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H_2O_2 AS A REGULATOR OF VASCULAR CONTRACTION

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Abstract: The reactive oxygen species, hydrogen peroxide (H_2O_2), hydroxyl radical (*OH) and superoxide anion (O_2*$^-$), participate as signal molecules in the paracrine regulation of vascular wall function. The role of H_2O_2-producing enzymes' (NADPH oxidases) activation and the involvement of H_2O_2 in arterial smooth muscle contraction under physiological and pathological conditions are discussed in details. Pleiotropic signal pathways as activation of Ca^{2+} entry through L-type and non-L-type voltage gated Ca^{2+} channels, different types K^+ channels, COX-derived thromboxane A_2 receptor agonist and NADPH oxidase-derived superoxide, as well as stimulation of MAP kinase, Rho kinase and soluble guanylate cyclase, play a key role in the amplification of H_2O_2 triggered signal mechanisms. Thus, H_2O_2 may either increase or decrease the artery contraction depending on the species and vascular bed by multiple intracellular targets. The increasing amount of new data gives rise to the conception of the important role of H_2O_2 as a regulator of vascular functions in health and under different pathological conditions like diabetes, atherosclerosis, obesity, aging, etc. The participation of oxygen containing reactive nitrogen species as peroxynitrite (ONOO*$^-$) and nitric oxide (NO) in vascular function is also reviewed.

The reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and superoxide anion (O_2^{*-}) are not only reactive by-products of metabolism, but also participate as signal molecules in the paracrine regulation of vascular wall function (Santiago et al. 2013). An important source of H_2O_2 are oxidative reactions that initially produce O_2^{*-} by several cellular enzymes − NADPH oxidase, xanthine oxidase, nitric oxide synthase, lipoxygenase and enzymes of the respiratory chain of mitochondria (Brandes & Kreuzer, 2005; Fukai & Ushio-Fukai, 2011). In many cases a generation of O_2^{*-} by NADPH oxidases triggers the production of ROS by other enzymes (Landmesser et al., 2003). Thus, O_2^{*-} can be transformed in H_2O_2 by superoxide dismutases (SOD) (Fukai & Ushio-Fukai, 2011). On the other hand, nitric oxide (NO) can be rapidly inactivated by a reaction with O_2^{*-} leading to the appearance of the strong oxidant peroxynitrite ($ONOO^-$).

Different NADPH oxidase isoforms are expressed in the circulatory system and the radical generation in response to identical stimuli may vary between cell types (for review see Brandes & Kreuzer, 2005). NADPH oxidases of vascular beds are activated by different mechanisms. G-protein-coupled receptor for angiotensin II was the first GPCR discovered to increase NADPH oxidase activity in vascular smooth muscle cells via a mechanism that involves stimulation of protein kinase C (PKC), Src kinase, EGF receptor trans-activation, and subsequent activation of phosphoinositol-3 kinase that further activates Rac (Agrawal et al., 2004). The small GTPase Rac is activated by many other cardiovascular signal molecules (Marikovsky et al., 2002), including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), tumour necrosis factor alpha (TNF-α), thrombin and lysophosphatidyl choline (Brandes & Kreuzer, 2005), and in all these cases plays a common role in NADPH oxidase activation (Price et al., 2002).

SOD enzymes are the major antioxidant defence systems against O_2^{*-}, which convert O_2^{*-} into H_2O_2. There are three SOD isoforms in mammals. SOD1, the major intracellular SOD that exists as a 32 kDa homodimer in the cytosol or in the intermembrane space of mitochondria. SOD2 is a mitochondrial manganese-containing homotetramer, which is localized in the mitochondrial matrix (Fridovich & Freeman, 1986), and SOD3 is a secretory extracellular Cu/Zn-containing SOD anchored to the extracellular matrix via binding to heparan sulphate proteoglycans, collagen, or fibulin-5 (for review see Fukai & Ushio-Fukai, 2011). SOD3 is the major SOD in the vascular extracellular space and its amount is high in blood vessels (Ookawara et al. 1998). Vascular SOD3 is localized mainly between the endothelium and the muscle layer and its major source is smooth muscle cells (Stralin et al., 1995). SOD3 plays a crucial role in preventing the destruction of endothelial NO (Fukai & Ushio-Fukai, 2011) and thus SOD is important for the NO-mediated vascular relaxation. Pathophysiology of atherosclerosis, aging, diabetes and others are associated with a decline in the availability of endothelium-derived NO (Kojda et al., 1999). SOD3 is well-expressed in fat cells (Ookawara

et al. 1998) and fibroblasts (Marklund, 1990) that are main cellular component of vascular adventitia and are involved in the adventitial regulatory function (Eringa et al. 2012, Gollasch 2012; Kwan et al., 2010).

The endothelial, adventitial and smooth muscle cells generate ROS, which are important for the human health because these molecules are involved in the pathogenesis of many cardiovascular disorders. ROS, generated by endothelium include superoxide (O_2^{*-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (*OH) as well as oxygen containing reactive nitrogen species (RNS) as peroxynitrite ($ONOO^{*-}$) and NO (Li et al. 2004). H_2O_2 (10 mM) induces Ca^{2+}- and MLC_{20} phosphorylation-independent contraction in pulmonary and systemic arterial and venous smooth muscle (Pelaez et al., 2000). In rabbit mesenteric artery H_2O_2 increases concentration-dependently (3-300 μM) the synthesis of prostaglandins that hyperpolarize the smooth muscle cell membrane by activating ATP-sensitive K^+ channels (Itoh et al., 2003). It was suggested that in this tissue SOD increased the acetylcholine-induced, endothelium-dependent relaxation most probably by H_2O_2-enhanced action of NO in the smooth muscle layer. In rat proximal coronary artery pre-constricted with the thromboxane A_2 agonist U44619, H_2O_2 (3-300 μM) increases the force of contraction in a concentration-dependent manner. Distal segments of the same artery relax in the presence of H_2O_2 (30-300 μM) while rat mesenteric artery contracts in the presence of 1 and 3 μM H_2O_2 and strongly relaxes at 10 μM H_2O_2 (Santiago et al., 2013). H_2O_2 is an endothelium-dependent mediator in rat coronary arteries that activates smooth muscle Ca^{2+} entry through L-type and non-L-type voltage gated Ca^{2+} channels, COX-derived thromboxane A_2 receptor agonist and NADPH oxidase-derived superoxide, as well as stimulates the MAP kinase and Rho kinase pathways (Santiago et al. 2013). H_2O_2 is a transferable endothelium-derived hyperpolarizing factor mediating the flow-induced dilation in human coronary arterioles via large K_{Ca} channel activation of the smooth muscle cells (Liu et al. 2011). Recently, Wong et al. (2014) presented data for the participation of small and intermediate K_{Ca} channels in H_2O_2 and endothelium-dependent relaxation of female porcine coronary arteries. In rat tail artery, H_2O_2 increases the force of contraction in the presence of intact endothelium. This effect is mediated by activating of thromboxane A_2 receptors and by increasing the contribution of α_2-adrenergic receptors (Reardon et al. 2013). Perivascular adipose tissues from coronary and mesenteric arteries augment contractions to KCl (20 mM) of swine coronary artery by a potential dependent mechanism (Owen et al., 2013). In arteries pre-contracted with 20 mM KCl from lean animals with PVAT, H_2O_2 (1 mM) relaxes the studied vessels to a lesser extent than the same kind of vessels isolated from obese animals. Additionally, adventitial fat decreases the dilatory effect of H_2O_2 on coronary arteries in lean pigs to a less extent, if compare to the same vessels from obese pigs (Owen et al., 2013). Perivascular adipose tissue of rat aorta produces H_2O_2 which relaxes isometric preparations of this artery by an endothelium-independent

activation of soluble guanylate cyclase and Ca^{2+}-activated K^{+}-channels of smooth muscle cells (Gao et al., 2007). Therefore, H_2O_2 may either increase or decrease the contraction of arteries depending on the species and vascular bed by diverse regulatory mechanisms and multiple intracellular targets.

Chronic high glucose level causes a rise in ROS derived mainly from glycolytic and mitochondrial metabolism (Brownlee, 2001). Modulation of the cellular effects of high glucose on oxidant-sensitive pathways may have important implications for the regulation of proximal insulin signal transduction in the tissues of patients with hyperglycaemia due to type 1 or type 2 diabetes mellitus (Wu et al., 2005). In contrast, insulin-induced ROS involve an activation of NADPH oxidases (Mahadev et al., 2004). In human adipocytes insulin activates the plasma membrane NADPH oxidase by $G_{\alpha i2}$ (Krieger-Brauer et al. 1997). Thus, insulin initiates a rapid production of H_2O_2, which causes the oxidative inhibition of thiol-dependent protein-tyrosine phosphatases, enzymes that regulate the insulin action cascade and enhances the tyrosine phosphorylation of proteins in many cell types (Mahadev et al. 2001a, b). Therefore, under conditions of high glucose, the generation of cellular oxidant molecules is enhanced, and this is potentiated in the presence of insulin that stimulates by several signal pathways, including protein kinase C (Wu et al., 2005). Additionally, a crosstalk between adrenaline and insulin signalling pathways with a link to H_2O_2 mediation was identified in isolated adipocytes (de Piña et al. 2008). Three experimental evidences were considered: (i) insulin stimulates a plasma membrane NADPH oxidase to increase the production of H_2O_2 in adipocytes (Mahadev et al. 2001b); (ii) H_2O_2 mimics insulin action in some of its effects (Visentin et al. 2003); and (iii) H_2O_2 inhibits the activation of cyclic AMP-activated protein kinase (PKA) by a stable cyclic AMP (cAMP) analogue in isolated adipocytes (Gaudiot, et al.2000) thus preventing the physiological action of cAMP on the enzyme (de Piñaet al. 2008). Therefore, by the generation of H_2O_2, insulin regulates PKA activation in adipose cells. The mechanism of this H_2O_2 effect most probably involves oxidation of Cys residues in PKA holoenzyme, as revealed by the use of an H_2O_2-insensitive PKA catalytic subunit (Humphries et al. 2005).

In conclusion, H_2O_2 in vascular wall influences the arterial smooth muscle contraction by pleiotropic mechanisms. An increasing body of data supports the notion of the important role of H_2O_2 as a regulator of vascular functions in health and under different pathological conditions.

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