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Redox Control of Vascular Smooth Muscle Function

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THIS FORUM EDITION of *Antioxidants & Redox Signaling* highlights several issues related to the impact of reactive oxygen (ROS) and nitrogen species (RNS) on vascular smooth muscle (VSM) biology, with special emphasis on cellular signaling. VSM cells represent an important component of the vascular wall. Situated in the medial layer of blood vessels, VSM cells are normally quiescent and express a differentiated phenotype that serves to generate and maintain vascular tone (19). In contrast, many vascular diseases, including atherosclerosis, postangioplasty restenosis, in-stent restenosis, posttransplant coronary arteriopathy, and vascular hyporesponsiveness during sepsis, are associated with dysfunctional VSM function. Responding to inflammatory cues, VSM cells may switch to a proliferative, migratory, and synthetic phenotype that underlines many features of these conditions. At the same time, many if not all of these conditions are associated with a change in redox balance, and ROS and RNS production. The implications of these changes are increasingly studied within the context of vascular biology and, more specifically, of VSM signaling.

A feature common to many signaling pathways in eukaryote cells is their ability to induce the intracellular production of ROS and RNS that are required for proper functioning and integration of cell-signaling pathways. Seminal work by Sundaresen *et al*. almost 15 years ago revealed that the transitory increase in hydrogen peroxide (H_2O_2) induced by platelet-derived growth factor (PDGF) was necessary to obtain full activation of extracellular signal-regulated kinases (ERKs) (24). This first study was performed in VSM cells. Since then, great strides have been made in elucidating the mechanisms by which ROS are produced in VSM cells, their mechanism of action with respect to VSM signaling pathways, and their role in health and disease in general. Similarly, studies over the past 30 years have revealed that binding of the free radical nitric oxide (NO) to the heme-group of soluble guanylate cyclase (sGC) stimulates the conversion of GTP to cGMP, the intracellular messenger molecule that ultimately mediates many NO-dependent cellular activities (26). The cGMP-dependent effects of NO are mediated through different cGMP-sensitive proteins including serine/threonine cGMP-dependent kinases (PKGs) or, in some cases, through cross-activation of cAMP-dependent protein kinases on excessive production of cGMP (6, 15). In smooth muscle cells, the NO/cGMP/PKG pathway contributes to relaxation (18) and smooth muscle–specific gene expression (1, 29). Inhibition of VSM cell migration and proliferation may be attained through cGMP-dependent pathways but also in the absence of sGC if sufficient amounts of NO are provided (9). More recent studies are now focusing on delineating the implications of cGMP-independent NO (and other RNS) pathways on VSM function (25).

A key approach to delineating the role of ROS and RNS in VSM signaling has been the characterization of specific intracellular sources including NADPH oxidase (Nox) and nitric oxide synthase (NOS). Although the expression of nNOS and iNOS in VSMs has been established for some time, new isoforms of Nox have been discovered over recent years, in addition to the phagocytic $gp91^{phox}$ (Nox2) (10). These include Nox1, 4, and 5, and their expression in VSM cells are now known to vary between species, vascular beds, and normal and diseased vessels (2). The recent availability of new transgenic mouse strains, at least for Nox1, is now allowing a more-detailed elucidation of the physiologic relevance of these isoforms within the context of vascular disease (11, 16). A current issue is to understand the molecular mechanism by which different Noxs may coexist in VSM cells and integrate distinctly different functions. The review article by San Martín and Griendling extensively covers this issue within the context of VSM migration and ROS signaling, an area of research that has been understudied relative to other aspects of ROS and VSM biology (20). The research article by Miller *et al*. illustrates one possible mechanism by which the specificity of ROS signaling in VSM may be derived from the compartmentalization of ROS formation and formation of specific ligand–receptor complexes (17). They show that TNF-α–mediated ROS production in cultured VSM cells requires Nox1-mediated production of endosomal ROS. In contrast, the thrombin-induced ROS production is not endosomal, but still requires the activation of Nox1, in this case, through trans-activation of the EGF receptor. The specific molecular mechanisms that would explain such dichotomy are yet to be uncovered.

The other primary source of ROS (and RNS) is the mitochondrial electron-transport chain (ETC). The physiological importance of the ETC in modulating VSM function is clearly delineated in hypoxic pulmonary vasoconstriction (HPV), a process during which pulmonary arteries constrict in response to hypoxic exposure (23). The Schumacker laboratory proposed in 1998 that ETC-derived ROS represent an upstream signal in the response to hypoxia (3). This initial work was extended over the years by the demonstration that the increase in ROS in pulmonary smooth muscle is necessary to

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trigger the release of Ca^{2+} from the sarcoplasmic reticulum to induce contraction. The review article by Wang and Zheng provides a thorough review of this field and extends to important questions related to the role of alternate sources of ROS, including Nox and the redox activation of specific cellular targets, such as protein kinase C (27). Two research articles in this Forum add to the role of ETC-derived ROS in regulating intracellular Ca^{2+} in pulmonary artery smooth muscle cells (PASMCs). In the first one, Desireddi *et al*. studied the role of ROS signaling in the hypoxic response in PASMCs in mouse precision-cut lung slices (7). This approach allows the study of ROS signaling in a normal cell–cell environment in contrast to isolated cell-culture experiments and, combined with a ratiometric indicator of ROS (RoGFP), they demonstrate a direct association between hypoxic ROS production and changes in intracellular Ca^{2+} in this *in situ* model. In the second one, Chi *et al*. demonstrate the utility of the same ratiometric ROS sensor in evaluating the effect of prolonged hypoxia on ROS production and its potential relation to Ca^{2+} sensitization (4). The relation between redox-sensitive pathways and Ca^{2+} signaling extends beyond that of HPV and PAMCs. VSM cells are endowed with channels, pumps, and transporters that regulate intracellular Ca^{2+} concentration to control contraction and other VSM functions, including cell migration and proliferation. Because of the recent advances in Ca^{2+} and ROS signaling in VSM, it is now possible to envision that these two systems are well integrated. A thorough review of the research in this area is provided by Trebak *et al*. in this Forum issue (25). These authors also expand on the concept that reactive species derived from NO may share many common biochemical pathways with ROS in modulating Ca^{2+}-dependent VSM function.

It is generally implied that the functional effect of ROS (and RNS) is a direct one, serving as intracellular mediators to affect intracellular signaling pathways that are involved in modulating VSM-cell function, such as migration and proliferation. However, it is becoming evident that pathologic increase in ROS/RNS production by VSM cells also is associated with indirect paracrine and autocrine effects leading to the redox-dependent production of secretory molecules, including growth factors, chemokines, and cytokines. In this Forum, Satoh *et al*. summarize a decade-long work by the Berk laboratory to delineate one such pathway (21). They elucidated a novel pathway in which the immunophilin cyclophilin A is expressed and secreted by VSMs in a redox-dependent manner to promote VSM cell growth, endothelial cell apoptosis and vascular inflammation. The therapeutic implications of such pathway related to ROS production and vascular disease are further highlighted by their recent observation that cyclophilin A also increases ROS production in the vasculature and contributes to abdominal aortic aneurysms (22).

Last, an important theme from this Forum is the impact of aging on the molecular mechanisms that underlie vascular diseases and its relation to redox regulation of VSM function. Age-related changes in VSMs have now been documented, and epidemiologic studies have clearly established an association between aging and the increased prevalence of cardiovascular diseases (28). In this issue, Li and Fukagawa thoroughly review the literature, examining specifically the association between aging, gene regulation, and redox control in VSMs (14). This group has been instrumental in delineating age-associated changes in VSM function related to the Akt/FOXO3a and ERK1/2 and the implications of redox balance in this context (12, 13).

It is clear that VSM cells exhibit large phenotypic variations at different developmental stages, in diseased vessels, and under normal conditions, depending on the blood vessel type and vascular bed (19). Understanding the contribution of redox active systems to the mechanisms underlying phenotypic plasticity of VSM cells should be an important endeavor. One recent example of such contribution is the observation that Nox4 is required to maintain VSM cells in a differentiated phenotype (5). As such, the development of cell- or tissue-specific molecular strategies aimed at manipulating redox signaling elements *in vivo* in VSMs will be of importance.

Finally, the large majority of animal studies and some human studies support a role for ROS and RNS in vascular disease (8). It is hoped that a further understanding of the molecular mechanisms that control redox-dependent signaling pathways in VSMs will contribute to refining and directing redox-based therapeutic strategies.

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Abbreviations Used

cGMP = cyclic guanosine monophosphate
EGF = epidermal growth factor
ERK = extracellular signal-regulated kinase
ETC = electron-transport chain
FoxO = forkhead box O
H_2O_2 = hydrogen peroxide
HPV = hypoxic pulmonary vasoconstriction
NO = nitric oxide
NOS = nitric oxide synthase
Nox = NADPH oxidase
PASMCs = pulmonary artery smooth muscle cells
PDGF = platelet-derived growth factor
PKG = cGMP-dependent protein kinase
RNS = reactive nitrogen species
ROS = reactive oxygen species
sGC = soluble guanylate cyclase
TNF-α = tumor necrosis factor α
VSM = vascular smooth muscle

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