HHS Public Access

Author manuscript

J Cardiovasc Pharmacol. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

J Cardiovasc Pharmacol. 2015 April ; 65(4): 308–316. doi:10.1097/FJC.0000000000000192.

Role of the Sympathetic Autonomic Nervous System in Hypoxic Remodeling of the Fetal Cerebral Vasculature

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Abstract

Fetal hypoxia triggers compensatory angiogenesis and remodeling through mechanisms not fully elucidated. In response to hypoxia, hypoxia inducible factor drives expression of cytokines that exert multiple effects on cerebral structures. Among these, the artery wall is composed of a heterogeneous cell mix, and exhibits distinct patterns of cellular differentiation and reactivity. Governing these patterns are the vascular endothelium, smooth muscle (SM), adventitia, sympathetic perivascular nerves (SPN) and the parenchyma. Whereas an extensive literature details effects of non-neuronal factors on cerebral arteries, the trophic role of perivascular nerves remains unclear. Hypoxia increases sympathetic innervation with subsequent release of norepinephrine (NE), neuropeptide-y (NPY) and adenosine triphosphate (ATP), which exert motor and trophic effects on cerebral arteries and influence dynamic transitions among smooth muscle phenotypes. Our data also suggests that the cerebrovasculature reacts very differently to hypoxia in fetuses and adults, and we hypothesize that these differences arise from age-related differences in arterial smooth muscle phenotype reactivity and proximity to trophic factors, particularly of neural origin. We provide an integration of recent literature focused on mechanisms by which SPN mediate hypoxic remodeling. Our recent findings suggest that trophic effects of SPN on cerebral arteries accelerate functional maturation through shifts in SM phenotype in an age-dependent manner.

Keywords

Cerebral Arteries; Chronic Hypoxia; Neuropeptide Y; Perivascular Sympathetic Innervation; Smooth Muscle Phenotype; Vasotrophic Effects

INTRODUCTION

Rates of premature births are increasing globally owing to numerous different causes that vary from country to country (1, 2). Despite this heterogeneity, a common feature among causes of premature birth, including gestational diabetes, preeclampsia (3) and placental insufficiency (4) involves varying severities of hypoxia (5). Exposure to reduced levels of

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oxygen induces multiple intrinsic compensatory mechanisms geared towards preserving oxygen delivery, particularly to the fetal brain and heart (6–8). Persistent exposure to hypoxia eventually overwhelms these intrinsic compensations and subsequently results in pathophysiological changes in the structure and function of many different tissues (9–11). In many cases, fetuses survive initial hypoxic insults but acquire increased long-term risks for altered cerebral and / or cardiovascular homeostasis (12–15). Increased long-term vulnerabilities to coronary, cerebrovascular, and even metabolic diseases secondary to such in utero fetal insults has been defined as fetal programming (14, 16).

Given that the brain has a high oxygen and metabolic demand with no commensurate reserves, its vasculature promptly undergoes angiogenesis and remodeling during hypoxic episodes (17–19). Several structural (18) and functional (20–22) changes in the cerebral vascular network define these remodeling processes. In addition, immaturity of the fetal cerebral vasculature increases the extent of remodeling upon exposure to decreased oxygen tension (10, 23, 24). Because of the highly heterogeneous mix of cells present in the medial layer of the artery wall and their innate plasticity, their reactivity to hypoxia varies significantly with artery type, size, and location (25, 26). Several studies have further suggested that shifts in smooth muscle phenotype are critically important components of vascular remodeling (27–31).

Under normoxic conditions HIF-1α is synthesized but rapidly ubiquitinated and targeted for proteosomal degradation (32). However, during hypoxia, the oxygen regulated HIF-1α-subunit gets stabilized, accumulates and dimerizes with the constitutively expressed HIF-1β-subunit. The HIF dimer then triggers a cascade of events that culminate in the transcription of multiple genes that encode numerous proteins including several angiogenic cytokines (33) (34). Coupling between HIF and angiogenic factors such as erythropoietin (EPO), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and their respective receptors serve to maintain the supply of oxygen and fuels to all cells (34). Cellular metabolic status and survival during hypoxia depend heavily on how successfully these compensatory changes increase vascular density, oxygen delivery and metabolic adaptation to hypoxia.

Previous work characterizing the various cell types in the artery wall reveals that these cells are tightly and uniquely organized into distinct phenotypic categories. In turn, the characteristics of these cells vary in relation to their relative distances to sources of various trophic factors coming from either the parenchyma or the lumen (35). Given that fetal vascular smooth muscle cells are largely immature and subject to high rates of differentiation, smooth muscle cells in all major phenotypic categories including migratory, proliferative, synthetic, and contractile can be found in the fetal arterial wall (Figure 1). In contrast, adult smooth muscle cells are more highly differentiated, at least under normoxic and non-pathologic conditions (29). Extravascular tissue cells, such as neurons and glia in the brain parenchyma, serve as a major source of vasotrophic factors that influence vascular differentiation and adaptation to hypoxia (35–38).

Another major source of vasotrophic factors is the vascular endothelium, which can exert both autocrine and paracrine effects on cerebrovascular smooth muscle and thereby

contribute to vascular adaptation to chronic hypoxia (39–41). In addition, vasotrophic factors such as VEGF can exert direct angiogenic and remodeling effects on vascular smooth muscle cells during exposure to low oxygen tensions (42, 43). During hypoxic insults or vessel injury, adventitial fibroblasts can also migrate into the intima as myoblasts, which transform into myocytes and finally differentiate into smooth muscle cells (44, 45). Additional progenitor cells have also been suggested to migrate into the vascular medial layer during hypoxic exposure where they transform and differentiate into smooth muscle cells (46, 47).Other fetal stresses, such as maternal food restriction, also can exert trophic influences that alter the structural and functional characteristics of cerebral arteries (48). During maternal food restriction in particular, stress-induced maternal glucocorticoid release can downregulate mediators of fetal angiogenesis and remodeling, including VEGF and its receptors (49). Further studies have implicated glucocorticoids as mediators of smooth muscle redifferentiation toward a non-contractile phenotype, as indicated by an increased colocalization of smooth muscle alpha actin with non-muscle myosin heavy chain II (48).

In addition to humoral mechanisms, a broad variety of evidence supports a major role for the sympathetic autonomic nervous in hypoxic cerebrovascular remodeling (50–52). Sympathetic nerves express receptors for VEGF (53) that stimulate proliferation and differentiation of neural cells consequently increasing the release of vasotrophic factors (53) (54). This review focuses on the role of the sympathetic autonomic system in hypoxic remodeling, particularly in the fetal cerebral vasculature. The main hypothesis addressed here is that the sympathetic perivascular innervation contributes to both structural and functional hypoxic remodeling of the fetal cerebrovasculature through a combination of adrenergic and non-adrenergic mechanisms.

Neuronal Pathways of Hypoxic Remodeling

Hypoxia upregulates sympathetic perivascular innervation in fetal cerebral arteries

Cerebral blood flow is regulated by numerous factors, including locally produced metabolites, circulating neurohormones, intrinsic myogenic mechanisms, and perivascular nerves (55–57). Under hypoxic conditions, the contribution from perivascular sympathetic nerves can become increasingly important, particularly at the limits of cerebral autoregulation (58). Correspondingly, hypoxic acclimatization can significantly increase the density of the sympathetic innervation (50, 59–61). Consistent with these and other findings (62–64), reactivity to electrical nerve stimulation after 110 days of altitude hypoxic acclimatization was significantly increased in fetal cerebral arteries, even though it was significantly depressed in adult cerebral arteries (Figure 2). This high degree of age-dependence could be accounted for by differences in sympathetic nerve density, neurotransmitter content, quantal release (64), reuptake capacity, cleft width or rates of neurotransmitter degradation (65). Alternatively, dynamic transitions between synthetic and contractile phenotypes during hypoxia may also be age-dependent (29, 66–69). In support of this latter possibility, adult vascular smooth muscle is more resistant to extraneous factors that induce phenotypic transformation, whereas fetal smooth muscle typically exhibits enhanced sensitivity to vasotrophic factors (70–72). Closely related to this concept is the finding that sympathetic nerve endings are typically more diffusely distributed throughout

the medial smooth muscle layer in fetal than in adult cerebral (73) and non-cerebral (74) arteries.

To further explore the effects of postnatal maturation and chronic hypoxia on the characteristics of the sympathetic cerebrovascular innervation, we have recently examined immunoreactivity for tyrosine hydroxylase (TH) and dopamine beta hydroxylase (DβH), the rate-limiting enzyme in the biosynthesis of norepinephrine (Figure 3). In adult cerebral arteries, immunostaining for both TH and DβH revealed well-developed nerve terminals at the medial-adventitial junction with long axes of medial smooth muscle nuclei oriented circumferentially around the lumen. In contrast, immunostaining of fetal arteries demonstrated an adrenergic innervation that was diffuse, was less well organized, and extended significantly into the medial layer. Although smooth muscle cells were more abundant in the fetal arteries, they were more poorly organized and their nuclei were more heterogeneously shaped and oriented than in adult arteries. In the adventitial layer, the density of cell nuclei, which presumably includes fibroblasts and other smooth muscle precursor cells, was relatively sparse in adult arteries but much greater in fetal arteries (Figure 3). These age-related differences in the patterns of cell size and organization in the artery wall emphasize the important effects of maturation on arterial structure and function. These differences also reinforce the view, particularly in fetal arteries, that smooth muscle cells of all major phenotypes including migratory, proliferative, synthetic and contractile, (29) are simultaneously present together with adrenergic nerves in both mature and immature cerebral arteries (Figure 1). Together, these results are highly consistent with the findings that activation of perivascular adrenergic nerves is far more efficacious in adult than in fetal cerebral arteries (75).

Preliminary studies recently undertaken in our laboratory have examined the effects of chronic hypoxia on the abundance and organization of the sympathetic perivascular innervation in ovine fetal cerebral arteries. These studies suggest, through measurements of both immunostaining and immunoblotting for DβH, that chronic hypoxia dramatically increases the density of sympathetic innervation in ovine cerebral arteries, as reported in other preparations (50). Whereas our model is unique in that hypoxia is imposed in a large mammal by acclimatization at high altitude (3820m) for 110 days (43, 68), the results add further support to the concept that chronic hypoxia induces expansion, and presumably efficacy, of the perivascular sympathetic innervation.

Sympathetic perivascular nerves influence cerebrovascular structure and function

The sympathetic autonomic nervous system is a key regulator of cerebral blood flow (76), particularly under conditions of hypoxia and hypercapnia (77). Sympathetic activation during hypoxia can significantly increase contractile tone, decrease arterial diameter, and enhance wall thickness (78). Such increases in wall thickness may afford protection against elevated wall stress in cerebral blood vessels (79). Importantly, the character and extent of the sympathetic innervation pattern is highly dynamic (80) and subject to modulation during the physiological processes of ageing and development as well as pathophysiological processes such as hypertension and chronic hypoxia (64, 81). In turn, sympathectomy promotes remodeling of the extracellular matrix and promotes redifferentiation of smooth

muscle toward non-contractile phenotypes (82). These findings are highly consistent with our preliminary findings that chronic hypoxia upregulated contractile responses to sympathetic activation in fetal cerebral arteries (Figure2).

Sympathetic nerves can also exert potent long-term trophic effects on arterial structure and function (78, 83–85). Some of these changes include increases in arterial wall stiffness secondary to either surgical or chemical sympathectomy in multiple models (86, 87) suggesting that sympathetic nerves mediate preferential decreases in the ratio of collagen to elastin (88). Whereas adult subjects appear relatively resistant to effects of surgical or chemical denervation, fetuses respond more robustly via marked changes in arterial wall composition (71) and distensibility (86). Importantly, most changes in arterial structure and function resulting from sympathectomy are directly attributable to changes in the release, reuptake and degradation of the multiple neurotransmitters released by the sympathetic perivascular innervation (89).

Norepinephrine released from sympathetic nerves exerts trophic effects on arterial smooth muscle

Norepinephrine is the main neurotransmitter released from post-ganglionic sympathetic nerve terminals that innervate vascular smooth muscle cells. In turn, hypoxic upregulation of sympathetic nerve activity increases the local release and activity of norepinephrine (89, 90). The immediate effects of norepinephrine involve induction of contraction in most artery types, including cerebral arteries (91, 92). In contrast, the long-term effects of sympathetic nerve activation include trophic effects of arterial smooth muscle that alter the function of contractile proteins (93) and electrical behavior of the smooth muscle membrane (94). Not surprisingly, the magnitude of norepinephrine induced trophic effects on the artery wall correlate with mass of norepinephrine released (90), access to its receptors (95), adrenergic receptor type and density (92, 96, 97), rates of reuptake, and synaptic cleft width (98)} (64, 99, 100). As indicated by denervation studies, the sympathetic perivascular innervation also influences artery wall thickness, rates of hyperplasia and hypertrophy, and remodeling of the adventitial matrix (72). Norepinephrine released from sympathetic nerves also can exert paracrine trophic effects and induce secretion of other trophic factors (101) that alter arterial phenotype in the cerebral vasculature (102). Together, these results highlight the important role of the sympathetic perivascular innervation, and norepinephrine in particular, on the maturation and differentiation of arterial smooth muscle (54).

Neuropeptide-Y (NPY) released from sympathetic nerve terminals exerts trophic effects on arterial smooth muscle

In addition to norepinephrine, sympathetic perivascular nerves release other vasoactive compounds, and careful analysis reveals that adrenergic receptors cannot mediate all the effects of sympathetic nerve activation (103–105). To probe the potential involvement of molecules other than norepinephrine in the effects of sympathetic stimulation on cerebral arteries, we recently examined the effects of transmural stimulation on cerebral artery contractions before and after depletion of norepinephrine with guanethidine. Our results demonstrated a significant guanethidine, non-adrenergic component that in fetal but not adult arteries was significantly greater than the norepinephrine component and was

enhanced by hypoxia (Figure4). These findings suggest, at least in fetal cerebral arteries, that non-adrenergic factors released from sympathetic nerves may play an important role in hypoxic cerebrovascular remodeling.

Aside from norepinephrine, Neuropeptide-Y (NPY) is the most potent vasoconstrictor released from sympathetic post-ganglionic nerve terminals (106–108). Composed of 36 amino acids NPY exerts direct vasoconstrictor effects through activation of Y1 receptors (109) on many blood vessel types (103, 110), including cerebral arteries (103, 106) (110–113). Although NPY does not initiate phasic contractions or induce spontaneous rhythm contractility, it can enhance the tone and frequency of phasic contractions produced by norepinephrine (114). NPY also potentiates the vasoconstrictor effects of norepinephrine (115) and is an important mediator of the contractile response to high frequencies of sympathetic nerve stimulation (116).

In addition to its acute contractile effects, NPY also has potent trophic and mitogenic effects on arterial smooth muscle (104). Of the 6 known subtypes of NPY-Y receptors, NPY exerts its most potent trophic effects through activation of its Y-1 receptors, which appear the subtype preferentially expressed by vascular smooth muscle (109, 117). Activation of Y1 receptors, in turn, promotes both angiogenesis and remodeling via cellular adhesion, migration, proliferation, and differentiation in the cerebral vasculature during periods of hypoxia (106). In addition, however, activation of Y2 and Y5 receptors can also promote angiogenesis (117). Through actions on these receptors, NPY can promote phenotypic transformation of arterial smooth muscle in both mature and immature arterial smooth muscle (118).

Adenosine Triphosphate (ATP) released from sympathetic nerve terminals exerts trophic effects on arterial smooth muscle

Together with norepinephrine and NPY, sympathetic nerves also release adenosine triphosphate (ATP) (105, 119–121). The idea that norepinephrine, NPY and ATP are released simultaneously (119, 122, 123) has evolved over the years into the more recent concept that ATP is released much earlier than norepinephrine (124, 125). In this way, ATP induces the initial phasic vasoconstriction and norepinephrine initiates more slowly developing and longer-lasting tonic contractions (124–127). ATP effects are largely mediated by the P2 class of purinergic receptors, which are coupled to the smooth muscle interior through either ion channels (P2X) or G-protein coupled receptors (P2Y), both of which can be found in cerebral arteries (52, 128). In smooth muscle, both classes of P2 receptors can mediate short-term effects on contractile tone, and also potent long-term mitogenic and trophic effects (104). Depending on the vessel type and receptor activated, ATP can promote cellular proliferation, migration or apoptosis (52, 121). These effects must be transduced very rapidly, however, because ATPases (124, 125, 129) and ADPases (130) are concurrently released with ATP and truncate its duration of effects. Even so, the characteristics of ATP render it a strong candidate for contribution to cerebrovascular remodeling, particularly under conditions of hypoxia that promote expansion of the sympathetic perivascular innervation. The exact role played by ATP in this capacity, however, remains largely unexplored in cerebral arteries of any age.

Overview

Vasotrophic factors that govern the growth and differentiation of cerebrovascular smooth muscle arise from many sources, including the neurons and glia of the brain parenchyma (131, 132), smooth muscle progenitors (133–137) and immune cells (138, 139) that reside in the adventitia, smooth muscle cells acting through autocrine and paracrine mechanisms in the medial layer (140), and the vascular endothelium (141). Adding to this rich mixture are perivascular nerve endings at the adventitial-medial border that release an abundance of vasotrophic factors including norepinephrine, NPY and ATP (52, 106, 142). The release of all these factors is enhanced by fetal hypoxia, due to concomitant increases in VEGF, which has potent growth-promoting effects on the sympathetic innervation (54) (Figure 5). The combined trophic actions of norepinephrine on α1-adrenergic receptors, NPY on Y1 receptors, and ATP on P2X/P2Y receptors all promote the contractile differentiation of vascular smooth muscle toward the contractile phenotype, particularly in immature arteries that are phenotypically highly plastic (101, 104, 143, 144). These effects can enhance fetal cerebral artery contractility, but also attenuate artery stiffness (87), probably through mechanisms that depress the ratio of collagen to elastin (88). Independent of the sympathetic innervation, fetal hypoxia can also increase wall thickness, increase stiffness, and depress contractility (6), indicating that the fetal cerebrovascular response is an integration of many different and highly dynamic influences. Chief among these is the sympathetic nervous system, which acts in a highly age-dependent manner to influence the structural and functional maturation of fetal cerebral vasculature, particularly under conditions of hypoxia.

ACKNOWLEDGMENTS

The work reported in this manuscript was supported by USPHS Grants HL54120, HD31266, HL64867, NS076945 and the Loma Linda University School of Medicine.

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Figure 1. The Continuum of Vascular Smooth Muscle Phenotypes

The medial layer of arteries consists of a highly heterogeneous mix of cells of diverse origins. Many but not all smooth muscle cells begin as adventitial fibroblasts. These fibroblasts initially differentiate into myofibroblasts and then into smooth muscle myocytes that migrate through the medial layer. Migratory myocytes can then transform into proliferative, synthetic and contractile smooth muscle in response to growth factor stimulation. These patterns of differentiation are not terminal, and can be reversed when certain growth and stress factors are introduced in the local environment. In this manner, the artery wall is highly dynamic and heterogeneous in terms of both its structural and functional characteristics.

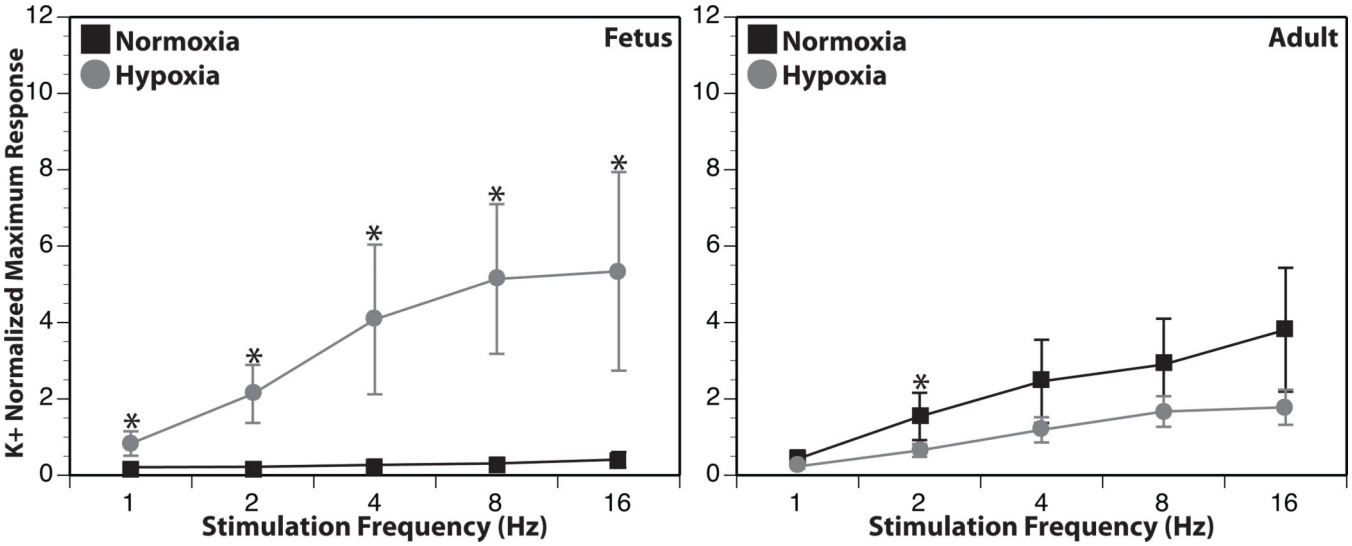

Figure 2. Chronic hypoxia modulates contractile responses to transmural nerve stimulation in an age-dependent manner

Following 110 days of hypoxic acclimatization, reactivity to electrical nerve stimulation in ovine fetal cerebral arteries was significantly enhanced compared to normoxic controls. Conversely, hypoxic acclimatization modestly depressed contractile reactivity to nerve stimulation in adult cerebral arteries. Results are presented as mean ± SEM. For fetal normoxic (FN), fetal hypoxic (FH), and adult normoxic (SNC) groups, N=16. For the adult hypoxic group (SHC), N=21.

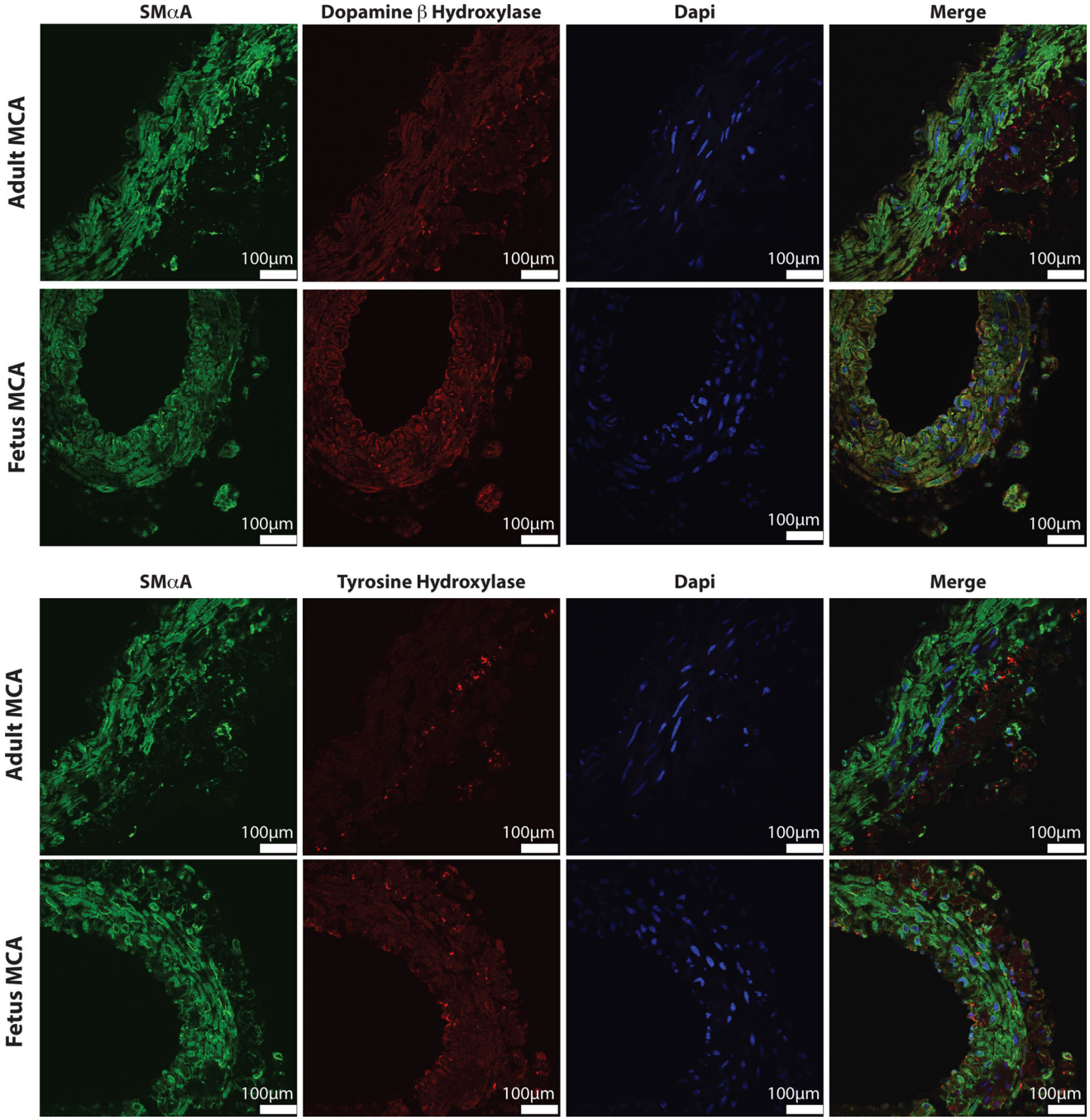

Figure 3. Dopamine βhydroxylaseand tyrosine hydroxylase staining demonstrate major developmental differences

Immunofluorescent staining for both dopamine β hydroxylase (DβH) and tyrosine hydroxylasein ovine middle cerebral arteries revealed distinct and well-developed neuronal terminals at the medial-adventitial border. In adult arteries, long axes of smooth muscle cell nuclei were oriented circumferentially and adventitial cells were relatively sparse. In contrast, within fetal arteries the neuronal terminals were much more diffuse with extensions well into the medial layer. In addition, adventitial cell density was much greater than in adult arteries and smooth muscle nuclei were more abundant but less organized in fetal arteries.

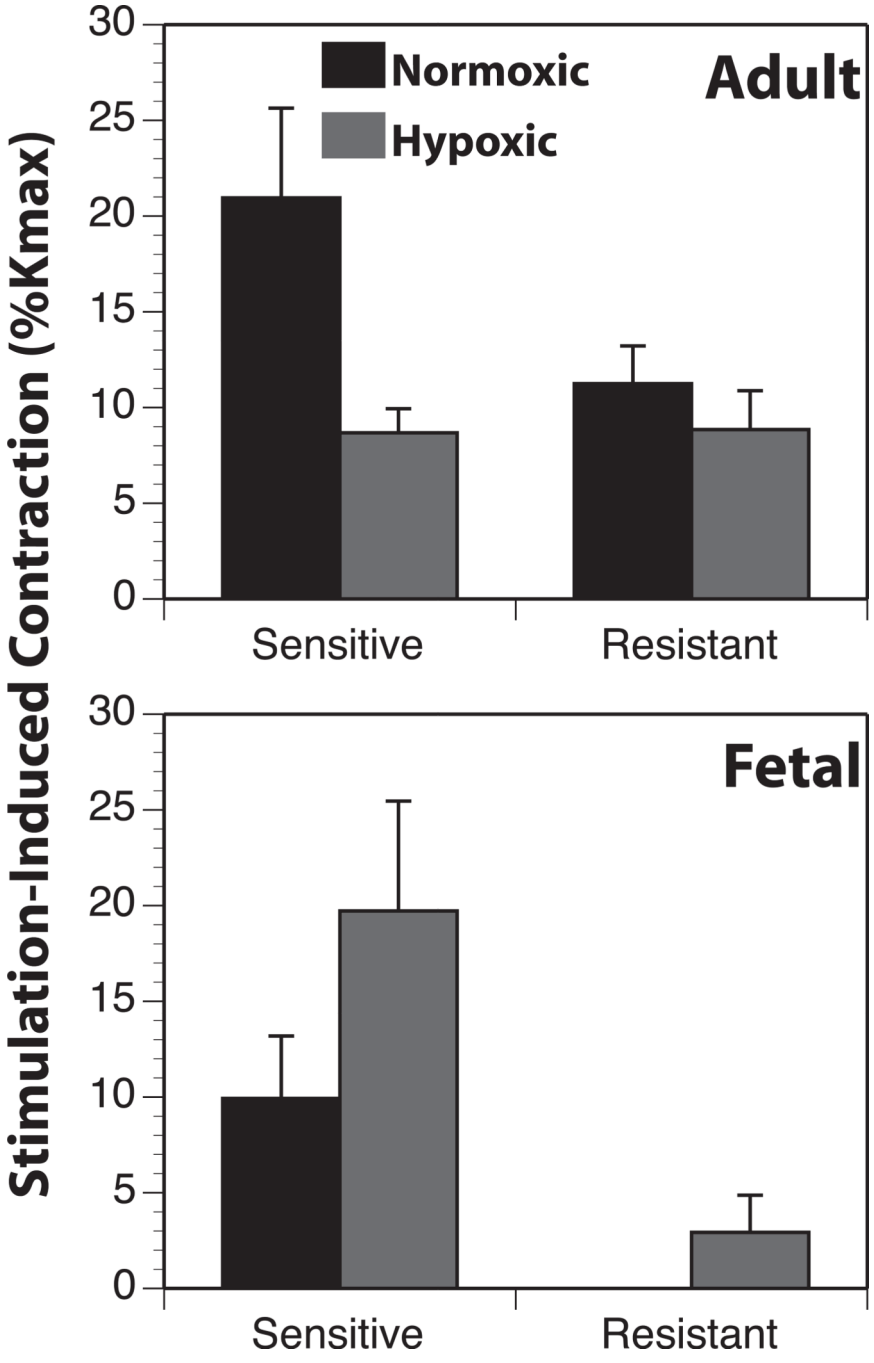

Figure 4. Chronic hypoxia enhances guanethidine-resistant contractions in fetal arteries

To test the possible involvement of non-adrenergic factors in modulation of arterial reactivity during hypoxic acclimatization, electrical nerve stimulation was applied before and after catecholamine depletion with guanethidine. The guanethidine-sensitive (GS) component was an index of the adrenergic contribution to arterial reactivity whereas the guanethidine resistant (GR) component was an index of the sympathetic release of a contractile and potentially trophic molecule other than NE. In adult arteries, hypoxia decreased only the GS component. Conversely, in fetal arteries hypoxia increased both the

GR and GS components, suggesting that hypoxia preferentially enhanced release of a non-adrenergic transmitter from sympathetic perivascular nerves in fetal arteries. Results are presented as mean ± SEM.

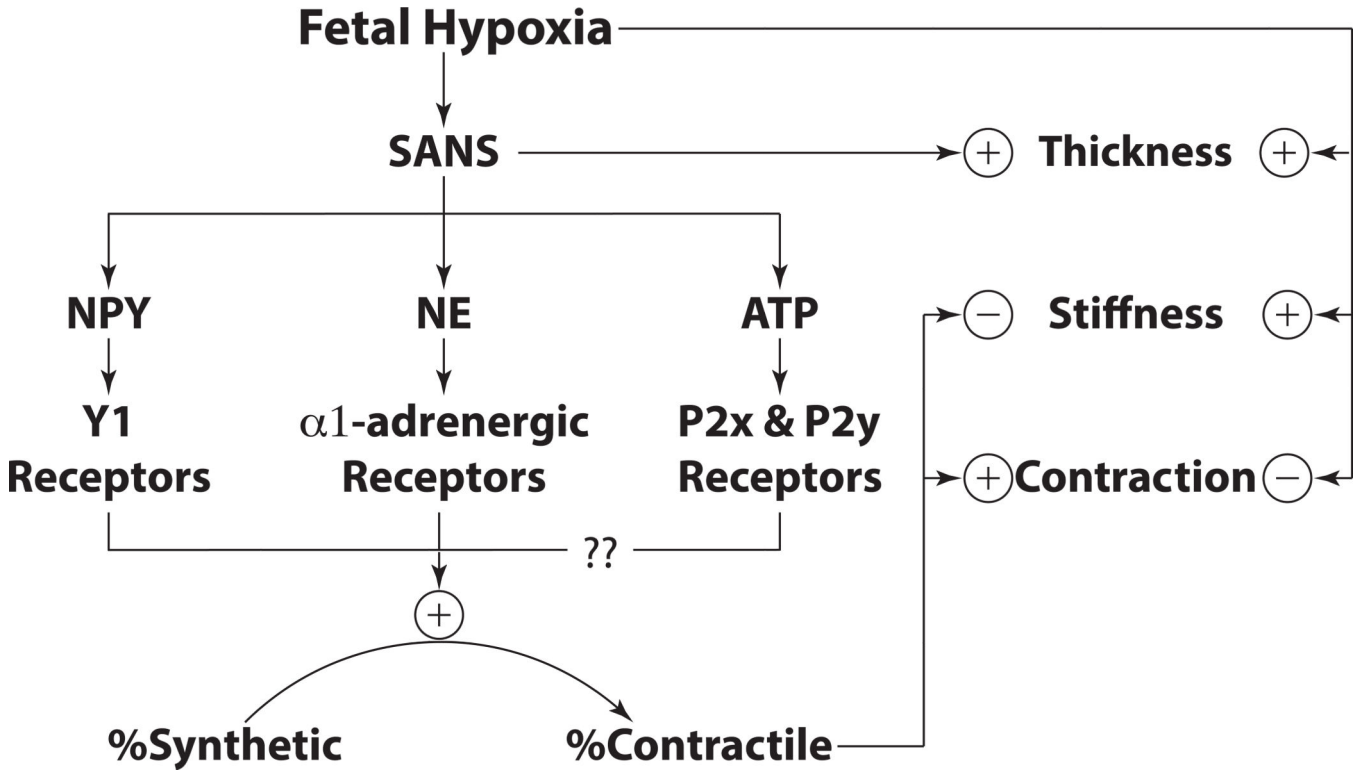

Figure 5. Overview Schematic

Hypoxic acclimatization induces the release of trophic factors such as VEGF, which stimulates growth and expansion of the sympathetic perivascular innervation. These nerves, in turn release NE, NPY and ATP, all of which act through their respective receptors to promote contractile differentiation of smooth muscle. These phenotypic changes enhance contractility but depress stiffness, as shown by denervation experiments. Independent of the sympathetic nerves, hypoxia can increase wall thickness and stiffness while also depressing contractility. Owing to these opposing effects, the final influence of hypoxia on artery structure and function depends on the balance between nerve-dependent and nerve-independent mechanisms.