The roles of nitric oxide (NO) in the heart have been studied intensely ever since the first report nearly 2 decades ago showing that endogenous NO modulates cardiac myocyte function.1 All 3 NO synthase (NOS) isoforms have been found in mammalian heart tissues (see reviews in Massion et al2 and Belge et al3). A complex array of myocyte and nonmyocyte cells in the heart express NOS isoforms, and the local generation of NO and reactive oxygen species (ROS)4 may exert both autocrine and paracrine effects on cellular function. Not only is the endothelial isofrom of NOS (eNOS, or NOS3) robustly expressed in the endothelial cells of the cardiac vasculature, but eNOS is also present within cardiac myocytes, where the enzyme associates with the scaffolding/regulatory protein caveolin-3 in T tubules in plasmalemmal caveolae.5 The neuronal NOS (nNOS, or NOS1) is also expressed in cardiac myocytes, where the enzyme appears to be localized in the sarcoplasmic reticulum and modulates phospholamban phosphorylation.6 Although both eNOS and nNOS appear to be physiologically expressed in cardiac tissues, the inflammation-related NOS isofrom (iNOS, or NOS2) only appears in the heart after immunoinactivation; iNOS may modulate the decline in myocardial function seen in sepsis.7 There have been innumerable studies of the cardiac effects of various NOS inhibitors, NO-donating drugs, and NO itself.8 The cardiac phenotypes of NOS “knockout” mouse models have been characterized extensively, and mice lacking 1, 2, or all 3 NOS isoforms have been generated and characterized.8 In addition, the roles of NOS substrates, cofactors, and allosteric modulators have been explored exhaustively in diverse cardiac model systems. Emerging from this broad range of experimental evidence is an increasingly clear view that NO and NOS are key determinants of cardiac function. Yet the molecular mechanisms and pathways whereby this ubiquitous, reactive, and pluripotent signaling system modulates cardiac contraction and relaxation remain incompletely defined.

Purified NOS can produce O2−/H11002 when the key NOS cofactor BH4 is oxidized to form dihydrobiopterin (BH2); importantly, O2−/H11002 production from purified NOS is not substantially affected by the NOS substrate arginine or by most arginine-based NOS inhibitors.11,12 Since the discovery of O2−/H11002 production from purified NOS, the term “NOS uncoupling” has become broadly used to describe any of a variety of experimental systems in which an increase in ROS production is seen in cells or tissues that contain NOS. Yet is this extrapolation from the catalytic properties of purified NOS to the complex cellular milieu entirely justified? Sometimes yes, sometimes no: It is not clear that O2−/H11002 formation in cells containing NOS perforce represents NOS uncoupling. Some studies have inferred a role for NOS uncoupling when observing effects of arginine or arginine

The Complexities of Coupling Nitric Oxide Synthase Pathways in the Heart

Thomas Michel, MD, PhD

NO Way to Relax

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

From the Cardiovascular Medicine Division, Brigham and Women’s Hospital, Harvard Medical School, Boston, Mass.

Correspondence to Thomas Michel, MD, PhD, Cardiovascular Division, Brigham and Women’s Hospital, Harvard Medical School, 75 Francis St, Thorn Bldg, Room 1210A, Boston, MA 02115. E-mail thomas_michel@harvard.edu

(Circulation. 2010;121:484-486.)

© 2010 American Heart Association, Inc.

Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIR.0b013e3181d1e24e
modulates both physiological and pathophysiological responses of the mitochondria (Figure) as a consequence of normal cellular oxidative metabolism. Molecular oxygen is, of course, the ultimate source for ROS in most mammalian cells, \( \text{H}_2\text{O}_2 \), and the highly unstable superoxide \( \text{O}_2^- \). The major source for ROS in most mammalian tissues is not “uncoupled” NOS; rather, ROS principally derive from mitochondria (Figure) as a consequence of normal cellular oxidative metabolism. Molecular oxygen is, of course, the ultimate acceptor of the electrons that flow through mitochondria, yielding \( \text{H}_2\text{O} \) as a final product. But along the way, a 1-electron reduction of \( \text{O}_2 \) can yield (sequentially) the ROS superoxide \( \text{O}_2^- \), hydrogen peroxide \( \text{H}_2\text{O}_2 \), and the highly unstable hydroxyl radical (\( \text{HO}^- \)) before \( \text{H}_2\text{O} \) is finally formed. It is estimated that up to 1% of the oxygen that is consumed in cellular respiration leads to the formation of such ROS, a fact that has important implications for NOS and NO signaling in many cellular systems. The most abundant and stable of these ROS in most mammalian cells, \( \text{H}_2\text{O}_2 \), is a key signaling molecule that promotes eNOS activation in endothelial cells. \( \text{O}_2^- \) is rapidly reduced by superoxide dismutases to form \( \text{H}_2\text{O}_2 \), but \( \text{O}_2^- \) can also rapidly interact with NO to form peroxynitrite, which itself is a reactive oxidant molecule with important cellular effects. NO can also interact directly with mitochondrial electron transport proteins, and under some conditions, it may influence cellular respiration and ATP synthesis in the heart. There are other physiological interactions between NOS pathways and ROS in cardiovascular tissues that may play key roles in oxidative metabolism. For example, eNOS appears to play a role in mitochondrial biogenesis, and NO and NO adducts appear to modulate cardiac mitochondrial respiration. Recently, eNOS was reported to exert a tonic inhibitory effect on \( \text{H}_2\text{O}_2 \) synthesis in cultured endothelial cells. That study reported an increase in endothelial cell \( \text{H}_2\text{O}_2 \) synthesis after NOS enzyme inhibition, a situation sometimes ascribed to NOS “uncoupling.” Yet small interfering RNA–mediated eNOS knockdown in endothelial cells also led to enhanced \( \text{H}_2\text{O}_2 \) synthesis, which argues strongly against eNOS serving as the \( \text{H}_2\text{O}_2 \) source in these cells. Indeed, the increase in \( \text{H}_2\text{O}_2 \) synthesis after eNOS knockdown was blocked by small interfering RNA–mediated knockdown of the AMP-activated protein kinase and related proteins in the AMP-activated protein kinase pathway, which implicates cellular phosphorylation pathways in at least some of the NOS-related changes in ROS formation (see Figure). Although the implications of these observations for organismal \( \text{H}_2\text{O}_2 \) homeostasis have not yet been established, such findings raise caveats about ascribing ROS production to NOS uncoupling without pursuing additional analyses. Moreover, NOS uncoupling can be influenced by factors independent of BH4. For example, posttranslational modifications may play a role in NOS-dependent \( \text{O}_2^- \) synthesis: Chen and colleagues have shown that phosphorylation of eNOS at serine 1177 increases \( \text{O}_2^- \) synthesis by the enzyme, whereas eNOS phosphorylation at threonine 495 does not. These findings suggest that some of the effects of BH4 oxidation on NOS-dependent ROS synthesis may reflect changes in the NOS phosphorylation state. Several of the studies that have explored the pathways whereby BH4 ameliorates diastolic dysfunction or endothelial dysfunction have provided evidence that implicates the renin-angiotensin system in the pathophysiological response. Angiotensin II modulates ROS production in diverse cardiovascular tissues and has been shown to stimulate eNOS-derived \( \text{O}_2^- \) production, which in turn is suppressed by BH4. The vascular dysfunction seen in models of diabetes, hypercholesterolemia, and hypertension is associated with enhanced oxidation of BH4 to BH2 and can be restored in vivo by BH4 supplementation. Moens and colleagues have elegantly documented that administration of BH4 can ameliorate the cardiac hypertrophy and myocardial fibrosis seen in a model of pressure overload, in association with an improvement in oxidative stress. These investigators provided evidence that indicated that the effects of BH4 were due to effects on NOS pathways in cardiac myocytes but did not involve activation of cGMP-dependent pathways in the heart. The study by Silberman et al provides evidence implicating...
the renin-angiotensin system in the diastolic dysfunction observed in this model system, accompanied by changes in phospholamban phosphorylation in cardiac myocytes that may reflect the involvement of oxidant-modulated phosphorylation pathways in the lusitropic response. Unraveling the complex relationships between BH4 metabolism and the numerous physiological and pathophysiological pathways in the vascular wall and in the heart, including the renin-angiotensin system, NOS catalysis, cardiac function, cellular redox balance, myocardial hypertrophy, and endothelial signaling, all remain active and important areas of investigation.

The central observation of the report by Silberman et al,9 namely, that BH4 and related redox pathways play a key role in cardiac diastolic function, represents an important addition to our growing appreciation of the salutary biological effects of BH4 in cardiovascular homeostasis.23,24,27 These observations have formed the basis for several clinical trials that have been exploring the therapeutic effects and safety of BH4 and related compounds in cardiovascular disease states. A clinical trial of the BH4 analog 6R-BH4 (sapropterin) for treatment of systemic hypertension failed to show efficacy for the drug, according to information released by the drug’s manufacturer (see www.bmn.com), but many other trials of BH4 and related compounds are ongoing. Current phase II clinical trials of BH4 and related compounds (listed at www.clinicaltrials.gov) include studies of endothelial dysfunction, peripheral vascular disease, sickle cell disease, coronary artery disease, systemic hypertension, endothelial dysfunction, pulmonary artery hypertension, chronic kidney disease, and cardiac diastolic function. These clinical trials are likely to provide critical information on the safety and efficacy of BH4 in cardiovascular therapeutics. At the same time, insights are emerging from ongoing basic research studies into the molecular mechanisms that control BH4-modulated cellular pathways, a confluence of research efforts that represents an exemplar of clinical science traveling from bench to bedside and back again.

Disclosures

None.

Sources of Funding

This work was supported in part by National Institutes of Health grants GM56259, HL46457, and HL48743 to Dr Michel.

References


Key Words: Editors ▪ tetrahydrobiopterin ▪ nitric oxide synthase ▪ free radicals ▪ diastole ▪ myocytes ▪ oxygen
NO Way to Relax: The Complexities of Coupling Nitric Oxide Synthase Pathways in the Heart
Thomas Michel

Circulation. 2010;121:484-486; originally published online January 18, 2010; doi: 10.1161/CIR.0b013e3181d1e24e

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/121/4/484

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/