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COMMENTARY

Endothelium-derived hyperpolarizing factor and hypertension

Jun-ichi Oyama and Koichi Node

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Hypertension is highly prevalent worldwide including Japan and is one of the major cardiovascular risk factors. Regulation of vascular tone is essential to maintain organ perfusion and cardiovascular homeostasis. Vasodilation in response to increased flow is the function of the normal endothelium through the secretion of nitric oxide (NO), prostacyclin (PGI_2) and endothelial-derived hyperpolarizing factor (EDHF) to promote the relaxation of vascular smooth muscle cells.[1,2] Reduction in these mechanisms contributes to aspects of endothelial dysfunction in several diseases, including hypertension, atherosclerosis and diabetes. Increased levels of reactive oxygen species including superoxide anions and hydrogen peroxide are recognized in patients with hypertension and contribute to vascular damages and dysfunction.[3] Therefore, impaired endothelial function results in impaired vasodilation and increased blood pressure and may be a common pathway to hypertension, which is influenced by the imbalance of oxidative stress and endothelial bioactivity.[4] EDHF is known to have an important role predominantly in modulating vasomotor tone, especially in resistance microvessels, whereas in the large conduit vessels, such as the aorta, endothelium-dependent responses are selectively mediated by NO.[5] As small resistance vessels contribute to regulate vascular resistance, EDHF is supposed to be important in pathogenesis of hypertension. As loss of NO was compensated by the upregulation of EDHF in hypertensive rats, the role of EDHF might be much more important for the patients with hypertension.[6] Prysyazhna *et al.*[7] revealed that single atom mutation in protein kinase G (PKG) eliminated oxidant sensing induced by hydrogen peroxide (H_2O_2), a major component of EDHF, which leads to hypertension.

Dr Jun-ichi Oyama and Dr K Node are at the Department of Cardiovascular Medicine, Saga University, Saga, Japan
E-mail: junoyama@cc.saga-u.ac.jp

EDHF AND HYDROGEN PEROXIDE

EDHF causes vascular relaxation by opening Ca^{2+}-activated K channels and then hyperpolarizing membrane potential of vascular smooth muscle. EDHF is synthesized not only upon stimulation by agonists but also by shear stress, and its synthesis and release are stimulated by an increase in intracellular calcium in the endothelium, although calcium-independent endothelial cell hyperpolarization has also been reported. NO and vasodilator prostaglandins elicit hyperpolarization of underlying vascular smooth muscle and NO may activate large conductance K_{Ca} channels in some blood vessels; however, those responses to NO and vasodilator prostaglandins are largely inhibited by the inhibition of ATP-sensitive potassium (K_{ATP}) channels. Importantly, substantial endothelium-dependent hyperpolarization exists even after the blockade of the synthesis of NO and vasodilator prostaglandins. Therefore, EDHF is thought to be different substances from prostaglandins or NO, and it is classically defined that EDHF-mediated responses are the endothelium-dependent responses (relaxations and hyperpolarizations) after the blockade of the synthesis of vasodilator prostaglandins and NO.

Although the nature of EDHF has not been fully elucidated, different EDHFs could exist depending on species, blood vessels and the size of blood vessels with different hyperpolarizing mechanisms involved.[8,9] Since the first report for the existence of EDHF, several candidates have been proposed for the nature of EDHF, including epoxyeicosatrienoic acids, metabolites of arachidonic P450 epoxygenase pathway, K ions and electrical communication through myoendothelial gap junctions.[10–15] Shimokawa[16] demonstrated that H_2O_2 is an EDHF in mouse and human mesenteric arteries and in porcine and canine coronary microvessels. They also demonstrated that endothelial Cu,Zn-superoxide dismutase has an important role in the synthesis of H_2O_2 in mouse and human mesenteric arteries and that endothelial NO synthase system is involved in the synthesis of H_2O_2/EDHF. Therefore, redox-sensitive regulatory pathway and responses to oxidative stress may be crucial in functional properties with EDHF. It has been accepted that H_2O_2 is a major component of EDHF in several vascular beds in multiple species, including human and H_2O_2 derived from the endothelium possess the protective role against cardiovascular system. However, the precise mechanisms for regulation and function of H_2O_2 in response to oxidative stress remain unclear.

HYDROGEN PEROXIDE AND PKG-I-ALPHA

PKG-I-alpha forms an interprotein disulfide linking its two subunits in cells exposed to exogenous H_2O_2, which directly activated the kinase and the affinity of the kinase for substrates it phosphorylates was enhanced by disulfide formation. This activation represents an alternate mechanism for regulation besides the classical activation of NO-cyclic guanosine monophosphate (cGMP) pathway. This mechanism underlies cGMP-independent vasorelaxation in response to oxidants in the cardiovascular system and provides a molecular explanation for how hydrogen peroxide can operate as an EDHF.

In this manuscript, the authors demonstrated the importance of PKG-I-alpha

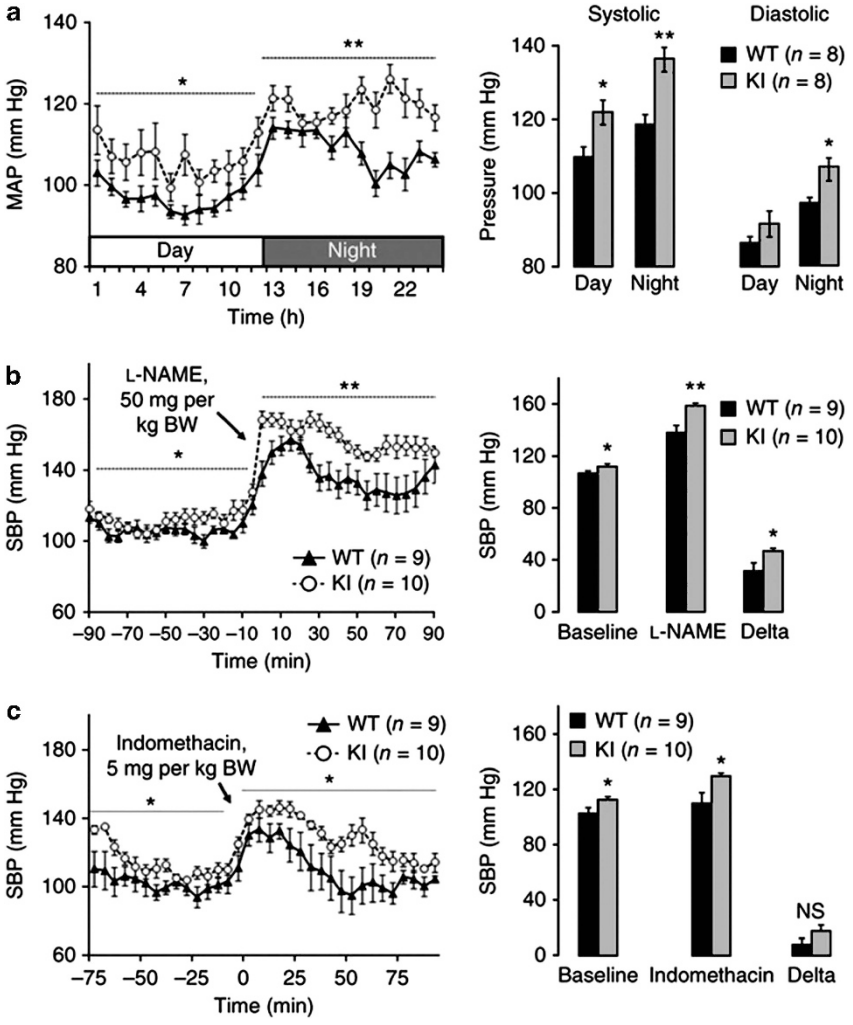

Figure 1 Knock-in (KI) mice are hypertense compared with wild type (WT) littermates.[7] (**a**) *In vivo* telemetric blood pressure monitoring comparing KI mice with WT littermate controls during the day and night. Data are presented as mean arterial pressure over time or as the time-averaged mean systolic and diastolic pressure in WT and KI mice. (**b**) Comparison of the blood pressure response to intraperitoneal injection of L-NAME in WT and KI mice. (**c**) Comparison of the blood pressure response to intraperitoneal injection of indomethacin in WT and KI mice. $^{*}P<0.05$, $^{**}P<0.01$. Reprinted by permission from Macmillan Publishers Ltd. Nature Medicine, copyright 2012.

oxidation on EDHF mechanism and regulation of blood pressure. The authors have generated a knock-in (KI) mouse expressing the PKG-I-alpha C42S mutation ('redox-dead' version of PKG-I-alpha) in mice and compared the vasorelaxation response of mesenteric arteries from wild-type (WT) and KI mice to H_2O_2. They demonstrated that H_2O_2 induced hyperpolarization and formed dimer of PKG-I-alpha in WT murine mesenteric vessels, which were blocked in KI mice. Vasorelaxation in responses to acetylcholine with or without combination of NO synthase inhibition and cyclooxygenase inhibition based on EDHF protocol was recognized in WT but not in KI mice in consistence with disulfide formation of PKG-I-alpha.

The blood pressure in KI mice was higher than that in WT mice, measured by telemetric monitoring *in vivo* (Figure 1). Moreover, decreased cardiac output was found in KI mice without any changes of heart weight, size of vessels and fibrosis, which could be explained by an adaptive mechanism to limit hypertension. As activation of PKG phosphorylates potassium channels to cause vessel hyperpolarization and relaxation, the findings presented by Prysyazhna *et al.*[7] are interesting and certain could suggest that single atom substitution of PKG causes hypertension. The polymorphisms of $p22^{phox}$ subunit of NADPH oxidase is frequently recognized among hypertensive patients, which suggests a possible role of these single-nucleotide polymorphisms in the development of hypertension.[17] However, it is still uncertain that this substitution of PKG-I-alpha is important for the patients with hypertension and processes the crucial role in hypertensive disease in human.

In conclusion, the emerging role of EDHF in the pathophysiology and pathogenesis of hypertension needs to be further elucidated in clinical fields.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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