Hypertension Research (2013) 36, 930–931

www.nature.com/hr

COMMENTARY

RhoGEF-mediated vasoconstriction in hypertension

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Hypertension Research (2013) **36,** 930–931; doi:10.1038/hr.2013.101; published online 22 August 2013

Arterial hypertension is a common health problem that is a major risk factor for many diseases, including myocardial infarction and stroke.[1,2] The pathogenesis of arterial hypertension and the basic mechanisms of blood pressure control are still insufficiently understood. An increase in the concentration of cytoplasmic free Ca^{2+} is thought to be the major trigger for contraction in smooth muscle (Figure 1).[3] This increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) induces Ca^{2+} binding to calmodulin (CaM), and the Ca^{2+}–CaM complex activates myosin light chain kinase to phosphorylate the myosin light chain (MLC) of myosin II. The degree of MLC phosphorylation is the determining factor in smooth muscle contraction: MLC phosphorylation promotes smooth muscle contraction, whereas MLC dephosphorylation, following a reduction in $[Ca^{2+}]_i$, results in muscle relaxation.

It has been revealed that the Ca^{2+} concentration does not always parallel the state of MLC phosphorylation and contraction. The extent of MLC phosphorylation induced by agonists is higher than that caused by a depolarization-induced increase in the Ca^{2+} concentration, so-called Ca^{2+} sensitization.[4] It has been proposed that an additional pathway increases the level of MLC phosphorylation and the degree of contraction independently of the Ca^{2+} concentration. Subsequent studies have revealed that ROCK (also known as Rho-kinase), a major downstream effector of the small GTPase RhoA, has a crucial role in the Ca^{2+} sensitization of smooth muscle contraction.[5,6] ROCK phosphorylates the myosin phosphatase target subunit (MYPT) and inactivates myosin light chain phosphatase, thereby increasing MLC phosphorylation followed by smooth muscle contraction. Accumulating evidence indicates that ROCK inhibitors may be a new treatment for vascular disorders caused by Ca^{2+} sensitization, including hypertension and coronary vasospasm.[7]

The small GTPase RhoA is a molecular switch that cycles between an inactive, GDP-bound state (GDP-RhoA) and an active, GTP-bound state (GTP-RhoA; Figure 2).[8] When cells are stimulated with agonists, GDP-RhoA is converted to GTP-RhoA by Rho guanine nucleotide exchange factors (RhoGEFs), which stimulate the GTP-GDP exchange reaction. GTP-RhoA then interacts with its specific targets, such as ROCK. GTPase-activating proteins act as negative regulators by accelerating the intrinsic GTPase activity of RhoA and converting it back to GDP-RhoA. Although a RhoA/ROCK pathway has an important role in the Ca^{2+} sensitization of smooth muscle, the mechanisms through which RhoA is activated in vascular smooth muscle cells (VSMCs) under conditions of hypertension are unknown. In this commentary, we would like to introduce two studies that suggest a role of RhoGEFs in hypertension.

To study the role of RhoGEF12 (also known as leukemia-associated RhoGEF, LARG) in salt-sensitive hypertension, Wirth *et al.*[9] treated mice with deoxycorticosterone acetate (DOCA) salt. DOCA-salt treatment induced a large increase in blood pressure in wild-type mice within a few days, and this increased hypertensive response was strongly reduced in mice with RhoGEF12 deficiency.

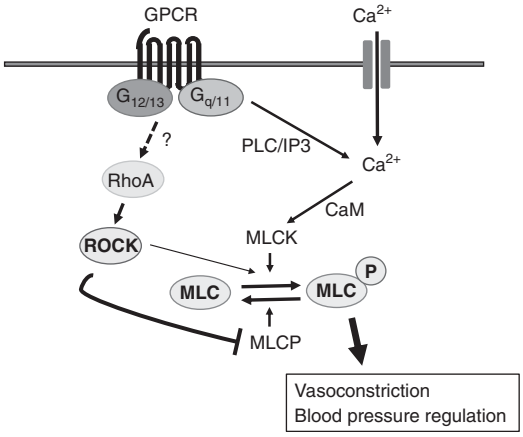

Figure 1 Ca^{2+} signaling and sensitization in vascular smooth muscle cell (VSMC) contraction. Increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) enhance the binding of Ca^{2+} to calmodulin (CaM), and the Ca^{2+}–CaM complex activates myosin light chain kinase (MLCK) to phosphorylate the myosin light chain (MLC) of myosin II, causing smooth muscle contraction (Ca^{2+} signaling). ROCK, a downstream effector of RhoA, phosphorylates the myosin phosphatase target subunit (MYPT) and inactivates myosin light chain phosphatase, thereby increasing the phosphorylation of MLC followed by smooth muscle contraction (Ca^{2+} sensitization). GPCR, G protein-coupled receptor; IP3, inositol-1,4,5-trisphosphate; MLCP, myosin light chain phosphatase; PLC, phospholipase C. A full color version of this figure is available at the *Hypertension Research* journal online.

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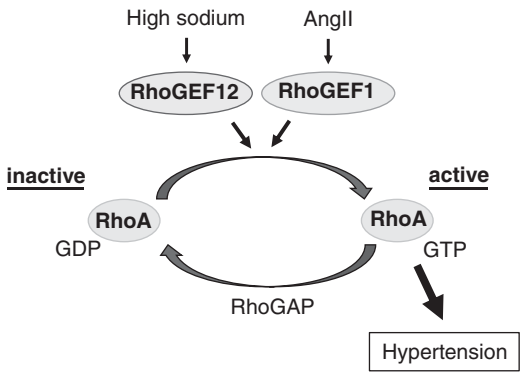

Figure 2 RhoGEFs activate RhoA signaling in hypertension. GDP-RhoA (inactive form of RhoA) is converted to GTP-RhoA (active form of RhoA) by Rho guanine nucleotide exchange factors (RhoGEFs), which stimulate the GTP-GDP exchange reaction. Abnormal activation of RhoA in vascular smooth muscle cells causes hypertension. RhogGEF12 activates RhoA-mediated vasoconstriction in salt-induced hypertension, and RhoGEF1 accelerates vasoconstriction in Ang II-induced hypertension. A full color version of this figure is available at the *Hypertension Research* journal online.

Interestingly, loss of RhoGEF12 did not affect basal blood pressure. RGS domain-containing RhoGEFs, such as RhoGEF1, 11 and 12, are known to bind the G_{12} subfamily ($G_{12/13}$) of heterotrimeric G proteins and be activated. Wirth *et al.*[9] studied the roles of G proteins in DOCA-salt-induced hypertension in mice with smooth muscle-specific $G_{q/11}$ and $G_{12/13}$ deficiency and proposed that RhoGEF12 is activated by $G_{12/13}$ in VSMCs. Both the $G_{q/11}$- and $G_{12/13}$-RhoGEF12-mediated signaling pathways in VSMCs were required for vasoconstriction in salt-induced hypertension; however, only $G_{q/11}$-mediated pathways were necessary to maintain basal blood pressure. These findings suggest that the $G_{12/13}$-RhoGEF12 pathways are potential targets for the treatment of salt-induced hypertension, as they may not affect normal pressure regulation.

Angiotensin II (Ang II) is known as a main mediator of the renin–angiotensin–aldosterone system, which regulates vasoconstriction.[10] Inhibition of the Ang II type 1 (AT1) receptor suppresses the activation of RhoA and ROCK in hypertensive rats, and Ang II infusion increases the activity of RhoA and ROCK in arteries. Guilluy *et al.*[11] have demonstrated that RhoGEF1 (also known as p115RhoGEF or Lsc) is activated by Ang II in VSMCs using affinity pull-down assays with a nucleotide-free RhoA mutant, a method designed to precipitate active pools of Rho-interacting proteins. Minipumps chronically releasing Ang II were placed into wild-type and smooth muscle-specific RhoGEF1-deficient mice to study the role of RhoGEF1 in Ang II-induced hypertension. Basal blood pressure was similar in wild-type and RhoGEF1-deficient mice. Ang II increased blood pressure within 24 h in wild-type mice, but RhoGEF1 deficiency reduced the Ang II-mediated increase in blood pressure. DOCA-salt-induced hypertension, in contrast, was not affected by RhoGEF1 deficiency. Guilluy *et al.*[11] suggested that $G_{q/11}$, not $G_{12/13}$, controls RhoGEF1 activity through phosphorylation of RhoGEF1 by Jak2 in VSMCs stimulated with Ang II. These data indicate that $G_{q/11}$-RhoGEF1 signaling has a crucial role in Ca^{2+} sensitization in Ang II-induced hypertension.

These studies show that RhoGEF pathways induce Ca^{2+} sensitization in VSMCs in response to hypertensive stimuli such as Ang II and salt. Interestingly, RhoGEF1 or RhoGEF12 deficiency reduces vasoconstriction only under the conditions of hypertension and does not affect basal blood pressure. More than 70 RhoGEFs are known in mammalian cells, and there is a high probability that some triggers, such as Ang II and salt, activate specific RhoGEF signaling pathways in hypertension. RhoGEF pathways are potential targets for selective therapeutic approaches to reduce vasoconstriction in hypertension without affecting normal pressure regulation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Staessen JA, Bianchi G. Essential hypertension. *Lancet* 2003; **362**: 1009–1010.
2 Turin TC, Murakami Y, Miura K, Rumana N, Kita Y, Hayakawa T, Okumura T, Okayama A, Ueshima H. NIPPON DATA80/90. Hypertension and life expectancy among Japanese: NIPPON DATA80. *Hypertens Res* 2012; **35**: 954–958.
3 Somlyo AP, Himpens B. Cell calcium and its regulation in smooth muscle. *FASEB J* 1989; **3**: 2266–2276.
4 Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. *Nature* 1994; **372**: 231–236.
5 Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamomori B, Feng J, Nakano T, Okawa K, Iwamatsu A, Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 1996; **273**: 245–248.
6 Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Imui J, Maekawa M, Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 1997; **389**: 990–994.
7 Shimokawa H, Rashid M. Development of Rho-kinase inhibitors for cardiovascular medicine. *Trends Pharmacol Sci* 2007; **28**: 296–302.
8 Schmidt A, Hall A. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev* 2002; **16**: 1587–1609.
9 Wirth A, Benyo Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S, Orsy P, Horvath B, Maser-Gluth C, Greiner E, Lemmer B, Schuetz B, Gutkind JS, Offermanns S. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. *Nat Med* 2008; **14**: 64–68.
10 Mori H, Yamamoto H, Ukai H, Yuasa S, Nakajima K, Mikawa T, Niizuma M, Hirao K, Umemura S, COMPATIBLE Study Group. Comparison of effect of angiotensin II receptor blocker on morning home blood pressure and cardiorenal protection between morning administration and evening administration in hypertensive patients: the COMPATIBLE study. *Hypertens Res* 2013; **36**: 202–207.
11 Guilluy C, Brégeon J, Toumaniantz G, Rolli-Derkinderen M, Retailleau K, Loufrani L, Henrion D, Scalbert E, Bril A, Torres RM, Offermanns S, Pacaud P, Loirand G. The Rho exchange factor Arhgef1 mediates the effect of angiotensin II on vascular tone and blood pressure. *Nat Med* 2010; **16**: 183–190.