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Kertas Ulasan/*Review Articles*

The Role of Reactive Oxygen Species in Pressure-Dependent Myogenic Tone

(Peranan Spesis Oksigen Reaktif dalam Tonus Miogenik yang Bergantung dengan Tekanan)

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ABSTRACT

Myogenic tone is the response of the vascular smooth muscle to an increase in intraluminal pressure with vasoconstriction and with vasodilation when the pressure is decreased. Such myogenic tone contributes a level of physiological basal tone in response to neurohumoral stimuli. In spite of myogenic tone discovery by Sir William Bayliss 100 years ago, questions still remain regarding the underlying signaling mechanism of the myogenic response. Studies have shown that increased intraluminal pressure or wall tension leads to membrane depolarization, voltage-operated calcium channel (VOCC), stretch-activated cation (SAC) channels, extracelullar matrix (ECM) and actin cytoskeleton. Recently, evidence has shown a potential role for reactive oxygen species (ROS) as a key signalling mediator in the genesis of myogenic tone. The identification of the primary mechanosensors in the initiation of pressure-dependent myogenic tone is essential as these components could be potential therapeutical targets in the future.

Keywords: Microcirculation, ROS, myogenic tone

ABSTRAK

Tonus miogenik adalah respon vasokonstriksi otot licin vaskular ke atas peningkatan tekanan intralumina dan respon vasodilatasi pula ke atas penurunan tekanan intralumina. Kompensasi tonus miogenik menyumbang kepada pengawalaturan tonus basal dalam keadaan fisiologi terhadap rangsangan neurohormon. Walaupun Sir William Bayliss telah menemui tonus miogenik pada 100 tahun yang lalu, namun mekanisme signal di sebalik respons miogenik masih belum dikenal pasti secara mendalam. Kajian lepas telah menunjukkan bahawa peningkatan tekanan intralumina ataupun ketegangan dinding vaskular menyebabkan depolarisasi membran, rangsangan kanal kalsium voltan-tergantung, kation ketegangan-terangsang, matrik ekstraselular dan sitoskeleton aktin. Bukti telah menunjukkan potensi sepsis oksigen reaktif (ROS) sebagai perantara signal semasa mekanisme tonus miogenik. Pengenalpastian dan pemahaman mengenai mekanosensor primary yang merangsang permulaan tonus miogenik bergantung-tekanan adalah penting sebagai potensi terapeutik di masa hadapan.

Kata kunci: Mikrosirkulasi, ROS, tonus miogenik

AUTOREGULATION IN RESISTANCE ARTERIES

In the cardiovascular system, blood vessel function is related to its structure. Specifically, major arteries of the trunk are the elastic conductance arteries with large diameter and low resistance. On the contrary, distal arteries are muscular arteries of small diameter and high resistance that are critical for blood flow or pressure within the microcirculation system. Under physiological conditions, the viscoelasticity of the large arteries results in pulsatile pressure and flow so that the microvasculature can mediate blood supply steadily. The microvessels proximal to the arterioles with lumen diameter less than 200 μm were defined by Mulvany and Aalkjaer (1990) as the vessels offering the greatest resistance. The resistance value can vary depending for the entire vascular system, for the vascular network in selected organs and for individual vessels (Zweifach 1991), therefore, the size of resistance arteries may vary and be more than 200 μm diameter. Resistance arteries have the ability to alter their diameter independent of neural and hormonal control, in order to keep a relatively constant blood flow. The efficiency of autoregulation varies from tissue to tissue with those organs most vital for survival, e.g. brain, heart, kidney, demonstrating the most marked autoregulatory capacity. A number of local intrinsic control mechanisms have been proposed to account for the homeostatic properties of autoregulation, which are the combination of metabolites such as lactate and adenosine, flow-dependent regulation and myogenic tone (Hill et al. 2006; Henrion 2005).

Pressure-dependent myogenic tone in small resistance arteries is defined as the intrinsic property of vascular smooth muscle (VSM) cells to respond to changes in transmural pressure. Sir William Bayliss was the first to discover myogenic tone in 1902 when he recorded large increases in the volume of a dog's hind limb following the release of brief aortic occlusion (Bayliss 1902). Bayliss considered the blood volume increase response too rapid to

be mediated by accumulation of metabolites and concluded that a significant component of vascular tone could be modulated by changes in intravascular pressure. Bjorn Folkow (1949) supported Bayliss's theory demonstrating that denervated preparations of blood vessels developed pressure-dependent vascular tone and autoregulation of blood flow was neurohormonal-independent (Folkow 1949). However, in spite of more than 100 years of myogenic tone study, the knowledge of the exact primary sensors to the signalling mechanisms that allow changes of intraluminal pressure to be converted accordingly into appropriate arterial diameter, remain incomplete and controversial. This has been a key challenge and a true understanding of the underlying mechanism is important in order to tackle the treatment of the pathological states.

PRESSURE-DEPENDENT MYOGENIC TONE MECHANISMS

Myogenic tone shows an inverse relationship between pressure and diameter, where an increase of intraluminal pressure causes a decrease in diameter, and vice versa. Moreover, the strength of the myogenic response varies depending on the diameter and the particular vascular bed. In the hamster cheek pouch, relative myogenic responsiveness increased with decreasing vessel size in second- and third-order arterioles, whereas fourth-order arterioles were substantially less responsive than third-order arterioles (Davis 1993). Relative myogenic tone varies between different vascular beds, with rat cerebral (Gokina et al. 2005) and skeletal muscle vessels (Coats 2010) exhibiting more prominent myogenic response than small mesenteric vessels (Chin et al. 2007). Differences in the strength of the myogenic response between vessels with similar diameter have also been observed within the network of a vascular bed. For example, there was a weak myogenic response in subendocardial but a strong myogenic response in subepicardial porcine arteries (Kuo et al. 1988).

According to the reviews by Hill et al. (2006) and Khavandi et al. (2009), myogenic behaviour can be divided into three phases. The first consists of myogenic or basal tone development, associated with a large increase in L-type VOCC-mediated membrane depolarization and $[Ca^{2+}]_i$. The second phase is known as myogenic reactivity, where further constriction happens in response to an increase in intraluminal pressure. The $[Ca^{2+}]_i$ concentration is maintained at this phase, but $[Ca^{2+}]_i$ sensitization of the mechanical apparatus is thought to occur. The third phase is called forced dilation, when the wall of the artery is unable to maintain a constriction against mounting pressure. There are a number of candidates that could be mediating myogenic response, however there is no agreement on whether it is the vessel wall tension (Davis et al. 1992, Dunn & Gardiner 1997, Brekke et al. 2002), stretch-activated cation (SAC) channels (Davis et al. 1992), VOCC (McCarron et al. 1997), extracelullar matrix (ECM) (Davis et al. 2001) or actin cytoskeleton (Cipolla et al. 2002). Knowledge about the cellular mechanisms underlying myogenic tone is important and could be exploited for therapeutic intervention, as myogenic responsiveness is commonly altered in vascular diseases. Therefore, it is crucial to understand the stimuli, sensor, signal transduction pathways involving second messengers, regulation of contractile proteins and finally the adjustment for vasomotor tone.

ION CHANNELS

Putative plasma membrane ion channels functioning as primary mechanosensors in mediating arteriolar myogenic vasoconstriction include VOCC (Kotecha & Hill 2005; Knot & Nelson 1998), SAC (Luchessi et al. 2004), TRPC (Mederos y Schnitzler et al. 2008), large conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) (Dong et al. 2009; Cheranov & Jaggar 2006), voltage-gated potassium channel (K_v) (Sobey et al. 1998) and Ca^{2+}-activated chloride channels (Cl_{Ca}) (Dimitropoulou et al. 2001).

A plethora of evidence has shown that the pressure-dependent myogenic response is mediated by an increase of membrane depolarization and $[Ca^{2+}]_i$ in VSM, accompanied by the classical Ca^{2+}-calmodulin–induced myosin light chain (MLC) phosphorylation (Harder 1984; Davis & Hill 1999; Hill et al. 2001; Schubert et al. 2008). The pioneering study by Harder (1984) was the first to show that an increase in pressure caused depolarization and therefore an increase in Ca^{2+} influx and vasoconstriction, within the cerebral artery. A subsequent study showed that the increased Ca^{2+} influx occurred in parallel with the decrease in diameter (Knot & Nelson 1998). Incubation in a Ca^{2+}-free solution or the administration of the Ca^{2+}-entry blocker diltiazem, led to a complete loss of myogenic tone (Bevan 1982). The Ca^{2+} influx has been shown to depend primarily on L-type VOCC which can be inhibited by nifedipine (Kotecha & Hill 2005; Coats et al. 2001; Scotland et al. 2004; VanBavel et al. 1998).

The primary events that lead to an increase of $[Ca^{2+}]_i$ are the release of Ca^{2+} from intracellular stores and transmembrane influx. However, a study found the internal ryanodine-sensitive Ca^{2+} store to be insignificant in myogenic constriction, indicating that CICR is a minor contributor to the response (McCarron et al. 1997). Recently, extracellular Ca^{2+} has been reviewed by Smajilovic and Tfelt-Hansen (2007) for eliciting the role as a first messenger through a G-protein coupled receptor (GPCR), namely the Ca^{2+}-sensing receptor (CaR). In subcutaneous arteries, the CaR was found to modulate pressure-dependent myogenic tone, while PKC was acting as its negative regulator (Ohanian et al. 2005). However, the CaR paradigm is relatively new in the area of myogenic tone and more investigation is needed to clarify its function. Another recent study that supports GCPR as a

mechanosensor is where $G_{q/11}$-coupled receptor, a GCPR, was activated and stimulated β-arrestin recruitment in response to increased pressure (Mederos y Schnitzler et al. 2008). Subsequently, this process lead to TRPC-mediated membrane depolarization and enhanced myogenic constriction in renal and cerebral arteries.

Although Ca^{2+} has been shown as a necessary key signalling component in myogenic constriction, some studies have shown that only a minimal rise in $[Ca^{2+}]_i$ is required to elicit myogenic constriction (D'Angelo et al. 1997; VanBavel et al. 1998). Therefore, Ca^{2+} is not the only determinant of steady-state constriction and there are Ca^{2+}-independent regulatory systems involving protein kinase C (PKC), Rho/Rho kinase, protein tyrosine kinase and cytoskeleton, that accompany myogenic constriction (Lagaud et al. 2002; Gokina et al. 2005; Massett et al. 2002; Jarajapu & Knot 2002).

INTRACELLULAR SECOND MESSENGERS

There has been a growing body of evidence that shows Ca^{2+} sensitivity regulation integrates with Ca^{2+}-independent mechanisms in the myogenic constriction, via intracellular second messengers signalling in VSM. Pressure or stretch acts as the stimulus for G proteins, which then stimulate the membrane enzyme phospholipase C (PLC) that specifically hydrolyzes phosphatidylinositol-4,5-biphosphate (PIP_2). PIP_2 is split into two second messengers, diacylglycerol (DAG) and inotisol-1,4,5-triphosphate (IP_3) (Narayanan et al. 1994). Both IP_3 and DAG have been shown to increase with the level of intraluminal pressure and to remain elevated for the duration of the pressure increase (Narayanan et al. 1994). The increase in the level of intracellular IP_3, induced by stretch, may be responsible for part of the pressure-induced increase of $[Ca^{2+}]_i$ (Jarajapu & Knot 2002). The increase in DAG, which is an activator of protein kinase C (PKC), implicates the participation of PKC in the myogenic response (Gokina et al. 1999; Wesselman et al. 2001). Indeed, the PKC activator, phorbol-12,13-dibutyrate (PDBu) was found to increase the level of Ca^{2+}, and this effect was abolished by nifedipine, an L-type Ca^{2+} channel blocker (Lin et al. 1998; Ohanian et al. 2005). This suggests that the PKC-enhancing effect by PDBu was through the activation of L-type Ca^{2+} channels and this could increase Ca^{2+} sensitivity resulting in myogenic constriction (Lin et al. 1998; Gokina et al. 1999). Consistent with the fact that PKC phosphorylates mitogen-activated protein kinase (MAPK), it has been shown that inhibition of MAPK reduced the Ca^{2+} sensitivity and hence, attenuated the pressure-dependent myogenic tone (Massett et al. 2002; Lagaud et al. 1999). Another potential pathway regulating myogenic tone is the RhoA/Rho kinase signaling pathway. Results have shown that RhoA/Rho kinase pathway to be activated by mechanical stretch, leading to an increase in intracellular $[Ca^{2+}]$ sensitivity. This could potentially inhibit myosin phosphatase and/or activate actin cytoskeleton polymerization, followed by increased myogenic tone (Gokina et al. 2005; Jarajapu & Knot 2005). Another interesting key candidate in modulating myogenic tone is the epidermal growth factor receptor (EGFR). A study in mesenteric arteries has shown pressure/stretch to stimulate metalloproteinases 2/9 (MMP-2/9), leading to endogenous HB-EGF (heparin-binding EGF-like growth factor) release and EGFR transactivation (Lucchesi et al. 2004). This mechanism was specific to myogenic tone as contractions to ANG II and KCl were independent of EGFR transactivation. The underlying mechanism within the VSM was not investigated, but it was speculated that MMP-2/9 activation may involve integrins (Belmadani et al. 2008) or SACs (Scotland et al. 2004), and the downstream signalling of EGFR activation may be PKC-dependent (Jarajapu & Knot 2005) or through Nox/ROS (Csanyi et al. 2009).

CYTOCHROME P-450

The cytochrome P-450 (CYP) enzymes metabolize endogenous arachidonic acid (AA) and are referred to as the third pathway of AA metabolism, the others being cyclooxygenases and lipooxygenases. The role of CYP has been reviewed as a candidate mediator in signaling events culminating in pressure-dependent myogenic tone in renal, skeletal muscle and pulmonary arteries (Terashvili et al. 2006; Frisbee et al. 2001; Kaley 2000; Parker et al. 2005). Phospholipase A_2 (PLA_2) is an enzyme which hydrolyzes phospholipids and produces AA. This AA can be further metabolized to produce autocrine vasoconstrictor 20-HETE (20-hydroxyeicosatetraenoic acid) and vasodilator 11,12-EET (11,12-epoxyeicosatrienoic acid), which could enhance ROS release. Stimulation of VSM cells by stretch, pressure and flow could trigger CYP to release 20-HETE, which inhibits the opening of Ca^{2+}-activated K^+ (K_{Ca}) channels in VSM, causing depolarization, and eventually increases Ca^{2+} entry and promotes myogenic constriction (Frisbee et al. 2001). As for 11,12-EET, it exhibits biological activities opposite to 20-HETE; where it vasodilates blood vessels and stimulates hyperpolarization via K_{Ca} channels in VSM cells (Imig 1999). Hence, the dual counterbalancing activity of these two AA metabolites may present a mechanism for balanced regulation of myogenic tone regulation.

REACTIVE OXYGEN SPECIES

Reactive oxygen species and oxidative stress have been synonymous in pathological conditions for decades. Recently, a growing body of evidence has shown that ROS could play a role in regulating pressure-dependent myogenic tone (Lecarpentier 2007; Keller et al. 2006). Besides ROS, another emerging new candidate is the actin cytoskeleton (Faraci 2006). Figure 1 summarizes the overall putative mechanotransduction pathways of pressure-dependent myogenic tone regulation.

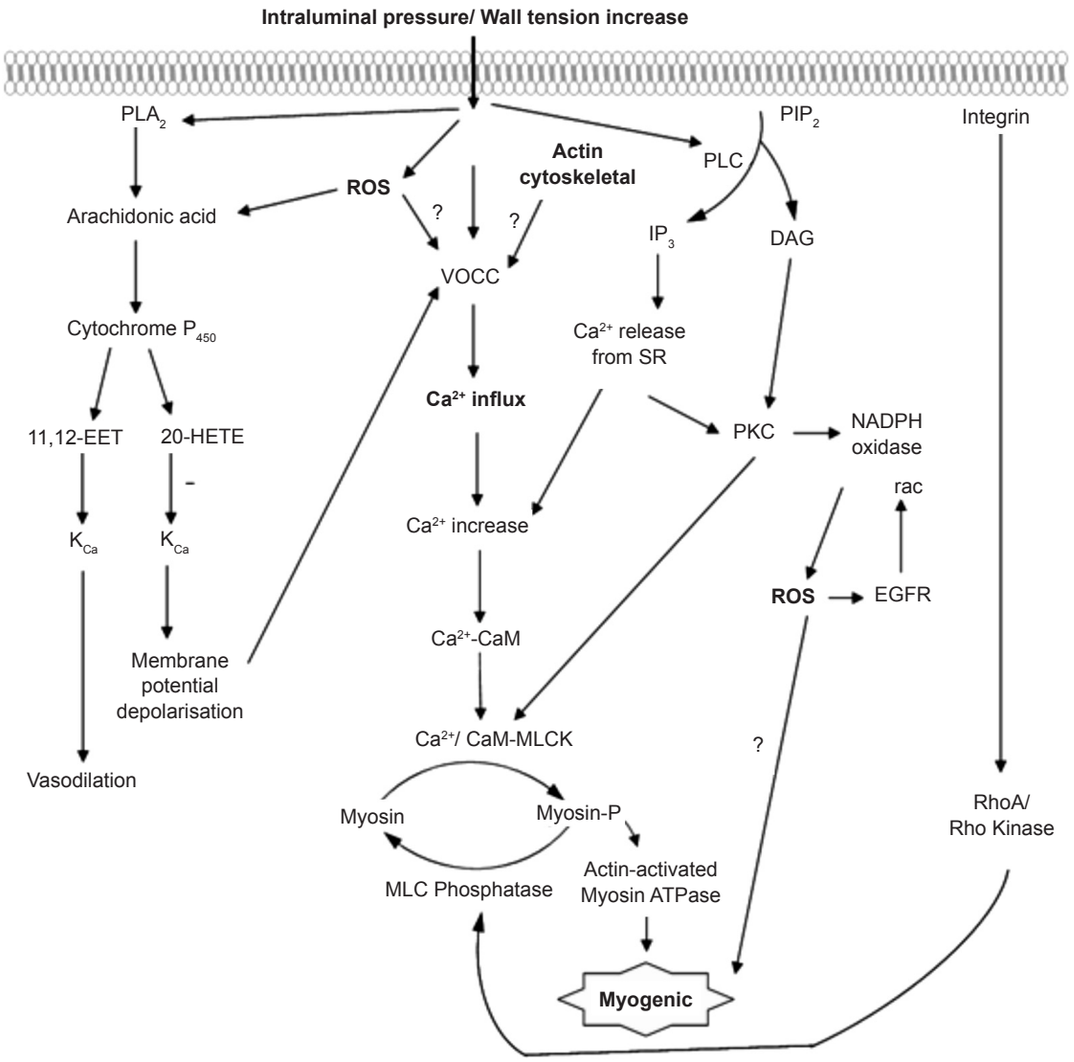

FIGURE 1. Overview of possible pathways involved in the myogenic response. The possible pathways may involve ion channels, intracellular second messengers, cytochrome P450 and the newly arised new candidates, ROS and actin cytoskeleton. PIP_2 (phosphatidylinositol (4,5)-bisphosphate); PLC (phospholipase C); IP_3 (inositol trisphosphate); DAG (diacylglycerol); PKC (protein kinase C); VOCC (voltage-operated calcium channel); PLA_2 (phospholipase A_2); 20-HETE (20-hydroxyeicosatetraenoic acid); KCa (calcium-activated potassium channel); Ca^{2+} (calcium); CaM (calmodulin); MLCK (myosin light-chain kinase); myosin-P (myosin-phosphate); SR (sarcoplamic reticulum)

REACTIVE OXYGEN SPECIES IN CARDIOVASCULAR HEALTH AND DISEASES

REACTIVE OXYGEN SPECIES AND OXIDATIVE STRESS

ROS are classically considered as a toxic by-product of metabolism in oxidative stress (Taniyama & Griendling 2003; Dworakowski et al. 2006). When ROS is produced excessively, overwhelming of endogenous antioxidant systems and/or during downregulation of antioxidant systems, oxidative stress occurs (Griendling & Fitzgerald 2003; Taniyama & Griendling 2003; Dworakowski et al. 2006). Dysregulation due to oxidative stress has been shown to contribute substantially to the progression of cardiovascular diseases such as hypertension (Schiffrin 2004; Intengan et al. 1999), diabetes (Sachidanandam et al. 2007), hypercholesterolaemia (Ishikawa et al. 2004), cardiac hypertrophy (Lang 2002) and myocardial ischaemia (Ferrari et al. 2004).

The term 'oxidative stress' may be an over-simplification as it is used to cover the diverse and complex role of ROS in physiological and pathophysiological conditions. Over the past decade, studies have shown that ROS at relatively low cellular levels may influence metabolism in physiological conditions (Lecarpentier 2007; Keller et al. 2006) and may be involved with the normal regulation of vascular tone and structure (Faraci

2006). ROS may have direct and indirect effects on VSM, and there are studies suggesting that both relaxation and contraction of vascular muscle may occur, depending on the tissue model and the physiological circumstances.

METABOLISM OF ROS

ROS includes free radicals such as superoxide anion (O_2^-) and hydroxyl radical (OH^-), and nonradical species such as hydrogen peroxide (H_2O_2) (Hamilton et al. 2004). Superoxide dismutase (SOD) metabolizes O_2^- to H_2O_2. There are three isoforms for SOD: CuZn-containing cytosolic SOD1, Mn-containing SOD2 and CuZn-containing extracellular SOD3. Peroxidases such as catalase and glutathione peroxidase further metabolize H_2O_2 to O_2 and water. H_2O_2 can also be transformed to OH^- and O_2^- to peroxynitrite ($ONOO^-$) (Faraci 2006; Miller et al. 2006; Hamilton et al. 2004). Almost all types of vascular cell produce O_2^- and H_2O_2 (Lyle & Griendling 2005). Primarily, ROS within a blood vessel is produced by the membrane-bound enzyme Nox (Griendling et al. 1994), but ROS has been shown to also be produced from adventitial fibroblasts (Pagano et al. 1997), cyclooxygenase (Sobey et al. 1998; Miller et al. 2006) as well as eNOS (Matoba et al. 2000). There are many stimuli which activate Nox, including ANG II (Mehta and Griendling, 2007), ET-1 (An et al. 2007), shear stress (Hwang et al. 2003), tumor necrosis factor-α (TNF-α) (Al-Mehdi and Fisher 1998), metabolic factors including hyperglycaemia, insulin (Dworakowski et al. 2006), hypercholesterolemia (Ishikawa et al. 2004), and vascular endothelial growth factor (VEGF) (Abid et al. 2007).

O_2^- is highly reactive, but it has a very short half-life and poor cell permeability. Therefore, the impact of O_2^- on vascular function could be instant but short-lasting. The reaction of O_2^- with EDNO is very rapid, 3 times faster than the dismutation of O_2^-, resulting in reduced NO bioavailability and increased $ONOO^-$. Conversely, H_2O_2 is relatively stable and highly diffusible between cells. Therefore, H_2O_2 is regarded as one of the most important ROS molecules for modulating vascular function. It has been reported as an EDHF in the mesenteric artery (Matoba et al. 2000) and partially mediates flow-dependent vasodilatation in coronary arterioles (Miura et al. 2003). Figure 2 shows the general metabolism of ROS and the associated complications in cardiovascular diseases.

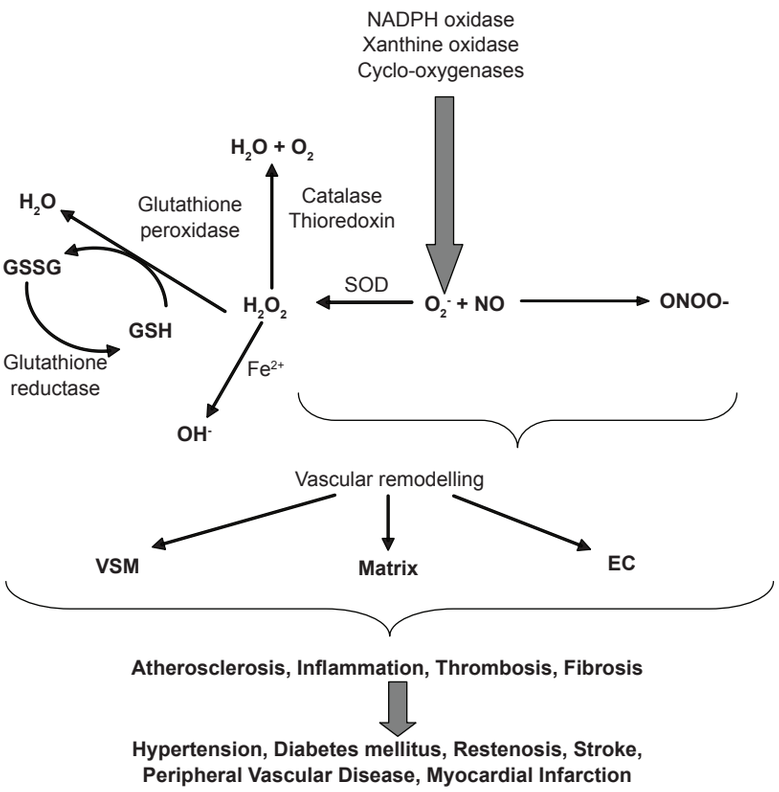

FIGURE 2. Generation of O_2^- and H_2O_2 from O_2 in vascular tissue and the associated complications in cardiovascular diseases. GSSG, oxidized glutathione; GSH, reduced glutathione; SOD, superoxide dismutase; ONOO-, peroxynitrite; NO, nitric oxide; VSM, vascular smooth muscle; EC, endothelial cells

NADPH OXIDASE SUBUNITS

The Nox in the vasculature consists of $p22^{phox}$, $p47^{phox}$ and novel homologues of $gp91^{phox}$, which are NOX1, NOX2 and NOX4 (Seshiah et al. 2002; Cave 2008). These isoforms are different between cell types and they have separate locations within the cell. The evidence is becoming increasingly clear that the individual Nox isoforms have delineated roles within the cell and are linked with specific downstream effects (Cave et al. 2005; Cave 2008).

ANG II AS AN NADPH OXIDASE/ ROS ACTIVATOR

ANG II stimulates Nox and ROS activity in all cells within the vascular wall (Griendling & Ushio-Fukai 2000; Pedruzzi et al. 2004; Gonzalez et al. 2008). ANG II not only stimulates Nox-dependent O_2^- formation in VSM cells but also in cardiac, endothelial, mesangial cells and adventitial fibroblasts (Zhang et al. 1999; Pedruzzi et al. 2004; Mehta & Griendling 2007). In rabbit aortic adventitial fibroblasts, induction of Nox subunit $p67^{phox}$ expression was caused by ANG II-induced O_2^- (Pagano et al. 1998). In rat VSM, subunit $p22^{phox}$ has been shown to be a component in the ANG II-induced hypertrophy (Ushio-Fukai et al. 1996). A member of a new family of $gp91^{phox}$, NOX1, showed a role in mediating ANG II-induced O_2^- formation via redox signalling in VSM cells (Lassegue et al. 2001). Angiotensin AT_1 receptors (AT_1R) are believed to be mainly located in the VSM cells and to induce vasoconstriction (Maeso et al. 1996; Touyz & Schiffrin 2000), while angiotensin AT_2 receptors (AT_2R) mediate endothelium-dependent vasodilation (Carey et al. 2000).

In addition to stimulating Nox to release ROS, ANG II has also been found to stimulate various downstream signalling pathways in relation to pressure-dependent myogenic tone. For instance, intraluminal pressure facilitated by ANG II may activate AT_1R which in turn causes transactivation of the platelet-derived growth factor (PDGF), then through the Ras-Raf pathway activates extracellular signal-regulated kinase 1/2 (ERK1/2) (Matrougui et al. 2000, Eskildsen-Helmond & Mulvany 2003). However, how the mechanical loading can cause such acute and rapid activation is still unknown. Suggested pathways may include the PLC-IP_3 pathway resulting in increase of $[Ca^{2+}]_i$ and Ca^{2+}-dependent ERK1/2 stimulation, or the PLC-DAG pathway whereby PKC can activate ERK1/2 (Eskildsen-Helmond & Mulvany 2003). Other interesting supporting findings were that AT_1R stimulation could initiate G-to-F actin and strengthen the cytoskeletal structure (Martinez-Lemus 2008) or it could activate TRPC-mediated membrane depolarization and cause myogenic tone (Mederos y Schnitzler et al. 2008).

ROS IN THE BIOLOGY OF VASCULAR TONE

The first direct evidence that endogenous ROS can act as a signalling molecule was found by Kontos et al. (1984). Their study found that dilation by topical sodium arachidonate and bradykinin was inhibited by catalase and SOD in cat cerebral artery. Thus, O_2^- and H_2O_2 or radical derivatives such as OH^- were likely to be the mediators from sodium arachidonate and bradykinin that caused vasodilation. Since then, a growing body of evidence has demonstrated ROS as potent intracellular and intercellular second messengers to modulate many downstream signalling molecules via $[Ca^{2+}]_i$-dependent and $[Ca^{2+}]_i$ -independent pathways and eventually cause substantial interplay in myogenic response.

The major effect of O_2^- was thought to be peripheral vasocontraction by its reaction with EDNO, as shown widely in oxidative stress. It is now known that in cerebral arteries, O_2^- can cause both constriction and dilation (Wei et al. 1996; Didion & Faraci 2002). The K^+ channel blocker, tetraethylammonium (TEA), reduced cerebral relaxation to O_2^-, suggesting that K_{Ca} channels are involved (Wei et al. 1996). H_2O_2 has also been shown to induce both vasorelaxation and vasoconstriction, depending on the vascular bed and the pre-constrictor agent. H_2O_2 from the endothelium may act as a vasodilatory EDHF in murine (Kimura et al. 2002), human mesenteric arteries (Matoba et al. 2002) and human coronary arterioles (Miura et al. 2003). At higher concentration (>1 mM), H_2O_2 has been found to cause vasoconstriction followed by vasodilatation (Miller et al. 2005). Dilatation of rat cerebral arterioles in response to exogenous H_2O_2, or endogenous bradykinin-derived H_2O_2 is mediated by activation of K_{Ca} channels (Sobey et al. 1997). In other studies, mitochondrial-derived ROS dilated cerebral arteries by increasing K_{Ca} channel activity via Ca^{2+} sparks (Xi et al. 2005), whereas H_2O_2 dilated cerebral arterioles by activating ATP-sensitive K^+ channels (Wei et al. 1996). Thus, activation of K^+ channels may be a major mechanism of dilatation in response to H_2O_2 in the microcirculation. In a study on canine basilar arteries, different types of exogenous ROS elicit different characteristics of contraction, whereby H_2O_2 showed the fast onset and a transient contraction, O_2^- showed slow onset and a transient contraction, and OH^- showed a sustained contraction (Tosaka et al. 2002). This shows the importance of identifying the types of radical and nonradical species in the pathological mechanisms as each ROS could play a different role.

ROS

From the study of ROS in cultured cells to vascular tone, the interest of its role in modulating pressure-dependent myogenic tone has been growing. Exogenous H_2O_2 was found to increase myogenic tone in tail arterioles (Nowicki et al. 2001) and mesenteric arteries (Lucchesi et al. 2005). In addition, endogenous ROS (H_2O_2) was produced from Nox after dipheleneiodonium (DPI) and N-acetylcysteine (NAC) treatment, and mice lacking rac1 and $p47^{phox}$ showed inhibited myogenic tone (Nowicki et al. 2001). Another supporting study showed myogenic tone was dependent on mitochondria-derived ROS (H_2O_2) during cold (28°C),

which stimulated RhoA/Rho kinase signalling and smooth muscle α_{2C}-adrenoceptors, a mechanism that could be relevant to Raynaud's pathophysiology (Bailey et al. 2005). Although H_2O_2 caused constriction in the study, other studies showed that it could also act as a vasodilator (EDHF) (Miura et al. 2003; Matoba et al. 2002; Drouin et al. 2007). The dual effects of H_2O_2 were also observed in a study by Luchessi et al. (2005) where low concentrations of exogenous H_2O_2 caused vasodilation when the K^+ channel was compromised by KCl, and the opposite effect was seen when KCl was removed in pressurized mesenteric arteries. H_2O_2 also exhibits a biphasic effect, causing vasoconstriction at low concentrations and vasodilation at high concentrations, also possibly mediated via K^+ channels (Cseko et al. 2004). With these observations, it could be possible that in pathophysiological conditions where K^+ channel activity is altered, a transition from the physiological vasodilatory effect of H_2O_2 to a contractile effect may play an important role in increasing peripheral resistance and pathogenesis of hypertension (Cseko et al. 2004). In hamster gracilis muscle, Sk1/S1P was shown to mediate pressure-dependent myogenic tone by stimulating the Rac/Nox/O_2^- pathway (Keller et al. 2006). Inhibition of a specific Nox subunit was shown to reduce pressure-dependent myogenic tone, but it did not affect the $[Ca^{2+}]_i$, which suggested that ROS is playing a modulatory rather than obligatory role in regulation of Ca^{2+} sensitivity (Keller et al. 2006). This observation is very important as Sk1/S1P could be a potential therapeutic target, although there should be a balance for the beneficial formation of O_2^- as a physiological signalling molecule.

The role of ROS has also been investigated in diseased animal models. NOX2-derived O_2^- may contribute to the enhanced myogenic response in renal afferent arterioles in spontaneously hypertensive rats (SHR), but not in normal rats (Ren et al. 2010). According to this study, the role of ROS is complex, because on one hand, increased O_2^- in afferent arterioles could add to hypertension development. But on the other hand, after hypertension is established, O_2^--induced myogenic enhancement could provide protection from glomerular barotrauma and injury in SHRs. In streptozotocin-induced diabetic rats, cerebral myogenic tone was increased in parallel with decreased large conductance BK_{Ca} channel activity and increased H_2O_2 (Dong et al. 2009). Rotenone, an inhibitor of the mitochondrial electron transport chain complex I, partially reversed the myogenic response, H_2O_2 levels and BK_{Ca} effect. This indicates that BK_{Ca} channels may be related to the inhibitory effects of rotenone on ROS (H_2O_2) production and the myogenic tone of diabetic rats (Dong et al. 2009).

The important role of ROS as a signalling molecule in pressure-dependent myogenic tone is indisputable. However, there arises a significant dilemma in determining the borderline of the beneficial and the hazardous effect of ROS. Additionally, ROS members are highly diverse and able to elicit multiple effects within the same tissue. Paravicini & Sobey (2003) have highlighted the importance of the delicate balance between O_2^- and H_2O_2 mediated by SOD when studying vascular oxidative stress. Therefore, it is essential to understand the exact mechanism categorized in order to determine how ROS can be 'good' or 'bad' signalling molecules.

POTENTIAL THERAPEUTIC IMPLICATIONS OF ARTERIAL MYOGENIC TONE

Considering our current knowledge of myogenic tone regulation, an ability to adjust vascular resistance through specific modulation of myogenic mechanism would enable normal neurohormonal mechanisms to interact with the therapeutically treated tone. Therefore, identifying the primary mechanosensors in the initiation of pressure-dependent myogenic tone is essential as these components could be targets for drug development. For example, in situations in which the cardiovascular system is depressed in shock and low-flow states, a drug increasing myogenic tone would be beneficial. On the contrary, in hyperdynamic conditions, reduction of drug-induced myogenic tone would be needed.

The possibility of the adventitia, especially the adventitial fibroblast, being a potential platform for intravascular local drug delivery is very intriguing. Work in the future should focus on understanding ways to control adventitial fibroblast activation, to reduce unwanted changes in vascular structure. As the adventitial fibroblasts contain Nox subunits, the development of specific inhibitors of Nox would provide pharmacological tools to completely elucidate the roles of these isoforms in vascular physiology and pharmacology, particularly, in pressure-dependent myogenic tone. To date, there is only one such compound i.e. plumbagin that has been reported to inhibit Nox/O_2^- production by NOX4 in cultured kidney embryonic cells (Ding et al. 2005). However, this compound was found to be carcinogenic and whether this compound selectively inhibits NOX4 activity or acts merely as a ROS scavenger was not investigated. Rey et al. (2001) designed a Nox inhibitor known as gp91ds-tat, which competitively binds to $p47^{phox}$ and prevents the assembly of the enzyme. To date, this peptide has been said to be the most successful Nox inhibitor because of its selectivity. However, it is uncertain if gp91ds-tat inhibits other Nox-containing isoforms of Nox, such as NOX1 or NOX4. In conclusion, it is essential to understand the specific Nox subunit source of ROS within the adventitial fibroblasts as it may become a potential therapeutic target in treating vascular diseases.

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