NO with no NOS in ischemic heart

Raymond K. Kudej, Christophe Depre *

Cardiovascular Research Institute, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry New Jersey, New Jersey Medical School, 185 South Orange Avenue, MSB G-609, Newark, NJ, United States

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See article by Martin et al. [5] (pages 46–55) in this issue.

The seminal observation by Furchgott and Zawadzki [1] of a paradoxical vasoconstrictive effect of acetylcholine in arteries deprived of endothelium paved the way for the rapid discovery of nitric oxide (NO) and of the mechanisms of NO generation by a specific family of isoforms known as NO synthases (NOS). The role of NO in the endothelium was characterized as a predominant mechanism of vasodilation through the production of cyclic GMP (cGMP) in vascular smooth muscle cells. The rapid progress of this research in endothelial cells automatically led to the question whether the cardiac cell itself could be a source of NO.

It was found that the three characterized isoforms of NOS are expressed in cardiac myocytes [2]. However, the role of NO in the myocardium, and the regulation of its production, seems much more complex than what was described before in the endothelium, and includes both cGMP-dependent and -independent mechanisms. Myocardial NO is involved in cellular processes as diverse as cardiac contractility, Ca\(^{2+}\) handling, mitochondrial respiration, substrate metabolism, generation of radical oxygen species, and ischemic preconditioning among others (see Fig. 1). In addition, the mechanisms of action of NO are diverse and sometimes contradictory. For example, depending on the experimental setting, blocking cardiac NOS can be either beneficial or deleterious for the ischemic heart [3,4]. Part of this complexity comes from the fact that NO production in the heart is performed by the three different isoforms (endothelial, inducible and neuronal) that differ in their properties, both qualitatively and quantitatively, for several reasons. First, the sub-cellular distribution of these enzymes varies (see Fig. 1). Whereas the endothelial NOS is described in plasma membrane microdomains known as caveolae, the inducible isoform is mainly soluble, and the neuronal isoform is bound to Ca\(^{2+}\)-regulatory proteins of the sarcolemma and of the sarcoplasmic reticulum [2]. Both the inducible and neuronal isoforms are also present in mitochondria. Another reason for the diversity stems from the fact that the three NOS isoforms have different kinetic properties (the inducible isoform producing much more NO than the constitutive enzymes), and that the biological effect of NO is largely dependent on its concentration. A third reason is that the activity of NOS can be manipulated by post-translational modifications (see Fig. 1), as exemplified by the endothelial NOS, which is activated both by binding to the heat shock protein Hsp90 and upon phosphorylation by Akt.

Adding to this diversity, the study by Martin et al. [5] published in this issue of Cardiovascular Research further confirms a provocative pathway by which cardiac NO can be produced independently from NOS in ischemic heart, which supports previous observations of this phenomenon in different cardiac preparations [6,7]. Numerous studies have shown that NO production increases in the heart submitted to ischemia. Although it was assumed that this production of NO would originate from NOS [8], it is worth reminding that the complex enzymatic reaction catalyzed by NOS requires O\(_2\) as a substrate, a molecule which, by definition, is cruelly lacking during myocardial ischemia. The authors devoted a large part of their study to the characterization and validation of an interesting and reliable method to measure this production of NO in the interstitium of the ischemic myocardium. The study, of course, generates more questions than it may answer, including the mechanisms, localization and role of NOS-independent NO production. As for the mechanism involved, Martin et al. postulate several possibilities that largely relate to increased nitrite...
degradation in a context of altered redox status that accompanies the acidosis of myocardial ischemia. Several studies, performed in various tissues including the heart, have shown that nitrite may serve as a generator of NO under the proper conditions, and it is very likely to be the case in the present setting [9]. Another question relates to the site of production of this pool of NO. This question is more difficult to address because both the myocardial and vascular compartments are susceptible to generate NO without NOS in the appropriate conditions. Considering the fact that NO diffuses rapidly, the question also relates to the buffering mechanisms that will prevent this NO production from being excessive.

A more important question that comes to mind relates to the role of this "no-NOS" NO. It remains unknown whether this pool of NO follows a specific purpose, or whether it is the consequence of a chemical serendipity that maintains NO production in the absence of oxygen. It is likely that this source of NO has specific goals, considering the conditions in which it is produced, i.e., acidosis and altered redox potential. There is a possibility that, under such conditions, NO will generate peroxynitrite, which is regarded as a highly toxic molecule [10]. However, the reader should be reminded, as pointed out by the authors in their Introduction, that a burst of NO production during ischemia may also activate pro-survival mechanisms, such as the modulation of caspase activity or the opening of ATP-dependent K+ channels. Another potential mechanism of cardioprotection could result from the capacity of NO to block specific exchangers, such as the Na+/H+ and the Na+/Ca2+ exchangers (see Fig. 1). A previous observation reporting this NOS-independent production of NO from nitrite in the ischemic heart confirms its potential cytoprotective role [7].

Finally, intact cardiac sympathetic nerves have been shown to be beneficial during myocardial ischemia and reperfusion via a mechanism that involves the generation and disposition of NO and reactive oxygen species [11]. It is interesting to note that the increase in interstitial NOS-independent NO concentration during ischemia demonstrated in the study by Martin et al. mirrors the nonexocytotic catecholamine release from sympathetic nerves during prolonged ischemia [12]. Initially, the metabolic alterations induced by ischemia reduce catecholamine release from central sympathetic stimulation. However, following more prolonged ischemia (> 10 min), local nonexocytotic norepinephrine release prevails. Therefore, cardiac sympathetic nerves may, either directly or indirectly, also be involved in the generation of NOS-independent NO during ischemia.

In summary, the study by Martin et al. reopens a frequently overlooked Pandora’s box in this area of research, i.e., the possibility of producing NO during ischemia independently of NOS activation. The specific purpose of producing NO with no NOS in that context will certainly be the objective of future investigations. As mentioned above, previous reports point to the potential cytoprotective effect of such mechanism. Although the authors relied on a chemical inhibitor to exclude the role of NOS, a biological rather than a pharmacological approach might be preferred to further examine the role and regulation of NOS-independent NO production.
in the heart. Several knockout mouse models with NOS deletion are available that might provide some help in these investigations. However, further characterization of the role of both NOS-dependent and -independent NO generation during ischemia should also include translation into clinical practice, e.g., the benefits or risks of hemoglobin-based oxygen carriers, which scavenge NO, during episodes of myocardial ischemia [13].

References


