

EDITORIAL COMMENT

Hydrogen Peroxide and Metabolic Coronary Flow Regulation*

John M. Canty, JR, MD, FACC,†‡§

Vijay S. Iyer, MD, PhD†§

Buffalo, New York

Myocardial perfusion is closely matched to increases in myocardial metabolism, because the extraction of oxygen is near maximal at rest (1). The net vascular resistance to coronary blood flow represents the integration of systolic compressive effects that vary throughout the cardiac cycle, with microvascular resistance networks that adjust tone in response to local physical factors (intraluminal pressure and luminal shear stress), vasodilator metabolites, autocooids, and adrenergic tone. The local microcirculatory resistance adjustments overcome compressive forces to match transmural variations in myocardial oxygen supply to demand in the steady state. Studies in conscious animals have demonstrated considerable redundancy in the mediators responsible for integrative coronary flow regulation such that multiple pathways need to be impaired before flow is altered at normal coronary pressures (2). In contrast, the importance of individual pathways (e.g., endothelial nitric oxide) can be unmasked by evaluating coronary regulation distal to a severe stenosis (3,4). Despite intensive study, however, the precise mediators responsible for initiating metabolic coronary vasodilation remain unknown (1).

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Flow-mediated vasodilation in resistance arteries and arterioles is a generalized mechanism that can match segmental resistance of the local coronary microcirculation to meet changes in downstream demand that occur during increased myocardial metabolism. Although this is governed by local shear stress and is endothelium dependent, the

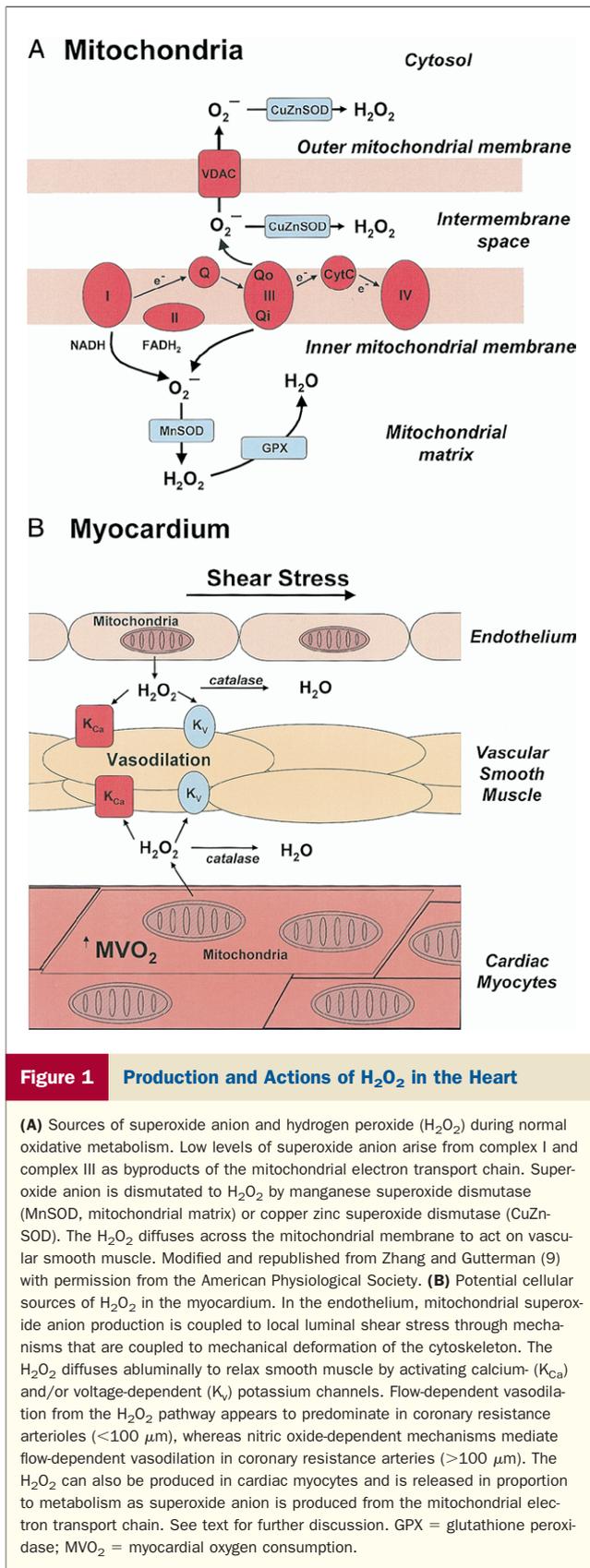
mechanisms responsible for flow-mediated vasodilation vary among different organs. Even within the heart, mediators vary in different vascular segments as well as with the magnitude and pulsatile characteristics of flow (5–7). Whereas initial studies focused upon the role of nitric oxide as an endothelium-dependent vasodilator, there is increasing evidence that endothelium-dependent hyperpolarizing factors (EDHFs) are important in some microcirculatory segments. Hyperpolarizing mechanisms vary with age, species, and coexisting disease processes that up-regulate this pathway when nitric oxide-dependent mechanisms are deficient and are particularly important in coronary arterioles isolated from patients (8). A number of different mediators have been identified as candidate EDHFs, including epoxyeicososatreinoic acid metabolites and hydrogen peroxide (H_2O_2) formed from the dismutation of superoxide anion.

Reactive oxygen species have traditionally been viewed as deleterious byproducts of metabolism, but there is accumulating evidence that at lower concentrations, they also serve as important cellular signaling molecules in the coronary endothelium as well as in cardiac myocytes (9). Superoxide anion is continually released from mitochondria at a number of points in the electron transport chain as a byproduct of oxidative metabolism (Fig. 1). This unstable radical is converted to H_2O_2 via superoxide dismutase (SOD) localized within the mitochondria (MnSOD) or the extracellular space (CuZnSOD). Matoba et al. (10) were the first to demonstrate that H_2O_2 is an endothelium-derived hyperpolarizing factor in mice. Subsequent studies by Miura et al. (8,11) demonstrated that H_2O_2 was an endothelium-dependent hyperpolarizing factor that was responsible for flow-mediated vasodilation in isolated human coronary arterioles and could be blocked by the scavenger catalase. Liu et al. (12) demonstrated that H_2O_2 produced in response to flow was a byproduct of complex I and complex III of the endothelial mitochondrial electron transport chain.

In this issue of the *Journal*, Yada et al. (13) present an extensive series of in vivo experiments that demonstrate the importance of H_2O_2 in adjusting coronary resistance vessel tone during pacing-induced increases in myocardial oxygen consumption in open-chest anesthetized dogs. Measurements of coronary flow and myocardial oxygen consumption were coupled with microcirculatory measurements of arteriolar diameter during pacing. As previously demonstrated (5), the vasodilation to pacing was not affected by inhibiting nitric oxide synthase with L-nitro monomethyl arginine. Both catalase, which inactivates H_2O_2 by converting it to water, and inhibiting adenosine with 8-sulfophenyltheophylline (8-SPT) blunted the arteriolar vasodilation to pacing (arterioles $<100 \mu m$) but did not affect larger resistance arteries ($>100 \mu m$). Collectively, the results extend previous observations during autoregulation (14) and suggest a differential mechanism for flow-induced vasodilation with nitric oxide predominating in

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From the Departments of †Medicine and ‡Physiology and Biophysics, Veterans Administration Western New York Health Care System, Buffalo, New York; and the §Center for Research in Cardiovascular Medicine, State University of New York, Buffalo, New York. Supported by the Veterans Administration, the National Heart, Lung, and Blood Institute (HL-55324, HL-61610), and the Albert and Elizabeth Rekatte Fund.



large resistance arteries and H₂O₂-mediated hyperpolarization predominating in small arterioles.

How does H₂O₂ cause vasodilation? A number of studies have demonstrated that H₂O₂ opens calcium-activated potassium channels (K_{Ca}) to cause smooth muscle relaxation. Activation of K_{Ca} channels causes efflux of potassium from within vascular smooth muscle, resulting in membrane hyperpolarization and closure of voltage-dependent Ca²⁺ channels. The reduction in Ca²⁺ influx leads to vascular relaxation. Experimental support implicating K_{Ca} channels include in vivo as well as in vitro studies using pharmacologic blockade, where relaxation and smooth muscle hyperpolarization can be blocked by antagonists such as tetraethylammonium (TEA). Merkus et al. (15) demonstrated that blocking K_{Ca} channels with TEA attenuates metabolic coronary vasodilation during exercise in conscious pigs (15). It is also possible that other potassium channel subtypes contribute to this response. For example, Rogers et al. (16) demonstrated that H₂O₂ activates voltage-dependent potassium channels which can be selectively blocked using 4-aminopyridine to inhibit the vasodilation to pacing in vivo. Whereas pharmacologic approaches are limited by drug specificity for certain channel subtypes, the available evidence supports the notion that both subtypes of potassium channels may be involved in mediating the effects of H₂O₂. The molecular interactions between H₂O₂ and potassium channels leading to vasodilation are currently under investigation.

The major limitation of the study of Yada et al. (13) is that the specific cellular source of H₂O₂ cannot be clearly identified. This is admittedly difficult in vivo, but the in vitro assessment of free radical production provided suggests that this arises in the arteriolar endothelium. Because hyperpolarization can occur in isolated arterioles removed from the myocardium, endothelial shear stress may be the primary stimulus (Fig. 1). The precise mechanism coupling shear stress to increased superoxide production and H₂O₂ in endothelial cells has not been established, but it may arise from mechanical deformation of the cytoskeleton. If the actions of H₂O₂ arise solely from shear stress, a different downstream metabolic mediator would still be needed to trigger the initial release of H₂O₂ in response to increases in tissue perfusion. The present data suggest that this could be adenosine, because arteriolar dilation during increased metabolism was blocked by 8-SPT. Smaller arterioles, beyond digital camera resolution, could also be the segment responsive to initial metabolic stimuli.

An alternative explanation for the link between H₂O₂ and metabolic flow regulation, proposed by Saitoh et al. (17), is that H₂O₂ arises from cardiac myocytes rather than the endothelium. This is attractive in coupling the production of this mediator to myocardial metabolism, because production of H₂O₂ is directly proportional to the production of superoxide anion as energy is produced via the mitochondrial electron transport chain. Saitoh et

al. (17) demonstrated that superoxide anion production is increased in proportion to increases in cardiac metabolism using electron paramagnetic spectroscopy in isolated cardiac myocytes, leading to a concomitant increase in H_2O_2 production in vitro. In vivo, increases in metabolism elicited by pacing or catecholamine infusion were accompanied by increases in myocardial H_2O_2 levels and perfusion. Blocking the effects of H_2O_2 using 4-aminopyridine to block voltage-dependent potassium channels shifted relationships among oxygen consumption, coronary flow, and coronary venous pO_2 , confirming physiologically relevant effects on the regulation of myocardial perfusion.

A cardiomyocyte origin of H_2O_2 could also explain why metabolic flow regulation continues in disease states associated with intrinsically reduced myocardial metabolism and coronary flow at rest, such as heart failure (18) and hibernating myocardium (19). The lower metabolism and set point for superoxide release would attenuate baseline H_2O_2 production yet preserve the relation between relative increases in metabolism and H_2O_2 . These in vivo observations, in conjunction with the results of Yada et al. (13), strengthen support for a broad role for H_2O_2 in metabolic coronary flow recruitment. Although additional studies are needed to clarify the independent role that H_2O_2 produced from endothelial cells (coupled to shear stress) versus myocytes (coupled to metabolism) plays in normal and pathophysiologic states, we now appear closer to identifying the critical link between myocardial metabolism and coronary blood flow.

Reprint requests and correspondence: Dr. John M. Canty, Jr., Division of Cardiovascular Medicine, State University of New York at Buffalo, Biomedical Research Building, Room 347, 3435 Main Street, Buffalo, New York 14214. E-mail: canty@buffalo.edu.

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