Curr Hypertens Rep (2011) 13:122–128
DOI 10.1007/s11906-011-0187-x

Cell Signaling of Angiotensin II on Vascular Tone: Novel Mechanisms

Aurelie Nguyen Dinh Cat · Rhian M. Touyz

Published online: 29 January 2011

Abstract Angiotensin II (Ang II) is a pleiotropic hormone that influences the function of many cell types and regulates many organ systems. In the cardiovascular system, it is a potent vasoconstrictor that increases peripheral vascular resistance and elevates arterial pressure. It also promotes inflammation, hypertrophy, and fibrosis, which are important in vascular remodeling in cardiovascular diseases. The diverse actions of Ang II are mediated via AT_1 and AT_2 receptors, which couple to many signaling molecules, including small G proteins, phospholipases, mitogen-activated protein (MAP) kinases, phosphatases, tyrosine kinases, NADPH oxidase, and transcription factors. In general, acute Ang II stimulation induces vasoconstriction through changes in the intracellular free calcium concentration $[Ca^{2+}]_i$, whereas long-term stimulation leads to cell proliferation and proinflammatory responses. This review focuses on signaling processes of vasoconstriction and highlights some new mechanisms regulating the contractile machinery in controlling vasomotor tone by Ang II, including RhoA/Rho kinase, transient receptor potential (TRP) channels, reactive oxygen species, and arachidonic acid metabolites.

Keywords RhoA · Arachidonic acid metabolites · TRP channels · Renin angiotensin system · Vasoconstriction · Vasodilation

A. N. D. Cat · R. M. Touyz (✉)
Canada Research Chair in Hypertension, Kidney Research Centre, OHRI/University of Ottawa,
451 Smyth Road,
Ottawa K1H 8M5 Ontario, Canada
e-mail: rtouyz@uottawa.ca

Introduction

Regulation of blood flow, local hemodynamics, and blood pressure occur acutely through vasomotor responses and chronically through adaptive arterial structural remodeling. Of the many factors that regulate vascular function and structure is angiotensin II (Ang II), the major bioactive peptide of the renin-angiotensin system (RAS). Ang II, produced systemically and locally within the vascular wall, is a potent vasoactive peptide that also stimulates vascular smooth muscle cell growth, inflammation, and fibrosis through myriad signaling pathways [1–3]. Accordingly, Ang II plays an important physiological role in maintaining vascular tone by regulating immediate vasoconstriction and a pathophysiological role in cardiovascular diseases such as hypertension, atherosclerosis, and heart failure, conditions that are associated with endothelial dysfunction, vascular hyperreactivity, and structural remodeling.

Ang II exerts its diverse actions via two G protein–coupled receptors (GPCRs), Ang II type 1 receptors (AT_1R) and type 2 receptors (AT_2R). The AT_1R mediates most of the (patho)physiological actions of Ang II. The AT_2R is associated with antiproliferative, pro-apoptotic, and vasodilatory actions of Ang II and tends to counteract effects of the AT_1R [2]. Signaling pathways induced by Ang II/AT_1R involve interactions with several heterotrimeric G proteins coupled to second messengers and cytosolic proteins, including phospholipase C (PLC), phospholipase A2 (PLA2), and phospholipase D (PLD) [1]. In addition Ang II/AT_1R regulates the activation of NADPH oxidase through the activation of many receptor and nonreceptor tyrosine kinases and serine threonine kinases, important in cell growth and hypertrophy. NADPH oxidase is a major source of vascular reactive oxygen species (ROS) involved in redox signaling and activation of pro-inflammatory

transcription factors [4, 5•] and stimulation of small G proteins such as Ras, Rac, and RhoA [1–4, 5•]. Although the primary vascular cell target of Ang II is smooth muscle, it also influences the endothelium by modulating production of nitric oxide (NO) and ROS [5•] and by influencing the many ion channels expressed in endothelial and vascular smooth muscle cells, including transient receptor potential (TRP) cation channels involved in regulating vascular tone [6, 7].

Ang II has a diverse array of vascular functions. Acute Ang II stimulation causes vasoconstriction and a rapid rise in blood pressure, whereas chronic Ang II stimulation leads to vascular smooth muscle cell proliferation and structural remodeling, important in sustained blood pressure elevation. Many excellent papers have focused on Ang II signaling involved in vascular remodeling, which will not be further detailed here [2–4]. The present review discusses the role of Ang II in the regulation of vascular tone, focusing on some novel signaling pathways (Fig. 1).

Angiotensin II–Induced Contraction Through Classic G Protein–Dependent Signaling: A Synopsis

Ang II is a potent vasoconstrictor that mediates effects through vasoconstriction, mediated by G protein–sensitive signaling pathways, where Ang II/AT_1R couples to small G proteins that activate downstream effectors, including PLC, PLD, and PLA2. PLC activation produces inositol-1-4-5-trisphosphate (IP3) and diacylglycerol (DAG) [8]. IP3 in turn mediates sarcoplasmic reticular release of Ca^{2+} to increase the intracellular free calcium concentration $[Ca^{2+}]_i$, the major trigger for contraction. Stimulation of Ca^{2+} influx through Ang II–activated Ca^{2+}

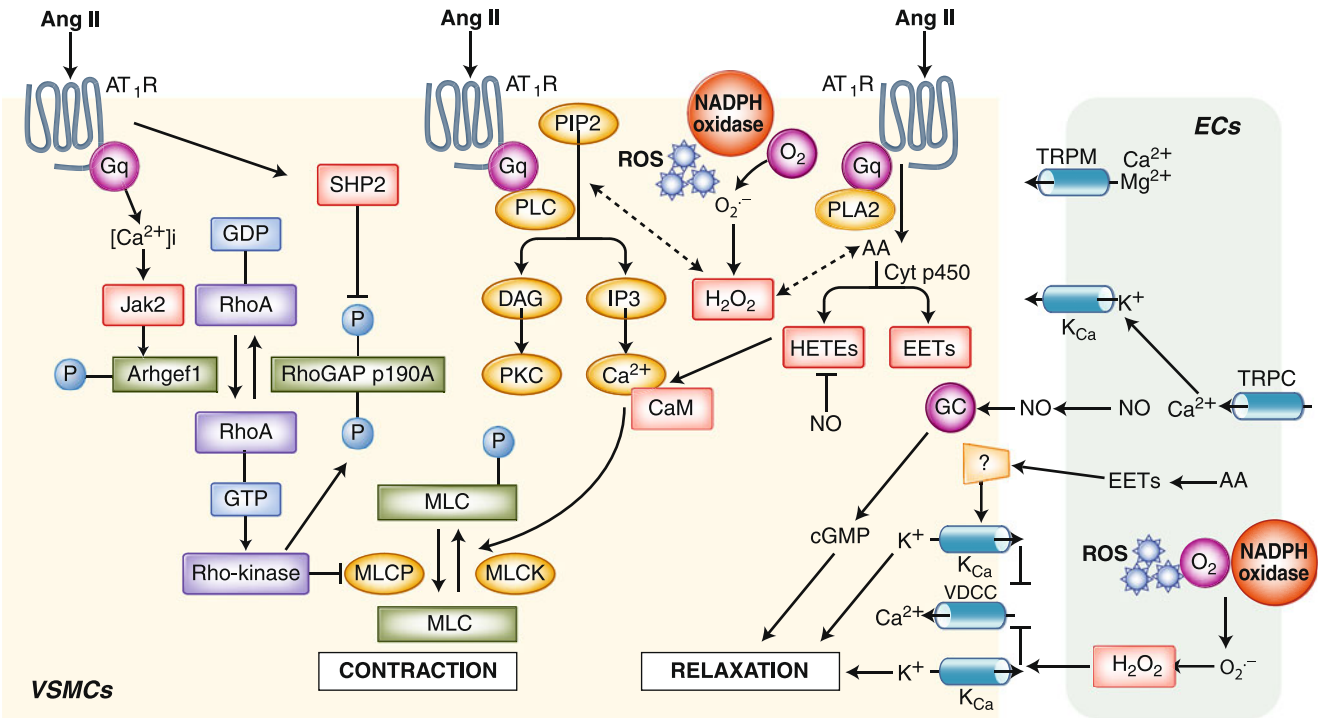

Fig. 1 Angiotensin II (Ang II)–mediated activation of Ang II type 1 receptors (AT_1R) regulates vasomotor tone through multiple mechanisms. Ang II binds to its AT_1R, which couples to heterometric Gq proteins, to activate phospholipase C (PLC), leading to generation of second messengers, inositol-1-4-5-trisphosphate (IP3) and diacylglycerol (DAG), resulting in increased intracellular free calcium concentration $[Ca^{2+}]_i$, which triggers phosphorylation of myosin light chain (MLC) and stimulation of contraction. Ang II also induces contraction through the RhoA/Rho kinase pathway, which increases Ca^{2+} sensitivity by inhibiting the myosin light chain phosphatase (MLCP). Ang II/AT_1R stimulates production of arachidonic acid (AA)–derived hydroxyeicosatetraenoic acids (HETEs) and formation of NADPH oxidase-derived reactive oxygen species (ROS), which stimulate contraction. Hydrogen peroxide (H_2O_2) induces vasodilation through the opening of Ca^{2+}-activated K^+ channels (K_{Ca}). Activation of transient receptor potential (TRP) cation channels by Ang II in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) influences Ca^{2+}-activated K^+ channels to modulate contraction and relaxation. ?: unknown factor; Arghef1: Rho guanine nucleotide exchange factors (GEF) p115; CaM: calmodulin; EETs: epoxyeicosatrienoic acids; GC: guanylate cyclase; Jak2: janus kinase; MLCK: myosin light chain kinase; NO: nitric oxide; p: phosphorylation state; P: phosphate group; PIP2: phosphatidylinositol 4,5 biphosphate; PLA2: phospholipase A2; RhoGAP p190A: Rho GTPase activating proteins (GAP) p190A; SHP2: Src homology region 2 domain containing phosphatase-2 (SHP2); TRPC: calcium transient receptor potential; TRPM: melastatin transient receptor potential; VDCC: voltage-dependent calcium channels

channels also contributes to the pool of cytoplasmic Ca^{2+}. Increased $[Ca^{2+}]_i$ induces Ca^{2+}-calmodulin binding, which activates the myosin light chain kinase (MLCK), promoting interaction of myosin II with actin and enhanced cross-bridge cycling with consequent contraction [9].

PLD activation by Ang II results in hydrolysis of phosphatidylcholine to choline and phosphatidic acid, which is rapidly converted to DAG, resulting in sustained PKC activation and associated sustained vasoconstriction [8]. In addition to the "classic" PLC-dependent and PLD-dependent pathways, ERK1/2 and tyrosine kinases, typically involved in growth signaling, influence Ang II–stimulated vascular contraction, an effect mediated through changes in $[Ca^{2+}]_i$ and the intracellular pH (pH_i) [10]. A number of other signaling mechanisms have recently been identified that also play an important role in the regulation of vascular tone by Ang II, including the RhoA/Rho kinase pathway, TRP channels, ROS, and arachidonic acid metabolites (HETEs, EETs), which are discussed later.

The RhoA/Rho-Kinase Signaling Pathway and Angiotensin II

Activation of RhoA and its downstream target Rho-kinase is increasingly being recognized as an important mechanism of vasoconstriction by Ang II and accordingly has been implicated in the pathophysiology of hypertension [11, 12]. Small guanosine triphosphatase (GTPase) Rho proteins are active when bound to GTP and inactive when bound to guanosine diphosphate (GDP). Activation is mediated by guanine nucleotide exchange factors (GEFs), which displace the GDP dissociation inhibitor (GDI) and promote release of GDP in exchange for GTP. GTPase activating proteins (GAPs) stimulate the intrinsic hydrolysis of GTP and lead to rapid conversion of Rho proteins to their inactive state, bound to GDP and GDI [12]. RhoA, a member of the Rho family of small GTPase binding proteins, is abundantly expressed in vascular smooth muscle cells and is well known to participate in arterial smooth muscle contraction via phosphorylation of myosin light chain (MLC) and sensitization of contractile proteins to Ca^{2+} [13]. In vascular smooth muscle cells (VSMCs), Ang II/AT_1R increases RhoA activity [14] via the $G_{12/13}$ family of G proteins, as well as G_q [15]. Therefore RhoGEFs sensitive to $G_{12/13}$, such as Arhgef1 (p115RhoGEF), Arhgef12 (LARG), or Arhgef11 (PDZ-RhoGEF), may mediate Rho activation [11, 12]. Recent evidence indicates that in VSMCs, Ang II/AT_1R specifically induces phosphorylation of Arhgef1 by the tyrosine kinase Jak2 [16]. In vivo, specific deletion of Arghef1 in smooth muscle does not modify blood pressure, but Ang II-induced contraction in aortic rings is inhibited in mice with inactivation of Arhgef1 in smooth muscle, whereas responses to other vasoconstrictors (norepinephrine and endothelin-1) are unchanged [16]. This model demonstrates that Ang II-AT_1R-Gq-Arhgef1-RhoA signaling is strongly implicated in the development of hypertension induced by Ang II and by NG-nitro-L-arginine methyl ester (L-NAME). Interestingly, the study of the constitutive deletion of Arhgef12 in mice showed that endothelin-1-ETA receptor-$G_{12/13}$-Arhgef12-RhoA signaling may be implicated in the development of deoxycorticosterone acetate (DOCA)-salt–induced hypertension [17].

Not only is activation of a regulator of G-protein signaling (RGS) domain-containing RhoGEF important, but inactivation of the RhoGAP is also a crucial aspect of the RhoA/Rho-kinase cascade stimulated by GPCRs, as shown by the recent identification in cultured vascular smooth muscle cells of the tyrosine phosphatase SHP2, as a novel negative regulator of RhoGAP (p190A). SHP2 is necessary to maintain basal p190A activation and consequently a low RhoA/Rho-kinase activity in rat aortic smooth muscle cells [18, 19]. SHP2 regulation by Ang II through AT_1R occurs in a redox-sensitive manner [20•]. Under certain conditions (possibly when the AT_2R is upregulated), Ang II can inhibit RhoA activity to induce vasodilation. This occurs through AT_2R-mediated Ste20-related kinase (designated SLK)-induced phosphorylation of Ser188 of RhoA [21•].

In rodents, Ang II-induced hypertension exhibits increased vascular RhoA/Rho kinase activation, without marked changes in expression [14]. This is associated with increased activity of Arhgef1, implicated to be important in RhoA hyperactivation, vasoconstriction, and hypertension [17]. Pharmacologic inhibition of Rho kinase with fasudil or Y27632 suppresses acute pressor responses of Ang II, but does not reduce blood pressure chronically, further supporting the role of RhoA/Rho kinase in acute vasoconstriction, rather than in mechanisms associated with adaptive vascular remodeling that occur chronically with Ang II infusion [12].

Transient Receptor Potential Channels and Vascular Cell Function

Transient receptor potential (TRP) channels are present in both endothelial and vascular smooth muscle cells and contribute to vasomotor control in many vascular beds. They are nonselective, cation-permeable channels, most of which are permeable for Ca^{2+} and constitute a large family of 28 mammalian TRP-related proteins, divided into six subfamilies: the classic TRPCs (TRPC1–7), the vanilloid receptor TRPs (TRPV1–6), the melastatin TRPs (TRPM1–8), the mucolipins (TRPML1–3), the polycystins (TRPP1–

3), and ankyrin transmembrane protein 1 (TRPA1). TRP channels have recently been reviewed [7, 22], so this review will not be detailed. Specific TRP channels are activated by different stimuli, such as vasoactive agents, oxidative stress, mechanical stimuli, and heat [23]. Multiple TRP isoforms are relevant for the regulation of vascular contractility, including TRPC1, TRPC3, TRPC4, TRPC5, TRPC6, TRPV1, TRPV4, TRPM4, TRPM7, TRPP2, and TRPA1 [23]. The contribution to systemic blood pressure regulation has been evaluated only for four TRP isoforms so far: TRPC6, TRPV1, TRPV4, and TRPM4.

Vascular smooth muscle cells express mainly TRPC1, TRPC4, and TRPC6 [23]. TRPC channels open in response to stimulation of plasma membrane receptors that activate PLC, such as Ang II/AT_1R, triggering transmembrane Ca^{2+} influx. Aortic rings from TRPC4 knockout mice displayed impaired endothelium-dependent relaxation in response to acetylcholine. This study demonstrated a direct functional link between endothelial TRPC4 channels and vasomotor tone control [24]. Activation of TRPC3 and TRPC6 channels leads to myocyte depolarization, which stimulates Ca^{2+} entry via voltage-dependent Ca^{2+} channels leading to vasoconstriction [25]. TRPC6 is involved in pressure-induced vascular smooth muscle cell depolarization and vasoconstriction of rat cerebral arteries [25]. TRPC6 has also been identified as a mechanosensor that regulates myogenic vasoconstriction. This effect occurs through ligand-independent activation of AT_1R [26]. In the heart, activation of TRPC7 channels by Ang II is associated with apoptosis and cardiac failure in Dahl salt-sensitive rats [27].

TRPV4-mediated Ca^{2+} entry in endothelial cells is important for steady-state production of NO and for vasoconstriction and vasodilatation of peripheral blood vessels. TRPV4 is regulated by many factors including Ang II/AT1R. Mechanisms linking AT1aR and TRPV4 have recently been demonstrated, where they form a constitutive heterodimer in the membrane [28•]. Moreover β-arrestin 1 interacts with TRPV4 to fine-tune TRPV4-mediated Ca^{2+} influx and $[Ca^{2+}]_i$. Constitutive interaction and cross-talk between TRPV4, AT_1R, and β-arrestin 1 represents a novel vascular regulatory mechanism that ensures rapid and efficient signaling through close proximity of signaling molecules.

Magnesium (Mg^{2+}) is the second most abundant intracellular cation and is involved in the regulation of vascular tone by counteracting effects of Ca^{2+} and through modification of the many ATP-sensitive enzymes involved in the contractile/dilatory machinery in endothelial cells and vascular smooth muscle cells [29]. Decreased Mg^{2+} concentration is associated with endothelial dysfunction, increased reactivity, enhanced contractility, and elevated blood pressure [29, 30]. TRPM6/TRPM7 cation channels have recently been identified as important regulators of Mg^{2+} homeostasis. In a mouse model of hypomagnesemia, TRPM7 was found to be important in endothelial function [31•]. Ang II regulates vascular TRPM7 acutely by inducing phosphorylation and chronically by increasing expression at the mRNA and protein levels [29, 32]. The (patho)physiological significance of this activity awaits further clarification.

TRPM4 and TRPM5 are also implicated in myogenic tone through changes in Ca^{2+} influx. TRPM4 and TRPM5 are highly selective for monovalent cations, and activation of TRPM4 currents in arterial myocytes elicits membrane depolarization, activation of voltage-dependent calcium channels, and vasoconstriction. Recent studies showed the possibility that regulation of TRPM4 activity by PKC could play an important role in the control of myogenic tone under normal conditions and could contribute to disrupted arterial function during pathophysiological situations [33•]. TRPM4 expression is increased in spontaneously hypertensive rats (SHR). Interestingly, TRPM4-deficient mice develop hypertension in a RAS-independent, catecholamine-dependent manner, so TRPM4 has been suggested to limit blood pressure increase [34].

Dual Effects of Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a stable, nonradical, reactive oxygen species produced in endothelial and vascular smooth muscle cells; it acts as a signaling molecule in the regulation of vascular function [35]. Ang II is a potent inducer of vascular H_2O_2 generation, in large part through activation of vascular NADPH oxidases (Noxs), including Nox1, Nox2, Nox4, and Nox5 [36]. The role of H_2O_2 in modulating vascular tone is complex, as studies show conflicting results on vasomotor tone: H_2O_2 can exert either a contractile or a relaxant response depending on the cellular and enzymatic source of H_2O_2, the intracellular compartmentalization of H_2O_2, the vascular bed, and the contractile state [37, 38]. Several mechanisms contribute to H_2O_2-induced vasoconstriction, including an increase in $[Ca^{2+}]_i$ through regulation of L-type Ca^{2+} channels [39, 40•], generation of arachidonic acid metabolites with vasoconstrictor activity, and a direct Ca^{2+}-independent tonic effect on the vascular smooth muscle contractile apparatus. Vascular overexpression of catalase in mice reduced the pressor response to vasoconstrictor agents and decreased blood pressure, suggesting the importance of endogenous H_2O_2 as a vasoconstrictor and regulator of blood pressure. H_2O_2 interacts with PLC, PKC, and phosphoinositide 3-kinase, which may contribute to molecular mechanisms underlying H_2O_2-induced vasoconstriction.

H_2O_2 also exerts vasodilatory effects in vascular cells and has been suggested to be an endothelium-derived

hyperpolarizing factor [41, 42]. H_2O_2 hyperpolarizes and dilates arteries through the opening of Ca^{2+}-activated K^+ channels [43]. Cardiac myocytes can modulate coronary vascular tone through H_2O_2, purinergic components (adenosine and ADP), and Ang II, especially in ischemic conditions. H_2O_2 released from cardiac myocytes induced vasodilatory effects and Ang II released from cardiac myocytes exhibited vasoconstrictor effects in the coronary circulation in response to oxygen supply [44•]. Mechanisms of H_2O_2-mediated vasodilation are complex but probably involve regulation of K^+ channels through direct actions and indirect actions via second messengers.

Endothelial Signaling of Ang II/AT_1R: The Role of HETEs/EETs in Vascular Tone Regulation

Arachidonic acid (AA) is metabolized to 20-hydroxyeicosatetraenoic acids (20-HETEs), epoxyeicosatrienoic acids (EETs), and dihydroxyeicosatetraenoic acids (DiHETEs). These eicosanoids are involved in many diverse physiological and pathophysiological functions, as well as in the regulation of vascular tone and blood pressure. Whereas EETs are recognized as lipid vasodilators that share many NO vascular protective properties, 20-HETE is a potent vasoconstrictor, associated with activation of PKC, Rho kinase, and MAP kinase [45]. Upregulation of 20-HETE production contributes to increased oxidative stress, endothelial dysfunction, vasoconstriction, and peripheral vascular resistance associated with Ang II-induced hypertension [46, 47•].

EETs and DiHETEs counterregulate vasoconstrictor actions of HETEs by mediating vasodilatation through inhibition of Ca^{2+}-activated K^+ channels and by modulating TRP channel activity [47•, 48, 49•]. Because of their vasodilatory and vascular-protective actions, increasing EET production may be an attractive therapeutic strategy for the management of cardiovascular disease [49•].

Conclusions

The mechanical functional response of vascular smooth muscle to Ang II is the summation of vasoconstrictor and vasodilator signals that are integrated at the level of the contractile machinery, mainly through the phosphorylation or the dephosphorylation state of the regulatory light chains of the MLC, which depends on the activity of two key enzymes, the kinase MLCK and the phosphatase MLCP. In addition to changes in $[Ca^{2+}]_i$ through classic G protein–coupled, receptor-mediated activation of PLC and PLD, it is now clear that many signaling molecules, including RhoA/Rho kinase, TRPs, ROS, EETS/HETEs, and others, play a role in Ang II–mediated regulation of vascular tone. Exactly how the pathways network and how signaling molecules interact to control vasoconstriction and vasodilation remain unclear. A greater understanding of the processes that regulate temporal and spatial aspects of contraction, together with the mechanism through which signaling pathways network, will facilitate development of new therapeutic agents to better control vascular tone in vascular disease. Such innovations are already under way, as evidenced by development of novel Rho kinase inhibitors, Nox inhibitors, and EET and HETE modulators. Inhibition of the RAS with ACE inhibitors, AT_1R blockers, and direct renin inhibitors, together with potentially beneficial actions of AT_2R agonists, are also effective strategies to regulate vascular tone, particularly in cardiovascular pathologies.

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Recently published papers of interest have been highlighted as:
- Of importance

1. Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev. 2000;52:639–72.
2. Mehta PK, Griendling KK. Angiotensin II cell signalling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol. 2007;292:C82–97.
3. Lemarié CA, Schiffrin EL. The angiotensin II type 2 receptor in cardiovascular disease. J Renin Angiotensin Aldosterone Syst. 2010;11(1):19–31.
4. Higuchi S, Ohtsu H, Suzuki H, et al. Angiotensin II signal transduction through the AT1 receptor: novel insights into mechanisms and pathophysiology. Clin Sci (Lond). 2007;112:417–28.
5. • Garrido AM, Griendling KK. NADPH oxidases and angiotensin II receptor signaling. Mol Cell Endocrinol. 2009;302(2):148–58. *This is a comprehensive review on Ang II signaling through NADPH oxidase activation.*
6. Di A, Malik AB. TRP channels and the control of vascular function. Curr Opin Pharmacol. 2010;10(2):127–32.
7. Earley S, Brayden JE. Transient receptors potential channels and vascular function. Clin Sci (Lond). 2010;119(1):19–36.
8. Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med. 2010;2(7):247–57.
9. Berridge MJ. Smooth muscle cell calcium activation mechanisms. J Physiol. 2008;586(Pt 21):5047–61.
10. Touyz RM, El Mabrouk M, He G. Mitogen-activated protein/extracellular signal-regulated kinase inhibition attenuates angiotensin II-mediated signaling and contraction in spontaneously

hypertensive rat vascular smooth muscle cells. Circ Res. 1999;84 (5):505–15.
11. Loirand G, Guerin P, Pacaud P. Rho kinases in cardiovascular physiology and pathophysiology. Circ Res. 2006;98:322–34.
12. Loirand G, Pacaud P. The role of Rho protein signaling in hypertension. Nat Rev Cardiol. 2010;7(11):637–47.
13. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature. 1997;389(6654):990–4.
14. Seko T, Ito M, Kureishi Y, et al. Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. Circ Res. 2003;92:411–8.
15. Gohla A, Schultz G, Offermanns S. Role for G12/G13 in agonist-induced vascular smooth muscle cell contraction. Circ Res. 2000;87:221–7.
16. Guilluy C, Bregeon J, Toumaniantz G, et al. The Rho exchange factor Arhgef1 mediates the effects of angiotensin II on vascular tone and blood pressure. Nat Med. 2010;16:183–90.
17. Wirth A, Benyó Z, Lukasova M, et al. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt induced hypertension. Nat Med. 2008;14:64–8.
18. Bregeon J, Loirand G, Pacaud P, et al. Angiotensin II induces RhoA activation through SHP2-dependent dephosphorylation of the RhoGAP p190A in vascular smooth muscle cells. Am J Physiol Cell Physiol. 2009;297(5):C1062–70.
19. Mori K, Amano M, Takefuji M, et al. Rho-kinase contributes to sustained RhoA activation through phosphorylation of p190A RhoGAP. J Biol Chem. 2009;284:5067–76.
20. • Tabet F, Schiffrin EL, Callera GE, et al. Redox-sensitive signaling by angiotensin II involves oxidative inactivation and blunted phosphorylation of protein tyrosine phosphatase SHP-2 in vascular smooth muscle cells from SHR. Circ Res 2008;103 (2):149–58. *This study shows that Ang II regulates SHP2 through redox-sensitive processes that influence SHP2 oxidation and phosphorylation.*
21. • Guilluy C, Rolli-Derkinderen M, Loufrani L, et al. Ste20-related kinase SLK phosphorylates Ser188 of RhoA to induce vasodilation in response to angiotensin II type2 receptor activation. Circ Res 2008;102(10):1265-74. *This study elucidates novel mechanisms whereby RhoA is regulated by Ang II.*
22. Pedersen SF, Owsianik G, Nilius B. TRP channels: an overview. Cell Calcium. 2005;38:233–52.
23. Inoue R, Jensen LJ, Shi J, et al. Transient receptor potential channels in cardiovascular function and disease. Circ Res. 2006;99:119–31.
24. Freichel M, Suh SH, Pfeifer A, et al. Lack of an endothelial store-operated Ca2+ current impairs agonist-dependent vasorelaxation in TRP4–/– mice. Nat Cell Biol. 2001;3:121–7.
25. Brayden JE, Earley S, Nelson MT, et al. Transient receptor potential (TRP) channels, vascular tone and autoregulation of cerebral blood flow. Clin Exp Pharmacol Physiol. 2008;35 (9):1116–20.
26. Mederos y Schnitzler M, Storch U, Meibers S, et al. Go-coupled receptors as mechanosensors mediating myogenic vasoconstriction. EMBO J. 2008;27:3092–103.
27. Satoh S, Tanaka H, Ueda Y, Oyama J, Sugano M, Sumimoto H, et al. Transient receptor potential (TRP) protein 7 acts as a G protein-activated Ca2+ channel mediating angiotensin II-induced myocardial apoptosis. Mol Cell Biochem. 2007;294:205–15.
28. • Arun K, Shukla AK, Kim J, Ahn S, Xiao K, Shenoy SK, Liedtke W, Lefkowitz RJ. Arresting a Transient Receptor Potential (TRP) Channel β-arrestin 1 mediates ubiquitination and functional down-regulation of TRPV4. J Biol Chem 2010;285:30115-25. *This study demonstrates that AT_1R, TRPV4, and β-arrestin 1 form a functional complex that may regulate vascular contraction.*
29. Touyz RM, He Y, Montezano AC, et al. Differential regulation of transient receptor potential melastatin 6 and 7 cation channels by ANG II in vascular smooth muscle cells from spontaneously hypertensive rats. Am J Physiol Regul Integr Comp Physiol. 2006;290:R73–8.
30. He Y, Yao G, Savoia C, et al. Transient receptor potential melastatin 7 ion channels regulate magnesium homeostasis in vascular smooth muscle cells: role of angiotensin II. Circ Res. 2005;96:207–15.
31. • Inoue K, Xiong ZG. Silencing TRPM7 promotes growth/proliferation and nitric oxide production of vascular endothelial cells via the ERK pathway. Cardiovasc Res 2009;83:547–57. *This study highlights the importance of TRPM7 in the regulation of endothelial cell function.*
32. Earley S, Waldron BJ, Brayden JE. Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. Circ Res. 2004;95:922–9.
33. • Gonzales AL, Garcia ZI, Amberg GC et al. Pharmacological inhibition of TRPM4 hyperpolarizes vascular smooth muscle. Am J Physiol Cell Physiol 2010;299(5):C1195-202. *This study demonstrates that TRPM4 is an important regulator of smooth muscle cell membrane depolarization and arterial constriction in response to changes in intraluminal pressure.*
34. Mathar I, Vennekens R, Meissner M, et al. Increased catecholamine secretion contributes to hypertension in TRPM4-deficient mice. J Clin Invest. 2010;120(9):3267–79.
35. Lucchesi PA, Belmadani S, Matrougui K. Hydrogen peroxide acts as both vasodilator and vasoconstrictor in the control of perfused mouse mesenteric resistance arteries. J Hypertens. 2005;23(3):571–9.
36. Montezano AC, Burger D, Ceravolo GS, Yusuf H, Montero M, Touyz RM. Novel Nox homologues in the vasculature: focusing on Nox4 and Nox5. Clin Sci (Lond). 2011;120(4):131–41.
37. Suvorava T, Lauer N, Kumpf S, et al. Endogenous vascular hydrogen peroxide regulates arteriolar tension in vivo. Circulation. 2005;112(16):2487–95. 18.
38. Ardanaz N, Pagano PJ. Hydrogen peroxide as a paracrine vascular mediator: regulation and signaling leading to dysfunction. Exp Biol Med. 2006;231:237–51.
39. Touyz RM. Reactive oxygen species as mediators of calcium signaling by angiotensin II: implications in vascular physiology and pathophysiology. Antioxid Redox Signal. 2005;7(9–10):1302.
40. • Amberg GC, Eraley S, Glapa SA. Local regulation of arterial L-type calcium channels by reactive oxygen species. Circ Res 2010;107(8):1002-10. *This paper demonstrates the novel findings that Ang II induces discrete sites of ROS generation resulting in oxidative activation of PKCα; these in turn promote local sites of enhanced L-type Ca^{2+} channel activity, resulting in increased Ca^{2+} influx and contraction. These findings link Ca^{2+} and ROS in Ang II-mediated vasoconstriction.*
41. Shimokawa H, Matoba T. Hydrogen peroxide as an endothelium-derived hyperpolarizing factor. Pharmacol Res. 2004;49(6):543–9.
42. Matoba T, Shimokawa H, Nakashima M, et al. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest. 2000;106:1521–30.
43. Miura H, Bosnjak JJ, Ning G, et al. Role of hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ Res. 2003;92:e31–40.
44. • Kaneshiro T, Saitoh S, Machii H, et al. Metabolic regulation of coronary vascular tone: role of hydrogen peroxide, purinergic components, and angiotensin. Eur J Pharmacol 2010;645:127–34. *This comprehensive review discusses the role of reactive oxygen species and Ang II in the regulation of vascular tone.*

45. Campbell WB, Falck JR. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. Hypertension. 2007;49:590–6.
46. Williams JM, Murphy S, Burke M, et al. 20-hydroxyeicosatetraeonic acid: a new target for the treatment of hypertension. J Cardiovasc Pharmacol. 2010;56(4):336–44.
47. • Yaghini FA, Song CY, Lavrentyev EN, et al. Ghafoor HU, Fang XR, Estes AM, Campbell WB, Malik KU. Angiotensin II-induced vascular smooth muscle cell migration and growth are mediated by cytochrome P450 1B1-dependent superoxide generation. Hypertension 2010;55(6):1461–7. *This study demonstrates that Ang II regulates vascular smooth muscle cell function through arachidonic acid metabolites that signal through redox-sensitive pathways.*
48. Michaelis UR, Fleming I. From endothelium-derived hyperpolarizing factor (EDHF) to angiogenesis: epoxyeicosatrienoic acids (EETs) and cell signaling. Pharmacol Ther. 2006;111:584–95.
49. • Campbell WB, Fleming I. Epoxyeicosatrienoic acids and endothelium-dependent responses. Pflugers Arch 2010;459 (6):881–95. *This excellent review highlights mechanisms whereby EETs and HETEs modulate vascular tone through effects on endothelial function.*