Mitochondrial NOS is upregulated in the hypoxic heart: implications for the function of the hypertrophied right ventricle

Jayan Nagendran and Evangelos D. Michelakis

Pulmonary Hypertension Program, University of Alberta, Edmonton, Canada

CARDIAC METABOLISM AND MITOCHONDRIAL function regulate energy utilization and thus cardiac performance in both normal and disease states. Changes in the fuel utilization of the heart during development and under disease states have been described extensively, and metabolic modulation of the heart is an evolving area of therapeutics (18).

Nitric oxide (NO) can affect heart function in multiple ways, although NO-mediated regulation of metabolism via the newly described mitochondrial nitric oxide synthase (mtNOS) is less studied and understood. In 1994, Cleeter et al. (5) showed that NO can inhibit mitochondrial respiration by reversibly competing for the O2 binding site on complex IV (cytochrome c oxidase) of the electron transport chain. This leads to uncoupling of respiration from oxidative phosphorylation and mitochondrial membrane hyperpolarization [increased membrane potential (∆Ψm)] (2). As fuels (carbohydrates and lipids) come into mitochondria, they enter the tricarboxylic acid cycle, forming electron donors; in turn, these donate electrons that are passed down a redox gradient from complex I to IV (Fig. 1). During that process, H+ is pumped out of the inner membrane, creating an electrical gradient known as ∆Ψm. As the H+ is returning back into the mitochondrial matrix, the stored energy of the ∆Ψm is used by the ATP synthase to produce ATP and deliver it to the cytoplasm. In addition to the regulation of respiration and energy production, NO binding to complex IV can also regulate apoptosis: the resulting increase in ∆Ψm increases the opening threshold of the voltage-gated megachannel through which proapoptotic mediators can leak into the cytoplasm and induce apoptosis (the mitochondrial transition pore), thus suppressing apoptosis. Although extracellular or cytoplasmic NO is highly diffusible and can easily reach the mitochondria, it first must go through a number of redox macro- and microenvironments. Hence the finding that NO can be produced “on the spot” by a mtNOS can have important implications for our understanding of myocardial function in health and disease.

In this issue of the American Journal of Physiology: Heart and Circulatory Physiology, Zaobornyj et al. (22) report that mtNOS activity is significantly upregulated in rat hearts from animals exposed to the chronic hypoxia of high altitude. They also show that sildenafil reverses this mtNOS upregulation. This is an important observation that might have implications in 1) cardiac ischemia (severe hypoxia) or anoxia, and 2) cardiac hypertrophy, since the rat heart, as the human heart, responds to hypoxia-induced pulmonary hypertension with significant right ventricular (RV) hypertrophy (RVH). It is uncertain whether the increase in mtNOS activity that the authors reported was a direct result of hypoxia (and thus occurred in both ventricles) or an adaptive response to RVH (and thus restricted within the RV). Unfortunately, the authors did not study the two ventricles separately. Because most of the heart mass in the hypoxic hearts came from the hypertrophied RV, it is reasonable to speculate that this was at least a feature of RVH. This is in keeping with Kanai et al. (11) who showed that mtNOS activity was increased in the diseased myocardium of a cardiomyopathy model of dystrophin-deficient knockout mice. Nevertheless, the authors’ findings are important, showing that a dynamic regulation of mtNOS occurs in the transition from healthy to diseased myocardium. There are several other examples of such a dynamic regulation of signaling in the RV.

In contrast to the diseased left ventricle (LV), there is a paucity of papers published on the diseased RV, despite the fact that it represents a critical problem in everyday clinical practice. The hypertrophied RV (in contrast to the hypertrophied LV, LVH) cannot respond adequately to an increased afterload. For example, as the afterload increases in the LV (i.e., systemic hypertension), LVH can support a compensated asymptomatic state, with stable cardiac output for decades. In contrast, even smaller increases in the RV afterload (i.e., pulmonary hypertension) lead to RV failure, suppression of cardiac output acutely (for example, in a large pulmonary embolus or peroperatively in heart/lung transplant surgery), and very limited survival of only a few years for acquired pulmonary arterial hypertension (PAH) (20).

Therefore, the mechanisms regulating RV contractility and long-term metabolic remodeling need to be studied, since they cannot be merely extrapolated from the LV. Indeed, the LV and RV are inherently different, even in their embryology (21). In hypoxia-induced RVH, there are metabolic transcriptional differences that occur between the two ventricles (1). While in few circumstances RVH may be adaptive (as seen in the neonatal RV that is normally hypertrophied in utero), most often RVH is maladaptive (as seen in RVH from acquired PAH). The differences between physiological and pathological RVH remain unknown. However, there are recently described molecular features of RVH that appear to be directly relevant to RV performance and contractility (Fig. 1). RVH is characterized by a marked increase in the ∆Ψm (14), a marked increase in phosphodiesterase-5 (PDE5) expression and activity (13), and increased glucose uptake [measured by 18-fluorodeoxyglucose (FDG)-positron emission tomography (PET) imaging] (16). Drugs that inhibit these processes, for example, dichloroacetate (DCA), which reverses the increase in ∆Ψm, or sildenafil, which inhibits PDE5, can optimize performance by increasing RVH contractility (13, 14). Improved performance is associated with a reversal of the metabolic remodeling (i.e., decreased glycolysis) as shown by a decrease in glucose uptake (16). Here we suggest that the described increase in mtNOS activity might be involved in all of these processes and may be central to the biology of RVH.

Address for reprint requests and other correspondence: E. D. Michelakis, Dept. of Medicine (Cardiology) Univ. of Alberta, Edmonton, Canada (e-mail: evangelos.michelakis@capitalhealth.ca).
Pyruvate dehydrogenase kinase upregulation in RVH: A brake in RVH performance. Recent evidence suggests that the metabolic adaptations in RVH are based on mitochondrial remodeling (14). There is a dynamic and “dose-dependent” increase in the ΔΨᵢ (i.e., the more the RVH, the higher the ΔΨᵢ) (14) (Fig. 1). Interestingly, these changes are also observed in cancer (3, 4). The mechanism for the increase in ΔΨᵢ is based on an upregulation of glycolysis, which is seen in both RVH (6) and cancer (8). The “gate-keeping” enzyme coupling the cytoplasmic glycolysis to the mitochondrial glucose oxidation is pyruvate dehydrogenase kinase (PDK). PDK phosphorylates and inhibits pyruvate dehydrogenase to decrease the conversion of pyruvate to acetyl-CoA, and thus limit the entry of carbohydrates in the mitochondria and the tricarboxylic acid cycle. The decreased entry of pyruvate in the mitochondria results in increased glycolysis (and lipid oxidation via the Randle cycle) (18). Because glycolysis is much less efficient energetically than glucose oxidation, the cardiomyocyte compensates by significantly increasing glucose uptake (18). Thus the shift toward glycolysis in RVH is observed by increased glucose uptake shown by FDG-PET imaging (Fig. 1); this metabolic shift normalizes when RVH resolves (16). An inhibitor of PDK, DCA, is shown to reverse the mitochondrial remodeling in both RVH (14) and cancer (3). DCA decreases ΔΨᵢ toward the normal levels and increases coupling of glycolysis to glucose oxidation in both cancer (3) and RVH, where it also leads to increased RVH contractility (14). Interestingly, because PDK is much less active in the normal compared with the hypertrophied RV, DCA does not have significant effects in the normal RV (14).

PDE5 upregulation in RVH: A brake in RVH performance. In human and rat RVH, there is a significant upregulation of PDE5 expression and activity (Fig. 1) (13). Acute PDE5 inhibition by sildenafil leads to increased contractility in RVH, in a manner similar to DCA (13). A potential mechanism for the increase in contractility by sildenafil may be linked to decreased ΔΨᵢ, since PDE5 inhibition also leads to opening of mitochondrial ATP-dependent K⁺ (KᵢATP) channels to depolarize ΔΨᵢ (15), and cGMP-mediated inhibition of phosphodiesterase-3 (PDE3) to activate protein kinase A (PKA) to increase RVH contractility (13). Mitochondrial nitric oxide synthase (mNOS) might also be a therapeutic target, since its inhibition might lead to decreased ΔΨᵢ and increased contractility, like with DCA and sildenafil. sGC, soluble guanylate cyclase; NO, nitric oxide; PKG, protein kinase G.

Fig. 1. Left: I, acutely treated right ventricle (RV) biopsies with the mitochondrial membrane potential (ΔΨᵢ)-specific stain tetramethylrhodamine methyl ester (TMRM) (where red fluorescence suggests higher ΔΨᵢ) show that ΔΨᵢ is significantly increased in RV hypertrophy (RVH) compared with the normal RV (13); II, immunohistochemistry of RV biopsies showing increased phosphodiesterase-5 (PDE5) expression (green) in RVH compared with normal RV (13). DAPI, 4,’6-diamidino-2-phenylindole. (Nuclei are stained blue by DAPI in both I and II); III, positron emission tomography (PET) images showing increased RV 18-fluorodeoxyglucose (FDG) uptake in a patient with RVH from pulmonary artery hypertension (PAH; left) compared with the patient with regression of RVH following treatment of PAH with epoprostenol (left) (16). LV, left ventricle. Right: schematic of mitochondrial remodeling and the effects of potentially RV-specific targets and therapies in RVH (see text). Dichloroacetate (DCA) inhibition of pyruvate dehydrogenase kinase (PDK) allows for entry of pyruvate in the mitochondria for glucose oxidation and subsequent depolarization of ΔΨᵢ, which is coupled with increased RVH contractility (14). Sildenafil inhibition of PDE5 allows for both opening of ATP-dependent Kᵢ (KᵢATP) channels to depolarize ΔΨᵢ (15), and cGMP-mediated inhibition of phosphodiesterase-3 (PDE3) to activate protein kinase A (PKA) to increase RVH contractility (13). Mitochondrial nitric oxide synthase (mNOS) might also be a therapeutic target, since its inhibition might lead to decreased ΔΨᵢ and increased contractility, like with DCA and sildenafil. sGC, soluble guanylate cyclase; NO, nitric oxide; PKG, protein kinase G.
mtNOS upregulation in RVH: A brake in RVH performance?. The role of mtNOS was originally uncertain, since there were concerns of contaminated mitochondrial preparations or a lack of specificity for most antibodies against mtNOS as they all cross-react with other nitric oxide synthases (NOS), including endothelial NOS and neuronal NOS (nNOS) (12). However, more recent data support mtNOS as an independent form of NOS. The discovery of mtNOS was first described in purified mitochondria from rat liver (9). It was later recognized that mtNOS was an α-isofrom of nNOS with two posttranslational modifications that are thought to help target mtNOS to the mitochondria and regulate enzymatic activity (7). This strategic location of mtNOS in the “heart of the heart” (i.e., in the heart mitochondria) allows for tight regulatory control of mitochondrial function and thus energy regulation and cardiac performance and apoptosis (10). The authors’ finding that mtNOS can be upregulated in RVH can provide a potential mechanism for the increased ΔΨm that we observed in RVH (14). In certain tissues, the decreased utilization of O2 caused by NO’s inhibition of complex IV may allow for O2 to diffuse to other tissues (perhaps further away from blood vessels), allowing for NO-O2 gradients and minimizing tissue hypoxia (19). However, in the heart, which is a significant consumer of oxygen, inhibition of complex IV may be detrimental, at least in terms of contractility. The inhibition of respiration may exacerbate the shift of metabolism away from mitochondrial glucose oxidation toward cytoplasmic glycolysis. Thus the increased presence of mtNOS in RVH correlates with both the hyperpolarized ΔΨm and increased glucose uptake.

Perhaps the switch to a glycolytic phenotype with hyperpolarized ΔΨm lends itself to a “cancer-like” state of apoptosis resistance, adapting long-term to the stress of hypoxia and increased afterload, but at the cost of suboptimal energy production and contractility, at least acutely. Reversal of this mitochondrial and metabolic remodeling by DCA and sildenafil improves RVH contractility, and this suggests that the same might be true with inhibition of mtNOS in RVH. Indeed, Kanai et al. (11) showed that inhibition of the upregulated mtNOS led to increased contractility in the cardiomyopathic mouse heart. This is in keeping with our previous work, since mtNOS leads to an increase in ΔΨm (2). Zaobornyj et al. (22) found that sildenafil decreased mtNOS activity in rat hearts, in agreement with our previous work showing that sildenafil increases RVH contractility (13). Unfortunately, these conclusions are difficult to extrapolate from the current study, since sildenafil would also have primarily decreased RV afterload (by direct effects on the pulmonary vasculature) and thus lead to a secondary decrease in RVH. In other words, sildenafil, which is now used clinically to treat PAH, might decrease mtNOS not only by primary effects on the myocardium but also by decreasing the RV afterload, hence confounding the interpretation of the current results. Nonetheless, although mtNOS output is thought to be very low in the normal heart, in the diseased heart, its upregulation suggests that it might become a therapeutic target. In most conditions associated with RVH, the LV is unaffected and typically normal. For example, in pulmonary hypertension, the LV is normal while the RV is hypertrophied (20). Therefore, it is possible that, in pulmonary hypertension with subsequent RVH, mtNOS inhibitors might be RV-specific therapies, like DCA and sildenafil. RV-specific therapies are clearly sought after and needed in clinical practice.

Despite its limitations, the authors’ work is important in showing a dynamic upregulation of mtNOS in the diseased heart and suggesting that mtNOS may be a therapeutic target, particularly in the diseased RV. The fact that the output of mtNOS in the normal heart is small should not discourage active investigation of this pathway in the diseased myocardium. More studies are needed to extend the authors’ work and dissect the role of mtNOS in the hypoxic/ischemic vs. the hypertrophied heart and in the LV vs. the RV myocardium.

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