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New technologies for dissecting the arteriolar myogenic response

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The arteriolar myogenic response is crucial for the setting of vascular resistance and for providing a level of tone upon which vasodilators can act. Despite its physiological importance, questions remain regarding the underlying signaling mechanisms of the arteriolar myogenic response. Does an increase in pressure within an arteriole exert its effects via the extracellular matrix, an action on cell membranes and/or deformation of cytoskeletal structures? Recent advances in methodology, particularly involving sophisticated imaging approaches, are enabling the study of forces at single-cell and even subcellular levels. Atomic force microscopy (AFM) not only enables detection of cell morphology and stiffness but also allows discrete forces to be applied to single smooth muscle cells and subsequent responses to be observed. Importantly, the repertoire of approaches involving AFM can be expanded by using it in combination with other imaging approaches – including fluorescence imaging for cellular signals such as Ca^{2+}, and total internal reflectance fluorescence, fluorescence resonance energy transfer and confocal microscopy for probing cellular contact function. Combinations of these advanced imaging and nanomechanical approaches will be instructive to studies of intact vessels and the circulatory system in general.

Introduction

Vascular cells are continually exposed to mechanical forces, as typified by the transmural forces exerted by blood pressure and the shear stresses generated by the flow of blood (Figure 1). Importantly, cells of the vascular wall (both smooth muscle and endothelial) are able to respond to these stimuli by acutely activating signaling pathways to modulate vessel diameter while, in the longer term, altering the structure of the vessel wall, in part by modulation of biosynthetic pathways. Through the ability of these mechanically initiated signaling pathways, both short- and long-term alterations of vasomotor tone, local control of blood flow and vascular resistance can be affected. Although this article focuses predominately on pressure–stretch activation of vascular smooth muscle, the reader is referred to recent reviews of shear-stress-induced activation of endothelial cells [1–4].

Despite the importance of mechanically activated mechanisms of control of the microcirculation and vessel wall, a complete knowledge of the underlying cellular events has not been attained. With regard to arteriolar constriction resulting from an increase in intraluminal pressure (the 'myogenic response'), the initial description of this vascular response is generally credited to Bayliss in 1902 [5]. Bayliss observed a link between reduced intravascular pressure and increased regional blood flow, which he attributed to the reduced distending pressure acting on the blood vessel wall. Since then, advances in understanding have come mainly from studies of whole animals, exteriorized organs, microvascular preparations and isolated arterioles (for review, see Refs [6–8]). These approaches have provided convincing evidence that pressure-induced vasoconstriction contributes to the setting of peripheral vascular resistance, the autoregulation of blood flow and the regulation of capillary pressure.

Mechanistically and conceptually, in its simplest form, increased smooth muscle cell membrane tension (resulting from an increase in intraluminal pressure) is thought to lead to membrane depolarization, voltage-gated Ca^{2+} entry, activation of actomyosin interaction and contraction [7–10] (see Figure Ia in Box 1). Recently, the contributions of Ca^{2+} sensitization of the contractile proteins and cytoskeletal rearrangement have been proposed as additional

Glossary

Atomic force microscopy (AFM): method enabling the application and measurement of forces in the pN–nN range by precisely controlling the deformation of a thin cantilever. The cantilever carries a tip that is either sharp or a surface such as a bead, and can be functionalized with biological molecules that act as binding partners for the cell or surface being studied.
Fluorescence (Forster) resonance energy transfer (FRET): method using the non-radiative transfer of energy from a donor to an acceptor molecule. Energy transfer relies heavily on the distance separating the donor and acceptor molecules.
Interference reflection microscopy (IRM): an optical technique for detecting the topography of the side of a cell in contact with a planar substrate. IRM uses the zero-order reflection interference pattern of the cell basal plane to measure the separation (<100 nm) and areas of contact (focal adhesions or close contacts) between the cell and a glass substrate.
Laser tweezer: method that enables the application and measurement of forces in the pN range by manipulating a bead using laser beams (optical trap).
Mechanical activity: force generated through (smooth muscle) cell contractility or relaxation.
Paramagnetic bead: method enabling the application of force, through torque, on a pN–nN scale. Torque is provided by a magnetic field or magnetic needle.
RGD peptides: synthetic amino acid sequences containing arginine-glycine-aspartate, which are analogs of sequences found in ECM proteins. Binding of the RGD sequence to integrins initiates signaling cascades.
Stiffness: Young's modulus, a measure of the elasticity of a cell in response to the loading of a compression force.
Total internal reflectance fluorescence (TIRF): a microscopy technique that excites the cell-labeling fluorophores with an evanescent wave (near-field standing wave exhibiting exponential decay), enabling quantitative measurement of fluorescent labeling within ~200-nm distance from a glass substrate.

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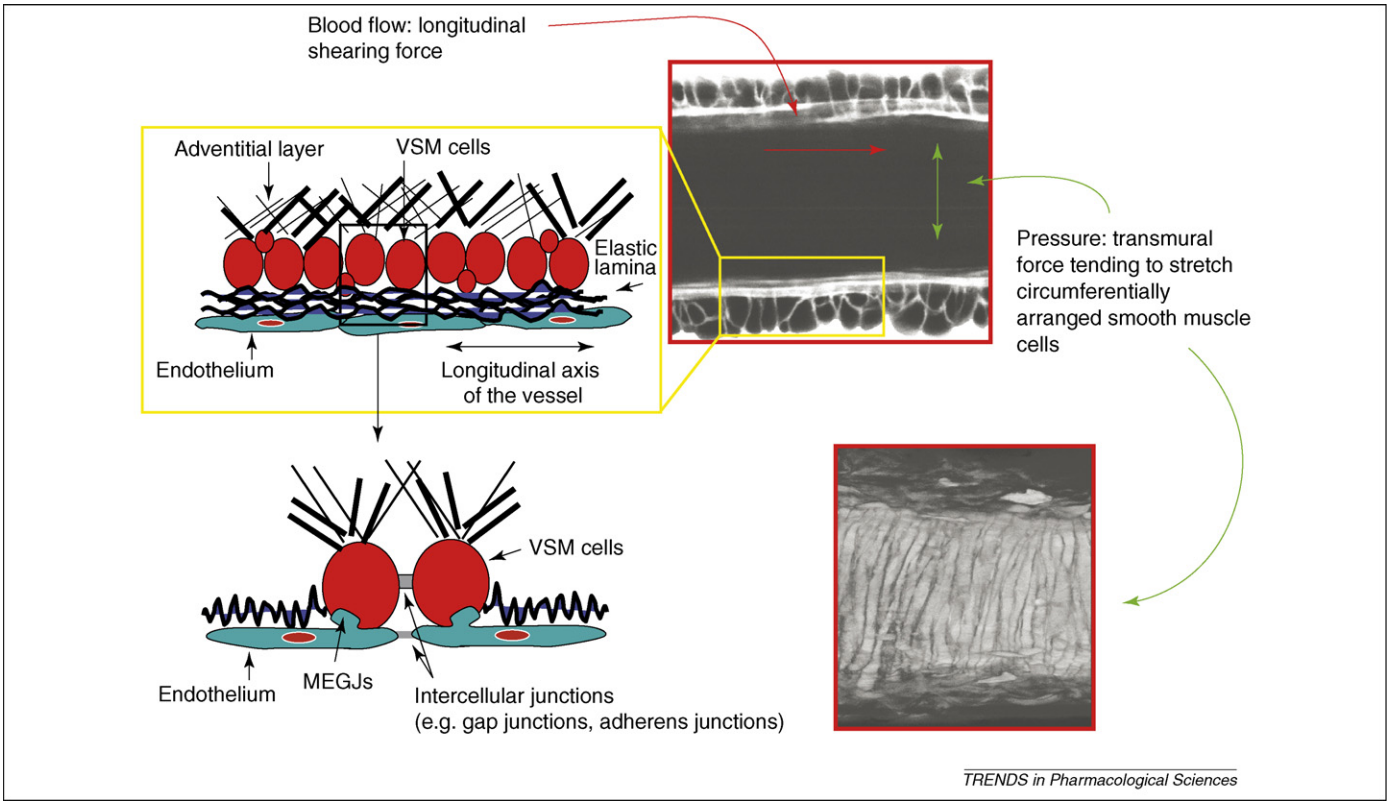

Figure 1. Mechanical forces and the vascular wall. The arteriolar wall is continually exposed to transmural and shearing forces as a result of the closed nature of the circulatory system. Complexity of the vessel wall occurs as a result of junctional contacts, both between vascular cells and with the ECM proteins. Gap junctional communication enables the vessel wall to act as a functional syncytium, facilitating the transfer of signals along – and perhaps through – the cells of the vessel wall. Contacts between cells and ECM proteins provide a mechanism for 'sensing' deformation caused by mechanical forces and, perhaps, initiating signal transduction mechanisms. Abbreviation: MEGJ, myoendothelial gap junction. Modified, with permission, from Ref. [24].

mechanisms that can modulate this pressure-dependent response [11–15]. Despite these advances in understanding, however, the exact molecular mechanisms through which a change in intraluminal pressure is sensed have remained elusive. In particular, the presence of a pressure-sensitive mechanosensor, or mechanically activated receptor, is yet to be conclusively demonstrated. It is our opinion, however, that developments in novel and sophisticated imaging techniques [e.g. atomic force microscopy (AFM) (see Glossary), fluorescence resonance energy transfer (FRET), total internal reflectance fluorescence (TIRF) microscopy, interference reflection microscopy (IRM) and multi-photon microscopy] – together with nanoscale- and picoscale-force measuring devices such as AFM, laser tweezers and paramagnetic microspheres, and approaches from disciplines such as nanomedicine and proteomics – are providing new tools for achieving this goal.

These developments in imaging and nanoscale-force measurement are enabling, in particular, the study of candidate mechanosensors, including the axis formed by

Box 1. Signaling events underlying the arteriolar myogenic response

Increased intraluminal pressure within an arteriole is thought to be detected by either cell stretch or an increase in wall tension [6,7]. This is conveyed through a direct effect on mechanically sensitive protein elements such as ion channels or the membrane lipid bilayer itself, or through interactions between extracellular elements (e.g. ECM proteins) and cell surface 'receptors' such as integrins. Activation of these pathways then leads to the opening of non-selective cation channels, membrane depolarization and the opening of voltage-gated Ca^{2+} entry [10,55]. Increased $[Ca^{2+}]_i$ results in Ca^{2+}/calmodulin-dependent activation of myosin light-chain kinase, phosphorylation of the 20-kDa myosin regulatory light chains and contractile interaction of actin and myosin [9,56] (Figure Ia). In addition to these events, it is evident that the mechanical stimulus drives other signaling Ca^{2+}-dependent and -independent events, including Ca^{2+} release, kinase activation and, perhaps, phosphatase inhibition. These pathways might contribute to modulation of the contractile response through alterations in Ca^{2+} sensitivity, Ca^{2+} handling, activation of ion channels such as BK_{Ca} and cytoskeletal rearrangements [11–14,57].

Although Figure Ia presents these events in a linear fashion, culminating in contraction, it is equally – if not more – likely that the mechanical stimulation that occurs during an increase in intravascular pressure results in parallel activation of multiple, perhaps interacting, pathways (Figure Ib). Such a situation would be expected if mechanical deformation of the membrane, as would occur during cell stretch, simultaneously applies force to varying mechanosensors or proteins whose activity is modulated by nanoscale forces. Interaction between such pathways would invariably occur on multiple levels. For example, myogenic contraction might decrease tension and the resultant driving force for Ca^{2+} entry, leading to a biphasic global Ca^{2+} signal; decreased tension might also relieve a stimulus for adaptive remodeling and structural modification of the vessel wall; Ca^{2+} entry, in addition to contraction, might activate opposing hyperpolarizing events, as proposed for the BK_{Ca} channel. Importantly, the separation of these events requires the ability to apply nanoscale forces precisely to the membrane, perhaps through specific ligand–receptor interactions.