Smooth muscle contractile diversity in the control of regional circulations

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Reho JJ, Zheng X, Fisher SA. Smooth muscle contractile diversity in the control of regional circulations. Am J Physiol Heart Circ Physiol 306: H163–H172, 2014. First published November 1, 2013; doi:10.1152/ajpheart.00493.2013.—Each regional circulation has unique requirements for blood flow and thus unique mechanisms by which it is regulated. In this review we consider the role of smooth muscle contractile diversity in determining the unique properties of selected regional circulations and its potential influence on drug targeting in disease. Functionally smooth muscle diversity can be dichotomized into fast versus slow contractile gene programs, giving rise to phasic versus tonic smooth muscle phenotypes, respectively. Large conduit vessel smooth muscle is of the tonic phenotype; in contrast, there is great smooth muscle contractile diversity in the other parts of the vascular system. In the renal circulation, afferent and efferent arterioles are arranged in series and determine glomerular filtration rate. The afferent arteriole has features of phasic smooth muscle, whereas the efferent arteriole has features of tonic smooth muscle. In the splanchnic circulation, the portal vein and hepatic artery are arranged in parallel and supply blood for detoxification and metabolism to the liver. Unique features of this circulation include the hepatic-arterial buffer response to regulate blood flow and the phasic contractile properties of the portal vein. Unique features of the pulmonary circulation include the low vascular resistance and hypoxic pulmonary vasoconstriction, the latter attribute inherent to the smooth muscle cells but the mechanism uncertain. We consider how these unique properties may allow for selective drug targeting of regional circulations for therapeutic benefit and point out gaps in our knowledge and areas in need of further investigation.

vascular smooth muscle; contractile; myosin; isoforms; renal; hepatic; pulmonary

THE DISTRIBUTION OF THE cardiac output to the regional circulations is determined by the local resistance to blood flow, a function that is dynamically regulated by the state of contraction of the vascular smooth muscle. Each regional circulation has unique requirements for blood flow and thus unique mechanisms by which it is regulated. As the resistance to flow through a vessel is inversely proportional to the fourth power of its radius, the primary resistance to blood flow, and thus site of regulation, is the small arteries and arteriolar network. It is well recognized that the large (conduit) and small (resistance) arteries are phenotypically and functionally distinct in both the systemic and pulmonic circulations (20, 29, 40, 110). The smooth muscle of large arteries and veins express the slow contractile gene program characteristic of the tonic phenotype. The smooth muscle of the small resistance arteries in the systemic circulation express components of the fast contractile gene program characteristic of the phasic phenotype. At its simplest smooth muscle contraction is determined by calcium influx, activation of myosin light chain (MLC) kinase (MLCK) and phosphorylation of myosin regulatory light chain, and relaxation by calcium efflux, regulation of myosin phosphatase (MP) activity, and dephosphorylation of myosin regulatory light chain (Fig. 1). While these same basic mechanisms apply to tonic and phasic smooth muscle, how they are controlled in each is quite distinct [reviewed in (53, 70)]. This review will focus on vascular smooth muscle phenotypic and functional differences within select circulations to highlight unique properties in the control of each regional circulation. Other aspects of unique control mechanisms of regional circulations, such as differences in controlling signals, are beyond the scope of this review [see recent reviews (5, 14, 15, 30)]. The significance of these differences with respect to the dysregulation of blood flow in disease and the potential for specificity in pharmacological targeting will also be discussed.

Smooth Muscle Contractile Phenotypes: Fast and Slow Gene Programs

It was almost 50 years ago that Somlyo and Somlyo (106) and Hermsmeyer (59) recognized functional differences between vascular tissues which they termed pharmacomechanical versus electromechanical coupling. In the intervening years, it became apparent that smooth muscle contractile function in the different organ systems is incredibly diverse and difficult to capture with a single simple classification scheme. The classification most relevant to organ system function is that of phasic versus tonic contractile activities which loosely corresponds to the dichotomy of electro- versus pharmacomechanical coupling. Phasic or fast rhythmic contracting smooth muscle is a
hallmark of the gastrointestinal and urinary systems that require highly coordinated unitary muscle function. Tonic or slow-sustained contracting smooth muscle is characteristic of the large arteries and veins involved in conduit and capacitance roles that function primarily as independent units. The advent of the molecular era of basic cardiovascular research in the 1990s led to the discovery of some of the molecular mechanisms underlying these contractile differences [reviewed in (40)]. In all muscle lineages (striated and smooth muscle), myosin isoforms are primary determinants of fast versus slow contractile properties (101). In smooth muscle, a 21 nucleotide alternative exon coding for a seven amino acid insert in the head of the myosin heavy chain was identified and conferred threefold increased myosin ATPase activity and shortening velocity in phasic smooth muscle (66) (Fig. 2A). Isoforms of the essential MLC (MLC17) generated by alternative splicing of a 39 nucleotide alternative exon were also identified and proposed to determine myosin and smooth muscle contractile kinetics (41, 54, 58, 78). These studies were predominately based on comparisons of tonic vascular smooth muscle and phasic visceral smooth muscle or experiments in vitro. In a landmark study, DiSanto and coworkers (28) showed that the fast isoforms of myosin heavy and light chain determine smooth muscle force production. NO, nitric oxide; E24, exon 24; P, phosphate.

A similar story has unfolded with regard to the expression of the regulatory proteins MLCK and MLC phosphatase (MLCP) that activate and deactivate the myosin motor, thereby mediating vasoconstriction and vasodilation, respectively. Several-fold increased expression and activity of MLCK and MLCP in phasic versus tonic smooth muscle were proposed to confer faster rates of contraction and relaxation (46) (Fig. 2B). Many of the signals that regulate vascular smooth muscle tone were subsequently shown to do so by controlling the activity of MP [reviewed in (47, 53)]. We and others have proposed that the regulated expression of two subunits of MP, the inhibitory subunit C-kinase potentiated protein phosphatase-1 inhibitor (CPI-17; PPP1R14a) and the regulatory subunit [PPP1R12a; myosin phosphatase targeting subunit 1 (Mypt1)], determine tissue and vessel-specific responses to vasoconstrictors and vasodilators (Fig. 2C). The level of expression of CPI-17 relative to MP is proposed to determine sensitivity to agonist-mediated inhibition of MP and thus force production [reviewed in (32)]. The alternative splicing of a 31 nt exon 24 (E24) generates isoforms of Mypt1 that contain or lack a COOH-terminal leucine zipper motif (LZ) that is required for nitric oxide (NO)/guanosine-cyclic monophosphate (cGMP)-mediated activation of MP and relaxation [reviewed in (40)]. In this review we will examine their patterns of expression of the
contractile machinery in different regional circulations and review progress that has been made in determining the role of unique smooth muscle contractile properties in conferring the unique properties of the highlighted regional circulations.

Renal Circulation

A unique aspect of the renal circulation is the arrangement of afferent and efferent arterioles in series separated by the glomerulus through which the blood is filtered (Fig. 3A). The tone of the afferent and efferent arterioles is a key determinant of the glomerular filtration rate and thus kidney function as well as renal vascular resistance and blood flow [reviewed in (85)]. Differences between the afferent and efferent arterioles are well described, whereas the significance of these differences remains conjectural. The afferent arteriole responds rapidly to changes in pressure and flow with a myogenic contractile response that autoregulates blood flow as well as a tubuloglomerular feedback mechanism that also autoregulates blood flow in the kidney. The myogenic response is characteristic of resistance arteries and absent in the tonic smooth muscle of conduit vessels. It is also absent in the efferent arteriole, which maintains tone and regulates glomerular filtration through changes in tone. Consistent with these functional differences, the fast (phasic) isoform of the smooth muscle-specific myosin heavy chain (MYH11) is expressed in the afferent arteriole, whereas only the slow (tonic) isoform is expressed in the efferent arteriole (104). The fast isoform is generated by the inclusion of a 21-nucleotide alternative exon (E8) in the myosin head (SM-B) and increases myosin ATPase activity and maximum velocity of shortening severalfold (Fig. 2A) (66). This correlates with the higher myosin ATPase activity and shortening velocity of agonist (angiotensin II and norepinephrine)-induced contractions of rat and mouse afferent versus efferent arterioles. However, inactivation of the fast (SM-B) exon did not equalize contraction velocities in mouse afferent and efferent arterioles, suggesting other undefined molecular differences as contributory to these functional differences (90). Isoforms of the actin filaments could also be involved in the functional differences between afferent and efferent arterioles. Expression of smooth muscle-specific α-actin has been demonstrated to be similar in afferent and efferent smooth muscle (67). Currently, there are no data regarding the functional differences noted in the afferent and efferent arterioles by regulating the activity of the MP. Indeed, Inscho and col-

Fig. 2. A: actin and myosin variants of the smooth muscle contractile apparatus. Alternative splicing of the myosin heavy and light chain transcripts generates isoforms with differential ATPase and shortening velocities. Variants of actin arise from separate gene products; however, the functional significance of these variants is unknown. B: phasic and tonic smooth muscle contractions and fast and slow gene programs. Phasic (fast) smooth muscle demonstrates relatively rapid cycling of force, whereas tonic (slow) smooth muscle demonstrates resting tone and slow modulation of force. C: isoforms of the myosin and MP subunits are generated by alternative splicing and specific to phasic and tonic smooth muscle. MLC, myosin light chain; F, fast; S, slow; E, exon; cGK, cGMP protein kinase.
leagues (64) demonstrated greater expression of the ROCK-1 versus ROCK-2 isoform in the afferent arterioles. There are no data regarding the relative expression of these isoforms in the efferent arterioles. The contribution of the ROCK signaling pathway to the regulation of afferent and efferent arteriolar function is controversial. Several studies have suggested equal contributions of the ROCK signaling pathway in afferent and efferent arterioles (19, 97). However, Nakamura and colleagues (84) suggest the inhibition of the ROCK pathway preferentially dilates the afferent arteriole.

There is also evidence for differences in ionic fluxes regulating the activation and deactivation of the contractile apparatus in afferent versus efferent arteriolar smooth muscle. Membrane depolarization and opening of voltage-gated calcium channels occurs with agonist or potassium chloride-induced force production in the afferent arterioles, whereas the efferent arterioles do not significantly depolarize and only release calcium from internal stores (16, 73). Similarly, the afferent arteriole is dilated by endothelium-derived hyperpolarizing factor (extracellular potassium) and hyperpolarization, whereas the efferent arteriole is not (21, 115). In other circulations, a greater role of endothelium-derived hyperpolarizing factor versus endothelium-derived relaxing factor (NO) in resistance versus larger conduit arteries has been attributed to increased expression of inward rectifier potassium channels (Kir2.1) (94). However, no difference in Kir2.1 expression was observed between afferent and efferent arterioles (21), and the molecular basis for these differing responses remains undefined. The large conductance calcium-activated potassium channel BKCa also appears to play more of a functional role in the afferent versus efferent arterioles, as chemical activators and inhibitors cause dilation and constriction of the former but not the latter in ex vivo preparations (34). BKCa is also a mediator of NO/cGMP-dependent vasodilation in the renal afferent arteriole (105). NO regulates afferent and efferent arteriole tone (105, 118), while it is controversial as to whether it has different roles or targets in the two vessels (87). Interestingly, NO/cGMP increases intracellular calcium in the afferent arteriole when BKCa is blocked (37) (the efferent arteriole was not studied), reminiscent of the ability of cGMP to induce phasic contractile activity (vasomotion) in resistance arteries, possibly through activation of chloride channels [reviewed in (49)], whereas NO/cGMP only relaxes conduit artery tonic smooth muscle. L-type calcium currents are evident in afferent but not efferent arterioles (17, 38, 72) [reviewed in (56)], correlating with the expression of the L-type channel subunit voltage-dependent calcium channel 1.2 (52).

However, there remains controversy as to the expression and activity of L- and T-type calcium channels in afferent and efferent arterioles, perhaps a function of regional (cortical vs. medullary) specialization within the kidney or differences between species. Prostanoids may also be involved in the functional differences between the afferent and efferent arteriole system. Thromboxane has been demonstrated to preferentially constrict the afferent arteriole with nominal effects on the efferent arteriole (55). Additionally, prostaglandin E2 dilates the efferent arteriole via the prostaglandin E2 receptor (63). Localized production of 20-hydroxyecicosatetraenoic acid seems to act as a vasoconstrictor to the afferent arteriole and modulates the myogenic response (45).

Fig. 3. Smooth muscle contractile diversity in regional circulations. A: afferent and efferent arterioles of the renal circulation are arranged in series and determine glomerular filtration rate. Afferent arterioles express components of the fast smooth muscle gene program involved in the myogenic contractile response, whereas efferent arterioles express a slow gene program and maintain tone and constant perfusion pressure. B: portal vein and hepatic artery are arranged in parallel to form a dual blood supply for delivery of oxygen and nutrients to the intestine. The low-pressure portal vein expresses a purely fast gene program and displays phasic contractility, whereas the high-pressure hepatic artery expresses a slow gene program and tonic contractile activity. The hepatic arterial buffer response (HABR) describes changes in blood flow through the hepatic artery to buffer changes in portal venous blood flow and maintain total blood flow to the liver constant. C: pulmonary arteries provide a low-pressure, low-resistance circulation for the oxygenation and delivery of blood to the heart. Pulmonary arteries constrict to hypoxia [hypoxic pulmonary vasoconstriction (HPV)] to match regional perfusion and ventilation and maximize oxygen uptake.
In summary, the afferent arteriole has properties typical of phasic smooth muscle and a resistance artery, whereas the efferent arteriole has properties more typical of a tonic smooth muscle. These paradigms, while useful, likely represent an oversimplification of their unique properties. A more complete analysis of the afferent versus efferent arteriole gene programs is hindered by their small size, inaccessibility, and mixed cell populations, as is true throughout the microcirculation (discussed further in Conclusions and Perspectives).

Efferent vasoconstriction increases glomerular capillary pressure and preserves glomerular filtration when renal perfusion is reduced, whereas afferent arteriolar constriction reduces glomerular pressures and protects the kidney from increases in perfusion pressure. Reduced renal perfusion and its effect on glomerular filtration and kidney function is a vexing problem in the management of heart failure and a major contributor to heart failure morbidity and mortality. Therapy directed at heart failure may exacerbate or improve the renal dysfunction for reasons that are unknown [reviewed in (8, 9)]. The L-type calcium channel blockers selectively dilate the afferent arteriole and thus improve glomerular filtration rate (1, 57), though whether this increased perfusion would improve the outcome is uncertain and confounded by effects on the heart and other blood vessels. Additionally, the action and efficacy of calcium channel blockers vary considerably in efferent arteriolar smooth muscle based on localization within the kidney (120). Pharmacological analysis of L- and T-type calcium channels in the kidney has been extensively reviewed elsewhere (56). Other vasodilator drugs that open potassium channels and inhibit LTCC via hyperpolarization, such as pinacidil (ATP-dependent potassium channel agonist) (96) and minoxidil (Kir6.1 agonist, as is diazoxide) (42), also preferentially dilate the afferent arteriole, as does hydralazine (42), a vasodilator that is a Kir6.1 agonist, as is diazoxide (42), a vasodilator commonly used to treat hypertension and heart failure. In contrast, inhibitors of angiotensin signaling cause balanced vasoconstriction of the efferent arteriole, as does hydralazine (42), a vasodilator commonly used to treat hypertension and heart failure. In contrast, inhibitors of angiotensin signaling cause balanced vasoconstriction of the afferent arteriole and the vessel is weak or inconsistent in the hepatic venous system and is weak or inconsistent in the hepatic arterial system.

Portal venous smooth muscle is unique among vascular tissues in that it exhibits spontaneous phasic contractile activity (Fig. 2B) and expresses exclusively the fast contractile gene program (24, 80, 81). This includes the fast isoforms (splice variants) of myosin heavy and light chains (79), and levels of the MLCK and MLCP, required for activation and deactivation of the myosin motor unit, that are severalfold higher compared with the tonic smooth muscle (Fig. 2C) (91). The MP targeting subunit Mypt1 is nearly exclusively the E24-included (fast) variant coding for the isoform that lacks the COOH-terminal LZ motif (91). The ratio of γ-actin to α-actin protein is higher in PV (45:55) compared with aorta, similar to other phasic smooth muscle, and increases further in pressure-overload hypertrophy (79). With regard to ion fluxes that activate and deactivate the contractile apparatus, there is limited description of the channels that are expressed and active in portal venous smooth muscle and limited comparisons with the tonic smooth muscle of the large vessels or other tissues and cell types. A number of voltage-dependent potassium channels (KCNQ) are expressed in cultured mouse PV myocytes (111). A splice variant of KCNQ1 (KCNQ1b) was identified that comprised 50% of transcripts and was not identified in heart or brain transcripts (86).

The role of the phasic contractile activity of the PV in the perfusion of the liver under normal conditions has not been experimentally tested. A reasonable speculation is that it serves as a low-pressure venous pump in series with the heart to propel blood to the liver. With regard to mechanisms, the fast isoforms of myosin and higher MLCK and MLCP expression and activity are likely required for the more rapid cycling of force production compared with tonic smooth muscle. PV, like other phasic smooth muscle, expresses the LZ-variant coding for the isoform of Mypt1 not activated by NO/cGMP, consistent with portal venous smooth muscle resistance to NO-mediated dilation in vivo (88) and ex vivo (36). With regard to ion fluxes, KCNQ1b variant contributes to the delayed outward rectifying potassium currents required for repolarization of this smooth muscle, whereas L-type calcium currents and internal stores provide calcium for force activation (4, 107). However, the source of the portal venous pacemaker current has not been defined with certainty. A calcium-activated chloride channel has been proposed to serve this function (26, 39). A different study proposed that a hyperpolarization-activating current pacemaker current carried by hyperpolarization-activated cyclic nucleotide-gated channel 2–4 triggers phasic contractile activity of rabbit PV myocytes (48). There is also evidence for and against dedicated pacemaker cells (interstitial cells of Cajal), as in the arterial buffer response (HABR), in which blood flow through the hepatic artery is adjusted in response to altered flow through the PV to maintain constant perfusion to the liver. The mechanism of the HABR is not defined but may involve adenosine (69). Perhaps relating to the HABR, resting blood flow is high as the liver receives ~25% of the cardiac output while comprising only ~2.5% of the total body weight, such that unlike other systemic beds, oxygen demand is primarily met by increased oxygen extraction rather than increased blood flow (12, 13), as for example in a model of endotoxic shock (98) [see review (31)]. In contrast to this cross regulation, autoregulation of blood flow does not occur in the portal venous system and is weak or inconsistent in the hepatic arterial system.

Splanchnic Circulation

The liver has a dual blood supply arranged in parallel in contrast to the kidney’s in-series arrangement (Fig. 3B). Similar to the kidney, this arrangement is integral to its function. The low-resistance/low-pressure portal vein (PV) provides ~75% of the blood flow to the liver for the purpose of first pass metabolism and detoxification of substances absorbed by the intestine [reviewed in (31, 114)]. The hepatic artery perfuses the liver under systemic pressures and provides ~25% of the blood flow to the liver, which unlike the portal venous blood is fully oxygenated. The perfusion of the liver by the low-pressure (6–10 mmHg) portal venous system reflects the low resistance and pressure (2–4 mmHg) of the sinusoidal bed. Another unique feature of the hepatic circulation is the hepatic-
Mypt1 E24a modulates in disease. In models of portal hypertension (91) and activity (vasomotion) and tonic contractions. Interestingly, the tractile patterns of these arteries including phasic contractile of fast and slow isoforms (splice variants) of the myosin heavy drug therapies problematic. Drugs that specifically target the changes in portal and systemic hemodynamics make vascular models of cirrhosis (65). These antithetical and complex increased role of NO in the splanchnic circulation in animal been determined but would be predicted to increase sensitivity increased, upstream mesenteric arterial resistance is low with resistance to blood flow and normally low portal pressures are decreased by the bradycardic agent ZD-7288 (Zeneca). It would be of interest to determine the effect of inhibition of phasic smooth muscle cell (SMC) contractile activity on blood flow in the portal and other regional circulations. However, ZD-7288 also increases basal tone in PV and other smooth muscle preparations (48), which along with its bradycardic effect on the heart would confound interpretation of its effects on vascular function in whole animal preparations.

Additionally, a role for prostanoids have been suggested in this phasic contractile activity as inhibition of the cyclooxygenase pathway decreases the amplitude of spontaneous phasic contractions in the PV (103). This vascular phasic pumping action is not unique to the PV as most small arteries exhibit some phasic contractile activity termed vasomotion (49, 92) and express components of the fast gene program, the functional significance of which is also not determined. In summary, the PV is unique among vessels as a prototypical phasic smooth muscle and may be useful for defining the full extent of differences between vascular fast and slow muscle gene programs.

Resistance vessels of the mesenteric circulation are the prototypical vessels to study vascular function as the small artery/arteriole network is an important determinant of systemic vascular resistance. The smooth muscle of first order-to-third order mesenteric arteries displays a phenotype intermediate to that of the phasic smooth muscle of the PV and tonic smooth muscle of the hepatic artery. This includes predominant expression of the Mypt1 E24a/LZ- isoform and mixtures of fast and slow isoforms (splice variants) of the myosin heavy light chains (91). This is consistent with the mixed contractile patterns of these arteries including phasic contractile activity (vasomotion) and tonic contractions. Interestingly, the gene program of the smooth muscle of mesenteric arteries also modulates in disease. In models of portal hypertension (91) and flow-induced vascular remodeling (93, 119), there are shifts to Mypt1 E24a-LZ- isoforms and slow gene program associated with increased sensitivity of vasorelaxation to NO donors and cGMP.

Increased pressure in the hepatic circulation, i.e., portal hypertension, is a vexing sequelae of liver injury and cirrhosis and is associated with high morbidity and mortality. While the resistance to blood flow and normally low portal pressures are increased, upstream mesenteric arterial resistance is low with portosystemic shunting of blood, resulting in a low systemic vascular resistance/high cardiac output state [reviewed in (114)]. Other regional circulations may also be affected, referred to as hepatorenal and hepatopulmonary syndromes, the bases of which are not well understood. In animal models of portal hypertension induced by ligature of the PV, there is reduced phasic activity of the PV (112) and partial switching from fast to slow smooth muscle gene programs in the PV and the upstream mesenteric arteries (91). The significance of this switch in the smooth muscle contractile phenotype has not been determined but would be predicted to increase sensitivity to NO/cGMP-mediated vasodilation, consistent with the increased role of NO in the splanchnic circulation in animal models of cirrhosis (65). These antithetical and complex changes in portal and systemic hemodynamics make vascular drug therapies problematic. Drugs that specifically target the portal hypertension would be especially desirable for use early

Pulmonary Circulation

The pulmonary circulation is unique in that resistance to blood flow is ~1/5th of that in the systemic circulation such that the entire cardiac output (minus the bronchial component) is delivered with low perfusion pressures (Fig. 3C). In addition to unique anatomic features, low vascular resistance may be due to the low basal tone of the pulmonary arterioles. This could reflect differences between signals that control tone in the pulmonary and systemic circulations (7, 18, 23, 71, 75), or differences within the vascular smooth muscle (43, 77, 113). In contrast to the systemic circulation, there appears to be low adrenergic tone at rest or with exercise in the pulmonary circulation (68, 108), in part accounting for the ability to accommodate increases in cardiac output with exercise with minimal increases in right heart pressures. It is also possible that the vascular smooth muscle response to neural or other signals is different from systemic arterioles and requires further investigation.

A particularly unique feature of the pulmonary circulation is hypoxic pulmonary vasoconstriction (HPV) (11, 109). HPV ensures that blood flows to the most ventilated regions of the lung to maximize oxygen uptake and thus delivery to the systemic tissues. In contrast, systemic arterioles dilate in response to reduced oxygen concentrations to maintain oxygen supply to the tissues. HPV is maintained in ex vivo preparations and isolated pulmonary artery SMCs (PASMCs), suggesting it is an intrinsic property of these cells [reviewed in (11, 109)]. Hypoxic contraction of PASMCs appears to involve calcium activation of MLCK (76, 83, 116) as well as increased calcium sensitivity of the myofilaments (25, 33, 61, 76), implicating inhibition of the MP, each of which are more prominent in distal (77, 102) versus proximal PASMCs (10, 74, 77), correlating with the magnitude of the HPV [reviewed in (11, 109)]. In contrast rat carotid artery SMCs relax to hypoxia and phosphorylation of myosin decreases (77), demonstrating that the sensor and mediator for hypoxic vasoconstriction are unique to and contained within the PASMCs. It has been suggested that hypoxic inhibition of voltage-potassium channels initiates HPV (95). These channels are enriched in distal versus proximal PASMCs (3, 11, 102), analogous to differences in Kir channel distribution in systemic circulations (2, 62, 82, 117). However, because these channel activities are also present in systemic arterioles, it seems less likely, though still possible, that they are responsible for the discordant hypoxic responses. A role for prostanoids has been suggested in mediating the HPV response in the pulmonary circulation. Chronic hypoxia increases expression of cyclooxygenase-2 and increases endothelium-independent thromboxane-induced contraction in pulmonary arteries (27), as well as sensitivity of pulmonary artery myocytes to thromboxane (60). The specific mechanisms for sensing and mediating HPV must be better

Review
defined before determining the molecular bases for the differing responses of pulmonary and systemic circulations.

Pulmonary arterial hypertension may be due to structural or functional changes in the blood vessels. “Muscularization” of the pulmonary arterioles (<300 μm) is thought to be a key contributor to the increased vascular resistance to blood flow (99). The drugs used to reduce vascular resistance in pulmonary hypertension are different from those used for this purpose in systemic hypertension, e.g. phosphodiesterase-5 inhibitors, endothelin receptor antagonists and prostaglandins in the former and α-adrenergic and calcium channel blockers in the latter [reviewed in (44)]. Whether this is merely a reflection of historical bias in drug development and testing or reflects true differences between pulmonary and systemic hypertensive vascular smooth muscle requires further study. Clearly a better understanding of differences between pulmonary and systemic microvascular smooth muscle, especially in disease, is needed to develop targeted therapies for hypoxic, inflammatory, and other causes of pulmonary hypertension.

Conclusions and Perspectives

Vascular smooth muscle is highly specialized both between and within regional circulations for organ-specific functions. This includes the afferent versus efferent arteriole in determining glomerular filtration in the kidney, PV and hepatic artery for delivery of oxygen, nutrients and toxins from the intestine, and pulmonary arterioles to maintain low vascular resistance and matching of perfusion to alveolar ventilation through hypoxic vasoconstriction. This diversity is evident in the variable expression of myosin isoforms that determine contractile kinetics, and regulatory proteins (MLCP and MLCK) that determine how the motor is activated and deactivated and responses to signaling pathways (Fig. 1). There is also ample evidence for differences in ion channels that determine calcium flux and activation and deactivation of the myosin motor. These differences fit into the paradigm of fast (phasic) versus slow (tonic) contractile phenotypes. It seems likely that the described differences represent the tip of the iceberg, and how much of vascular smooth muscle diversity fits within versus outside of this paradigm will only be known with more comprehensive studies of vessel-specific gene programs. In contrast to striated muscle there remains limited understanding of vascular smooth muscle contractile diversity, when and how it is generated, and how it modulates in disease. There also remains limited understanding of the effect of this contractile diversity on vascular function in different contexts. This no doubt reflects in part the dispersed nature of smooth muscle within “microstructures” of the vascular system. The modest investigation of smooth muscle phenotype within the vascular system is surprising given the many drugs used clinically to target smooth muscle contraction in vascular and the many other diseases characterized by increased smooth muscle tone. Newer technologies should facilitate rapid progress in defining smooth muscle contractile phenotypes in different circulations and their modulation in disease. One such technology is comprehensive analysis of gene expression, including arrays and more recently deep RNA sequencing (RNASeq). In RNASeq mRNAs present within a sample are exhaustively sequenced and matched back to the reference genome, providing both absolute counts of transcripts and isoforms generated by alternative splicing of exons. A second useful technology is reporter-based cell purification, as for example green fluorescent protein labeling of SMCs (89), facilitating sorting of a relatively purified population of SMCs from a tissue for downstream applications such as RNASeq. These approaches should overcome barriers to definition of smooth muscle gene programs in the physiologically relevant tissue for control of blood flow and pressure, the microvasculature, in developmental and disease contexts. It is our hope that this information, as well as studies of unique attributes of human genomes, will lead to more rationale and mechanistic drug targeting in individuals with sundry vascular diseases.

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AUTHOR CONTRIBUTIONS


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