Capitalizing on diversity: an integrative approach towards the multiplicity of cellular mechanisms underlying myogenic responsiveness

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Abstract

The intrinsic ability of resistance arteries to respond to transmural pressure is the single most important determinant of their function. Despite an ever-growing catalogue of signalling pathways that underlie the myogenic response, it remains an enigmatic mechanism. The myogenic response’s mechanistic diversity has largely been attributed to ‘hard-wired’ differences across species and vascular beds; however, emerging evidence suggests that the mechanistic basis for the myogenic mechanism is, in fact, ‘plastic’. This means that the myogenic response can change quantitatively (i.e. change in magnitude) and qualitatively (i.e. change in mechanistic basis) in response to environmental challenges (e.g. disease conditions). Consequently, understanding the dynamics of how the myogenic response capitalizes on its mechanistic diversity is key to unlocking clinically viable interventions. Using myogenic sphingosine-1-phosphate (S1P) signalling as an example, this review illustrates the remarkable plasticity of the myogenic response. We propose that currently unidentified ‘organizational programmes’ dictate the contribution of individual signalling pathways to the myogenic response and introduce the concept that certain signalling elements act as ‘divergence points’ (i.e. as the potential higher level regulatory sites). In the context of pressure-induced S1P signalling, the S1P-generating enzyme sphingosine kinase 1 serves as a divergence point, by orchestrating the calcium-dependent and -independent signalling pathways underlying microvascular myogenic responsiveness. By acting on divergence points, the proposed ‘organizational programmes’ could form the basis for the flexible recruitment and fine-tuning of separate signalling streams that underlie adaptive changes to the myogenic response and its distinctiveness across species and vascular beds.

1. Functional importance of myogenic autoregulation

In 1902, Sir William Bayliss discovered that transmural pressure elevation causes resistance arteries to ‘writhe like a worm’ (i.e. constrict).
This phenomenon, now known as the myogenic response (or ‘Bayliss effect’),\textsuperscript{5–12} is a primary mechanism mediating blood flow autoregulation: the continuous matching, over a wide pressure range, of blood supply to tissue nutrient and energy demand.\textsuperscript{13} Although the precise pressure range governed by autoregulation is subject to some dispute,\textsuperscript{14} the widely accepted human autoregulatory range is 60–150 mmHg. In addition to contributing to the control of tissue perfusion, the myogenic response also (i) protects fragile capillary beds from high transmural pressure,\textsuperscript{15} (ii) maintains capillary hydrostatic pressure at levels that minimize oedema formation,\textsuperscript{16} (iii) provides the partial constriction necessary for vasodilator responses, and (iv) contributes to the generation of peripheral resistance, which builds and maintains the systemic blood pressure.\textsuperscript{17,18} Any modification to the magnitude of the myogenic mechanism necessarily has widespread effects on peripheral microvascular resistance and as a consequence, impacts both tissue perfusion and blood pressure. Indeed, altered myogenic responsiveness is a hallmark of several vascular pathologies, including cardiomyopathy,\textsuperscript{19} heart failure,\textsuperscript{3,20} diabetes,\textsuperscript{21,22} hypertension,\textsuperscript{23,24} hypoxia,\textsuperscript{25} and stroke.\textsuperscript{26}

2. Myogenic signalling: fragmented knowledge about a complex response

On a simplistic level, the myogenic response is the conversion of an extracellular and primarily mechanical stimulus (transmural pressure imposes a circumferential stretch on the vessel wall that leads to altered wall tension) into biochemical intracellular signals within vascular smooth muscle cells. Recent reviews on the topic attest to the enormous complexity of these mechanisms.\textsuperscript{9–12} The myogenic response incorporates several putative sensor elements, including stretch-activated cation channels (mediating depolarization),\textsuperscript{27,28} membrane-bound enzymes (e.g. matrix metalloproteinases 2 and 9),\textsuperscript{29} the cytoskeleton,\textsuperscript{30} extracellular matrix elements (e.g. integrins),\textsuperscript{31,32} and stretch-sensitive G-protein-coupled receptors (GPCRs),\textsuperscript{33,34} which, in turn, activate classical calcium/calmodulin-dependent (e.g. G-proteins, phospholipase C, diacylglycerol, phospholipase A2, arachidonic acid metabolites, store-mediated calcium entry, ryanodine-, or inositol trisphosphate-stimulated intracellular calcium release mechanisms)\textsuperscript{6,35–39} and calcium-independent (e.g. protein kinase C (PKC), Rho/RhoA, Rho kinase)\textsuperscript{40–44} second messenger pathways. Significantly, these mechanisms may operate in series or in parallel and act cooperatively or in a mutually exclusive manner.\textsuperscript{5–12}

The already complex mechanistic nature of the myogenic response is marked by significant mechanistic heterogeneity (e.g. differences are observed across species, vascular beds, etc.): although this fact appears to be well-established and accepted within the literature (even taken for granted), few investigations have intentionally compared the level or mechanistic basis of the myogenic response in different resistance arteries. Several investigations have made discrete comparisons under non-pathological (i.e. naïve or sham) conditions and identified differences across branch order,\textsuperscript{1,45–47} gender,\textsuperscript{48,49} developmental stage/ageing,\textsuperscript{50–52} vascular bed\textsuperscript{2,3,47,51} and species.\textsuperscript{47} However, despite the clear lack of systemic assessment, the majority of myogenic variation is ‘nonchalantly’ attributed to differences in species and vascular beds. When combined with the lack of experimental standardization (buffers, equipment, procedures, etc.), directly comparing data across separate research groups is difficult, if not impossible.

Although the field has uncovered many mechanistic elements, our understanding of how these elements are actually coordinated within the myogenic response remains a major knowledge deficit. In this respect, attributing mechanistic differences to ‘heterogeneity’ evades consideration that ‘organizational programmes’ must determine which mechanisms operate under specific conditions. More directly, heterogeneity exists because ‘organizational programmes’ specifically and precisely tailor the mechanistic basis of the myogenic response, with remarkable spatial resolution (i.e. at minimum, to the level of branch order). This ‘lack of hard wiring’ makes the myogenic response flexible and highly adaptive mechanistically; indeed, the fact that myogenic reactivity persists in genetic deletion models (i.e. compensation) is further evidence that a higher level of control is present. Neglecting this higher level of regulation limits the value of our mechanistic schemes (i.e. they are ‘snap-shots’ pertinent only to very precise conditions) and largely accounts for our general failure to identify ‘universal’ mechanisms and ‘magic bullet’ therapeutic interventions. Thus, one of the emerging and pressing questions in the field is ‘how are the individual mechanisms that comprise the myogenic response orchestrated’?

Topical reviews have always faced the near-insurmountable task of distilling the complexity of myogenic mechanisms into straightforward and general concepts: this has resulted in over-simplifications that have later required correction. As an example, the myogenic response has been historically characterized as a ‘two-phase’ response, resulting from the sequential activation of signalling streams dominated initially by a rapid change in intracellular calcium (i.e. calcium-dependent mechanisms) and then subsequently by ‘slower’ calcium-independent signalling mechanisms that promote/maintain myosin light chain 20 (MLC\textsubscript{20}) phosphorylation (primarily via the inhibition of MLC\textsubscript{20} dephosphorylation; usually referred to as calcium sensitization).\textsuperscript{5–12} However, at least two lines of evidence argue against a sequential activation model: rapid myogenic vasoconstriction (in terms of diameter) can be (i) elicited in the absence of calcium changes\textsuperscript{53} and (ii) attenuated under conditions that inhibit calcium-independent mechanisms, but preserve calcium responses.\textsuperscript{12,43,54} Intuitively, activating both the calcium-dependent and -independent signalling streams simultaneously makes sense: it would allow for finer modulatory control at all phases of the response (since it would incorporate more inputs) and it would increase the maximal response capability (i.e. activating both streams would elicit more constriction than activating one stream alone).

The consequence of the ‘two-phase’ model is that it proposed an over-simplistic explanation for a temporal shift in mechanisms mediating vasoconstriction in the myogenic response. Although we now know that the calcium-dependent and -independent streams operate together, we still compartmentalize our knowledge into two distinct pathways (i.e. calcium-dependent and -independent).\textsuperscript{5–12} As a result, we still do not investigate the myogenic response from the perspective that the calcium-dependent and -independent mechanisms are regulated by processes capable of controlling and shifting their contributions; we therefore possess limited knowledge related to how shifts between the calcium-dependent mechanisms and calcium-independent mechanisms are orchestrated. Additionally, we frequently attribute mechanistic differences to ‘heterogeneity’, which avoids the complexity of an additional level of regulation.
3. Emerging viewpoint: how to organize diversity

This article promotes our viewpoint that higher level ‘organizational programmes’ dynamically dictate the fundamental mechanisms that mediate the myogenic response. The nature of these ‘organizational programmes’ is not known, but they would serve to ‘engage’ or ‘disengage’ primary myogenic signalling elements (Figure 1). The ability to fundamentally alter the myogenic response’s mechanistic basis (i.e. mechanistic plasticity) necessitates the existence of multiple regulatory elements (Figure 1). Since the calcium-dependent and -independent streams are simultaneously activated, these regulatory elements would need to integrate and control the relative contributions of the two streams. We will therefore refer to these regulatory elements as ‘divergence points’, because it accurately reflects their position and regulatory function within the myogenic signalling cascade (Figure 2).

One might expect that a plethora of candidate divergence points (points in the myogenic signalling pathway capable of ‘splitting’ a mechanically initiated signal into the calcium-dependent and -independent signalling streams) would be evident in the literature; this is, actually, not the case. Several general issues contribute to this deficiency, including: (i) the complexity of downstream signalling pathways, (ii) a lack of specific inhibitory tools that are necessary to precisely define mechanistic roles and (iii) mechanistic heterogeneity, which can spur the belief that certain mechanisms are not broadly applicable and thus, limit the attention certain mechanisms receive. However, it is the field’s lack of investigation into the higher levels of organization and integration that is primarily responsible: in essence, the field has not looked at signalling mechanisms from the viewpoint that ‘organizational programmes’, operating at a level above the commonly studied signalling mechanisms, dynamically dictate which mechanisms mediate the myogenic response. This viewpoint article will use S1P signalling, which is increasingly recognized as a crucial determinant of cardiovascular function, to illustrate the key aspects of our proposed mechanistic scheme. Our intention is to

**Figure 1** Although multiple divergence points potentially contribute to the myogenic response, some are ‘mechanistically silent’ (1). Organizational programmes can modify the myogenic response by: (1) engaging or disengaging divergence points, (2) increasing/decreasing a divergence point’s overall contribution and/or (3) shifting the relative contributions that a divergence point makes to the calcium-dependent and -independent signalling streams.

**Figure 2** Emerging evidence indicates that myogenic mechanisms are ‘plastic’: they can change both (A) quantitatively (i.e. in magnitude) and (B) qualitatively (i.e. change in the relative contribution of specific mechanisms). (A) Mouse posterior cerebral arteries normally display minimal myogenic tone (sham), but are recruited to the myogenic mechanism in the context of heart failure (see Yang et al.1). (B) Mouse mesenteric arteries isolated from mice after 1 week of heart failure (left panel) and after 2 weeks of heart failure (right panel) possess similar levels of myogenic tone. However, the effect of disrupting sphingosine-1-phosphate signalling (JTE013) is significantly greater at the later time point (~30 vs. ~100%), indicating the mechanistic basis of the myogenic response has changed (see Hoefer et al.2).
share two specific lessons that we have learned from investigating the role of S1P in the myogenic response.

First, we will review how S1P signalling patterns change under pathological conditions, which we assert is evidence that the myogenic response is mechanistically plastic. This aspect currently represents the Achilles’ heel with respect to therapeutic interventions, because while we possess extensive knowledge regarding the functional mechanisms that are capable of driving the myogenic response, we know very little about the regulatory level that governs and alters the mechanisms that actually operate.

Next, we will review the characteristics that make S1P signalling a prime regulatory element (i.e. a divergence point) for higher level ‘organizational programmes’ to act upon. Although there are two fundamental mechanisms in operation (calcium-dependent and -independent), they must be continuously integrated. These ‘organizational programmes’, therefore, are most likely to act upon specific elements capable of independently modulating calcium levels and calcium sensitivity. This second lesson demonstrates how S1P signalling patterns are able to achieve independent control for the calcium-dependent and -independent signalling streams.

3.1 Lesson 1: the myogenic response displays ‘mechanistic plasticity’

Even when caveats are considered (e.g. the dependence on species and vascular bed), the vast majority of investigations propose that specific mechanisms (composed of individual elements) mediate the myogenic response. This assumption has favoured study designs that investigate mechanisms in isolation, which necessarily underestimate the myogenic response’s plasticity and ability to substantively change mechanistically. In simplistic terms, our mechanistic views of the myogenic response are based on ‘snap-shots’ under rather specific experimental conditions.

As a new concept, we assert that the mechanisms underlying the myogenic response are not hard-wired; as such, all myogenic mechanisms described to date are potential contributors to the overall response with variable impact depending on the circumstances. Thus, specific physiological and pathological settings can shift the contributions of individual mechanisms\(^1\) to alter (i) the magnitude of myogenic responsiveness (a scenario that also includes the recruitment of ‘myogenically silent’ vascular regions into myogenic autoregulation)\(^2\) or (ii) the pattern of the operating mechanisms, without necessarily changing the magnitude of the response.\(^3\)

In the first scenario, we observed that ‘myogenically silent’ (proximal) mouse cerebral arteries become myogenically active in the pathological setting of heart failure (Figure 2A).\(^1\) We can presume that these arteries express many of the ‘catalogued’ sensor elements (e.g. ion channels, matrix proteins) and second messenger mechanisms (e.g. PLC, Rho signalling) that can drive both the calcium-dependent and -independent mechanisms of the myogenic response. Yet, under normal physiological conditions, these mechanisms do not operate (at least, not in the context of myogenic reactivity). During the pathological condition of heart failure, however, this ‘dormant mechanistic infrastructure’ is activated to promote myogenic responsiveness. Importantly, phenylephrine responses are the same under both conditions, indicating that the responsiveness to transmural pressure, rather than contractility in general, is impacted.

In the second scenario,\(^3\) we observed that the relative contribution of S1P signalling to the enhanced myogenic reactivity in mesenteric arteries isolated from mice with heart failure increases over time (Figure 2B). Specifically, in the early phases of the microvascular adaptation (1 week following heart failure), S1P receptor-dependent signalling is a modest contributor to the response (S1P receptor antagonism with JTE013 reduces the myogenic response \(\sim 30\%\)). However, after 2 weeks, S1P receptor antagonism ablates the myogenic response (i.e. S1P receptor-dependent S1P signalling account for \(\sim 100\%\) of the myogenic response), although the magnitude of the myogenic response does not change between weeks 1 and 2 (i.e. the pattern of operating mechanisms of the myogenic response dramatically changes, although the magnitude of the response itself does not). This is a clear indication that considerable and coordinated rearrangements in the molecular signalling that underlies the myogenic response must be present.

These two examples imply the existence of a signalling level capable of dynamically regulating both the sensitivity to transmural pressure (i.e. the magnitude of the myogenic response) and the selection and weighting of contributing mechanisms (i.e. the orchestration of the calcium-dependent and -independent signalling pathways that mediate the myogenic response). How this is achieved represents a key element in understanding how different physiological (e.g. exercise and pregnancy) and pathological settings alter myogenic reactivity.

In summary, investigations have searched for ‘specific’ myogenic mechanisms that are altered under pathological settings. While this is important, the described examples of the plasticity of pressure-induced molecular mechanisms of constriction suggest that it is equally important to focus on the signalling level that governs the relative contributions of distinct signalling pathways. All smooth muscle cells have the capability of being myogenically active and have the same ‘library’ of control mechanisms (hard-wired, in the sense that the genes are all present): whether they are myogenically active and which mechanisms are utilized will depend on ‘organizational programmes’ that dynamically control gene expression at the transcriptional and translational level, the activity of signalling elements (e.g. post-translational modifications) and the integration of distinct pathways (e.g. the regulation of divergence points).

3.2 Lesson 2: sphingosine-1-phosphate signalling as a model divergence point

Although the myogenic response is a well-researched entity,\(^5\)\(^\sim\)\(^12\) we possess only a rudimentary understanding of (i) how the calcium-dependent and -independent streams are actually coordinated and integrated to elicit the highly reproducible myogenic response, and (ii) the regulatory points that are recruited to determine the functional mechanisms that drive the myogenic response (i.e. the regulatory points that impart ‘mechanistic plasticity’). Signalling elements capable of controlling both calcium levels and calcium-independent mechanisms represent key ‘hot-spots’ (i.e. potential divergence points) that would control both the integration of the two signalling streams and the mechanistic basis of the myogenic response. S1P signalling is one prime candidate that is well suited for this task and we are only beginning to unravel how it operates in this role.

As a signalling mediator, S1P is known to control a diverse array of biological responses.\(^5\)\(^6\) It is synthesized by two rate-limiting kinases,
named sphingosine kinase 1 and 2 (Sphk1 and Sphk2) that phosphor-
ylate the highly abundant cell membrane lipid sphingosine. S1P pos-
sesses a peculiar signalling aspect, in that it can act as both an
intracellular second messenger and as an extracellular receptor
ligand, this attribute imparts S1P with an additional level of signal-
ling capability and complexity. S1P activates multiple second mes-
senengers signalling pathways (the small GTPases RhoA and Rac,
phospholipase C, and adenylate cyclase are a few examples) and
plays significant roles in both calcium mobilization and calcium sensitization, both of which play prominent roles in
the regulation of vascular smooth muscle cell contractility. It is not sur-
prising, therefore, that S1P regulates both vascular tone and myogenic reaction. Since disease processes are able to profoundly alter
S1P signalling via regulation of S1P signalling components, this pathway is potentially an important inspection point when
attempting to understand why and how the myogenic response
changes in pathological settings.

S1P signalling associated with the myogenic response appears to
rely primarily on the Sphk1 isoenzyme and requires pressure-
dependent Sphk1 translocation from a predominant cytosolic distri-
bution to the plasma membrane. There are several mechanisms
that control Sphk1 localization and target it to signalling domains
within the plasma membrane, Targeting Sphk1 to lipid rafts
appears to be particularly crucial for S1P’s signalling functions, suggesting that discrete signalling domains link S1P signalling to specific intracellular effectors. This fact is clearly evidenced by the discrep-
ant oncogenic effects when Sphk1 is membrane targeted by motifs that promote lipid raft accumulation (increased proliferation and cell cycle progression) vs. those that do not (decreased proliferation and delayed G0/G1 exit).

We have shown that pressure-dependent Sphk1 translocation relies on the phosphorylation of an ERK1/2 motif of the kinase. Blocking Sphk1 translocation (via mutation of its ERK phosphorylation motif) results in significant attenuation of both calcium mobilization and sensitivity; as a consequence, myogenic vasoconstriction is abol-
ished. Remarkably, while adding a myristoylation/palmitoylation motif to the mutated Sphk1 protein (i.e. forcing it to the plasma membrane) restores the calcium mobilization responses, it only partially recovers myogenic vasoconstriction (i.e. indicating that calcium sensitivity is still be reduced). Thus, the localization mechanism imparts Sphk1 with the ability to generate at least two distinct pools of S1P; these pools exert individual functions within the myogenic response by inde-
pendently controlling intracellular calcium levels and calcium sensitiv-
ity. Sphk1, therefore, represents a divergence point between the calcium-dependent and -independent streams that govern the myo-
genic response.

The precise nature of the two S1P pools is not known: one pool could be intracellular and the other extracellular, which we favour because S1P receptor antagonism attenuates myogenic vasoconstric-
tion, without an effect on pressure-stimulated calcium elevation. How-
ever, the two pools could also be localized to distinct plasma membrane signalling domains that are differentially targeted by the myristoylation/palmitoylation motif. Discrete, spatially restricted S1P signals (i.e. within a ‘signalosome’) allow S1P signalling to take ad-
vantage of (i) S1P’s dual functions as an intracellular second messen-
gen and extracellular ligand and (ii) S1P’s ability to activate at least three S1P GPCR subtypes (S1P1R, S1P2R, and S1P3R have been identi-
fied in vascular cells), each uniquely coupled to G-protein subunits (i.e. G1, Gq, and G12/13) and downstream effectors. The fact that S1P receptors can be heterologously desensitized adds yet another level of regulation. Clearly, a single molecule of S1P can have distinct signalling effects, depending on where and when it is synthesized. This principle not only underlies how S1P is capable of regulating individual elements within myogenic signalling streams (Figure 3), but also explains how S1P is able to simultaneously regulate so many distinct and fundamental cellular processes.

The ability of Sphk1 to independently control calcium-dependent and -independent mechanisms within the myogenic response makes it a ‘divergence point’ for the two streams; but in order to precisely regulate the relative contributions of the two streams (i.e. the shift between reliance on calcium-dependent mechanisms and calcium-
dependent mechanisms), feedback or cross-talk between the two streams would have to impact the individual S1P pools by altering Sphk1 activity/localization. Interestingly, the calcium-myristoyl switch protein named calcium and integrin-binding protein 1 interacts with Sphk1 via a calmodulin-binding motif. Thus, while calcium is not crucial for Sphk1 translocation/activation, it does have the capability of modulating Sphk1 translocation and, hence, S1P signalling. This is one potential mechanism that could assist Sphk1 in integrating the relative contributions of the calcium-dependent and -independent streams.

In summary, S1P signalling is one of presumably many potential ‘hot-spots’ that serve as a functional divergence point for calcium-
dependent and -independent mechanisms within the myogenic response (Figure 3). As observed in lesson 1, S1P’s mechanistic con-
tribution significantly shifts from being a non- or minor-contributor to a major-contributor. As a consequence, by studying S1P signalling, we have the opportunity to learn distinctly new aspects of myogenic regulation with respect to its adaptive nature (mechanistic plasticity) and the functional integration of the calcium-dependent and -independent streams.

4. Protein kinase C: a second putative ‘mechanistically plastic divergence point’

Since our proposed model necessitates the existence of multiple regulatory elements, S1P signalling cannot be the only mechanism capable of (i) functioning as a divergence point and (ii) altering the molecular composition of the myogenic response, by engaging or dis-
engaging as the primary mechanism. Accordingly, there is a relatively strong case that PKC, a family of 12 isoenzymes that share sequence homology and similar substrates, could represent a second myogenic divergence point. However, it must be initially noted that investiga-
tions into PKC’s myogenic involvement have generally been hindered by both poor inhibitor specificity and the fact that the PKC exists as several functionally distinct isozymes, which display non-uniform ex-
pression throughout the vascular tree. PKC has been shown to modulate both intracellular calcium levels (via actions on several ion channels) and calcium sensitivity during myogenic vaso-
constriction. However, consistent with our viewpoint that divergence points do not have to be universally active, PKC does not influence myogenic vasoconstriction under certain settings.

Like Sphk1 (which pressure-dependently translocates), PKC dis-
plays spatial-temporal targeting, with a kinetic consistent with myo-

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genic involvement. This property confers distinct PKC isoforms, which generally display minimal substrate specificity, with an ability to perform specific intracellular signalling functions (i.e. analogous to S1P exerting distinct effects based on location). In fact, Kashihara et al. have identified distinct roles for the PKC alpha (calcium sensitivity) and PKC delta (calcium influx) isoforms in the context of the myogenic response; Wesselman et al. have also potentially observed isoform-specific functions, but with the caveat that non-specific inhibitor effects could be confounding. Interestingly, the PKC alpha isoform, which appears to primarily modulate calcium sensitivity, is differentially activated by localized changes in intracellular calcium, a potential feedback link that could modify PKC activity/function in response to changing calcium. This mechanistic profile suggests that PKC may serve integrator functions within the myogenic response and can be considered a ‘divergence point; importantly, there is also compelling evidence that the role of PKC in modulating the myogenic response can be dynamically ‘replaced’ by another mechanism. Specifically, Su et al. observed a developmental shift in swine mesenteric arteries, where PKC activity is absolutely required for myogenic tone in arteries isolated from 1-day postnatal animals, but is not required in arteries isolated from 10-day postnatal animals; this functional shift in PKC-dependence correlates with a reduction in PKC alpha and PKC epsilon protein expression. These observations staunchly support our viewpoint that the mechanistic basis for the myogenic response is not hard-wired; they also suggest that the dynamic control of myogenic mechanisms is not limited solely to pathological adaptations (i.e. mechanistic plasticity serves a yet undefined developmental role).

5. G-protein-coupled receptors: a unique role as sensor and divergence point?

Few signalling mechanisms have reached the level of elucidation that would allow them to be categorized as divergence points. If we are to gain any further insight to support (or refute) our proposed model (Figure 1), future advancements related to myogenic Sphk1 and PKC signalling and importantly, new mechanisms, need to be assessed from the viewpoint that we have presented. One interesting emerging field that warrants our attention is the involvement of GPCRs in myogenic signalling.

Several GPCRs, including AT1R (angiotensin; Gq/11 and Gq/o coupled), ETAR (endothelin; Gq/11 and Gq/o coupled), H1R (histamine; Gq coupled), M3R (acetylcholine; Gq coupled), V1aR (vasopressin; Gq coupled), PTH1R (parathyroid hormone; Gq/11 and Gq coupled), FPR1 (formylpeptide; Gq/o coupled) and D2R (dopamine; Gq coupled), appear to activate in response to stretch. In the case of AT1R, mechanical stress clearly induces a distinct conformation compared with the ligand-bound form; this perhaps imparts an ability to transmit unique signals in response to stretch vs. ligands. Since this field is in its infancy, there is limited and rather indirect evidence the GPCRs function as mechanosensors in intact vessels. However, the ability to sense stretch and then directly couple to both calcium mobilization and calcium sensitization would be an attractive divergence point for myogenic regulation. Of note, GPCR mechanosensitivity is not a ubiquitous phenomenon:

![Figure 3](image-url)
the β2-adrenergic receptor (noradrenaline; G, coupled), for example, does not display this mechanosenstivity.33 Also, GPCR activation per se does not necessarily alter myogenic responsiveness.98 Nevertheless, it is tempting to contemplate that as this field expands, GPCRs will emerge as unique modulators of the myogenic response, functioning both as sensors and divergence points within myogenic signalling cascades.

6. Summary and perspectives
As highlighted by several recent reviews,8–12 the myogenic research field has assembled a substantial body of knowledge that delineates the contributions of specific signalling mechanisms to the myogenic response. However, because investigations have traditionally focused on specific effectors, rather than on the higher levels of organization, our overall understanding is fragmented. We propose that ‘organizational programmes’ dynamically dictate the myogenic mechanisms that operate under specific circumstances (Figure 1): this higher level framework could account for the broad ‘mechanistic heterogeneity’ that is experimentally observed and, more importantly, represent a governing level of regulation that has the power to fundamentally change the activity of specific myogenic mechanisms in response to environmental challenges (e.g. pathological settings; Figures 1–2). The exact nature of these proposed ‘organizational programmes’ remains to be determined and therefore, we can only speculate about its elements, how they are controlled and what their relevant signalling inputs are.

Intuitively, local mediators (e.g. cell signals) and conditions (e.g. mechanical strain) influence the ‘organizational programmes’ under physiological and pathological settings. Using vascular smooth muscle cell phenotypic switching (between a synthetic and contractile phenotype) as a template, potential modulatory factors could include transcriptional (i.e. cell signalling)99,100 epigenetic,101,102 microRNA,103,104 mechanical,105,106 structural–matrix interactions,107 and endothelial influences.108,109 Genetic110,111 and cell origin influences112–114 would superimpose an additional level of complexity and variation; these additional influences perhaps explain some of the differences across species and vascular bed.

Evidence that (i) sympathotony alters basilar artery smooth muscle cell phenotype in vivo115 and (ii) norepinephrine alters cultured vascular smooth muscle cell phenotype in vitro,116 form an attractive basis to suggest that, in addition to local mechanisms, central nervous control mechanisms may also influence these ‘organizational programmes’. Indeed, a link between sympathetic input and smooth muscle phenotype may explain why arteries in cardiac transplants (which are necessarily denervated) display an ‘intrinsic defect in vascular smooth muscle contraction’ and attenuated myogenic vasoconstriction.16 It is intriguing to speculate that the afferent sympathetic arm is connected to the proposed ‘organizational programmes’. Such a connection could ultimately link CNS sensory inputs relevant for MAP regulation to the modulation of myogenic responsiveness.

We propose that these ‘organizational programmes’ could choose from a ‘large catalogue’ of sensor elements and signalling mechanisms. Targeting a single mechanistic effector, therefore, is unlikely to yield a long-term therapeutic option. This may explain why treating hypertension, for example, is so challenging: the plasticity of the myogenic response imparts it with the ability to effectively adapt to (i.e. escape) the treatment strategy. If we can decipher the myogenic response’s higher order organization, these mechanisms could become the strategically preferred therapeutic targets.

Our viewpoint is that the next breakthrough, from a therapeutic perspective, will result from understanding the regulatory level that dictates which myogenic mechanisms operate in the response. Because our knowledge about this aspect is limited at present, there is no doubt that this is a daunting task. To narrow our mechanistic search, we propose that, at least initially, we concentrate on the signalling divergence points (which coordinate the calcium-dependent and -independent signalling streams), which are most likely targeted by these ‘organizational programmes’.

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