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SPOTLIGHT REVIEW

Emerging role of G protein-coupled receptors in microvascular myogenic tone

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Abstract Blood flow autoregulation results from the ability of resistance arteries to reduce or increase their diameters in response to changes in intravascular pressure. The mechanism by which arteries maintain a constant blood flow to organs over a range of pressures relies on this myogenic response, which defines the intrinsic property of the smooth muscle to contract in response to stretch. The resistance to flow created by myogenic tone (MT) prevents tissue damage and allows the maintenance of a constant perfusion, despite fluctuations in arterial pressure. Interventions targeting MT may provide a more rational therapeutic approach in vascular disorders, such as hypertension, vasospasm, chronic heart failure, or diabetes. Despite its early description by Bayliss in 1902, the cellular and molecular mechanisms underlying MT remain poorly understood. We now appreciate that MT requires a complex mechanotransduction converting a physical stimulus (pressure) into a biological response (change in vessel diameter). Although smooth muscle cell depolarization and a rise in intracellular calcium concentration are recognized as cornerstones of the myogenic response, the role of wall strain-induced formation of vasoactive mediators is less well established. The vascular system expresses a large variety of Class 1 G protein-coupled receptors (GPCR) activated by an eclectic range of chemical entities, including peptides, lipids, nucleotides, and amines. These messengers can function in blood vessels as vasoconstrictors. This review focuses on locally generated GPCR agonists and their proposed contributions to MT. Their interplay with pivotal $G_{q\text{-}11}$ and $G_{12\text{-}13}$ protein signalling is also discussed.

Keywords Myogenic tone • G protein-coupled receptors • $G_{q\text{-}11}$ • $G_{12\text{-}13}$ • Rho • TRP channels

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1. Introduction

The arterial microcirculation encompasses small arteries and arterioles of the vascular tree (10–100 μm in diameter) that are embedded within organs, as opposed to larger conduit arteries, which transport blood to organs. Arterioles carry blood to the capillaries and are responsible for the distribution of blood within tissues. The functions of microcirculation include the regulation of blood flow and tissue perfusion, blood pressure, fluid movement (swelling or oedema), oxygen and nutrient delivery, as well as the regulation of body temperature. According to Poiseuille's law, the laminar flow rate of an incompressible fluid along a tube is proportional to the fourth power of its radius. As a result, small changes in arterial radius strongly impact blood flow, peripheral vascular resistance, and mean arterial pressure. Microcirculatory tone is set by the sympathetic tone and neurohumoral vasoactive systems, but is largely dependent on its endogenous myogenic tone (MT), which relies on the intrinsic ability of small arteries to contract and reduce their diameter in response to increased internal pressure.[1,2] Under physiological conditions, MT represents a rapid response of blood vessels to counteract pressure increases in capillaries and avoid fluid leakage and tissue damage. Importantly, MT represents an efficient mechanism allowing a vascular bed to maintain its nutritive blood flow constant despite wide changes in arterial pressure. Although taking place in most tissues, MT is particularly pronounced in the kidney, the brain, and the heart. Renal blood flow autoregulation is achieved by the myogenic response and by tubuloglomerular feedback.[3,4] Cerebral blood flow autoregulation is critical in several disorders, including ischaemic disorders or Alzheimer's disease. Although cerebral blood flow can be measured using functional magnetic

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resonance imaging and positron emission tomography, the mechanisms involved in its autoregulation remain mainly puzzling. Vascular resistance in peripheral tissues is attributed to the resistance to flow offered by small arteries and arterioles, while in the cerebral circulation, large extra and intracranial arteries also contribute to vascular resistance.

Resetting MT to a normal level has been proposed as a valuable therapeutic target in several vascular pathologies.[5] In hypertension, 'resetting' MT not only limits blood pressure increase but also prevents inward eutrophic remodelling, which is thought to be linked to excessive vasoconstriction.[6] The mechanisms implicated in vasospasm following subarachnoid haemorrhage (SAH) also suggest that limiting MT could reduce the deleterious effects caused by blood flow cessation.[7] Abnormally elevated MT may underlie the increased vascular resistance in chronic heart failure[8] and diabetes.[9] In the long term, chronic activation of resistance artery mechanotransduction initiates mitogenic signalling as well as transcriptional activation, thus stimulating smooth muscle growth that can account for both hypertrophy and inward eutrophic remodelling that occurs in hypertension.[6] Therefore, targeting MT would limit not only exaggerated vascular constriction but also pathological arterial remodelling.[10]

The development of MT requires a complex mechanotransduction process in vascular smooth muscle cells (VSMCs). Although depolarization of the membrane potential and subsequent calcium entry is essential, the role of wall strain-induced formation of vasoactive mediators is less well established. We review here the cellular mechanisms of MT and we focus on locally generated mediators and their interplay with the pivotal $G_{q\text{-}11}$ $G_{12\text{-}13}$ intracellular signalling pathways.

2. Mechanisms underlying vascular myogenic contraction

The understanding of the specific mechanisms underlying MT can be organized into a linear sequence including (i) detection of vascular wall strain/stretch by mechanosensors; (ii) parallel or sequential transduction of the signal initiated by depolarization, ion channel modulation, Ca^{2+} increase, and protein phosphorylation; (iii) activation of actin–myosin interaction; and (iv) vasoconstriction.[2]

Membrane depolarization of VSMCs is necessary for MT to take place.[2] This depolarization depends on stretch-activated ion channels (SACs), which allow direct calcium entry and depolarization, inducing voltage-dependent calcium channel opening, and thus, a further increase in intracellular calcium concentrations. The molecular identity of SACs remains elusive. Transient receptor potential channels (TRP function will be detailed in Section 2.2.3) and the epithelial Na^+ channel ENac have been proposed to play a permissive role in these currents.[11,12] Membrane depolarization subsequently triggers a secondary Ca^{2+} entry through L-type voltage-gated calcium channels (VGCC).[2] Concomitantly, a negative feedback occurs through the opening of Ca^{2+}-activated potassium channels (BK_{Ca}), which leads to cell hyperpolarization.[13] Adding to this classical scheme, arguments exist in favour of integrins as sensors/transducers initiating the myogenic process.[14,15] More specifically, αvβ3 and α5β1 integrins were proposed to modulate cellular mechanotransduction,[16] likely through phosphorylation of VGCCs and BK_{Ca} ion channels.[17] Integrin activation could be the trigger for tyrosine phosphorylation occurring in the myogenic response.[18]

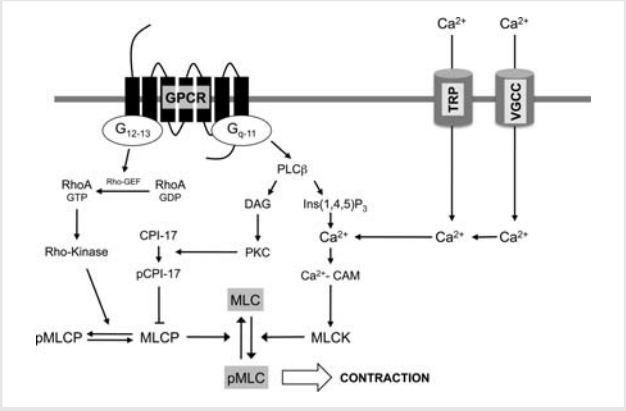

Figure 1 Intracellular signalling underlying vascular smooth muscle cells contraction. Contraction is induced by the increased phosphorylation of 20 kDa myosin light chain (MLC). Activation of G protein-coupled receptors (GPCR) triggers both Ca^{2+}-dependent phosphorylation of MLC and calcium facilitation inhibiting MLC dephosphorylation. $G_{q\text{-}11}$ protein activates phospholipase C (PLC) and induces intracellular Ca^{2+} rise through inositol (1,4,5) triphosphate ($Ins(1,4,5)P_3$)-sensitive endoplasmic reticulum stores. This adds to extracellular Ca^{2+} entry through voltage-gated calcium channels (VGCCs) and transient receptor potential (TRP) channels. The Ca^{2+}-Calmodulin complex (Ca^{2+}-CAM) induces the phosphorylation of MLCK. The second product of PLC, diacylglycerol (DAG), activates protein kinase C (PKC), which, through its substrate CPI-17, inhibits MLCP, and thus, MLC dephosphorylation. $G_{12\text{-}13}$ activates Rho guanine nucleotides exchanging factor (Rho-GEF) that activates RhoA protein through GDP exchange to GTP. GTP-bound Rho activates Rho-kinase which, through MLCP phosphorylation, inhibits MLC dephosphorylation.

Vascular contraction correlates with the phosphorylation state of 20 kDa myosin light chain (MLC) that drives myosin–actin interaction. The extent of phosphorylation depends on the intracellular-free calcium concentration ($[Ca^{2+}]_i$) and on mechanisms modulating the sensitivity of the contractile apparatus to calcium.[19] The degree of MLC phosphorylation is determined by myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) activity. The activation of MLCK is regulated by the Ca^{2+}-calmodulin complex formation, whereas MLCP activity, which constitutes the Ca^{2+}-sensitization pathway, is controlled by both Rho-kinase and protein kinase C (PKC)-dependent mechanisms[19] (*Figure 1*).

Hence, $[Ca^{2+}]_i$ is essential for the molecular mechanisms of vascular contraction. The regulation of $[Ca^{2+}]_i$ entry in VSMCs encompasses: (i) Ca^{2+} entry via VGCC (especially the L-type Ca^{2+} channel) and ligand-gated Ca^{2+}-channels; (ii) Ca^{2+} release from endoplasmic reticulum stores following phospholipase C (PLC) activation and inositol(1,4,5) triphosphate ($Ins(1,4,5)P_3$) release; (iii) Ca^{2+} entry via transient receptor potential (TRP) channels; (iv) Ca^{2+}-induced Ca^{2+} release (CICR) from endoplasmic reticulum stores; (v) Ca^{2+} entry through type 1 Na^+/Ca^{2+} exchanger (NCX1); and (vi) other Ca^{2+} channels such as GPCR-operated Ca^{2+} channels and tyrosine-kinase-activated Ca^{2+} channels. Current knowledge of $[Ca^{2+}]_i$ homeostasis in VSMCs has been reviewed recently.[20]

It was assumed for some time that transmembrane $[Ca^{2+}]_i$ influx is essential for VSMC contraction in response to stretch.[21,22] However,

Meininger *et al.*,[23] using real-time measurement of $[Ca^{2+}]_i$, pointed to the lack of a simple proportional relationship between calcium and contraction, and this was taken as evidence for alternative mechanisms regulating vascular contraction.

Calcium sensitization is clearly important for myogenic response. The Ca^{2+} requirement to sustain stretch-induced MT in the rabbit facial vein is much lower than that needed by equi-effective contractile agonists.[24] Furthermore, direct PKC activation increases pressure-induced force without increasing Ca^{2+} influx,[25] suggesting the existence of $[Ca^{2+}]_i$ increase-independent and PKC-dependent MLC phosphorylation pathways. One arm of this Ca^{2+} sensitization has indeed been attributed to the PKC substrate CPI17,[26] a 17 kDa peptide, whose phosphorylation enhances its ability to inhibit MLCP, and thus, MLC phosphorylation.[19] The other arm is dependent on the Rho/Rho-kinase signalling pathway.[27] In cerebral arteries, pharmacological inhibition of Rho-kinase reduces MT *in vitro*[28] and *in vivo*.[29] The contribution of Rho-dependent Ca^{2+} sensitization is enhanced during chronic hypertension.[29] While the molecular events leading to Rho activation remains to be fully characterized, it seems that RhoA interaction with caveolin-1 is essential for Rho-kinase activation.[30] This Ca^{2+} sensitization process probably regulates the interaction of MT with other constrictors, as initially described by the synergistic interaction between the sympathetic system and MT in cremaster muscle arteries.[31]

3. G protein-coupled receptors (GPCR) and MT

Vasoconstrictor hormones and autacoids acting through GPCRs exert their effect via activation of the $G_{q\text{-}11}$ and $G_{12\text{-}13}$ subclasses.[32] While $G_{q\text{-}11}$ protein leads to Ca^{2+} mobilization and PKC activation, $G_{12\text{-}13}$ protein activates the Rho pathway. Both pathways work in synergy during the myogenic process.

3.1 Contribution of locally generated GPCR agonists to MT

The mechanical stress imposed on the vessel wall following an increased intra-arterial pressure induces the local generation of an eclectic range of mediators including peptides, lipids, nucleotides, and amines. To date, evidence suggests that these mediators reinforce MT through interactions with intracellular second messenger and as autocrine/paracrine vasoconstrictors through Class1 GPCR activation. Proposed participation of locally generated GPCR agonists in MT is represented in *Figure 2*.

3.1.1 Endothelin-1

Endothelin-1 (ET-1) is released by endothelial cells in response to various stresses and its effect is mediated via the activation of two GPCR subtypes: ET_A and ET_B.[33] It is commonly accepted that in human vessels, ET_A receptors are mainly located on VSMCs, with ET_B receptors being present on endothelial cells. ET_B receptors, on the other hand, may play a role in the release of NO and PGI_2.[34]

In spontaneously hypertensive rats (SHRs), the enhanced MT of skeletal muscle arterioles is due to the production of endothelium-derived constrictor factors such as Prostaglandin H_2 (PGH_2)/thromboxane A_2 (TXA_2) and ET-1.[35] Both agents increase Ca^{2+} sensitivity of the contractile apparatus in arteriolar smooth muscle.[36] Similarly, ET_A antagonists abolish the exacerbated MT in tumour arterioles. This selectivity is associated with a large increase in ET-1 abundance in tumours and a higher ET_A receptor density in tumour vessels.[37] Indeed, oxidative stress increases ET-1 production in isolated and pressurized rat gracilis skeletal muscle arterioles. In these arteries, H_2O_2 increases MT through the production of ET-1.[38] Basal ET-1 release has also been reported to mediate MT in coronary arteries, and it has been suggested that the contribution of ET-1 in coronary artery MT would lead to a reduction in coronary vasodilatory reserve, and thus, increase susceptibility to ischaemia and arrhythmia.[39]

3.1.2 Arachidonic acid-derived metabolites

Arachidonic acid (AA) released after membrane phospholipid hydrolysis by phospholipases is the precursor of a number of bioactive metabolites such as leukotrienes, prostaglandins, TxA_2, epoxyeicosatrienoic acids (EETs), and hydroxyeicosatetraenoic acids (HETE). Many of these molecules are vasoactive, for instance, endothelial-derived prostacyclin induces vasodilation while TXA_2 and PGH_2 are potent vasoconstrictors.[40]

3.1.2.1 20-Hydroxyeicosatetraenoic acid

20-Hydroxyeicosatetraenoic acid (20-HETE) is synthesized by ω-hydroxylation of AA via cytochrome P-450 (CP450) 4A. The contribution of 20-HETE to MT has been described in renal,[41] coronary,[42] skeletal,[43] and mesenteric[44] arteries. Inhibition of 20-HETE synthesis reduces infarct size after cerebral ischaemia reperfusion injury (IRI),[45] whereas inhibition of 20-HETE activity decreases cerebral damage following stroke,[46] suggesting that targeting the 20-HETE pathway could have therapeutic benefits in IRI and vasospasm following SAH.

Evidence supporting a key role of 20-HETE in MT is the following: First, 20-HETE inhibits the opening of the large conductance BK_{Ca} channel, causing depolarization and increasing Ca^{2+} influx through L-type Ca^{2+} channels.[47,48] Second, the phosphorylation of myristoylated, alanine-rich PKC substrate (MARCKS), and the inhibition produced by PKC pseudosubstrate suggest that 20-HETE-induced inhibition of K^+ currents largely depends on PKC activation.[49] Third, 20-HETE activates the Rho pathway, leading to the phosphorylation of MLC and the sensitization of the contractile apparatus in coronary arteries.[50] Finally, 20-HETE interacts with TRPC6,[51] which appears to be a cornerstone of the myogenic process (Section 2.3.3.). Interestingly, 20-HETE-dependent vasoconstriction of cerebral arteries was reported to occur through G protein-coupled thromboxane prostanoid (TP) receptors.[52] Together with 20-HETE-dependent Rho activation, this observation raises the question of the contribution of this mediator as a TP agonist in addition to its well-known role as an intracellular second messenger.

3.1.2.2 TXA_2 and PGH_2

Stimulation of TXA_2/PGH_2 (TP) receptors elicits contraction of VSMCs.[53] In rat descending coronary artery, inhibition of prostaglandin synthesis or TP receptors blockade reduces MT.[54] In SHRs, increased MT is thought to be due to an enhanced production of endothelium-derived constrictor factors, primarily PGH_2.[55] Interestingly, this seems to be specific to hypertension because endothelial-derived vasorelaxant prostanoids oppose MT in normotensive animals.[56] An increased arteriolar MT accompanies microvascular growth in young rats through mechanisms that are sensitive to TP receptor blockade.[57] The role of TXA_2 or PGH_2 in reinforcing MT

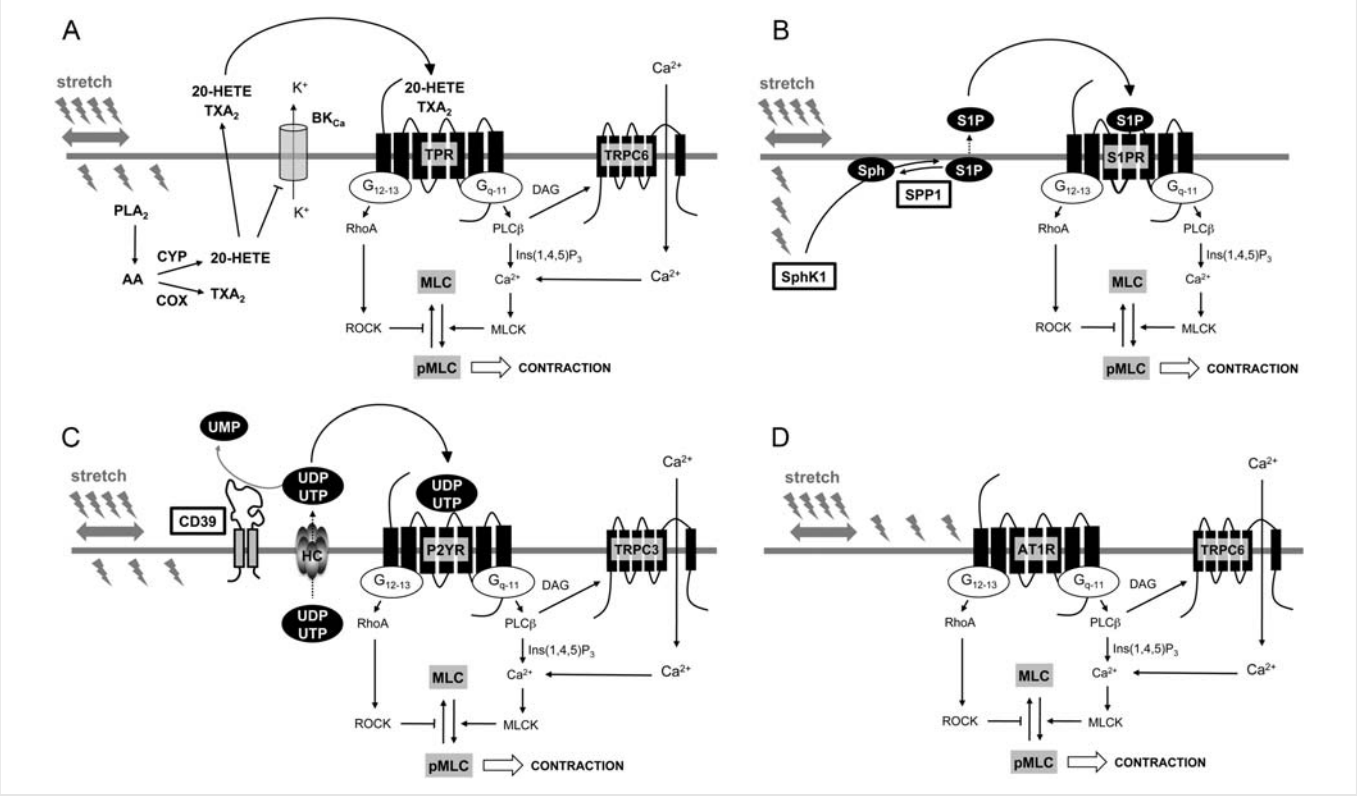

Figure 2 Proposed mechanism of local GPCR agonists in response to smooth muscle stretch. VSMC stretch activates the generation and release of several autacoids. (*A*) Phospholipase A_2 generates arachidonic acid (AA), which can be metabolized into thromboxane A_2 (TXA_2) and 20-hydroxyeicosatetraenoic acid (20-HETE) through the respective action of cyclooxygenase (COX) and cytochrome P450 (CYP) enzymes. 20-HETE inhibits calcium-activated potassium channels (BK_{Ca}), thus limiting VSMC hyperpolarization. Both TXA_2 and 20-HETE may act locally on an autocrine mode on VSMC TP receptor (TPR). (*B*) Sphingosine kinase-1 (SphK1) translocates to plasma membrane in response to stretch and phosphorylates sphingosine (Sph) to generate sphingosine-1-phosphate (S1P). This reaction is functionally antagonized by the sphingosine-1-phosphate specific phosphatase (SPP1). S1P activates S1P2 or 3 receptors (S1PR) to reinforce contraction. (*C*) Nucleotides are released in response to cell stretch,[72] most probably through membrane hemichannels (HC). Autocrine activation of purinergic receptor (P2YR) by uridine-5′-tri and/or diphosphate (UTP, UDP) reinforces contraction. UTP receptor-dependent activation of TRPC3 channel further increases Ca^{2+} rise. This effect of extracellular nucleotides is controlled by CD39 ectonucleotidase.[70] (*D*) AngiotensinII type 1 receptor (AT1R) has been proposed to be directly activated by stretch and to subsequently activate TRPC6 channels.[78]

during rapid juvenile growth also persists in hypertension. A proposed mechanism of action of TXA_2 in MT is presented in *Figure 2A*.

3.1.3 Sphingosine-1-phosphate (S1P)

S1P is a bioactive lipid with pleiotropic cellular effects such as proliferation, differentiation, and cell migration.[58] S1P exerts its effect through five GPCRs [S1P receptors 1–5] (previously endothelial differentiation gene-identified).[59] Vascular myocytes express S1P2 and/or S1P3 receptors that are involved in S1P-mediated constriction and *in vitro* SMC proliferation through the Rho pathway and p42/p44 mitogen activated protein kinases (MAPK), respectively.[60] Interestingly, some small-diameter arteries that develop MT (e.g. renal, mesenteric, and basilar arteries) are more responsive to exogenous S1P-mediated vasoconstriction compared with large-diameter conduit arteries.[61,62] Cellular synthesis of S1P is limited under unstimulated conditions through the spatial separation of the sphingosine kinase 1 (Sphk1) enzyme in the cytosolic compartment from its membrane substrate, sphingosine. Bolz *et al.*[63] proposed that arterial myocytes generate S1P in the vascular wall, which can then participate in MT. This mechanism implicates stretch-dependent relocation of Sphk1 and sphingosine phosphorylation. Locally generated S1P enhances vascular contraction through S1P2/Rho-kinase activation in an autocrine/paracrine mode (*Figure 2B*). Accordingly, the S1P2 receptor antagonist JTE-013 potently inhibits MT of cremaster arterioles.[64] A second enzyme, the S1P-specific phosphatase (SPP1), functionally antagonizes S1P effects. By dephosphorylating S1P, SPP1 opposes the effect of Sphk1 and, consequently, constitutes a negative regulator of myogenic and resting microvascular tone.[65] These molecular mechanisms suggest that a finely tuned mechanism of S1P receptor agonist formation and elimination may constitute regulatory steps of the myogenic response.

3.1.4 Extracellular nucleotides

Beside their well-characterized role in cellular metabolism, nucleotides can be released extracellularly following non-lytic mechanisms.[66] Extracellular nucleotides exert both vasodilator and vasoconstrictor effects through EC and VSMC membrane-bound P2-type receptors. P2 receptors are divided into ligand-gated P2X ion channels (P2X1–7) and G protein-coupled P2Y receptors (P2Y1,2,4,6,11–14).[67] ATP activates all P2X receptors, whereas P2Y receptors are differentially activated by ATP, ADP, UTP, UDP, or UDP-glucose.[68]

Ectonucleotidases from the ENTPDase family actively hydrolyse juxtacellular nucleotides and terminate activation of P2 receptors.[69] Interestingly, invalidation of the dominant vascular NTPDase (CD39) results in a facilitated contractile response to exogenous nucleotides (prolonged bioavailability) but also in enhanced MT in resistance arteries.[70] Nucleotides exert a P2 receptor-dependent potent vasoconstriction in mouse mesenteric arteries[70,71] and the difference in MT may be attributable to the release of nucleotides upon cell stretching following intraluminal pressure increases. Accordingly, cellular mechanical stretch is a well-known nucleotide release process[72] (*Figure 2C*). VSMCs themselves may be a source of uracil nucleotides as vascular smooth muscles have a low intracellular ATP/UTP ratio. It has also been reported that pyrimidines are released by perfused rat hind limbs in response to constrictor agents (noradrenaline, vasopressin, AngII).[73] Finally, P2 receptor antagonists inhibit MT in mouse mesenteric arteries.[70,74] Preliminary observations suggest that P2Y6 receptors may underlie this amplification process.[75] Interestingly, another study reported a similar autocrine loop involving P2Y6 receptor activation in cardiomyocytes which, in this case, participates in the development of cardiac fibrosis.[76] It is noteworthy that the proposed mode of action of nucleotides is quite similar to the mechanisms suggested for the action of S1P (*Figure 2B*). Further investigations are required to characterize the role of nucleotide release and the activation of specific P2 receptors in MT. Noteworthy is the suggestion that the ionotropic P2X1 ATP receptor participates in pressure-dependent autoregulation in kidney afferent arterioles,[77] suggesting that locally released nucleotides can act on both ionotropic and metabotropic P2 receptors to modulate MT.

3.2 Agonist-independent activation

The angiotensin II type 1 (AT_1) receptor was the first GPCR implicated to be mechanosensitive.[78] Using angiotensinogen-deficient mice and a neutralizing antibody directed against angiotensin II, Zou *et al.* reported that mechanical activation of AT_1 receptor in cardiac myocytes is agonist-independent.[79,80] Direct mechanosensitivity of AT1 receptors has been shown more recently in rat aortic A7r5 cells. These authors have also shown that MT of cerebral and renal resistance arteries was strongly reduced by an inverse AT_1 receptor agonist independently of AngII secretion[79,80] (*Figure 2D*). This hypothesis is different from autocrine activation by locally generated GPCR agonists that have been discussed above such as S1P, 20-HETE, and nucleotides. However, both indirect and direct evidence exists for the local generation of AngII.[81–83] Importantly, functionally relevant changes in local AngI to AngII conversion are not necessarily reflected by detectable changes in circulating AngII.[84] Thus, although activation of AT_1 receptors is involved in MT, the question related to the requirement for local AngII generation for AT_1 receptor activation remains a matter of debate. It is noteworthy that direct mechanical activation of AT_1 receptor leading to TRP channels opening was observed in transfected cells, and it remains to be determined whether these observations are physiologically relevant.

Recently, additional vasoactive GPCR agonists, including apelin, motilin, neuromedin U, and urotensin-II, have emerged.[85] To date, no data have shown that they had a role in MT. However, considering the central role of GPCRs to trigger and/or amplify MT, it would be interesting to evaluate the contribution of these agents and their receptors in MT.

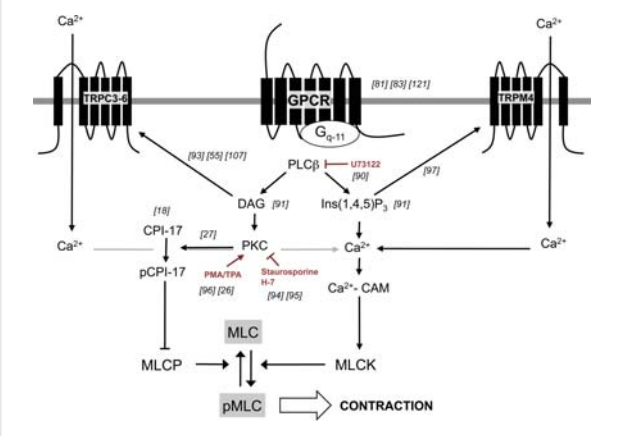

Figure 3 Implication of the $G_{q\text{-}11}$ transduction pathway in the myogenic process. There is a strong rationale regarding the participation of the different elements of the $G_{q\text{-}11}$ pathway in MT. Pharmacological, genetic, and electrophysiological approaches allowed to highlight that both intracellular Ca^{2+} stores mobilization, Ca^{2+} entry through TRP channels, and calcium facilitation pathway can be triggered through $G_{q\text{-}11}$ pathway activation. Abbreviations: CPI-17 indicates 17 kDa protein kinase C substrate phosphatase inhibitor; DAG, diacylglycerol; MLC, 20 kDa myosin light chain; MLCK, 20 kDa myosin light chain kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; Staurosporine: [9*S*-(9α,10β,11β,13α)]-2,3,10,11,12,13-Hexahydro-10-methoxy-9-methyl-11-(methylamino)-9,13 -epoxy-1*H*,9*H*-diindolo[1,2,3-*gh*:3′,2′,1′-*lm*]pyrrolo[3,4-*j*][1,7]benzodiazonin-1-one, protein Kinase C inhibitor. TPA, 12-O-tetradecanoylphorbol-13-acetate; TRP, transient receptor potential channel. U7122: 1-[6-[[(17β)-3-Methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione, phospholipase C inhibitor.

3.3 GPCR activation acts in synergy with myogenic contraction signal transduction pathways

Vasoconstrictor hormones and autacoids acting through GPCRs exert their effects through $G_{q\text{-}11}$ and $G_{12\text{-}13}$ proteins activation,[32] and these G protein subclasses are thought to modulate MT.

3.3.1 $G_{q\text{-}11}$- PLCβ pathway

A large body of evidence suggests that the different elements of the $G_{q\text{-}11}$ transduction pathway act as MT enhancers, including G protein-coupled receptor itself, PLCβ, PKC, and Ins(1,4,5)P_3-dependent Ca^{2+} mobilization (*Figure 3*).

It has been almost 20 years since the first report showing that MT is sensitive to pharmacological inhibition of PLC,[86] implicating G protein modulation of mechanotransduction through pathways superimposed on basal MT. This report was followed by a study by Narayanan *et al.*[87] showing pressure-dependent Ins(1,4,5)P_3 and DAG accumulation in dog renal arteries. Following $G_{q\text{-}11}$-coupled GPCR activation, Ca^{2+} mobilization resulting from Ins(1,4,5)P_3 binding to its endoplasmic reticulum receptors adds to the overall $[Ca^{2+}]_i$ increase, thus reinforcing VSMC contraction. A recent study suggests that Ins(1,4,5)P_3 can increase $[Ca^{2+}]_i$ independently of intracellular Ca^{2+} stores.[88] This alternative mechanism requires a co-coordinated interaction of Ins(1,4,5)P_3 receptors, TRPC3 channels, and voltage-dependent

Ca^{2+} channel activation (*Figure 3*). Several TRP channels, potential components of the SAC involved in MT (i.e. TRPC3 and 6), are directly activated by DAG[89] (*Figure 2A*, *C*, and *D*), suggesting that SAC-dependent non-selective cation currents may be partly dependent on $G_{q\text{-}11}$ through DAG formation. Moreover, MT is sensitive to pharmacological inhibition of PKC,[90,91] whereas PKC activators, such as TPA or PMA, potentiate MT.[92] PKC exerts its influence at different levels in MT. First, it increases sensitivity of the contractile apparatus to calcium,[25] possibly through the intermediate C protein kinase inhibitor of phosphatase (CPI17),[26] from which the phosphorylated form inhibits MLCP (*Figure 3*). Second, PKC phosphorylates and activates the TRPM4 channel, which is thought to be a key component of the SAC current.[93] Based on studies using purified G proteins reconstituted into phospholipids vesicles, Gudi *et al.*[94] proposed that G proteins are directly sensitive to shear stress through changes in membrane bilayer physical properties. So far, such direct mechanical activation of a G protein was not reported in MT.

The role of $G_{q\text{-}11}$-coupled receptors as direct mechanosensors has recently been reviewed.[95] Membrane stretch induces agonist-independent activation of AT_1 receptors, which subsequently signals TRPC channels in a $G_{q\text{-}11}$ and PLC-dependent manner.[78] Antagonists and inverse agonists of AT_1 receptors prevent this activation. Consistent with a contribution of this mechanism to MT, contraction of cerebral and renal arteries in response to increasing intraluminal pressure is reduced by AT_1 receptor inverse agonists. According to this hypothesis, membrane stretch sensing by GPCRs is not only independent of the presence of agonist activation but can also be substituted by other (any) $G_{q\text{-}11}$-coupled receptors.[78]

Kinases from the mitogen-activated protein kinases (MAPK), whose activity depends on GPCR signalling, also play a role in MT. Massett *et al.*[96] reported that whereas PKC and p42/44 MAP kinases are involved in both MT and agonist-induced contraction, p38 MAP kinase appears to be specifically involved in MT in rat gracilis muscle arterioles. Nevertheless, using a model that allows the application of stretch with or without the development of MT, we have shown that the MAPK p42/44 are activated by stretch but are not involved in MT.[97] Similarly, using the same model, we have shown that p38 is only minimally involved in MT, although activated by wall stretch.[98] Nevertheless, the latter study shows that the RhoA and Rho-kinase play a key role in MT.

3.3.2 $G_{12\text{-}13}$-Rho pathway

Although the role of the Rho pathway in MT has been clearly shown, the involvement of GPCRs in Rho activation in response to pressure increases is not known. In VSMCs, GPCRs coupled to $G_{12\text{-}13}$ proteins activate the RhoA pathway and cause MLC phosphorylation[99] through indirect inhibition of its phosphatase (*Figure 1*). Interestingly, locally generated GPCR agonists are proposed to participate in MT (Section 2.1) and the corresponding GPCRs seem to couple preferentially to $G_{12\text{-}13}$ proteins in arterial SMCs. This was shown for TXA_2, endothelin-1,[32] S1P,[63] and P2Y uracyl nucleotides agonists,[100] suggesting that locally generated $G_{12\text{-}13}$-coupling GPCR agonists can trigger Rho activation.

Adding to the complexity of the scheme are the RhoA guanine nucleotide exchange factors (GEFs), which act as molecular switches to activate Rho. Specific GPCRs agonists may couple to distinct GEFs. Indeed, a recent work identified p115RhoGEF as specifically activated by AngII after binding to the AT1 receptor.[101] The p63RhoGEF is directly coupled to and is activated by G_q protein establishing a link between $G_{q\text{-}11}$ and the Rho pathway.[102] p63RhoGEF has been recently shown to mediate AT1 receptor-dependent RhoA activation in VSMCs, including the subsequent cellular proliferation and contraction.[103] Considering the central role of RhoA in vascular contraction, the contribution of GEFs and other modulators of Rho activity, such as guanine dissociation inhibitors (GDIs) and GTPase-activating proteins (GAPs), in MT needs to be further investigated.

3.3.3 GPCRs act in synergy with TRP activation

As TRP are polymodal sensory ion channels involved in a number of mechanosensory systems,[104] they are primary candidates for a role as SACs involved in Ca^{2+} entry in MT[105,106] and possibly in MT.[107] First, TRPC6 antisense oligonucleotides reduce pressure-induced depolarization, cationic currents, and MT in intact cerebral arteries,[108] suggesting their participation in stretch-activated currents. Surprisingly, TRPC6-deficient mice display an exacerbated MT and vascular contraction to adrenergic agonists, likely due to compensatory TRPC3 over-expression and is reversible by TRPC3 silencing.[109] This result suggests a possible redundancy between TRPC channels.[110] Nevertheless, down-regulation of TRPC3 did not affect MT,[111] leaving open the question of the direct contribution of TRPC3 and 6 in MT.

TRPM4 have properties very similar to that of the native SACs identified in isolated cerebral artery myocytes. Myocytes depolarization and MT were both attenuated in cerebral arteries treated with TRPM4 antisense oligonucleotides.[112] Although the activation mode of TRPM4 channels remains unknown, intracellular ATP, PKC-dependent phosphorylation, and calmodulin binding are required.[113] TRPM4 is a voltage-dependent channel,[114] making it likely that its activation results from TRPC6-dependent Ca^{2+} influx and depolarization.

Finally, a role for TRPP1 and TRPP2 in pressure sensing was recently reported. TRPP2 inhibits SACs through a specific interaction with TRPP1.[115] Hence, the TRPP1/TRPP2 ratio is critical, through filamin A coupled to the actin cytoskeleton, to convert intraluminal pressure to local bilayer tension into an increase in SAC mechanosensitivity. A specific decrease in the TRPP1 level in mouse SMCs induces a large decrease in MT, suggesting a critical role for the TRPP1/TRPP2 balance.

Several studies suggest that GPCRs interact with TRPs. *Table 1* summarizes similarities between GPCR and TRP activation. GPCR-dependent TRP activation is well documented for DAG-sensitive TRPC3-6-7 channels.[89] Mechanosensitive cation channels and TRPCs are activated by DAG and attenuated by PLC inhibitors.[116] Knockdown of TRPC3 with specific small-interfering RNA significantly reduces AngII-dependent calcium influx.[117] Similarly, the down-regulation of arterial TRPC3 expression with antisense oligodeoxynucleotides decreased cerebral arteries depolarization and vasoconstriction in response to the P2Y receptor agonist UTP.[111] ATP and UTP, probably acting through $P2Y_2$ receptors, induce TRPC3/7 channel opening. Uracyl nucleotides activation of neuronal PC12 cells increases TRPC5 currents, suggesting a general coupling of P2Y receptors to TRPC channel opening.[118]

4. Conclusion/discussion

Reduced levels of MT occur in depressed cardiovascular conditions such as shock, resulting in organ perfusion failure, whereas exaggerated MT contributes to increased peripheral resistance in diseases,

Table 1 Synergy between TRP and GPCR signalling

TRP	GPCR ligand activation	PLCβ activation	DAG sensitivity	PKC activation	PtdIns(3,4,5)P3 sensitivity	$[Ca^{2+}]_i$ sensitivity	Knockout phenotype
TRPC1	S1P[123]; ET-1	+[123]	NI	NI	+[124]	+[125]	Normal[126]
TRPC3	UTP/P2Y[111]	+[127]	+[89]	NI	NI	+[127]	NI
TRPC6	AT1R[80]	+[80]; −[128]	+[89]	NI	NI	+[105]	Increased vascular contraction, MT; elevated blood pressure[109]
TRPM4	NI	NI	–	Increase Ca^{2+} sensitivity[129]	NI	+[114]	Normal (Vennekens and Nilius, unpublished results)

References reporting the interaction between GPCR signalling and TRPs proposed to be involved in MT are listed (NI, not investigated).

such as hypertension, type-2 diabetes, and SAH. Pharmacological control of MT would allow resetting of inappropriate vascular resistance, and consequently, alter the actions of other neurohumoral control mechanisms.[5] Identification of the cellular and molecular determinants of MT is essential to enable such interventions. MT integrates a complex set of cellular and molecular process acting in synergy to produce vascular contraction. Parallel (amplifier) or in series (initiator) positioning of GPCRs in MT is not clearly known. There are numerous data to support the hypothesis that GPCRs could initiate the myogenic process: first, the generation of GPCR agonists in response to stretch; second, their ability to trigger TRP channels opening through DAG formation and PKC activation; third, the intrinsic property of some $G_{q\text{-}11}$-coupled GPCR exhibiting mechanosensitive properties. On the other hand, a recent study shows that GPCRs modulate TRP currents without affecting their mechanosensitivity nor MT,[119] suggesting that these GPCRs are not involved in the triggering of MT but rather act as amplifiers. Such parallel and synergistic interaction was proposed for adrenergic receptor stimulation that complement MT.[31] Also opposing this hypothesis is the short time of the myogenic response in cerebral arteries (in the millisecond range) that barely fits with agonist generation and GPCR activation (that takes several seconds). Noteworthy, as previously proposed, is that the determinants of MT may vary along the arteriolar tree and this may be true for the global contribution of GPCRs. In addition, the contribution of specific GPCRs/mediators in the myogenic process may depend on both the expression level of receptors and the local generation of the appropriate agonist. A good knowledge of the pharmacology of territory-specific arterial constriction may give insights concerning the potential contribution of specific GPCR in MT. Parallel contribution of GPCRs in the MT would act as backup in case other pathways are compromised.

A fundamental question concerns the means of GPCR activation during the myogenic response. Both ligand-dependent and -independent receptor activation have been proposed. Mechanical activation initiated by a conformational change of the receptor is discernible from agonist-bound activation.[80] Indeed, AT_1 receptors could be sensitive to mechanical stimuli.[78] In apparent contradiction with these data is that membrane stretch releases autacoids such as nucleotides,[70] S1P,[63] and 20-HETE.[120] Quantification of these molecules, particularly AngII, is hampered by the absence of sensitive detection methods suitable for use in resistance arteries *in situ*. However, some local generation of AngII in the microvasculature has been reported,[81–83] making it difficult to fully rule out an agonist-dependent activation of AT_1 receptor. Another possibility is that mechanoperception is a property shared by many GPCRs expressed in VSMCs and may depend on their expression level. It would be interesting to evaluate $G_{12\text{-}13}$ protein and eventually other G protein ($G_{i/o}$, G_s, or G_z) susceptibility to mechanical stimulation and Rho pathway activation. Finally, it is conceivable that both ligand-dependent and -independent receptor activation may coexist.

The RhoA-Rho-kinase pathway appears to be a major component of the calcium facilitation pathway in VSMC, and thus, could be the focal point of different GPCRs involved in MT. Its greater contribution in hypertensive pathology has prompted many groups to propose specific RhoA pathway targeting as a possible antihypertensive approach.[121] It seems reasonable to propose that an increase in pressure activates a sequence of events starting with the opening of ionic channels, the 'stretch-activated channels', together with the release of vasoactive autacoids, which are most likely sensitive to stretch. Both processes are fast responding, thus allowing the initial depolarization needed to rapidly increase wall force. In parallel, or with a small shift in time, mechanosensitive GPCRs or activated by stretch-induced agonists released (i.e. 20HETE, S1P, nucleotides) activate the RhoA-Rho-kinase pathway and, consequently, increase calcium sensitivity of the contractile apparatus. This latter would allow maintaining a new level of force over time. In this regard, the study performed by Cipolla *et al.*[122] showing that the ratio of F to G actin rises when pressure increases in resistance arteries, leading to higher MT, is important, especially since this ratio is determined by RhoA-Rho-kinase activation.[121] The large variety of GPCRs described as potentially activated by stretch, directly or not, certainly reflects some degree of tissue and/or species specificity of MT. In addition, in most, if not all, diseases associated with disturbed MT, GPCR expression level, and sensitivity to vasoconstrictor (i.e. endothelin, TXA_2) change. Knowing more precisely which GPCRs are involved in each tissue would allow the design of more selective treatments or the prevention of some side effects. Thus, a more in-depth knowledge of the precise sequence of events involved in MT in each tissue or organ is probably the key for more selectivity and less side effects in cardiovascular diseases and other disorders affecting blood flow perfusion.

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