Control and Coordination of Vasomotor Tone in the Microcirculation

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1. Introduction

The blood vascular system consists in a complex network of vessels that is mainly intended to provide oxygen and nutrients to all individual cells of peripheral tissues and help to dispose metabolic wastes. Several distinct functional compartments can be distinguished in the vascular network: arteries, arterioles, capillaries, venules and veins. Conduit arteries (diameter, 1 to several millimeters) carry blood away from the heart through a divergent arborescence that reaches and penetrates into the tissues via the feed arteries (diameter, 100 to 500 µm) (Davis et al., 1986; Segal, 2000, 2005). These muscular vessels give rise to the arterioles (diameter, < 100 µm), which control and coordinate the blood flow distribution in such a way that each capillary is correctly supplied at the proper pressure (Mulvany, 1990; Segal, 2005). This part of the vascular network composed of arterioles, capillaries and venules is embedded within the organ irrigated and is called microcirculation (Davis et al., 1986; Segal, 2005; Lockhart et al., 2009). Finally, veins carry blood back to the heart through a convergent arborescence.

In general, the vascular wall of arteries consists of an outer tunica adventitia, a central tunica media, and an inner tunica intima. The adventitia mainly contains connective tissue, fibroblasts, mast cells, macrophages, and nerve axons. Although the amount of the wall taken up by adventitia varies with the vascular territory, it is directly proportional to the size of the vessel (Gingras et al., 2009). The media is comprised of circumferentially arranged smooth muscle cells and is bounded on the luminal side by a well-defined internal elastic lamina. An external elastic lamina may also be present between the media and the adventitia or even within the media in larger vessels such as the aorta, but this structure is fragmented in small arteries and absent in arterioles (Mulvany, 1990; London et al., 1998). The number of smooth muscle cell layers decreases with decreasing vessel diameter and, in arterioles, only an unbroken monolayer of smooth muscle cells is found (Davis et al., 1986; Mulvany, 1990; Segal, 2005). In contrast, the structure of the intima is similar in all blood vessels and is formed by a smooth, continuous single layer of endothelial cells that lines the inner surface of the vessels (Mulvany, 1990). These cells are very thin (2 µm thick) and elongated (10 to 20 µm wide and 100 to 150 µm long, in arterioles), and are oriented parallel to the longitudinal axis of the vessel (Haas & Duling, 1997).
Correct supply of blood to the tissues relies on the ability of the vascular system to adjust the resistance of each vessel by controlling its lumen diameter, which is, in turn, a function of the level of tone of the vascular smooth muscle (i.e. vasomotor tone). As blood vessels are complex structures that must work as an unit, control of vasomotor tone depends on the fine synchronization of function of the different cellular components of the vessel wall, mainly smooth muscle cells and endothelial cells (Segal, 2000; Figueroa et al., 2004; Segal, 2005; Figueroa & Duling, 2009). Such synchronization and coordination is accomplished by an intricate system of radial and longitudinal cell-to-cell communication (Beach et al., 1998; Figueroa et al., 2004; Rummery & Hill, 2004; Segal, 2005; Figueroa & Duling, 2009; Bagher & Segal, 2011). In addition, arterioles in the microcirculation form a complex network, and then, the changes in the luminal diameter of different arteriolar segments must also be coordinated to regulate blood flow distribution and peripheral vascular resistance (Figueroa et al., 2004; Rummery & Hill, 2004; Segal, 2005; Figueroa & Duling, 2008). It has typically been assumed that most of the total resistance to blood flow resides on the arterioles. However, it has become apparent that as much as 50% of the precapillary resistance lies proximal to the arterioles (Davis et al., 1986; Mulvany, 1990; Segal, 2000), which situates the feed arteries at a key point for controlling vascular function and highlights the importance of the functional communication between arterioles and feed arteries in the regulation of blood flow distribution.

It is widely recognized that the endothelium plays a critical role controlling function of the vessel wall by the release of paracrine molecules such as nitric oxide (NO), prostaglandins (PGs) and also by the activation of the signaling mechanism known as endothelium-derived hyperpolarizing factor (EDHF) (Moncada et al., 1991; Busse et al., 2002; Feletou & Vanhoutte, 2007; Vanhoutte et al., 2009). However, another mechanism of communication that has emerged as a key pathway to command and coordinate the vascular wall function is the direct cell-to-cell communication via gap junctions (Sando et al., 2003; Figueroa et al., 2004, 2006). In addition, it is important to note that K+ channels expressed in the endothelium and smooth muscle cells play a central role in the control of vasomotor tone by paracrine or gap junction-mediated signaling mechanisms (Jackson, 2005).

2. Membrane potential and vascular K+ channels

In contrast to endothelial cells, Ca2+ is a signal for contraction in smooth muscle cells. In smooth muscle cells of blood vessels the L-type voltage-dependent Ca2+ channels play a central role controlling the vasomotor tone (Jackson, 2000). Changes in membrane potential modulate the opening of these Ca2+ channels. Thereby, depolarization produces a Ca2+ influx that leads to vasoconstriction and, on the contrary, hyperpolarization leads to a reduction in intracellular Ca2+ concentration and, subsequently, vasodilation (Jackson, 2000, 2005). In this context, K+ channels play a pivotal role in vascular function by controlling the membrane potential of both endothelial and smooth muscle cells. The main K+ channels expressed in resistance vessels, from a functional point of view, are: the ATP-sensitive K+ channels (KATP), inward rectifying K+ channels (Kir) and Ca2+-activated K+ channels (KCa) of small (SKCa), intermediate (IKCa) and large (BKCa) conductance (Jackson, 2000, 2005). KATP and Kir are expressed in both endothelial and smooth muscle cells (Quayle et al., 1996; Jackson, 2000, 2005; Ko et al., 2008), whereas BKCa are mostly found in smooth muscle cells (Jackson, 2005; Ko et al., 2008), but, on occasion, these K+ channels have also been described
in endothelial cells (Papassotiriou et al., 2000; Wang et al., 2005). In contrast, SKCa and IKCa are expressed exclusively in endothelial cells (Jackson, 2000; Kohler et al., 2000; Nilius & Droogmans, 2001; Eichler et al., 2003; Taylor et al., 2003; Brahler et al., 2009).

All these K+ channels play critical roles in the regulation of vascular function. KATP channels are opened at rest, and then, are very relevant in the control of smooth muscle membrane potential and vasomotor tone in basal unstimulated conditions (Jackson, 1993, 2000). Interestingly, KIR are typically closed at resting conditions, but are activated by hyperpolarization of membrane potential and by increments in extracellular K+ concentration ([K+]o) smaller than 20 mM (Jackson, 2005; Jantzi et al., 2006; Smith et al., 2008). Although BKCa channels are involved in the response to several vasomotor stimuli, the most relevant function of these K+ channels is the tonic control of vasomotor tone by buffering the smooth muscle cell depolarization. The increase in intracellular Ca2+ concentration associated to smooth muscle depolarization activates local Ca2+ transients (i.e. Ca2+ sparks) that result from the opening of tightly clustered ryanodine receptor channels located at extensions of sarcoplasmic reticulum. Ca2+ sparks activate a BKCa-dependent hyperpolarizing current that opposes the smooth muscle depolarization, and thereby, regulates the magnitude of the vasoconstriction (Jaggar et al., 1998; Gollasch et al., 2000; Gordienko et al., 2001; Lohn et al., 2001). SKCa and IKCa channels play a central role in the endothelial cell control of vasomotor tone and peripheral vascular resistance (Busse et al., 2002; Eichler et al., 2003; Taylor et al., 2003; Si et al., 2006; Brahler et al., 2009). However, probably the most recognized function of these K+ channels is their participation in the EDHF signaling (see below) (Busse et al., 2002; Vanhoutte, 2004).

3. Paracrine signaling in the vessel wall

One of the most well-characterized mode of communication in the vessel wall is the production of paracrine signals by endothelial cells such as PGs, NO and EDHF (Vanhoutte, 2004; Vanhoutte et al., 2009). The role of these signaling pathways in vascular physiology has been extensively studied and there are several recent reviews that address their involvement in vascular function in normal conditions and disease (Feletou & Vanhoutte, 2009; Vanhoutte et al., 2009; Rafikov et al., 2011). In this section, we will address the most relevant aspects of these signals in relation to the control of vasomotor tone in physiological conditions.

3.1 Prostaglandins

PGs are a family of bioactive lipids derived from arachidonic acid (AA or 5,8,11,14-eicosatetraenoic acid), which, in turn, is generated by the enzyme phospholipase A2 (PLA2) from phospholipids of the cell membrane in a Ca2+-dependent manner (Simmons et al., 2004; Fortier et al., 2008). The metabolism of PGs is complex and depends on the hydrolysis of AA by the enzymes cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) to form the unstable endoperoxide derivative, prostaglandin G2 (PGG2), and subsequently, prostaglandin H2 (PGH2) (Simmons et al., 2004). PGH2 is the parent compound of all PGs, which are synthesized by specific enzymes: prostaglandin I2 synthase (PGIS), prostaglandin E2 synthase (PGES-1), prostaglandin D2 synthase (PGDS), prostaglandin F2 synthesis (PGES-2), and thromboxane A2 synthase (TBXAS-1) that catalyze the production of
prostacyclins (PGI₂), PGE₂, PGD₂, PGF₂α and thromboxane A₂ (TXA₂), respectively (Simmons et al., 2004; Gryglewski, 2008). The presence of the different PG synthases varies from tissue to tissue. Finally, PGs are released to the extracellular space and exert their physiological effects by acting on specific membrane receptors (Norel, 2007), as depicted in Figure 1. Then, the production of prostanoids is triggered by an increase in intracellular Ca²⁺ concentration and the key reaction of this complex enzymatic cascade is catalyzed by the enzymes COXs (Figure 1).

Fig. 1. Biosynthetic pathway of prostaglandins (PGs). An increase in intracellular Ca²⁺ concentration activates the production of arachidonic acid (AA) by phospholipase A2 (PLA2) from cell membrane phospholipids. The enzymes cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) convert AA into the endoperoxide PGH₂, which is then metabolized by several synthases to PGs PGD₂, PGE₂, PGF₂α, TXA₂ and PGI₂ (prostacyclin). Each PG acts on specific membrane receptors located in endothelial and/or smooth muscle cells. The transduction pathways activated by PGs are also depicted in the figure.

COX-1 and COX-2 are very similar and show a 60% homology. However, COX-1 is expressed constitutively, whereas the expression of COX-2 is inducible, since the levels of this COX isoform are very low in normal conditions and its expression increases in response to pro-inflammatory stimuli (Simmons et al., 2004). Consistent with this, in normal physiological conditions, vascular endothelial and smooth muscle cells express COX-1 (Vanhoutte, 2009). In these cells, COX-1 mainly leads to the production of PGI₂, which
induces the relaxation of smooth muscle cells by the stimulation of IP receptors (Figure 1) (Gryglewski, 2008; Vanhoutte, 2009). In contrast to COX-1, expression of COX-2 in normal blood vessels is very low (Crofford et al., 1994; Schonbeck et al., 1999). However, Topper et al. (Topper et al., 1996) found that laminar shear stress, but not turbulent flow, up-regulates the levels of COX-2 expression in cultures of vascular endothelial cells. Laminar shear stress is a highly relevant stimulus that is involved in the tonic control of vasomotor tone, which highlights the participation of COXs and PGs in the regulation of vascular function.

### 3.2 Nitric oxide

Probably, the most relevant intercellular communication signal in vascular physiology is the endothelium-dependent NO production. NO is a potent vasodilator synthesized by the enzyme NO synthase (NOS) (Moncada et al., 1991). The substrates for NOS-mediated NO production are the amino acid L-arginine, molecular oxygen and nicotinamide adenine dinucleotide phosphate (NADPH). Three isoforms of NOS have been described: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) (Moncada et al., 1991; Alderton et al., 2001). The enzyme expressed in endothelial cells (eNOS) is the main NOS isoform found in the vascular system in normal conditions. The NO released by endothelial cells elicits the relaxation of the underlying vascular smooth muscle cells mainly through the initiation of the signaling cascade cGMP/PKG by activation of soluble guanylate cyclase, which has been ascribed as the primary receptor of NO (Moncada et al., 1991). Although certainly the cGMP-dependent signaling pathway has several targets in the vessel wall, the relaxation induced by NO is mainly associated with a reduction in the Ca^{2+} sensitivity of smooth muscle contractile machinery (Bolz et al., 1999; Bolz et al., 2003).

Consistent with the importance of NO in vascular function, the activity of eNOS is finely regulated at transcriptional and posttranscriptional level (Fleming & Busse, 2003). Although eNOS was initially characterized as a Ca^{2+}-dependent enzyme and binding of the complex Ca^{2+}-calmodulin plays a central role in the activation of eNOS, NO production is also modulated by phosphorylation and protein-protein interactions (Mount et al., 2007; Rafikov et al., 2011). In this context, the sub-cellular targeting of eNOS is a key process in the regulation of NO production. Two functional pools of eNOS have been identified in vascular endothelial cells: one associated to Golgi complex and other located at caveolae, a subset of invaginated plasmalemmal rafts where the function of key signaling proteins is coordinated (Govers & Rabelink, 2001; Goligorsky et al., 2002; Michel & Vanhoutte, 2010), which provides eNOS with a special proximity to signaling molecules, such as calmodulin, Ca^{2+} channels, BK_{ca} channels and plasma membrane Ca^{2+} pumps (Darby et al., 2000; Wang et al., 2005). Although both pools of eNOS have been demonstrated to be functional, it is widely recognized that the integrity of caveolae is critical for the control of Ca^{2+}-mediated activation of NO production. In caveolae, eNOS is found in an inhibitory association with caveolin-1, an integral membrane protein of this signaling microdomain, and the interaction of eNOS with calcium-calmodulin releases the enzyme from its inhibitory association with caveolin-1 (Govers & Rabelink, 2001; Goligorsky et al., 2002; Michel & Vanhoutte, 2010).

The eNOS localization at caveolae seems to be essential for the regulation of eNOS function by controlling L-arginine substrate supply. Typically, regulation of L-arginine availability has been under-appreciated, since intracellular L-arginine concentration is saturating from
The perspective of eNOS kinetics ($K_m = \sim 5 \mu M$) (Harrison, 1997). However, several reports indicate that increments in extracellular L-arginine levels can enhance NO production in endothelial cells (Zani & Bohlen, 2005; Kakoki et al., 2006), despite a saturating intracellular L-arginine concentration, which was termed as the “Arginine Paradox” (McDonald et al., 1997). This control of NO production by substrate suggests that intracellular L-arginine is not fully available for eNOS, whereas extracellular L-arginine is preferentially delivered to the enzyme. Consistent with this notion, NO production seems to be coupled to L-arginine uptake, because the main carrier that transports 60 – 80% of L-arginine across the plasma membrane of endothelial cells, the cationic amino acid transporter-1 (CAT-1), was found to co-localize with eNOS in caveolae (McDonald et al., 1997) (Figure 2). Interestingly, it was reported that eNOS interacts directly with CAT-1 in bovine aortic endothelial cells (BAECs), and apparently, the eNOS–CAT-1 association in addition to facilitate the delivery of extracellular L-arginine for NO generation, also enhances the eNOS enzymatic activity by increasing the activating phosphorylation of the enzyme at serine 1179 and 635, and by decreasing the association of eNOS with caveolin-1 (Li et al., 2005).

![Fig. 2. Local control of eNOS activity by L-arginine. eNOS synthesizes nitric oxide (NO) and the byproduct L-citrulline from L-arginine. The eNOS localization at signaling microdomains known as caveolae provides to this enzyme with a direct, local source of L-arginine. In caveolae, eNOS is in direct association with the main carrier of L-arginine in endothelial cells, the cationic amino acid transporter-1 (CAT-1), and is also associated with the enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) that regenerate L-arginine from L-citrulline.](image-url)
Another mechanism that has emerged as an important source of L-arginine supply for NO production is the regeneration of L-arginine from the other product of the eNOS-catalyzed reaction, L-citrulline. This regeneration is catalyzed by the enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL), which are mostly expressed in caveolae in endothelial cells (Flam et al., 2001; Solomonson et al., 2003) (Figure 2). Interestingly, addition of exogenous L-citrulline results in a larger increase in endothelial NO production than that observed with exogenous L-arginine, without a proportional increase in intracellular L-arginine (Solomonson et al., 2003), suggesting that recycling of L-citrulline to L-arginine is channeled directly to synthesize NO (Figure 2). In addition, it was estimated that under maximum stimulation of NO production with bradykinin, but not in unstimulated conditions, approximately 80% of the eNOS-catalyzed L-arginine was supplied by the recycling of L-citrulline (Solomonson et al., 2003). These findings indicate that eNOS activation is functionally coupled with the L-citrulline recycling system (ASS and ASL) in caveolae (Figure 2). Therefore, NO production seems to be regulated by a complex interaction between different pools of L-arginine, where direct channeling to eNOS of the L-arginine regenerated from L-citrulline by the coordinated action of the enzymes ASS and ASL is likely to play a central role (Figure 2).

3.3 Endothelium-derived hyperpolarizing factor

Although the development of knockout animals has demonstrated the importance of the multiple functions of NO along the whole vascular system, it has become apparent that the relevance of NO in the control of vasomotor tone depends on vessel size. Accordingly, NO is the primary endothelium-dependent vasodilator signal in large, conduit vessels (Shimokawa et al., 1996). However, an additional vasodilator component has also been identified in small resistance arteries and arterioles (Suzuki et al., 1992; Murphy & Brayden, 1995). In these vessels, blockade of NO and PG production only attenuates the response to endothelium-dependent vasodilators such as acetylcholine (ACh) or bradykinin (Vanhoutte, 2004). The relaxant pathway resistant to NOS and COX blockers is associated with smooth muscle hyperpolarization, and thereby, it was attributed to the release of an endothelium-derived hyperpolarizing factor (EDHF). The chemical nature of EDHF remains controversial and seems to depend on vessel size, vascular territory, and species (Vanhoutte, 2004). In this context, several EDHF candidates have been proposed, such as K+ ions (Edwards et al., 1998), epoxyeicosatrienoic acids (EETs) (Archer et al., 2003; Fleming, 2004), hydrogen peroxide (Shimokawa & Morikawa, 2005), and C-type natriuretic peptide (CNP) (Chauhan et al., 2003; Ahluwalia & Hobbs, 2005). However, in most cases, the EDHF-mediated smooth muscle hyperpolarization and vasodilation has been shown to be sensitive to simultaneous blockade of SKCa and IKCa (Doughty et al., 1999; Ghisdal & Morel, 2001; Crane et al., 2003; Eichler et al., 2003; Hilgers et al., 2006). Interestingly, these K+ channels have been reported to be located in two different subcellular domains. While SKCa channels are found in caveolae (Absi et al., 2007; Rath et al., 2009), IKCa channels were proposed to be expressed in the abluminal side of endothelial cells (Figure 3), facing Na+ pumps and Kir channels situated in smooth muscle cells (Edwards et al., 1998; Dora et al., 2008). Then, the opening of IKCa channels may increase the K+ ion concentration in the myoendothelial space, which may couple endothelial cell IKCa signaling to Na+ pump- and Kir channel-mediated smooth muscle hyperpolarization (Edwards et al., 1998; Dora et al., 2008) (Figure 3).
Fig. 3. K+ channel distribution and endothelium-dependent vasodilation. Ca2+-activated K+ channels of small (SK<sub>Ca</sub>) and intermediate (IK<sub>Ca</sub>) conductance may contribute to the vasodilation associated with an endothelium-mediated smooth muscle hyperpolarization (response typically attributed to an endothelium-derive hyperpolarizing factor, EDHF) by two pathways. First, the hyperpolarization induced by activation of SK<sub>Ca</sub> and IK<sub>Ca</sub> is transmitted electrotonically to the underlying smooth muscle cells (SMC) through the gap junctions located at discrete points of contact between endothelial and smooth muscle cells, structure known as myoendothelial junctions (MEJ). In addition, the small increase in extracellular K+ concentration (<20 mM) resulting from the opening of the IK<sub>Ca</sub> found at the abluminal side of endothelial cells (EC) may activate inward rectifying K+ channels (K<sub>ir</sub>) and Na+ pump in smooth muscle cells. Between SMC and EC is found the internal elastic lamina (IEL).

Notwithstanding the smooth muscle hyperpolarization is considered to be the hallmark of EDHF action (Vanhoutte, 2004), it is important to note that hyperpolarization of the vessel wall is not a unique characteristic of EDHF. In several vessel preparations, in addition to a reduced Ca2+ sensitivity of the contractile machinery, the NO-dependent vasodilation has also been associated with smooth muscle hyperpolarization (Cohen et al., 1997; Lang & Watson, 1998). Furthermore, consistent with a NO-mediated hyperpolarization, NO has been reported to activate BK<sub>Ca</sub>, K<sub>ir</sub> and K<sub>ATP</sub> channels on the smooth muscle cells and endothelial cells directly or through the activation of cGMP production (Bohotina et al., 1994; Abderrahmane et al., 1998; Lee & Kang, 2001; Si et al., 2002; Schubert et al., 2004). Therefore, NO and EDHF are not only complementary, but also additive and the effect of both
vasodilator components may be confounded. In this context, it is interesting to note that, as mentioned above, the EDHF-mediated response is typically studied in presence of NOS and COX blockers. However, NOS inhibition with analogues of L-arginine is a slow, time-dependent process and, on occasion, blockade of NO production with these drugs has been observed to be incomplete (Vanheel & Van de Voorde, 2000; Figueroa et al., 2001; Chauhan et al., 2003; Stoen et al., 2003; Stankevicius et al., 2006), and then, the residual NO production observed in presence of NOS inhibitors may contribute to the vasodilation associated with the smooth muscle hyperpolarization attributes to EDHF. In addition, the findings reported recently by Gaete et al. (Gaete et al., 2011) are another important point to take into account in the interaction between EDHF and NO. In this work Gaete et al., demonstrated that SKCa and IKCa channels control the Ca2+-dependent NO release, and thereby, the inactivation of these K+ channels is associated with an increase in NAD(P)H oxidase-mediated superoxide production, which leads to the inhibition of eNOS primarily by its phosphorylation at threonine 495 (Gaete et al., 2011). These findings highlight the relevance of these K+ channels in the control of vascular function and indicate that the participation of superoxide in the EDHF-mediated response associated to SKCa and IKCa channels must be evaluated.

Furthermore, the regulation of NO and EDHF is different depending on gender. In male animals, NO is the major endothelium-dependent vasodilator signal, but in female EDHF prevails over NO or PGI2 (Scotland et al., 2005). In this context, it is interesting to note that estrogen enhances the EDHF-mediated vasodilation in response to flow (Huang et al., 2001), which suggests that the EDHF-dependent signaling pathway may be more important in the control of blood pressure in female than in male animals. This idea was confirmed using an eNOS/COX-1 double knockout. Deletion of eNOS and COX-1 did not alter the mean arterial blood pressure in female mice, whereas the double knockout resulted in hypertension in male mice (Scotland et al., 2005). In these animals, the endothelium-dependent relaxation was intact in resistance vessels of female mice and was mediated by the smooth muscle hyperpolarization (Scotland et al., 2005), strongly supporting that EDHF plays a predominant role in the tonic control of blood pressure in female. These data suggest that EDHF rather than NO may underlie the higher resistance of premenopausal females to cardiovascular diseases such as hypertension.

4. Gap junction communication in the vascular function

Gap junctions are intercellular channels that directly connect the cytoplasm of neighboring cells, allowing the passage of current or molecules smaller than ~1.4 nm of diameter such as metabolites (e.g., ADP, glucose, glutamate and glutathione) or second messengers (e.g., Ca2+, cAMP and IP3) (Evans & Martin, 2002; Saez et al., 2003). These intercellular channels are made up by a protein family known as connexins (Cx), which are named according to their predicted molecular mass expressed in kDa. Connexin proteins have four transmembrane domains with the N- and C-termini located on the cytoplasmic membrane face. The radial arrangement of six connexins around a central pore makes a connexon or hemichannel, and the association in the plasma membrane of two hemichannels provided by adjacent cells forms an intercellular gap junction channel (Evans & Martin, 2002; Saez et al., 2003). It is noteworthy that independent hemichannels can also remain unpaired and functional, which have been recognized to release paracrine signals such as ATP, PGE2 or NAD+ (Goodenough & Paul, 2003; Cherian et al., 2005; Saez et al., 2005). The importance of
this mode of communication in the vasculature is just starting to be evaluated and, consistent with the participation of vascular hemichannels in paracrine signaling, human microvascular endothelial cell (HMEC-1) monolayers were found to release ATP through Cx43-formed hemichannels (Faigle et al., 2008).

At least twenty connexin isoforms have been described in mammals and one cell type may express more than one connexin (Saez et al., 2003). However, the expression of several connexins in one cell does not seem to be redundant, because gap junctions are not just simple channels that offer a low-resistance intercellular pathway, but connexins mediate highly specific cell-to-cell signaling pathways, and the molecular selectivity as well as subcellular localization differs among connexins (Saez et al., 2003; Figueroa et al., 2004; Locke et al., 2005). Thus, although these proteins may have some overlap in function, they work in concert (Simon & Goodenough, 1998; Figueroa et al., 2004, 2006; Haefliger et al., 2006) and, consequently, it has been observed that many times the function of one connexin cannot be replaced by other connexin isoform (White, 2003; Haefliger et al., 2006; Zheng-Fischhofer et al., 2006; Wolfle et al., 2007). In addition, hemichannels can be composed by one or a mixture of connexin proteins, which provides an additional mechanism for fine regulation of gap junction-mediated signaling processes (White & Bruzzone, 1996; He et al., 1999; Beyer et al., 2000; Cottrell et al., 2002; Moreno, 2004).

Five connexin proteins have been found to be expressed in the vasculature: Cx32, Cx37, Cx40, Cx43, and Cx45 (Severs et al., 2001; Figueroa et al., 2004; Haefliger et al., 2004; Okamoto et al., 2009). The expression of connexins in the different cell types of the vessel wall is not uniform and vary with vessel size, vascular territory, and species (van Kempen et al., 1995; van Kempen & Jongsma, 1999; Hill et al., 2002). In most cases, Cx43 is only observed in smooth muscle cells and has mainly been detected in brain vessels (Kruger et al., 2000; Li & Simard, 2001). In contrast, the expression of Cx32 and Cx37 seems to be restricted to the endothelium (Gabriels & Paul, 1998; van Kempen & Jongsma, 1999; Severs et al., 2001; Okamoto et al., 2009), but Cx37 has also been detected in smooth muscle cells (Rummery et al., 2002). Although Cx40 and Cx43 may be expressed in both cell types (Little et al., 1995; Gabriels & Paul, 1998; van Kempen & Jongsma, 1999; Severs et al., 2001), Cx40 is located predominately in endothelial cells (Gabriels & Paul, 1998; van Kempen & Jongsma, 1999) and Cx43 is the most prominent gap junction protein found in smooth muscle cells (van Kempen & Jongsma, 1999). It should be noted, however, that in mouse, Cx40 is expressed exclusively in the endothelium (de Wit et al., 2000; Figueroa et al., 2003; Figueroa & Duling, 2008).

In addition to connexins, another family of three members of membrane proteins named pannexins (Panxs 1-3) has been documented (Bruzzone et al., 2003). Apparently, pannexins only form hemichannels, and then, the main function of pannexin-based channels is paracrine or autocrine communication (Locovei et al., 2006). Although connexins and pannexins share a similar membrane topology, their amino acid sequences present only a 16% homology (Bruzzone et al., 2003). Only the expression of Panx-1 has been identified in blood vessels at the moment and recently this pannexin was found to be involved in the activation of the vasoconstrictor response mediated by $\alpha_1$-adrenoceptor stimulation (Billaud et al., 2011).

4.1 Gap junctions in vascular smooth muscle

Coordination of vasomotor signals among smooth muscle cells is critical for the function of blood vessels. As mentioned above, the contractile state of smooth muscle cells depends on
the cytoplasmic Ca²⁺ concentration and Ca²⁺ sensitivity of the contractile apparatus. Intracellular Ca²⁺ concentration is controlled by the smooth muscle cell membrane potential. Then, gap junctions play a central role integrating the smooth muscle cell function because these intercellular channels synchronize changes in both membrane potential and intracellular Ca²⁺ between adjacent smooth muscle cells (Christ et al., 1991; Christ et al., 1992; Christ et al., 1996).

In addition, gap junction communication of vascular smooth muscle cells seems to be involved in the development of myogenic vasomotor tone in resistance arteries (Lagaud et al., 2002; Earley et al., 2004). Interestingly, the participation of gap junction in this process is not related to synchronization of Ca²⁺ signaling, but rather to earlier signaling events such as coordination of the smooth muscle cell-depolarization or directly the mechanosensitivity of the vascular smooth muscle. This notion is supported by the fact that the gap junctions and connexin hemicannels inhibitors Gap27 (a connexin mimetic peptide) or 18α-glycyrrhetinic acid, in addition to block Ca²⁺ influx and vasoconstriction in mesenteric resistance arteries, also prevented the pressure-induced smooth muscle cell depolarization (Earley et al., 2004). It is important to note that Gap27 and 18α-glycyrrhetinic acid are two well-known gap junction blockers, but they also block connexin-formed hemicannels, which indicates that hemicannels may also be involved in the development of the myogenic response. In any case, the involvement of Cx43-based channels in the control of vasomotor tone is consistent with the finding that tensile stretch increased the expression of this connexin as well as gap junction intercellular communication in vascular smooth muscle cells (Cowan et al., 1998). Interestingly, this response was mediated by the formation of reactive oxygen species (Cowan et al., 1998; Cowan et al., 2003), which has been reported to contribute to the initiation of the myogenic constriction in mouse-tail arterioles (Nowicki et al., 2001).

Cx43 has also been involved in the regulation of cell proliferation and migration in the vasculature (Polacek et al., 1997; Yeh et al., 1997; Kwak et al., 2001), which can be appreciated in Cx43-deficient smooth muscle cells. Damage of carotid artery by vascular occlusion or wire injury resulted in an increase in neointima and adventitia formation in smooth muscle cell Cx43 specific knockout mice as compared to wild type animals (Liao et al., 2007), suggesting an accelerated growth of smooth muscle cell with the Cx43 deletion, which was further confirmed using cultured cells. Nevertheless, in apparent opposition to these findings, Chadjichristos et al. (Chadjichristos et al., 2006) show that in heterozygous Cx43 knockout mice the neointimal formation was reduced. However, in those animals, Cx43 was reduced from all cell types expressing Cx43 and the experiments included a high-fat diet, which may have influenced the result by either vascular adaptive response to the diet or complex interactions between different cell types. Although the participation of Cx43 in neointimal formation demands further investigation, these data highlight the relevance of Cx43 in the feedback control pathways necessary for vascular morphogenesis.

4.2 Gap junctions in vascular endothelium

The endothelium plays a key role in the tonic control of blood pressure and the development of knockout animals of vascular connexins has disclosed that gap junction communication of endothelial cells is essential in the coordination and integration of
microvascular function. Vascular endothelial cells-specific deletion of Cx43 (VEC Cx43−/−) results in hypotension (Liao et al., 2001) and, in contrast, ablation of Cx40 produces a hypertension associated with an irregular vasomotion (de Wit et al., 2000; de Wit et al., 2003; Figueroa & Duling, 2008) and a dysregulation of renin production (Krattinger et al., 2007; Wagner et al., 2007). Although deletion of Cx37 does not appear to alter vascular function or blood pressure (Figueroa & Duling, 2008), several polymorphisms of this connexin have been associated with myocardial infarction, coronary artery disease and atherosclerosis (Boerma et al., 1999; Yamada et al., 2002; Hirashiki et al., 2003; Yamada et al., 2004). In mice, Cx40 and Cx37 are primarily expressed in the endothelium, which emphasizes the importance of the endothelial cell-gap junction communication in the control of cardiovascular homeostasis.

Although the mechanistic bases of the hypotension observed in VEC Cx43−/− are still unknown, the plasma levels of angiotensin I and II as well as NO were elevated in these animals (Liao et al., 2001), suggesting that a dysregulation of NO production may have been the responsible of the hypotension with the subsequent activation of the renin-angiotensin system. Also, it is interesting to note that shear stress up-regulates the expression of Cx43 in cultured endothelial cells (DePaola et al., 1999; Bao et al., 2000) and in the endothelium of rat cardiac valves (Inai et al., 2004), which suggests that Cx43 may be involved in the response to mechanical stimuli.

4.3 Gap junctions in smooth muscle-endothelium communication

Smooth muscle cells and endothelial cells have also been found to be electrically and metabolically connected by gap junctions located at discrete points of contact between the two cell types at the myoendothelial junction (MEJ) (Beny & Pacicca, 1994; Little et al., 1995; Emerson & Segal, 2000; Sandow et al., 2003). This heterocellular communication seems to play a pivotal role in the Ca2+-mediated responses induced by endothelium-dependent vasodilators, such as ACh. As mentioned above, these vasodilator responses are typically paralleled by hyperpolarization of the underlying smooth muscle cells (Emerson & Segal, 2000; Goto et al., 2002; Griffith, 2004), which has been attributed to the release of an EDHF (Vanhoutte, 2004; Feletou & Vanhoutte, 2009). However, the direct electrotonic transmission of a hyperpolarizing current from the endothelial cells to the smooth muscle cells via myoendothelial gap junctions may explain the EDHF pathway (Busse et al., 2002; Dora et al., 2003; Griffith, 2004). In this perspective, the increase in endothelial cell intracellular Ca2+ concentration activates SKCa and IKCa channels leading to the endothelium-dependent hyperpolarization of smooth muscle cells via gap junctions located at the MEJ (Busse et al., 2002; Crane et al., 2003; Eichler et al., 2003; Feletou et al., 2003) (Figure 3). Consistent with this hypothesis, the EDHF-dependent vasodilation has been reported to be prevented by connexin-mimetic peptides that are thought to specifically block gap junctions (De Vriese et al., 2002; Karagiannis et al., 2004; Chaytor et al., 2005) as well as endothelial cell-selective loading of antibodies directed against the carboxyl-terminal region of Cx40 (Mather et al., 2005). Interestingly, the gap junction-mediated EDHF signal might be controlled by NO through S-nitrosylation. Cx43-based channels can be activated by S-nitrosylation (Retamal et al., 2006). Cx43 and eNOS has been found to be express at MEJ and the activation of NO production in this microdomains leads to a S-nitrosylation-associated opening of Cx43-formed myoendothelial gap junction (Straub et al., 2011), which support the idea that EDHF and NO are not parallel, independent vasodilator components, but in contrast, they work in concert.
Flow (i.e. shear stress) is one of the most important stimuli involved in the tonic regulation of vasomotor tone. Although the response to shear stress is thought to be mediated primarily by NO, shear stress has also been reported to activate an EDHF-dependent vasodilator response (Watanabe et al., 2005), which suggests that a gap junction-mediated EDHF pathway may be involved in the tonic control of peripheral vascular resistance. Consistent with this idea, intrarenal infusion of connexin-mimetic peptides homologous to the second extracellular loop of Cx43 (\textsuperscript{43}Gap 27) or Cx40 (\textsuperscript{40}Gap 27) not only decreased basal renal blood flow, but also increased mean arterial blood pressure of rats, either in presence or absence of NOS and COX blockers (De Vriese et al., 2002), suggesting that connexin-mimetic peptides induced vasoconstriction by disrupting or reducing the response to a tonic vasodilator stimulus such as shear stress.

5. Conduction of vasomotor responses

Longitudinal conduction of vasomotor responses provides an essential means of coordinating changes in diameter and flow distribution among vessels of the microcirculation. Vasomotor signals spread along the vessel length through gap junctions connecting cells of the vessel wall, and thereby, participate in the minute-to-minute coordination of vascular resistance by integrating function of proximal and distal vascular segments in the microcirculation (de Wit et al., 2000; Figueroa et al., 2004, 2006). Although vasoconstrictor responses are thought to be conducted by smooth muscle cells (Welsh & Segal, 1998; Bartlett & Segal, 2000; Budel et al., 2003), the cellular pathway for conduction of vasodilator signals is more controversial and may be either exclusively by the endothelium (Emerson & Segal, 2000; Segal & Jacobs, 2001) or by both smooth muscle and endothelial cells (Bartlett & Segal, 2000; Budel et al., 2003). The cellular pathway for conduction of vasomotor responses has been studied by selectively damaging a short segment of endothelial cells or smooth muscle cells by injection of an air bubble via a side branch (Bartlett & Segal, 2000; Figueroa et al., 2007) or with a light-dye (fluorescein-conjugated dextran) treatment (Emerson & Segal, 2000). In feed arteries, selective damage of the endothelium completely blocked the ACh-induced conducted vasodilation (Emerson & Segal, 2000; Segal & Jacobs, 2001), but in arterioles, either damage of the endothelium or the smooth muscle did not affect the ACh-induced conducted responses (Bartlett & Segal, 2000; Budel et al., 2003), which led to the proposal that the cellular pathway for conduction of vasodilations depends on the functional location of the vessel in the microvascular network (Segal, 2005). However, the cellular pathway of vasodilator signals may also depend on the stimulus that initiated the response, because, in contrast to ACh, selective damage of the endothelium blocked the vasodilation induced by bradykinin in arterioles (Welsh & Segal, 1998; Budel et al., 2003).

Direct measurements of membrane potential have shown that conducted vasomotor responses are associated with rapid propagation (milliseconds) of an electrical signal along the vessel length (Xia & Duling, 1995; Welsh & Segal, 1998; Emerson & Segal, 2000). Because many observations have revealed an exponential decay of the conducted electrical signal, it was proposed that longitudinal spread of vasomotor responses reflects the passive, electrotonic conduction of changes in membrane potential via gap junctions connecting cells of the vessel wall (Pacicca et al., 1996; Welsh & Segal, 1998; Gustafsson & Holstein-Rathlou, 1999). Therefore, the decay of the conducted vasomotor responses along the vessel length
should be consistent with the length constant estimated from electrotonic potentials produced by current injection into the smooth muscle or endothelial cells of arterioles, which is between 0.9 and 1.6 mm (Hirst & Neild, 1978; Hirst et al., 1997; Emerson et al., 2002).

Conduction of vasoconstrictor responses typically behaves as predicted by the electrotonic model. However, a simple electrotonic model often fails to predict conduction of vasodilator signals initiated by endothelium-dependent stimuli, such as ACh or bradykinin. These signals have been reported to propagate for many millimeters without showing noticeable decay in magnitude (Emerson & Segal, 2000; Figueroa & Duling, 2008). In addition, the electrical length constant of ACh-induced hyperpolarization has been shown to be longer than that measured for current injection (Emerson et al., 2002) and the hyperpolarizing signal activated by ACh has been also reported to increase during the first 1000 µm of longitudinal conduction (Crane et al., 2004). The lack of decay of these responses suggests that a regenerative, energy-dependent mechanism underlies the conduction process, similar to that described in neurons. Consistent with this idea, electrical stimulation also activates a conducted, non-decremental endothelium-dependent vasodilation that was hypothesized to be mediated by a complex interplay between voltage-gated Na\(^+\) channels (Na\(_v\)) and T type, voltage-gated Ca\(^{2+}\) channels (T-Ca\(_v\)) (Figueroa et al., 2007). In this hypothetic model, Na\(_v\) channels underlie the conduction of the signal and T-Ca\(_v\) mediates the vasodilation. Interestingly, deletion of Cx40 selectively eliminates the regenerative component of the conducted vasodilation induced by ACh (Figueroa & Duling, 2008), bradykinin (de Wit et al., 2000) or electrical stimulation (Figueroa et al., 2003), leaving a decaying component consistent with the electrotonic model (Figueroa & Duling, 2008), which suggests that Cx40-based gap junctions provide the pathway for the intercellular propagation of the regenerative conducted component of vasodilator signals. Deletion of Cx37 did not affect conduction of vasodilator responses (Figueroa & Duling, 2008) and replacement of Cx40 by Cx45 did not restore the non-decremental component of the conducted vasodilation activated by ACh or bradykinin (Wolfle et al., 2007), supporting the idea that individual connexins have different functions.

The opening of K\(_r\) channels induced by the smooth muscle hyperpolarization may be an alternative hypothesis to explain the extended conduction of vasodilator responses. An intrinsic biophysical property of K\(_r\) channels is that they increase their activity upon cell hyperpolarization and it has been proposed that the activation of these K\(^+\) channels in the smooth muscle cells amplify the hyperpolarizing current initiated by ACh, thereby facilitating the conduction of this signal (Jantzi et al., 2006). However, as mentioned above, current-induced hyperpolarization decays faster than the response induced by ACh (Emerson et al., 2002), which argues against the participation of K\(_r\) alone in the non-decremental component of the conducted vasodilation, and suggests that further investigation is needed to elucidate the mechanisms involved in the conduction of vasomotor responses.

### 6. Neurovascular coupling

The brain has a very high metabolic demand and its activity depends on the communication between brain cells and local microvessels (i.e. neurovascular unit). Then, the function of
cerebral microcirculation must be coupled to neuronal activity, which is known as neurovascular coupling (Hawkins & Davis, 2005; Leybaert, 2005). In this case, however, vasomotor signals seem to be conducted by astrocytes as opposed to smooth muscle or endothelium (Anderson & Nedergaard, 2003; Zonta et al., 2003; Mulligan & MacVicar, 2004; Koehler et al., 2006; Metea & Newman, 2006; Takano et al., 2006). Tight spatial and temporal coupling between neuronal activity and blood flow is essential for brain function (Anderson & Nedergaard, 2003; Hawkins & Davis, 2005; Leybaert, 2005) and astrocytes are found in a strategic location between neurons and the microvasculature, with the astrocytic endfeet ensheathing the vessels. This spatial organization places the astrocytes in a key position to orchestrate the neurovascular coupling and an increasing body of evidence shows that the astrocyte transduces and conducts to the local microvasculature vasomotor signals generated by an increase in synaptic activity (Anderson & Nedergaard, 2003; Zonta et al., 2003; Mulligan & MacVicar, 2004; Metea & Newman, 2006; Takano et al., 2006) (Figure 4). As a result, astrocytes couple neuronal activation to vasodilation of local parenchymal arterioles (Figure 4), which, in turn, leads to an increase in blood-borne energy substrate that rapidly matches the enhanced metabolic demand (Anderson & Nedergaard, 2003; Hawkins & Davis, 2005; Leybaert, 2005).

Calcium seems to be the intracellular vasomotor signal of the astrocyte-mediated neurovascular coupling. Astrocytes express receptors for several neurotransmitters such as glutamate, GABA and ATP (Anderson & Nedergaard, 2003; Leybaert, 2005; Koehler et al., 2009), which can initiate Ca^{2+} signals (Figure 4). Then, the increase in neuronal activity results in an astrocytic calcium signaling that propagates through the astrocytic processes into the endfeet (Anderson & Nedergaard, 2003; Zonta et al., 2003; Mulligan & MacVicar, 2004; Metea & Newman, 2006; Takano et al., 2006) (Figure 4). Interestingly, astrocytes express gap junctions (Martinez & Saez, 2000; Saez et al., 2003; Retamal et al., 2006) and a calcium signal may propagate between neighboring astrocytes in a wave-like manner (Cornell-Bell et al., 1990; Nedergaard, 1994; Cai et al., 1998; Nedergaard et al., 2003), coordinating the neurovascular coupling in the local cerebral microcirculation (Anderson & Nedergaard, 2003; Zonta et al., 2003; Filosa et al., 2004; Mulligan & MacVicar, 2004). Some of the Ca^{2+}-dependent vasodilator mechanisms that may be activated at the astrocytic endfeet facing the vessel wall are the production of epoxyeicosatrienoic acid (EETs) by the cytochrome P450 epoxygenase and PGs by the COX enzyme (Anderson & Nedergaard, 2003; Zonta et al., 2003; Zonta et al., 2003; Filosa et al., 2004; Straub et al., 2006; Koehler et al., 2009), and also ATP release (Shi et al., 2008) via connexin or pannexin hemichannels (Figure 4). In addition, astrocytic endfeet express BK_{Ca} and Girouard et al. (Girouard et al., 2010) recently showed in mouse cortical brain slices that these K^{+} channels play a central role in neurovascular coupling through the release of K^{+} ion into the perivascular space (Figure 4). The small increase in local [K^{+}]_{o} (<20 mM) activates the K_{p} channels located in the smooth muscle cell membrane facing the endfeet, which leads to hyperpolarization, and subsequently, vasodilation (Girouard et al., 2010) (Figure 4). It is noteworthy that a higher increase in [K^{+}]_{o} would produce smooth muscle cell depolarization and vasoconstriction (Girouard et al., 2010).
Fig. 4. Astrocytes-mediated neurovascular coupling. Neurotransmitters may exit the synaptic cleft and activate receptors on astrocytes, which couple neuronal activity with astrocyte signaling. The activation of astrocyte receptors triggers a $\text{Ca}^{2+}$ wave that reaches the astrocytic endfeet, leading to the opening of large conductance $\text{Ca}^{2+}$-activated $\text{K}^+$ channels ($\text{BK}_{\text{Ca}}$). The $\text{K}^+$ ion release via $\text{BK}_{\text{Ca}}$ elicits a small increase in extracellular $\text{K}^+$ concentration (<20 mM) in the perivascular space that activates the $\text{K}_\text{ir}$ channels located in the smooth muscle cell membrane facing the endfeet, which, in turn, leads to hyperpolarization, and subsequently, vasodilation. The vessel wall hyperpolarization-mediated vasodilation is conducted to upstream arterioles, coupling function of proximal and distal vessels.

As described in the peripheral microcirculation (Segal & Kurjiaka, 1995; Segal, 2000), local vasodilation of cerebral arterioles must be communicated to upstream vascular segments to produce a functional increase of blood flow supply and effectively match the local metabolic demand (Cox et al., 1993; Iadecola et al., 1997). Although vasomotor responses have been observed to be conducted by the wall of cerebral arterioles (Dietrich et al., 1996; Horiuchi et al., 2002), it seems to be that astrocytes also play a central role in integrating function of local arterioles with upstream cerebral vessels involved in the neurovascular coupling. Pial arterioles are important upstream vessels of the parenchymal cerebral arterioles. It is important to note that pial arterioles overlie a thick layer of astrocytic processes, known as...
the glia limitans, which isolate these arterioles from the neurons that are located right below. Vasodilation of pial arterioles associated with neuronal activation was blocked by either selective elimination of astrocytes with L-α aminoacidipic acid treatment or the inhibition of Cx43-based channels with the specific connexin mimetic peptide gap-27 (Xu et al., 2008). In astrocytes, Cx43 may be found forming unpaired hemichannels or gap junction intercellular channels (Stout et al., 2002; Saez et al., 2003; Retamal et al., 2006). Thus, astrocytic Cx43-based channels could be involved in the coordination of calcium waves between astrocytes, or in the release of vasoactive factors such as ATP that can be metabolized to the potent vasodilator, adenosine (Shi et al., 2008)

7. Conclusion

Control of vasomotor tone relies on a complex interplay between NO, PGs, K+ channels and gap junction communication. It is typically thought that NO is the most relevant endothelium-dependent vasodilator signal, but, in resistance vessels and arterioles, K+ channels and gap junction communication between the cells of the vessel wall have emerged as major players in the tonic control and coordination of vascular function. While several K+ channels (e.g. BKCa, Kir and KATP channels) may contribute to the vasodilator response induced by NO, the endothelial cell K+ channels, SKCa and IKCa, seem to be involved in the fine regulation of eNOS activation. In addition, it has become apparent that NO production is also modulated by a delicate caveolar control of L-arginine supply. Although myoendothelial gap junction communication probably contributes to the EDHF signaling mediated by SKCa and IKCa channels, the strategic spatial organization of IKCa and Kir may also be involved in the intercellular transmission of an endothelium-initiated smooth muscle hyperpolarization. A similar organization, but between BKCa and Kir channels, is observed in the astrocyte-mediated neurovascular coupling. Connexin- and pannexin-based hemichannels are an attractive signaling mechanism that may be involved in the control of vascular function, but the study of hemichannels in resistance vessels is just beginning.

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The cardiovascular system includes the heart located centrally in the thorax and the vessels of the body which carry blood. The cardiovascular (or circulatory) system supplies oxygen from inspired air, via the lungs to the tissues around the body. It is also responsible for the removal of the waste product, carbon dioxide via air expired from the lungs. The cardiovascular system also transports nutrients such as electrolytes, amino acids, enzymes, hormones which are integral to cellular respiration, metabolism and immunity. This book is not meant to be an all encompassing text on cardiovascular physiology and pathology rather a selection of chapters from experts in the field who describe recent advances in basic and clinical sciences. As such, the text is divided into three main sections: Cardiovascular Physiology, Cardiovascular Diagnostics and lastly, Clinical Impact of Cardiovascular Physiology and Pathophysiology.

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