J Appl Physiol.* 66(3):1065–1070.1989; [PubMed: 2496082]
122. du Plessis AJ. Cerebral blood flow and metabolism in the developing fetus. *Clin Perinatol.* 36(3): 531–548.2009; [PubMed: 19732612]
123. Park TS, Van Wylen DG, Rubio R, Berne RM. Increased brain interstitial fluid adenosine concentration during hypoxia in newborn piglet. *J Cereb Blood Flow Metab.* 7(2):178–183.1987; [PubMed: 3558500]
124. Ment LR, Stewart WB, Duncan CC, Pitt BR, Cole JS. Beagle puppy model of perinatal cerebral infarction. Regional cerebral prostaglandin changes during acute hypoxemia. *J Neurosurg.* 65(6): 851–855.1986; [PubMed: 3772484]
125. Basu S, Barman S, Shukla R, Kumar A. Effect of oxygen inhalation on cerebral blood flow velocity in premature neonates. *Pediatric research.* 75(2):328–335.2014; [PubMed: 24226632]
126. Lou HC, Tweed WA, Davies JM. Preferential blood flow increase to the brain stem in moderate neonatal hypoxia: reversal by naloxone. *Eur J Pediatr.* 144(3):225–227.1985; [PubMed: 2996903]
127. Jones MD Jr, Traystman RJ. Cerebral oxygenation of the fetus, newborn, and adult. *Semin Perinatol.* 8(3):205–216.1984; [PubMed: 6429860]
128. Szymonowicz W, Walker AM, Yu VY, Stewart ML, Cannata J, Cussen L. Regional cerebral blood flow after hemorrhagic hypotension in the preterm, near-term, and newborn lamb. *Pediatric research.* 28(4):361–366.1990; [PubMed: 2235134]
129. McPhee AJ, Kotagal UR, Kleinman LI. Cerebrovascular hemodynamics during and after recovery from acute asphyxia in the newborn dog. *Pediatric research.* 19(7):645–650.1985; [PubMed: 3839577]
130. Koehler RC, Traystman RJ, Jones MD Jr. Influence of reduced oxyhemoglobin affinity on cerebrovascular response to hypoxic hypoxia. *Am J Physiol.* 251(4 Pt 2):H756–763.1986; [PubMed: 3766753]
131. Richardson BS, Rurak D, Patrick JE, Homan J, Carmichael L. Cerebral oxidative metabolism during sustained hypoxaemia in fetal sheep. *J Dev Physiol.* 11(1):37–43.1989; [PubMed: 2794387]
132. Laudignon N, Farri E, Beharry K, Rex J, Aranda JV. Influence of adenosine on cerebral blood flow during hypoxic hypoxia in the newborn piglet. *Journal of Applied Physiology.* 68:1534–1541.1990; [PubMed: 2347792]
133. Ment LR, Stewart WB, Duncan CC, Pitt BR. Beagle puppy model of perinatal cerebral insults. Cerebral blood flow changes and intraventricular hemorrhage evoked by hypoxemia. *J Neurosurg.* 65(6):847–850.1986; [PubMed: 3772483]
134. Pearce WJ, Ashwal S, Long DM, Cuevas J. Hypoxia inhibits calcium influx in rabbit basilar and carotid arteries. *Am J Physiol.* 262(1 Pt 2):H106–113.1992; [PubMed: 1733303]
135. Frascch MG, Muller T, Wicher C, et al. Fetal body weight and the development of the control of the cardiovascular system in fetal sheep. *J Physiol.* 579(Pt 3):893–907.2007; [PubMed: 17218361]
136. Rhee CJ, Fraser CD, Kibler K, et al. The Ontogeny of Cerebrovascular Pressure Autoregulation in Premature Infants. *Acta neurochirurgica Supplement.* 122:151–155.2016; [PubMed: 27165897]
137. Strandgaard S, Paulson OB. Cerebral autoregulation. *Stroke.* 15(3):413–416.1984; [PubMed: 6374982]
138. Jordan JD, Powers WJ. Cerebral autoregulation and acute ischemic stroke. *Am J Hypertens.* 25(9):946–950.2012; [PubMed: 22573015]
139. Hanigan WC, Bogner D. Cerebrovascular physiology in perinates with congenital hydrocephalus. *Childs Nerv Syst.* 26(6):775–780.2010; [PubMed: 20082196]
140. Hernandez MJ, Brennan RW, Bowman GS. Autoregulation of cerebral blood flow in the newborn dog. *Brain Res.* 184(1):199–202.1980; [PubMed: 7357418]

141. Ramaekers VT, Casaer P, Daniels H, Marchal G. Upper limits of brain blood flow autoregulation in stable infants of various conceptional age. *Early Human Development*. 24(3):249–258.1990; [PubMed: 2096074]
142. Pasternak JF, Groothuis DR. Autoregulation of cerebral blood flow in the newborn beagle puppy. *Biol Neonate*. 48(2):100–109.1985; [PubMed: 4041504]
143. Arnold BW, Martin CG, Alexander BJ, Chen T, Fleming LR. Autoregulation of brain blood flow during hypotension and hypertension in infant lambs. *Pediatric research*. 29(1):110–115.1991; [PubMed: 2000255]
144. Busija DW, Heistad DD, Marcus ML. Effects of sympathetic nerves on cerebral vessels during acute, moderate increases in arterial pressure in dogs and cats. *Circ Res*. 46(5):696–702.1980; [PubMed: 7363418]
145. Pearce WJ, Duckles SP, Buchholz J. Effects of maturation on adrenergic neurotransmission in ovine cerebral arteries. *Am J Physiol*. 277(4 Pt 2):R931–937.1999; [PubMed: 10516229]
146. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev*. 2(2):161–192.1990; [PubMed: 2201348]
147. Walsh MP, Cole WC. The role of actin filament dynamics in the myogenic response of cerebral resistance arteries. *J Cereb Blood Flow Metab*. 33(1):1–12.2013; [PubMed: 23072746]
148. Nakayama K, Tanaka Y. Stretch-induced contraction and Ca<sup>2+</sup> mobilization in vascular smooth muscle. *Biol Signals*. 2(5):241–252.1993; [PubMed: 8038857]
149. Davis MJ, Wu X, Nurkiewicz TR, et al. Integrins and mechanotransduction of the vascular myogenic response. *Am J Physiol Heart Circ Physiol*. 280(4):H1427–1433.2001; [PubMed: 11247750]
150. Sharif-Naeini R, Folgering JH, Bichet D, et al. Sensing pressure in the cardiovascular system: Gq-coupled mechanoreceptors and TRP channels. *J Mol Cell Cardiol*. 48(1):83–89.2010; [PubMed: 19345226]
151. Laudignon N, Beharry K, Farri E, Aranda JV. The role of adenosine in the vascular adaptation of neonatal cerebral blood flow during hypotension. *J Cereb Blood Flow Metab*. 11(3):424–431.1991; [PubMed: 2016349]
152. Park TS, Van Wylen DG, Rubio R, Berne RM. Brain interstitial adenosine and sagittal sinus blood flow during systemic hypotension in piglet. *J Cereb Blood Flow Metab*. 8(6):822–828.1988; [PubMed: 3192647]
153. Leffler CW, Busija DW, Beasley DG, Armstead WM, Mirro R. Postischemic cerebral microvascular responses to norepinephrine and hypotension in newborn pigs. *Stroke*. 20(4):541–546.1989; [PubMed: 2929031]
154. Chemtob S, Beharry K, Rex J, Varma DR, Aranda JV. Changes in cerebrovascular prostaglandins and thromboxane as a function of systemic blood pressure. Cerebral blood flow autoregulation of the newborn. *Circ Res*. 67(3):674–682.1990; [PubMed: 2397575]
155. Armstead WM, Mirro R, Busija DW, Desiderio DM, Leffler CW. Opioids in cerebrospinal fluid in hypotensive newborn pigs. *Circ Res*. 68(4):922–929.1991; [PubMed: 1672630]
156. Fletcher AM, Leffler CW, Busija DW. Effects of hypertension and sympathetic denervation on cerebral blood flow in newborn pigs. *Am J Vet Res*. 50(5):754–757.1989; [PubMed: 2729721]
157. Monin P, Feillet F, Hascoet JM, Vert P. Effect of sympathetic nervous system on cerebral blood flow in the newborn piglet. *Biol Neonate*. 58(4):192–199.1990; [PubMed: 2125504]
158. Edvinsson L, Owman C, Siesjo B. Physiological role of cerebrovascular sympathetic nerves in the autoregulation of cerebral blood flow. *Brain Res*. 117(3):519–523.1976; [PubMed: 990942]
159. Vesoulis ZA, Mathur AM. Cerebral Autoregulation, Brain Injury, and the Transitioning Premature Infant. *Front Pediatr*. 5:64.2017; [PubMed: 28421173]
160. Ong BY, Greengrass R, Bose D, Gregory G, Palahniuk RJ. Acidemia impairs autoregulation of cerebral blood flow in newborn lambs. *Can Anaesth Soc J*. 33(1):5–9.1986; [PubMed: 3948047]
161. Tweed A, Cote J, Lou H, Gregory G, Wade J. Impairment of cerebral blood flow autoregulation in the newborn lamb by hypoxia. *Pediatric research*. 20(6):516–519.1986; [PubMed: 3714361]
162. Gardiner RM. Cerebral blood flow and oxidative metabolism during hypoxia and asphyxia in the new-born calf and lamb. *J Physiol*. 305:357–376.1980; [PubMed: 6777488]

163. Busija DW, Leffler CW. Selective attenuation by perivascular blood of prostanoid-dependent cerebrovascular dilation in piglets. *Stroke*. 22(4):484–488.1991; [PubMed: 1902599]
164. Edvinsson L, Owman C, Sjoberg NO. Autonomic nerves, mast cells, and amine receptors in human brain vessels. A histochemical and pharmacological study. *Brain Res*. 115(3):377–393.1976; [PubMed: 184880]
165. Duckles SP, Banner W Jr. Changes in vascular smooth muscle reactivity during development. *Annu Rev Pharmacol Toxicol*. 24:65–83.1984; [PubMed: 6145388]
166. Hayashi S, Park MK, Kuehl TJ. Higher sensitivity of cerebral arteries isolated from premature and newborn baboons to adrenergic and cholinergic stimulation. *Life Sci*. 35(3):253–260.1984; [PubMed: 6748852]
167. Wagerle LC, Delivoria-Papadopoulos M. Alpha-adrenergic receptor subtypes in the cerebral circulation of newborn piglets. *Am J Physiol*. 252(6 Pt 2):R1092–1098.1987; [PubMed: 3035948]
168. Busija DW, Leffler CW. Eicosanoid synthesis elicited by norepinephrine in piglet parietal cortex. *Brain Res*. 403(2):243–248.1987; [PubMed: 3828821]
169. Busija DW, Leffler CW, Wagerle LC. Responses of newborn pig pial arteries to sympathetic nervous stimulation and exogenous norepinephrine. *Pediatric research*. 19(11):1210–1214.1985; [PubMed: 4069832]
170. Wagerle LC, Kurth CD, Roth RA. Sympathetic reactivity of cerebral arteries in developing fetal lamb and adult sheep. *Am J Physiol*. 258(5 Pt 2):H1432–1438.1990; [PubMed: 2337177]
171. Wagerle LC, Heffernan TM, Sacks LM, Delivoria-Papadopoulos M. Sympathetic effect on cerebral blood flow regulation in hypoxic newborn lambs. *Am J Physiol*. 245(3):H487–494.1983; [PubMed: 6412568]
172. Wagerle LC, Kumar SP, Delivoria-Papadopoulos M. Effect of sympathetic nerve stimulation on cerebral blood flow in newborn piglets. *Pediatric research*. 20(2):131–135.1986; [PubMed: 3945523]
173. Wagerle LC, Busija DW. Cholinergic mechanisms in the cerebral circulation of the newborn piglet: effect of inhibitors of arachidonic acid metabolism. *Circ Res*. 64(5):1030–1036.1989; [PubMed: 2495868]
174. Wagerle LC, Busija DW. Effect of thromboxane A<sub>2</sub>/endoperoxide antagonist SQ29548 on the contractile response to acetylcholine in newborn piglet cerebral arteries. *Circ Res*. 66(3):824–831.1990; [PubMed: 2306808]
175. Edvinsson L, Uddman R, Juul R. Peptidergic innervation of the cerebral circulation. Role in subarachnoid hemorrhage in man. *Neurosurg Rev*. 13(4):265–272.1990; [PubMed: 2126362]
176. Kawamura K, Sakata N, Takebayashi S. Neuropeptide Y- and vasoactive intestinal polypeptide-containing nerve fibers in the human cerebral arteries: characteristics of distribution. *Angiology*. 42(1):35–43.1991; [PubMed: 1992857]
177. Horgan K, O'Connor TP, van der Kooy D. Prenatal specification and target induction underlie the enrichment of calcitonin gene-related peptide in the trigeminal ganglion neurons projecting to the cerebral vasculature. *J Neurosci*. 10(7):2485–2492.1990; [PubMed: 2376783]
178. Maassenvandenbrink A, Chan KY. Neurovascular pharmacology of migraine. *Eur J Pharmacol*. 585(2–3):313–319.2008; [PubMed: 18423447]
179. Wu D, Wang J, Wang H, Ji A, Li Y. Protective roles of bioactive peptides during ischemia-reperfusion injury: From bench to bedside. *Life Sci*. 180:83–92.2017; [PubMed: 28527782]
180. Dejda A, Sokolowska P, Nowak JZ. Neuroprotective potential of three neuropeptides PACAP, VIP and PHI. *Pharmacol Rep*. 57(3):307–320.2005; [PubMed: 15985713]
181. Silva AP, Xapelli S, Grouzmann E, Cavadas C. The putative neuroprotective role of neuropeptide Y in the central nervous system. *Current drug targets CNS and neurological disorders*. 4(4):331–347.2005; [PubMed: 16101553]
182. Pearce WJ, Williams JM, Hamade MW, Chang MM, White CR. Chronic hypoxia modulates endothelium-dependent vasorelaxation through multiple independent mechanisms in ovine cranial arteries. *Adv Exp Med Biol*. 578:87–92.2006; [PubMed: 16927675]

183. Williams JM, White CR, Chang MM, Injeti ER, Zhang L, Pearce WJ. Chronic hypoxic decreases in soluble guanylate cyclase protein and enzyme activity are age dependent in fetal and adult ovine carotid arteries. *J Appl Physiol.* 100(6):1857–1866.2006; [PubMed: 16469937]
184. Pearce WJ, Khorram O. Maturation and differentiation of the fetal vasculature. *Clin Obstet Gynecol.* 56(3):537–548.2013; [PubMed: 23820122]
185. Marko SB, Damon DH. VEGF promotes vascular sympathetic innervation. *Am J Physiol Heart Circ Physiol.* 294(6):H2646–2652.2008; [PubMed: 18408130]
186. Lou HC, Lassen NA, Tweed WA, Johnson G, Jones M, Palahniuk RJ. Pressure passive cerebral blood flow and breakdown of the blood-brain barrier in experimental fetal asphyxia. *Acta Paediatr Scand.* 68(1):57–63.1979; [PubMed: 31759]
187. Watson CG, Dehaes M, Gagoski BA, Grant PE, Rivkin MJ. Arterial Spin Labeling Perfusion Magnetic Resonance Imaging Performed in Acute Perinatal Stroke Reveals Hyperperfusion Associated With Ischemic Injury. *Stroke.* 47(6):1514–1519.2016; [PubMed: 27143277]
188. Barrington KJ, Finer N, Pennaforte T, Altit G. Nitric oxide for respiratory failure in infants born at or near term. *Cochrane Database Syst Rev.* 1:Cd000399.2017; [PubMed: 28056166]
189. Lincoln TM, Dey NB, Boerth NJ, Cornwell TL, Soff GA. Nitric oxide–cyclic GMP pathway regulates vascular smooth muscle cell phenotypic modulation: implications in vascular diseases. *Acta Physiol Scand.* 164(4):507–515.1998; [PubMed: 9887973]
190. Semenza GL. Expression of hypoxia-inducible factor 1: mechanisms and consequences. *Biochem Pharmacol.* 59(1):47–53.2000; [PubMed: 10605934]
191. Owens GK. Molecular control of vascular smooth muscle cell differentiation and phenotypic plasticity. *Novartis Foundation symposium.* 283:174–191.2007; [PubMed: 18300422]
192. Spin JM, Maegdefessel L, Tsao PS. Vascular smooth muscle cell phenotypic plasticity: focus on chromatin remodelling. *Cardiovasc Res.* 95(2):147–155.2012; [PubMed: 22362814]
193. Pearce WJ, Doan C, Carreon D, et al. Imatinib attenuates cerebrovascular injury and phenotypic transformation after intracerebral hemorrhage in rats. *Am J Physiol Regul Integr Comp Physiol.* 311(6):R1093–r1104.2016; [PubMed: 27707720]
194. Jiang Y, Yin H, Zheng XL. MicroRNA-1 inhibits myocardin-induced contractility of human vascular smooth muscle cells. *J Cell Physiol.* 225(2):506–511.2010; [PubMed: 20458751]
195. Chuang TD, Pearce WJ, Khorram O. miR-29c induction contributes to downregulation of vascular extracellular matrix proteins by glucocorticoids. *Am J Physiol Cell Physiol.* 309(2):C117–125.2015; [PubMed: 26017148]
196. Cheng Y, Liu X, Yang J, et al. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ Res.* 105(2):158–166.2009; [PubMed: 19542014]
197. Parmacek MS. MicroRNA-modulated targeting of vascular smooth muscle cells. *J Clin Invest.* 119(9):2526–2528.2009; [PubMed: 19690387]