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eNOS phosphorylation: a pivotal molecular switch in vasodilation and cardioprotection?

Rakesh C. Kukreja* and Lei Xi

Division of Cardiology, Department of Internal Medicine, Virginia Commonwealth University, Richmond, VA 23298-0281, USA

Nitric oxide (NO) is a ubiquitous signaling molecule that regulates various cellular physiological and pathological events in virtually all vital organs. The synthesis of NO is catalyzed primarily by three isoforms of NO synthase (NOS), which were originally named after the tissues in which these enzymes were first characterized and cloned (i.e. nNOS - neuronal, iNOS - inducible or inflammation-related, and eNOS - endothelial) [1]. Accumulating evidence suggests that all of the three NOS isozymes are expressed and distributed in various cell types in the cardiovascular system far beyond their initial identified cell types and locations. Besides the extensive studies on transcriptional control of NOS expression and the Ca^{2+} -dependent activation of eNOS and nNOS, there has been an increasing recognition that post-translational modification of NOS may effectively modulate the enzyme activity which in turn tightly controls the cellular viability and function through NO production at the right time, right location, and right amount [2]. Among these post-translational regulation mechanisms of eNOS, the kinase-mediated protein phosphorylation plays a critical role [3,4]. Several studies were simultaneously published in 1999, which provided direct evidence that eNOS activity may be substantially modulated through phosphorylation by Akt/PKB (Protein kinase B)[5-8] or AMPK (AMP-activated protein kinase)[9]. More recently, there have been a number of studies on the regulatory role of eNOS phosphorylation in determining the enzyme activity, independently or interplaying with the sub-cellular localization of eNOS [10,11].

In the current issue of Journal of Molecular and Cellular Cardiology, Mount and colleagues have provided a timely update on multi-site eNOS phosphorylation, its active role in regulating NO synthesis under various cardiovascular physiological and pathological processes [12]. The authors reviewed the current knowledge on eNOS phosphorylation including a complex picture of the phosphorylation site-specific effects on eNOS enzyme activity. The discussion was particularly focused on each of the known eNOS phosphorylation sites (namely serine 114, 615, 633, 1177 and threonine 495) and their regulation by a number of kinases (including Akt/PKB, AMPK, PKA, PKC, PKG, and CaMK-2) in response to a variety of pathophysiological or pharmacological stimuli [12]. In addition, the authors discussed about the biological significance of eNOS phosphorylation in cardiovascular system and there is no need to repeat all the details in this editorial commentary. Nevertheless, as the authors pointed out, the current understanding of multi-

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***Address for Correspondence:** Rakesh C Kukreja, PhD Professor of Medicine, Physiology, Biochemistry and Emergency Medicine Eric Lipman Chair of Cardiology Division of Cardiology, Box 980281 Virginia Commonwealth University 1101 East Marshall Street, Room 7-046 Richmond, VA 23298, USA Telephone: (804) 828-0389 Fax: (804) 828-8700 E-mail: rakesh@vcu.edu.

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sites eNOS phosphorylation in the heart remains incomplete [12] and there is only limited information available concerning the eNOS phosphorylation sites other than serine 1177 in cardiomyocytes. This indeed requires further investigations in future.

Focusing on the role of eNOS in cardioprotection against ischemia-reperfusion injury, there are still few key issues remain unsolved. For instance, despite the well recognized role played by NO in triggering and/or mediating cardioprotection induced by ischemic or pharmacological preconditioning [13-17], the relative contribution of iNOS versus eNOS-derived NO to the NO-mediated cardioprotection is still unclear. Akt-mediated eNOS-Ser¹¹⁷⁷ phosphorylation was associated with the treatment of various cardioprotective modalities such as ischemic pre- and post-conditioning [18-20] and agents including insulin [21], statins [22-25], bradykinin [26], phosphodiesterase-5 (PDE-5) inhibitor, sildenafil (Viagra) [27,28] and peroxisome proliferator-activated receptor- γ (PPAR- γ) agonist, rosiglitazone [29]. A recent study also showed that sildenafil prolonged erection and increased the protein expressions of phosphorylated eNOS at serine 1177 and phosphorylated Akt at serine 473 in penis of aged rats [30]. These results suggest a potential role for eNOS phosphorylation in the induction of cardiovascular protection (Figure 1). On the other hand, genetic knockout of eNOS only partially attenuated the cardioprotective effects induced by classic ischemic preconditioning [31] or by acute pharmacological preconditioning with sildenafil [14]. These findings indicate that there is a redundant system controlled by iNOS and/or nNOS to exert the NO-mediated cytoprotective effects even when eNOS is completely knocked out. It would be interesting to clarify how the three NOS isozymes do interact or interplay among each other in order to achieve the cardioprotective phenotypes.

In addition to the potential cardioprotective effects triggered by eNOS phosphorylation, recent studies also indicated a detrimental or pro-inflammatory role of eNOS phosphorylation [32,33]. It has been shown that Akt-mediated eNOS phosphorylation played a causal role in the pathogenesis of endotoxic shock [33] as well as anaphylactic shock [32]. Under these pathological situations, genetic deletion of eNOS was proved to be protective against the lethal hypotensive reaction caused by eNOS-derived or -triggered overproduction of NO [32,33]. This is another typical example of the dual role of NO in cardiovascular pathophysiology.

In summary, it is anticipated that the review article by Mount et al. [12] would stimulate further investigations in delineating the precise role of eNOS phosphorylation in cardiovascular homeostasis and protection against pathological stressors. Recent studies have shown that many other vital biological processes such as cellular aging [34] and stem cell-based cellular regeneration and repair [35] are likely dependent upon eNOS and its phosphorylated status, which may represent new areas of research in cardiovascular biology and medicine.

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Figure 1.