Microvascular control of myocardial perfusion

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Abstract

Myocardial perfusion is closely linked to oxygen demand, which is met by regulation of the resistance to flow in small arteries and arterioles via smooth muscle tone. Adjustment of smooth muscle tone occurs by modulation of the cytosolic calcium concentration, via influx of calcium across the cell membrane through a variety of calcium channels and release of calcium from the sarcoplasmic reticulum by inositol trisphosphate and ryanodine receptors. In addition, the calcium sensitivity of the contractile apparatus is regulated by several signaling cascades that converge on different protein kinases. Among the local mechanisms responsible for coupling perfusion to demand, metabolic mediators are the most potent in decreasing coronary vascular resistance. pH, partial pressure of oxygen, partial pressure of carbon dioxide, and potassium concentration all have profound effects on perfusion, but the identity of the mediator responsible for metabolic dilatation under physiologic conditions remains elusive. Because metabolic mediators reach only the most distal precapillary arterioles in sufficient concentrations, additional mechanisms are required to induce a coordinated dilatory response of the upstream larger resistance vessels. These may include myogenic mechanisms – that is, active contraction of smooth muscle cells in response to increased stretch or, in the case of downstream dilatation, active relaxation in response to decreased luminal pressure. The decrease in resistance as a result of metabolic and myogenic mechanisms in small and midsize arterioles increases flow in the entire upstream system of larger resistance vessels, resulting in endothelium-dependent flow-induced dilatation of this vessel segment.
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Introduction

Coronary blood flow is closely matched to myocardial oxygen demand. This is achieved by alterations of vascular tone in the coronary vessels that contribute most to overall coronary resistance – that is, small resistance arteries and arterioles of the coronary microcirculation. The mechanisms involved in this regulatory response have elicited increasing interest in cardiologists in recent years, because accumulating evidence suggests that myocardial malperfusion may result, not only from stenoses in the large epicardial blood vessels, but also from inadequate dilatation or obstruction of microvessels. Some excellent reviews have addressed the putative role of coronary microvascular dysfunction in patients [1–3] and the

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techniques currently available for its diagnosis [4]. The pathogenetic mechanisms contributing to coronary microvascular dysfunction are not well understood, but may include extravascular components such as extramural compression, structural alterations in the microvessels such as luminal obstruction by microemboli, or functional deficits, including endothelial and smooth muscle cell dysfunction. Research aiming at a better understanding of the pathogenesis of coronary microvascular dysfunction and the development of therapeutic principles is necessarily based on knowledge of the physiological principles governing the microvascular control of myocardial perfusion in healthy individuals. Here, we will briefly review the major mechanisms contributing to the microvascular control of myocardial perfusion.

Regulation of smooth muscle tone

Myocardial perfusion is adapted to metabolic demand by regulation of the diameters of resistance vessel – that is, by regulation of vascular smooth muscle tone in small arteries and in arterioles. Therefore, we now briefly summarize the mechanisms involved in smooth muscle contraction and relaxation. For more detailed analysis, we recommend recent in-depth reviews on this topic [5–7].

Smooth muscle tone depends upon the phosphorylative state of the myosin light chain (MLC), in that phosphorylated MLC (MLC-P) interacts with actin filaments, inducing contraction, whereas dephosphorylated MLC does not, facilitating relaxation (*Figure 1*). The enzymes MLC kinase (MLCK) and MLC phosphatase (MLCP) determine the balance between MLC and MLC-P. Calcium induces contraction by binding to calmodulin*, which then forms a complex with MLCK, thus activating the enzyme and shifting the balance to MLC-P. In addition to cytosolic calcium concentration, the balance between MLC and MLC-P is modulated by phosphorylation of MLCK and MLCP, which alters the calcium sensitivity of the contractile system. Various protein kinases that are activated by G-protein-coupled receptor-dependent signaling pathways* or by signaling molecules that can enter the smooth muscle cell, such as nitric oxide, participate in the regulation of calcium sensitivity and thus of vascular smooth muscle tone.

Calcium enters the cell via a variety of calcium channels, including voltage-operated L-type and T-type calcium channels, which are activated by depolarization of the sarcolemma [6]. In addition, ligand-operated, store-operated, and stretch-sensitive calcium or unspecific cation channels may contribute to calcium entry. A second source of calcium entry is the intracellular calcium store, the sarcoplasmic reticulum. Inositol 1,4,5-trisphosphate (IP_3) is the second messenger of various G-protein-coupled receptors, among them the adrenergic α_1-receptor, which binds to and activates an IP_3-sensitive calcium channel, the IP_3 receptor in the sarcoplasmic reticulum membrane, inducing the release of calcium into the cytosol. Interestingly, at moderately enhanced cytosolic concentrations, calcium seems to enhance the effect of IP_3, forming a positive-feedback loop, which results in calcium-induced release of calcium. A calcium-sensitive calcium channel (the so-called ryanodine receptor) has been shown to be expressed in smooth muscle cells, but its actual contribution to the release of calcium from the sarcoplasmic reticulum remains unclear.

Removal of calcium from the cytosol is achieved by its re-uptake into the sarcoplasmic reticulum by the sarcoplasmic reticulum calcium pump, by calcium transport across the sarcolemma via another pump (Ca^{2+}/H^+-ATPase), which exchanges calcium for protons, and by a sodium–calcium exchanger, which depends upon the electrochemical gradient for the entry of sodium ions.

As mentioned above, cytosolic calcium concentration is not alone in determining smooth muscle tone. Several signaling cascades are involved in modulating the calcium sensitivity of the contractile fibers (*Figure 1*). A multitude of G-protein-coupled receptors convey signals into the cell, resulting in activation of different protein kinases. Protein kinase A (PKA), activated via, among others, β_2-adrenergic receptors, inhibits MLCK, facilitating relaxation. PKG, activated by, for example, natriuretic peptides or nitric oxide, activates MLCP, which also induces relaxation. In contrast, PKC and rho-kinase inhibit MLCP, thus inducing smooth muscle contraction. In addition, these signaling cascades may also modulate the conductivity of sarcolemmal ion channels, facilitating or
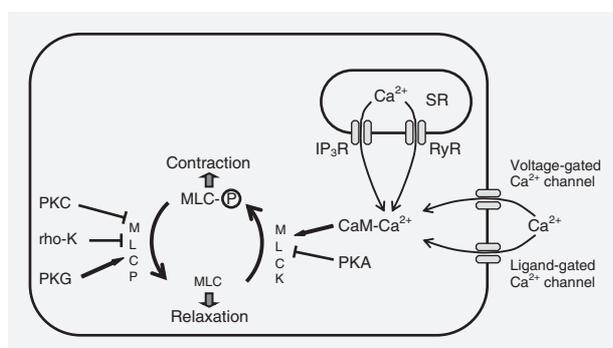

Figure 1. Schematic representation of the basic cellular mechanisms involved in the control of vascular smooth muscle tone. CaM, calmodulin; IP_3R, inositol trisphosphate receptor; MLC, myosin light chain; MLCK, MLC kinase; MLCP, MLC phosphatase; MLC-P, phosphorylated MLC; PKA, protein kinase A*; PKC, protein kinase C*; PKG, protein kinase G*; rho-K, rho-kinase*; RyR, ryanodine receptor*; SR, sarcoplasmic reticulum.*

inhibiting calcium entry, which contributes to the relaxing or constricting effects, although the quantitative contribution of this mechanism to the total relaxing or constricting effect is unknown.

Metabolic regulation of perfusion

In the coronary circulation, perfusion is particularly well-matched to metabolism, such that coronary venous oxygen tension remains essentially unchanged, even during marked changes in myocardial oxygen demand and consumption. Matching perfusion to metabolic demand is believed to be mediated mainly by locally acting mechanisms, including hypoxia, decreased pH, and increased concentrations of carbon dioxide, potassium, or adenosine, all of which induce vasodilatation of coronary resistance vessels [8–10]. The actual contribution of each of these mechanisms to the metabolic control of myocardial perfusion is, however, still unclear. Hypoxemia and hypercarbia both result in an increase in coronary perfusion, and a combination of the two exerts a synergistic effect, in that a high carbon dioxide concentration potentiates the effect of hypoxia, and vice versa, resulting in a more than additive effect [11]. However, such experiments do not reveal whether altered gas tensions induce vasodilatation directly or via release of a biochemical mediator such as adenosine. In addition, the observed effects explain only part of the increase in flow during increased metabolic demand, suggesting that other factors must be involved [10].

In 1963, two groups of investigators independently suggested adenosine to be the major metabolic dilating factor in the heart [12,13]. Since then, numerous studies have confirmed the potent dilatory effect of adenosine in the coronary circulation, but today it is widely accepted that adenosine becomes important mostly in pathophysiologic conditions such as hypoxia or ischemia, and contributes only little to physiologic metabolic control of flow [14]. Thus other factors must be more important. Among these, the involvement of ATP-sensitive potassium channels (K_{ATP}) in smooth muscle cells has long been favored, although the exact mode of activation remains unclear [8–10,15]. A decreasing ATP concentration in smooth muscle cells is, of course, a major activator of these channels, which then induces hyperpolarization and relaxation of smooth muscle cells. However, because of their much greater oxygen demand, myocardial muscle cells would be hypoxic long before ATP concentrations decrease in smooth muscle cells. Other factors released from cardiac muscle cells would be required to activate these channels, and, indeed, adenosine seems to exert its dilatory effect partly via activation of K_{ATP} channels [10]. At present,

experimental data can be interpreted either to support a major role of K_{ATP}^* channels in metabolic dilatation [8,15] or to reject it [10].

Numerous other mediators – such as prostaglandins, nitric oxide, or (EDHF) endothelium-derived hyperpolarizing factor* – have been investigated for a possible role in metabolic dilatation, but none of these, either alone or in combination with others, seems sufficient to explain the metabolic coupling of perfusion [10]. An attractive concept is feed-forward β_2-adrenergic-receptor-mediated dilatation of resistance vessels, because the same sympathetic stimulus that increases oxygen demand via β_1-receptors would also induce the increase in perfusion required to meet this oxygen demand. However, while β_2-mediated dilatation of microvessels is readily demonstrated, this mechanism seems to account for only about 25% of the total increase in blood flow during exercise [16]. In conclusion, although several mediators may be involved to a certain degree, the major mechanism responsible for matching coronary perfusion to metabolic demand remains elusive.

Myogenic response

Myogenic activity is an intrinsic property of vascular smooth muscle cells. Thus vascular smooth muscle contracts in response to increased transmural pressure and the resulting increase in circumferential wall tension. Consequently, any distension of the vessel wall is followed, within 20–60 s, by a sustained constriction. The extent of the contraction may result in constriction of the vessel to a final diameter that is considerably smaller than the baseline diameter. Conversely, decreased transmural pressure results in dilatation of the vessel. This mechanism has been observed in most vascular beds of the systemic circulation, and is generally considered to be most pronounced in renal, cerebral, and coronary resistance vessels. Myogenic activity stabilizes organ perfusion during alterations in systemic arterial pressure, and protects the capillaries from excessive changes in transmural pressure and, consequently, fluid filtration.

The mechanisms of myogenic responses seem to include activation of stretch-activated unspecific cation channels, and possibly also of chloride channels in the sarcolemma, inducing influx of calcium and depolarization, which is followed by further influx of calcium via voltage-sensitive calcium channels [17]. This response may be enhanced by calcium-induced release of calcium, and by concomitant activation of membrane-bound phospholipase C and release of IP_3 and diacylglyceride* from the phospholipids of the cell membranes. IP_3 would further increase cytosolic calcium concentration by release from intracellular

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stores, and diacylglyceride may increase the sensitivity of the smooth muscle contractile apparatus to calcium by activating PKC [8,17]. The exact mechanisms of the transduction of force into smooth muscle cells – that is, how increased stretch activates ion channels and PKC – remains to be elucidated. As the sarcolemma cannot bear enough force to activate stretch-sensitive ion channels without rupturing, force transduction has been suggested to be achieved via transmembrane adhesion molecules, integrins, which bind to extra-cellular matrix structures on the outside and to the actin cytoskeleton on the inside of the cell [17]. Negative-feedback mechanisms that protect blood vessels from stretch-induced spasms may involve voltage-activated and calcium-activated potassium channels, which would counter the depolarization of the cell membrane induced by the stretch-activated influx of cations [17].

Flow-induced dilatation

Endothelium-mediated vasodilatation in response to flow can be observed throughout the systemic and pulmonary circulation, in large arteries, in arterioles, muscular venules, and veins. The flow signal is transferred to the endothelial surface via wall shear stress, τ_W, which depends upon the volume flow rate, Q, blood viscosity, η, and vessel radius, r, according to the formula:

$$\tau_W = Q \times \eta \times 4/(r^3 \times \pi)$$

The exact mechanism by which increased wall shear stress is translated to an endothelial response – the mechanotransduction of this signal – has not yet been identified. Putative flow sensors include membrane proteins such as mechanosensitive ion channels, which may be activated directly or via mediation of the glycocalyx* at the endothelial surface [18,19], or membrane-bound G proteins [8,20]. In addition, mechanosensitive kinases may be activated by transmission of the mechanical stimulus to focal adhesion sites on the abluminal membrane by cytoskeletal actin fibers [21].

Among mechanosensitive ion channels, the family of transient receptor potential channels has attracted special interest. At least 18 different such channels have been shown to be expressed in endothelial cells, and several of these are obviously activated by shear stress. Some of these facilitate potassium efflux and hyperpolarization of endothelial cells – and, consequently, calcium entry as a result of the increased electrochemical driving force – whereas others function as shear-stress-sensitive calcium channels [22]. Calcium serves as a second messenger that directly activates endothelial nitric oxide synthase (eNOS) via calmodulin binding, but may also be involved in increased synthesis of prostaglandins (specifically, of prostacyclin), or release of an endothelium-derived hyperpolarizing factor [23]. The identity of this EDHF has not been determined conclusively, but potassium, calcium-activated potassium channels, and cytochrome P-450 metabolites of arachidonic acid are the major candidates [24,25]. The relative contribution of these endothelium-derived dilating mediators to flow-induced dilatation of coronary microvessels is controversial [8], but a prominent role of nitric oxide is unequivocally suggested.

However, increased calcium is not the only mediator of increased eNOS activity [26,27]. Calcium-independent mechanisms, which seem to be responsible for basal eNOS activity during constant shear stress, include, among others, tyrosine phosphorylation* of the enzyme and an increase in expression of protein as a result of increased transcription or mRNA stability.

The actions of the endothelial-dilating mediators on smooth muscle cells follow different pathways. Nitric oxide activates a soluble guanylate cyclase*, resulting in increased cyclic GMP concentration, which desensitizes the smooth muscle cell to calcium by PKG-mediated phosphorylation and activation of MLCP, but may also inhibit the entry of calcium through the sarcolemma. Prostacyclin, by binding to its receptor on the smooth muscle cell surface, activates a G_s protein, which then activates the adenylate cyclase–cyclic AMP–PKA signaling pathway. PKA desensitizes the smooth muscle cell to calcium by phosphorylation and inhibition of MLCK. In addition, a cyclic-AMP-mediated inhibition of calcium entry may contribute to the dilatory effect of prostacyclin. The major effect of the different putative EDHFs is hyperpolarization of the smooth muscle cells and the consequent inhibition of voltage-gated calcium entry channels [8].

Integration of metabolic dilatation with upstream mechanisms of diameter control

Increasing myocardial oxygen demand by pacing [28], as well as perfusion with dipyridamole [29], which increases tissue adenosine concentration, or with adenosine itself [30,31], all induce a similar pattern of vasodilatation across the different segments of the coronary vasculature, in that relative increases in diameter of the resistance vessels are inversely related to resting diameters. In other words, the relative diameter increase is greatest in the smallest precapillary arterioles.

This pattern of vasodilatation would be consistent with the assumption that metabolic mediators released from myocardial muscles would primarily
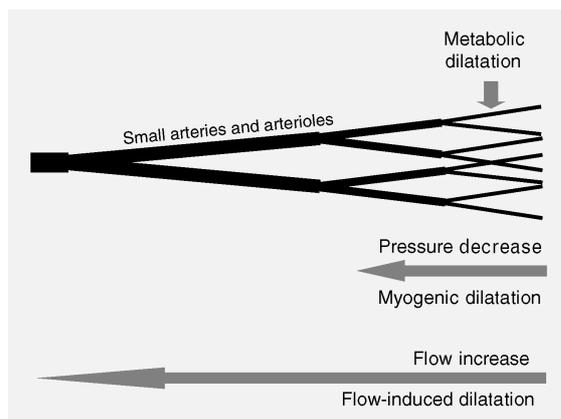
Figure 2. Model for the functional integration of myogenic and endothelium-dependent flow-induced control mechanisms into a coordinated response of the arteriolar vessel tree to increased metabolic demand. After direct dilatation of the most distally located precapillary arterioles in response to metabolic stimuli released from myocardial cells, upstream resistance vessels are indirectly recruited to dilate also. (Modified from Jones et al [9].)

reach these most peripheral arterioles, whereas larger upstream vessels with diameters in the range 50–300 μm remain largely unaffected. However, these upstream vessels contribute substantially to total coronary resistance [32], and full recruitment of the coronary flow reserve requires dilatation of these vessels also. A comprehensive model of how these upstream vessels could be recruited to a coordinated dilatory response of the entire arterial tree to increased metabolic demand was first suggested by Jones et al [9], and is presented in *Figure 2*, with some modifications. Metabolic mediators are released from myocardial cells and induce dilatation of the smallest precapillary arterioles, which also seem to be the most sensitive to these mediators. This in turn induces a decrease in pressure in the next upstream segment of midsize arterioles, inducing myogenic dilatation of these vessels. The resultant decrease in total resistance increases flow, which then induces flow-induced, endothelium-dependent dilatation within the entire upstream segment of larger arterioles and small resistance arteries. This model is supported by the observation, in isolated coronary arterioles, that larger vessels seem to react more sensitively to flow than do smaller vessels [33]; this is, however, in contrast with the findings of a recent in-vivo study in rats, in which acetylcholine, which is considered to activate mechanistic responses in endothelial cells that are similar to those induced by flow, had the strongest dilatory effect in the smallest precapillary arterioles [34]. Differences in species, the experimental model, or the mode of eliciting endothelium-dependent dilatation may account for these conflicting observations. Whether or not midsize arterioles are more sensitive to myogenic stimuli than smaller or larger vessels as

proposed by Jones et al [9] is unclear, because of a lack of experimental data. Preliminary data from our laboratory suggest that myogenic responses are maintained in small coronary arterioles, albeit only over a range of low transmural pressures [35], which would be consistent with the pressure profile in these vessels [32]. A similar observation has also been made in isolated skeletal muscle arterioles of hamster cheek pouch [36].

The actual contribution of a distinct mechanism to a vascular reaction depends, not only on the sensitivity of the respective microvascular segment to this mechanism, but also upon the actual exposure of the segment to the respective stimulus. For example, analyses using a comprehensive computer model of the coronary vasculature indicated that small arterioles are effectively shielded from changes in perfusion pressure by the strong myogenic responses of the upstream larger vessels [37]. In addition, flow-induced or myogenic effects on small arterioles may be masked by the potent effects of metabolic stimuli on this vessel segment.

In conclusion, the model of recruitment of upstream vessels to the coordinated response to a metabolic challenge remains valid, independent of the actual distribution of sensitivity to flow-induced dilatation and myogenic responses among different segments of the arteriolar tree.

*See glossary for definition of these terms. ■

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