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The Fetal Cerebral Circulation: Three Decades of Exploration by the LLU Center for Perinatal Biology

William J. Pearce, Ph.D.

Center for Perinatal Biology, Loma Linda University, School of Medicine, 92350 Loma Linda, CA, USA

William J. Pearce: wpearce@llu.edu

Abstract

For more than three decades, research programs in the Center of Perinatal Biology have focused on the vascular biology of the fetal cerebral circulation. In the 1980s, research in the Center demonstrated that cerebral auto-regulation operated over a narrower pressure range, and was more vulnerable to insults, in fetuses than in adults. Other studies were among the first to establish that compared to adult cerebral arteries, fetal cerebral arteries were more hydrated, contained smaller smooth muscle cells and less connective tissue, and had endothelium less capable of producing NO. Work in the 1990s revealed that pregnancy depressed reactivity to NO in extra-cerebral arteries, but elevated it in cerebral arteries through effects involving changes in cGMP metabolism. Comparative studies verified that fetal lamb cerebral arteries were an excellent model for cerebral arteries from human infants. Biochemical studies demonstrated that cGMP metabolism was dramatically upregulated, but that contraction was far more dependent on calcium influx, in fetal compared to adult cerebral arteries. Further studies established that chronic hypoxia accelerates functional maturation of fetal cerebral arteries, as indicated by increased contractile responses to adrenergic agonists and perivascular adrenergic nerves. In the 2000s, studies of signal transduction established age-dependent roles for PKG, PKC, PKA, ERK, ODC, IP3, myofilament calcium sensitivity, and many other mechanisms. These diverse studies clearly demonstrated that fetal cerebral arteries were functionally quite distinct compared to adult cerebral arteries. In the current decade, research in the Center has expanded to a more molecular focus on epigenetic mechanisms and their role in fetal vascular adaptation to chronic hypoxia, maternal drug abuse, and nutrient deprivation. Overall, the past three decades have transformed thinking about, and understanding of, the fetal cerebral circulation due in no small part to the sustained research efforts by faculty and staff in the Center for Perinatal Biology.

Keywords

Fetal calcium metabolism; Fetal cerebral circulation; Fetal endothelium; Fetal hypoxia; Fetal signal transduction

Correspondence to: William J. Pearce, wpearce@llu.edu.

1 Introduction

Through the 1970s, interest in the fetal cerebral circulation grew steadily. The foundational studies of Rudolf and colleagues [1] had firmly established the idea that cardiovascular homeostasis was negotiated very differently in the fetus than in the adult. A broad variety of reviews also advanced the idea that patterns of CNS damage in the human infant due to hypoxia and trauma were highly dependent on gestational age [2] and exhibited very different patterns of injury than observed in adults [3]. Interest in the fetal cerebral circulation was further stimulated by a growing number of cases involving open-heart surgery in human fetuses and neonates [4]. Together, these clinical priorities motivated numerous basic science studies of the fetal cerebral circulation. In 1971, Zamenhof and colleagues laid the groundwork for modern epigenetics with their studies of the trans-generational effects of maternal food restriction on brain growth in rat offspring [5]. The elegant and ground-breaking studies of Nuwayhid and colleagues clearly established that responses to adrenergic and cholinergic agonists were very different in the fetal and adult pulmonary circulation [6], and that in turn, autonomic regulation of the heart and lungs exhibited many unique characteristics in fetuses compared to adults [7]. Further work by Su and colleagues reinforced the view that adrenergic, cholinergic, and serotonergic vascular neuroeffector mechanisms were markedly different in fetal and adult arteries [8, 9]. Together, these early basic science studies set the stage for a rapid expansion of studies of the fetal cerebral circulation.

2 The 1980s: Studies of Cerebral Hypoxia and Autoregulation

As the decade of the 1980s opened, clinical interest in the fetal cerebral circulation was growing rapidly. In his now classical review in the New England Journal of Medicine, Volpe laid out the main features and issues related to neonatal intracranial hemorrhage [10]. Other prominent reviews focused on the increased incidence of intraventricular hemorrhage in premature infants [11] and the heightened vulnerability of the immature cerebral circulation to hypoxia and ischemia [12], and even strokes [13]. Whereas the progression of many of these insults to neonatal, hypoxic-ischemic encephalopathy was well recognized [14], no mechanisms responsible for the unique vulnerability of the fetal brain were clearly identified. As recognized in several major reviews, the lack of understanding of the etiology of fetal brain injury was attributed to the fact that most studies of this category of pathophysiology had been conduced in autopsy specimens, many of which were in advanced stages of disease [15]. With this realization came a new motivation to examine the structure and function of the fetal cerebral circulation using animal models.

Among the first investigators to use animal studies to implicate compromised cerebrovascular regulation as a cause of neonatal hypoxic-ischemic encephalopathy were Robert & Susan Vannucci [16], whose neonatal rat model of hypoxic ischemia has now been used in more than 1,000 publications. About the same time than the Vannucci model was being established, a fetal lamb model was introduced [17] that offered many advantages, the most notable of which was that it enabled chronic instrumentation. With the fetal lamb model, many important findings quickly accrued, include the connection between fetal asphyxia and vasogenic brain edema [17], the reduced efficiency of cerebral autoregulation

in the fetus ([18–20] and the vulnerability of fetal cerebral autoregulation to hypoxic insults [21]. Within this context, the Ashwal, Longo team at LLU were among the first to use the microspheres technique in a chronically catheterized fetal lamb preparation to simultaneously measure cerebral perfusion to more than 30 brain regions to establish that cerebral blood flow was highly heterogeneous in the fetal brain, and that vasodilatory responses to acute hypoxia lasted long after normoxemia was restored [22, 23]. The Ashwal, Longo team at LLU went on to establish the fundamental responses of the unanesthetized fetal cerebral circulation to hypercapnia, acidosis, hypotension [24], evoked auditory potentials [25], and calcium channel antagonists [26]. Together, these studies demonstrated that the fetal cerebral circulation was preferentially distributed to the brain stem. exhibited an attenuated hypercapnic reactivity, exhibited excellent coupling between local cerebral metabolism and local perfusion, and auto-regulated over a very narrow range of blood pressures, compared to adults.

The burst of studies of fetal autoregulation in the 1980s focused attention on fetal cerebrovasculature and its unique contractile characteristics. To better understand how the contractility of fetal cerebral arteries contributed to overall flow-metabolism coupling the fetal brain, the pial window technique was adapted for use in neonatal piglets and used in many studies to reveal that pial arteries received a functional sympathetic innervation at birth [27], were highly reactive to hypoxia and hypercapnia [28, 29], and were highly dependent on prostanoid metabolism for many vascular responses [30]. Despite the in vivo advantages of the pial window technique, however, this approach did not provide a clear definition of pial vascular reactivity independent of the adjacent brain tissue; all applications of exogenous substances influenced both the pial vasculature and the underlying brain parenchyma. For this reason, interest in isolated cerebral arteries, studied in vitro, grew rapidly. Although the study of isolated adult cerebral arteries began in the 1970s [31–33], it was not until the mid-1980s that fetal cerebral arteries were studied in vitro [34]. These early studies illustrated that the contractility of fetal cerebral arteries was highly dependent on gestational age such that responses to vasodilator prostanoids decreased with fetal age, whereas vasoconstrictor responses increased [34]. The first contribution in this area from LLU came from the team of Ashwal and Pearce [35], which was the first to demonstrate direct vasodilator effects of acute hypoxia on fetal cerebral arteries that varied with artery size and age.

3 The 1990s: Growing Interest in Fetal Cerebral Vascular Biology

3.1 Effects of Pregnancy on Cerebral Arteries

The rising interest in fetal cerebral arteries in the early 1990s quickly translated into a parallel interest in the effects of pregnancy on maternal cerebral arteries, particularly at LLU. The team of Hull, Longo, and Pearce produced a series of contributions that revealed that pregnancy-depressed reactivity to NO in maternal extracerebral arteries, but elevated it in cerebrals [36]. Further studies detailed the parallel effects of pregnancy on cGMP synthesis and endothelium-dependent relaxation [37] and also revealed that pregnancy-induced increases in contractility were depressed by chronic hypoxia [38]. Other studies advanced the idea that human placental arteries were responsive to exogenous NO, but not

to most endothelium-dependent vasodilators [39]. These early publications helped form a background against which additional studies of fetal cerebrovascular structure and function could be performed.

3.2 Effects of Postnatal Maturation on Cerebral Artery Structure and Contractility

The initial studies of isolated fetal cerebral arteries at LLU examined the effects of maturation on artery structure and function and were the first to measure wall thicknesses and active wall stresses in these small arteries [40]. These findings established that fetal carotid arteries matured both structurally and functionally much sooner than did cerebral arteries, and that fetal cerebral arteries had much smaller smooth muscle cells, had much larger extracellular space, and had greater water content than did adult cerebral arteries [41]. Interestingly, fetal arteries were more compliant, but more reactive to stretch [42] and aminergic agonists [43] than were adult arteries. This increased reactivity appeared attributable, at least in part, to a greater sensitivity to calcium in fetal cererbral arteries, as determined by indirect measurements [44]. This series of studies also introduced the ideas that cerebrovascular maturation decreases binding affinity for norepinephrine, and more so in 4th branch than 2nd branch middle cerebral arteries [45]. Parallel studies were also the first to show that maturation right-shifted dose-response relations for ATP-sensitive potassium channel activators, indicating that fetal cerebral arteries exhibited unique and highly reactive electrophysiological characteristics compared to adult arteries [46]. In addition, fetal cerebral arteries exhibited a depressed reactivity to electrical transmural stimulation, indicating that the cerebral sympathetic perivascular innervation was probably not fully functional at term [47]. Equally important, these early functional studies also suggested that cerebral arteries from the fetal lamb were a good model for studying the structural and functional characteristics of human infant cerebral arteries [48].

3.3 Effects of Postnatal Maturation on Signal Transduction in Cerebral Arteries

Owing in large part to the many publications in the early 1990s that the vascular endothelium was a major determinant of vascular reactivity [49], early studies of fetal cerebrovascular signal transduction at LLU focused on endothelium dependent responses, including reactivity to exogenous NO donors [50], which was greater in immature than in mature cerebral arteries. Corresponding measurements of rates of cGMP synthesis and turnover further revealed that basal cGMP was more than 5× greater in newborn than adult arteries, that rates of cGMP synthesis were 2× greater in newborn than adult arteries, and that rates of cGMP degradation were 50 % greater in fetal than adult arteries [51]; clearly a major component of age-related differences in endothelium-dependent relaxation was attributable to corresponding differences in cGMP metabolism.

Another focus of work at LLU in the 1990s was on calcium biology in the fetal cerebral vasculature. Initial studies suggested that for equivalent active stresses, immature cerebral arteries required greater calcium uptake than did adult arteries [52, 53]. This greater reliance on calcium uptake appeared due to reduced sensitivity to IP3 despite elevated levels of IP3 receptors in fetal compared to adult cerebral arteries [54, 55]. Subsequent measurements of myofilament calcium sensitivity in permeabilized preparations suggested that calcium sensitivity was greater in fetal than adult cerebral arteries, particularly following activation

of G-protein coupled receptors [56, 57]. Companion studies further demonstrated that 5HT-induced contraction of both fetal and adult cerebral arteries relied on activation of Rho-kinase but not PKC or PKA [58]. For norepinephrine-induced contractions, the influx of extracellular calcium through L-type calcium channels was also critically important for contraction, particularly in fetal cerebral arteries [59].

3.4 Effects of Hypoxia and Ischemia on the Fetal Cerebral Circulation

In regards to pathophysiology, the majority of studies of the fetal cerebral circulation at LLU in the 1990s focused on the effects of hypoxia. Whereas acute hypoxia clearly had the capacity to relax isolated fetal cerebral arteries [60] through endothelium-independent mechanisms [61], chronic hypoxia dramatically altered the structure and contractility of both fetal and adult cerebral arteries and attenuated the vasodilator efficacy of NO [62, 63]. In relation to norepinephrine, hypoxia also decreased adrenergic receptor density and NE-induced IP3 mobilization [64], but upregulated pre-synaptic adrenergic reactivity while simultaneously depressing post-synaptic adrenergic reactivity [65]. In relation to serotonin, hypoxic effects were less pronounced than for norepinephrine and were attributable largely to decreases in 5HT-reeeptor density [66].

Another area of focus of hypoxia studies at LLU in the 1990s was on the role of parenchymal ornithine decarboxylase as a sensor and/or mediator of the cerebral effects of hypoxia [67]. These unique studies revealed that acute hypoxia increases ornithine decarboxylase activity and polyamine concentrations in fetal brain [67] through pathways that can also be activated by-administration of CO to the mother [68]. Interestingly, these effects of hypoxia could be elicited even in newborn brain slice preparations [69], and appeared to be mediated by oxygen radicals [70], indicating the fundamental cellular nature of the effects of cerebral hypoxia.

At the whole animal level, work at LLU in the 1990s focused increasingly on the effects of cerebral ischemia [71]. Effort was invested to develop a middle cerebral artery occlusion model in SHR pups [72] and this preparation enabled elucidation of a neurotoxic role for NO following transient focal cerebral ischemia in the immature brain [73]. Subsequent studies of nNOS biochemistry revealed that nNOS abundance was heterogeneously distributed throughout the fetal brain, and that nNOS cofactor levels were not present at saturating conditions [74]. Correspondingly, cofactor supplementation studies indicated that local ischemic vulnerability was strongly influenced by both nNOS abundance and cofactor availability, and more so in immature than in mature brain [75, 76]. A key conclusion of these studies was that age-related and regional differences in ischemic vulnerability could be explained, at least in part, by differences in overall nNOS activity.

4 The 2000s: Calcium, cGMP, PKC, and Fetal CBF

The year 2000 ushered in an era of renewed enthusiasm for studies of cerebral development and maturation. Early studies revealed the presence of a novel fatty-acid binding protein in fetal brain with potential involvement in neuronal differentiation and axon growth [77]. Within the fetal vasculature, the expression of the key contractile protein smooth muscle alpha-actin proved to increase significantly as a function of maturity [78]. Systematic studies

identified all four known classes of potassium channels in fetal cerebral arteries and their involvement in both norepinephrine-induced [79], and 5HT-induced [80], contractions. Detailed studies of fetal and adult cerebrovascular BK channels further revealed that these channels were more sensitive to calcium in the fetus than in the adult [81], due at least in part to differential phosphorylation by PKG and PKA [82]. Together, these findings helped explain why BK channel activity was inherently greater in fetal than adult cerebral arteries [83].

At the level of G-protein coupled receptors, studies from the Longo lab revealed that the alpha-2 subtype of adrenergic receptor was chiefly prejunctional in both fetal and adult cerebral arteries, but the fetal cerebral arteries also expressed a significant post-synaptic population of these receptors [84]. For serotonin, ERK activation appeared as a significant downstream component of 5HT receptor activation, particularly in fetal arteries [85]. Incubation with dexamethasone also exhibited a time-dependent ability to attenuate 5HT-induced contractions through a cyclooxygenase-dependent pathway in fetal arteries [86]. Conversely, incubation with dopamine enhanced contractile responses to 5HT [87], suggesting that vasoactive compounds commonly administered to neonates in the NICU may have conflicting effects on cerebrovascular contractility.

4.1 The Role of Calcium in the Functional Maturation of Fetal Cerebral Arteries

Exploration of the contractile role of calcium in fetal and adult cerebral arteries proceed at a rapid pace throughout the 2000s. Studies of NE-induced contractions demonstrated less reliance on calcium release from the sarcoplasmic reticulum, and far greater reliance on calcium influx in fetal compared to adult cerebral arteries [88]. Direct measurements of intracellular calcium mass using isotope tracer methods revealed that fetal cerebral arteries have significantly less IP3-releasable calcium than adults, and that the ryanodine-releasable pool is relatively small in both age groups [89]. Correspondingly, immunoblotiing measurements demonstrated that L-type calcium channel density was greater in fetal than in adult cerebral arteries [90]. From a functional perspective, simultaneous measurements of wall calcium with Fura-2 photometry and artery diameter suggested that myofilament calcium sensitivity was lower in term fetal than adult cerebral arteries [91]. Because this result contradicted previous measurements indicating greater calcium sensitivity in immature arteries [56], this finding was very interesting. Ultimately, this inconsistency led to the hypothesis that not all smooth muscle cells in the artery wall are of the same phenotype, contractility, and calcium sensitivity. Thus, membrane permeabilization as used in the earlier study [56] would “clamp” calcium in all smooth muscle cells, regardless of phenotype. In contrast, Fura-2 photometry would record calcium signals from all cells, both contractile and non-contractile. This important hypothesis motivated detailed studies of smooth muscle phenotype in the cerebral artery wall, and has since appeared to be correct [92, 93]; compared to adult cerebral arteries, fetal cerebral arteries appear to contain a larger proportion of smooth muscle cells in the synthetic phenotype which exhibit a relatively high basal calcium that changes little in response to agonist stimulation.

To further explore relations between calcium concentration, myosin light chain phosphorylation, and contractile force, studies by Nauli et al. measured all three variables

simultaneously again using membrane permeabilized preparations [94]. These interesting studies enabled discrimination between “thick filament regulation,” which determined the relation between cytosolic calcium concentration and myosin light chain phosphorylation, and “thin filament regulation”, which determined the relation between myosin light chain phosphorylation and contractile force. With this approach, fetal cerebral arteries exhibited greater overall calcium sensitivity (calcium vs. force) than did adult cerebral arteries, even though calcium was less able to promote myosin light chain phosphorylation (thick filament regulation) in fetal compared to adult cerebral arteries. Conversely, the relation between phosphorylated (activated) myosin light chain and contractile force (thin filament regulation) was markedly upregulated in fetal compared to adult cerebral arteries. To test the physiological importance of these differences, reliance on calcium influx for myogenic tone in 2nd branch middle cerebral arteries was explored and found to be significantly greater in immature than mature cerebral arteries [95]. Separate experiments in which myofilament calcium sensitivity was measured directly in membrane permeabilized preparations corroborated the earlier findings [96], confirmed that fetal arteries exhibit depressed thick filament regulation and enhanced thin filament regulation, and suggested for the first time that myogenic stretch is more tightly coupled to myosin light chain phosphorylation in adult than in fetal arteries [97]. Owing to the important age-related differences in myosin light chain phosphorylation observed in fetal and adult arteries, further experiments were carried out to measure MLCK activity, in situ using a custom-built, rapid freeze apparatus [98]. Consistent with previous results, MLCK activity was markedly less in adult than in fetal arteries. Further studies in membrane permeabilized 2nd branch middle cerebral arteries were the first to confirm that immature cerebral arteries contain a greater proportion of non-contractile smooth muscle, and as a consequence rely more on myofilament Ca(2+) sensitization and Ca(2+) influx to maintain myogenic reactivity than do adult cerebral arteries [99].

4.2 Roles of PKC and cGMP in Cerebrovascular Maturation

In light of the important age-related differences in cerebrovascular calcium handling indicated by studies in the Center for Perinatal Biology, a variety of further studies were initiated to better understand what mechanisms were responsible, with particular emphasis on cytosolic kinases. In turn, inhibition of PKC augmented NE-induced IP3 and calcium responses in adult, but not fetal, cerebral arteries whereas PKC stimulation increased calcium in fetal but not adult arteries, suggesting a critical role of PKC in age-related differences in cerebrovascular calcium handling [100]. Application of the ERK inhibitor U0126 potentiated phenylephrine-induced contractions in fetal but not adult arteries, indicating an age-dependent role for ERK in modulation of contractility [101]. Subsequent studies revealed that PKC activation preferentially activated ERK2 in fetal arteries, and ERK1 in adult arteries, further implicating ERK in age-related differences in contractility [102]. This study also suggested that PKC activation increased Rho-kinase in fetal arteries, but activated CP1-17 and caldesmon in adult arteries; again, cytosolic kinases appeared to play a major role in age-related differences in cerebrovascular contractility.

Another kinase studied extensively in the 2000s was guanylate cyclase, which is the intracellular “effector” for many of NO’s actions within vascular smooth muscle [103]. A

series of systematic experiments by Nauli et al. [104] demonstrated that maturation of cerebral arteries attenuated the ability of cGMP to promote vasorelaxation. Further studies revealed that soluble guanylate cyclase was more abundant [105], and that cGMP was more potent as a vasorelaxant [106], in fetal than adult cerebral arteries. Further studies in membrane permeabilized preparations showed that cGMP attenuated myofilament calcium sensitivity more effectively in fetal than adult cerebral arteries [107]. Detailed studies of relaxation rates indicated that upon stimulation with NO. cGMP concentrations rise more rapidly in immature than mature cerebral arteries due to greater rates of phosphodiesterase activity in adult arteries [108]. Additional studies showed that maturational increases in endothelial vasodilator capacity [109] were attributable to age-dependent increases in NO release secondary to elevated eNOS specific activity [110] and abundance [111]. Together, these studies strongly implicated both guanylate cyclase and PKC as major determinants of age-dependent cerebrovascular reactivity.

4.3 Effects of Hypoxia on Fetal and Adult Cerebral Arteries

Studies of cerebrovascular pathophysiology also continued throughout the 2000s with a particular emphasis on hypoxia. Morphometric measurements indicated that chronic hypoxia increased endothelial cell density and smooth muscle cell size in fetal cerebral arteries, but had opposite effects in adult cerebral arteries [112]. Measurements of 5HT receptor density, binding affinity and IP3 generation showed that 5HT was more efficiently coupled to IP3 synthesis in fetal than adult arteries [113] and that acute hypoxia decreased 5HT receptor density and binding affinity much more in adult than in fetal arteries [114]. For norepinephrine contractions, chronic hypoxia depressed cytosolic calcium and overall contractility much more in adult than in fetal arteries, due in part to attenuation of potassium-channel mediated relaxation in fetal arteries [115]. In other studies, chronic hypoxia decreased cerebrovascular nNOS abundance, due presumably to decreased innervation by nitridergic nerves, particularly in immature arteries [116]. Ex vivo neurophysiological studies also demonstrated that chronic hypoxia can markedly attenuate calcium-induced calcium release, SERCA function, and subsequent norepinephrine release in fetal sympathetic neurons [117].

Regarding the NO/cGMP pathway, studies in the late 2000s indicated that chronic hypoxia depressed NO release via reduced eNOS specific activity without decreasing eNOS abundance [118]. In parallel, hypoxia also decreased soluble guanylate cyclase activity in fetal but not adult arteries without a change in mRNA abundance for guanylate cyclase [119]; hypoxia clearly affected some aspect of mRNA translation, suggesting the possible involvement of microRNAs. Overall, chronic hypoxia inhibited NO-induced vasodilation in both adult and fetal ovine cerebral arteries via decreased sGC activity [120]. Together, these results emphasized that chronic hypoxia induces a variety of both adaptive and maladaptive effects in fetal cerebral arteries including increased protein content, decreased IP3 synthesis and IP3 receptor expression, increased 5HT receptor affinity, decreased activity of ATP- and calcium-sensitive potassium channels, and decreased calcium-dependent myosin phosphorylation [121, 122]. As a whole, these findings led to the important hypothesis that fetal responses to chronic hypoxia may “program” the fetal cerebral circulation and thereby alter its function throughout postnatal life.

4.4 Fetal Cerebral Blood Flow, In Vivo

To compliment the many studies conducted using isolated cerebral arteries, Center faculty invested considerable effort to develop and test contractile mechanisms through measurements of cerebral blood flow in intact, unanesthetized, chronically instrumented fetal sheep [123]. This novel method helped illustrate the involvement of NO in hypoxic vasodilatation of fetal cerebral blood flow [124], the ability of only 4 min of umbilical cord occlusion to ablate cerebral autoregulation [125], the absence of ischemic preconditioning responses in fetal cerebral circulation responses to repeated cord occlusions [126], the indirect role of prostanoids in fetal cerebrovascular responses to hypoxia [127], the effects of hypercapnia on cerebral oxygenation in the fetus [128], the positive effects of maternal oxygen supplementation on fetal cerebral oxygenation [129], and the ability of the fetal brain to adapt to chronic hypoxia through modification of oxygen extraction [130]. Similar studies have also demonstrated the ability of maternal caffeine to decrease fetal cortical oxygen tensions [131], the potential effects of maternal hypocapnia, CO_2 supplementation, and hypercapnia on fetal cerebral oxygenation [132, 133], and finally, the tight coupling between ECoG state with cerebral oxygen consumption and cerebral blood flow in the term fetal brain under both normoxic and hypoxic conditions [134]. As a whole, this group of studies helps depict the fetal cerebral circulation as a highly dynamic system capable of adaptation and homeostasis mediated by mechanisms unique to the fetus. Clearly, the relation between cerebral metabolism and cerebrovascular resistance is tightly coupled in the fetal brain, but is mediated by mechanisms quite distinct from those in the adult cerebral circulation.

As an adjunct to studies of the fetal lamb cerebral circulation, efforts to develop new animal models to explore stroke and ischemia in the immature brain have also continued [135], although with less activity than during the 1990s. In terms of relevance to the human infant cerebral circulation, the Angeles group employed MRI techniques to assess brain injury in human infants. Most interestingly, NICU infants treated with opioids during the first week of life demonstrated less brain injury and better long-term neurologic outcomes than infants not treated. This study has important implications for the long-term effects of neonatal stress and pain, and suggest the possible involvement of epigenetic mechanisms [136].

5 The Current Decade: The Work Continues

Many of the research programs initiated in the 2000s remain active in the current decade. New ultrastructural studies of cerebrovascular morphology have revealed dramatic changes in the size and shape of the smooth muscle cells in fetal cerebral arteries, further suggest that cerebral arteries contain a structurally highly heterogeneous population of smooth muscle cells, and indicate that development of the extracellular matrix during late fetal development is highly dynamic [137]. Studies comparing patterns of gene expression in fetal, newborn, and adult cerebral arteries point to dramatic activity in genes regulating cell proliferation, growth, and assembly pathway genes during fetal development, but decreased activity in genes governing mitogen-activated protein kinase-extracellular regulated kinase, actin cytoskeleton, and integrin-signaling pathways [138]. Among the genes so regulated were those controlling expression of alpha-adrenoceptors; in fetal cerebral arteries both the

alpha-1B and alpha-1D adrenergic receptors were detected and observed to participate in contractile responses to adrenergic agonists [139].

Concerning the effects of chronic hypoxia, a recent microarray study has identified 38 fetal vascular genes upregulated more than twofold, and 9 genes downregulated more than twofold, in response to hypoxia [140]. Although the functional implications of these changes in gene expression remain unclear, these findings constitute an excellent foundation for further work. For example, hypoxic changes in the genes regulating expression of PKC may help to explain the observations that hypoxia potentiates PKC-mediated contractions more in adult than fetal arteries, but inhibits ROCK contribution to PKC-mediated contractions more in fetal than adult arteries [141]. These studies are ongoing.

Recently, another avenue of investigation has opened in the Center regarding cellular mechanisms of response to hypoxia, with particular emphasis on VEGF. In organ culture VEGF can alter the expression of contractile proteins, contractility and smooth muscle phenotypc [142]. Correspondingly, the vascular remodeling induced by chronic hypoxia [143] may involve the effects of VEGF on smooth muscle differentiation [144]. Given that hypoxic increases in circulating VEGF levels are transient, it is particularly interesting that the long-term effects of hypoxia on smooth muscle phenotype appear to be mediated, at least in part by an increased abundance of VEGF receptors in arterial smooth muscle [93]. Importantly, long term hypoxia through the actions of VEGF also transforms smooth muscle expression of myosin heavy chain informs, which helps explain the corresponding changes in contractility, particularly in fetal arteries [92]. Hypoxic activation of the VEGF pathway may also help explain the attenuating effects of hypoxia on PKG-mediated activation of BK channels in fetal arteries [145]. From many perspectives, VEGF appears to be a master coordinator of numerous vascular responses to chronic hypoxia, and perhaps other stresses as well [146].

Another new area of investigation in Center is focused on epigenetic mechanisms of fetal adaptation. In response to maternal food restriction (50 % total caloric intake of paired controls), a recent microRKA survey revealed dozens of types that were upregulated and downregulated with a broad variety of target genes [147]. Most interestingly, the patterns of change in microRNA were not the same in day-old offspring as in 12-monlhs old offspring, suggesting that maternal food restriction imposes some form of epigenetic programming throughout the fetal genome. In a more targeted study, maternal protein restriction induced in fetal brains an increased mRNA expression of angiotensinogen and angiotensin converting enzyme-1 (ACE-1), with a decrease in mRNA levels of angiotensin II type-2 (AT2) receptors [148]. This study also produced the very interesting result that the promoter regions of the ACE-1 gene were hypomethylated, and miR27a and miR27b, which influence the translation of ACE-1 mRNA, were also upregulated. Again, these results strongly suggest that fetal stress translates through epigenetic mechanisms into major alterations of cerebral structure and function. How these mechanisms influence cerebrovascular structure and function is a topic of ongoing investigation, but abundant reports in the recent literature strongly suggest, the involvement of epigenetic mechanisms in most major cerebrovascular pathologies [149].

6 Overview

The past three decades have transformed thinking about, and understanding of, the fetal cerebral circulation. Overall, it is clear that fetal cerebral arteries are in many ways structurally and functionally quite different than adult cerebral arteries. More importantly, the mechanisms that govern the composition and contractility of cerebral arteries are highly dynamic and involve multiple mechanisms operating at all levels of organization. At the whole animal level, circulating vasotrophic factors such as VEGF and angiotensin mediate the effects of environmental influences such as hypoxia and food restriction on cerebrovascular growth and differentiation. In general, the proliferative effects of these vasotrophic factors are more pronounced in fetal than adult smooth muscle. Many other growth factors are undoubtedly involved in these processes, and are under active investigation. At the tissue level, cerebrovascular smooth muscle alters membrane populations of receptor and ion channels, cytosolic kinases, and calcium handling, all of which culminate in a heightened sensitivity to aminergic agonists, decreased capacity for calcium release, and increased reliance on calcium influx for contraction in fetal compared to adult cerebral arteries. At the cytosolic level, myofilament calcium sensitivity and soluble guanylate cyclase are upregulated in the fetus but calcium release and PKC appear to be upregulated in the adult, at least in relation to their roles in NE-induced contractions. At the molecular level, fetal and adult tissues express very different patterns of genes and microRNAs, which help to explain why both structural and contractile protein compositions are so different in fetal and adult cerebral arteries. The functional implications of these differences remain under active investigation. As for most scientific programs of research, studies of the vascular biology of the fetal brain have produced more questions than answers. That said, it is clearly a worthy topic of continued investigation, particularly as recent work suggests that smooth muscle plays a critical role not only in regulating blood flow, but in the responses to, and recovery from, cerebral injury [150]. Without doubt, the Center for Perinatal Biology will continue their adventures in this arena, and with all the new genetic and analytical tools becoming available, the next 30 years might be even more interesting than the last.

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