Tetrahydrobiopterin (BH₄) is an essential cofactor for the normal enzymatic function of endothelial NO synthase (eNOS) to produce NO, because it is involved in the catalytic process of L-arginine oxidation. Insufficient BH₄ availability impairs this process, and the free radical superoxide anion (O₂⁻) is released rather than NO, a condition termed “eNOS uncoupling.” BH₄, eNOS, and NO levels physiologically increase in parallel, as seen in response to shear stress, the most important physiological stimulus for endothelial NO production. Shear stress increases BH₄ through a casein kinase 2–dependent phosphorylation of GTP cyclohydrolase-1, the enzyme catalyzing the first step in BH₄ synthesis. Blockade of GTP cyclohydrolase-1 by the specific inhibitor 2,4-diamino-6-hydroxypyrimidine (DAHP) results in eNOS uncoupling in endothelial cells exposed to shear stress. In transgenic mice overexpressing eNOS, a large portion of the enzyme is uncoupled because of BH₄ deficiency, leading to excessive endothelial formation of O₂⁻, which reacts rapidly to form peroxynitrite. Because BH₄ is highly sensitive to oxidation by the particular aggressive reactive oxygen species (ROS) peroxynitrite, in most cardiovascular disease states, BH₄ is depleted. Compelling evidence exists that eNOS uncoupling contributes to endothelial dysfunction in diabetes mellitus, atherosclerosis, and hypertension. NO is also an essential regulator of cardiac structure and function; however, only a few studies have investigated the role of BH₄ in this regard. Takimoto et al revealed the importance of BH₄ depletion for eNOS uncoupling and subsequent left ventricular hypertrophy in mice subjected to pressure overload. Exogenous BH₄ was able to recouple eNOS and reverse pre-established advanced hypertrophy. In addition, depletion of BH₄ and subsequent uncoupling of cardiac NO synthase appear to contribute to the development of diastolic dysfunction.

In the present issue of Hypertension, Ceylan-Isik et al report that eNOS uncoupling by BH₄ depletion through treatment with the GTP cyclohydrolase-1 inhibitor DAHP impaired myocyte and mitochondrial function in the heart (Figure). Their data clearly show that BH₄ depletion and subsequent O₂⁻ formation disrupt calcium handling and cardiomyocyte function. The data of Ceylan-Isik et al further indicate a crosstalk between ROS produced from uncoupled eNOS and the mitochondria, leading to increased mitochondrial ROS production and mitochondrial dysfunction. The importance of mitochondrial ROS formation has also been highlighted recently in endothelial cells in response to angiotensin II, which induced mitochondrial dysfunction via protein kinase C–dependent activation of NADPH oxidase, and a crosstalk between mitochondrial- and NADPH-oxidase–derived ROS was also found in nitroglycerin-triggered vascular dysfunction.

Cardiomyocyte-restricted overexpression of the ROS scavenger metallothionein prevented the DAHP-induced increase in O₂⁻ formation and preserved cardiomyocyte calcium handling and cardiac function. Because elevated blood pressure during DAHP treatment was not normalized by metallothionein overexpression, BH₄ depletion appears to impair cardiac structure and function independent of blood pressure changes. Metallothionein overexpression also prevented eNOS phosphorylation at threonine associated with eNOS uncoupling during BH₄ deficiency. An open question remains the relative contribution of other NO synthase isoforms affected by BH₄ deficiency. Interestingly, uncoupling of a putative mitochondrial NO synthase in cardiomyocytes was described recently to stimulate mitochondrial ROS production.

In summary, by BH₄ depletion using GTP cyclohydrolase-1 blockade, Ceylan-Isik et al clearly demonstrate that BH₄ deficiency and subsequent eNOS uncoupling disrupt cardiac structure and function, which is linked to mitochondrial dysfunction, indicating a crosstalk between uncoupled NO synthase and the mitochondria. Scavenging O₂⁻ through cardiomyocyte-restricted overexpression of metallothionein prevented most sequelae of BH₄ deficiency.

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From the Medizinische Klinik und Poliklinik I, Universität Würzburg, Germany.

Correspondence to Johann Bauersachs, Medizinische Klinik und Poliklinik I, Universität Würzburg, Josef-Schneider-Str 2, D-97080 Würzburg, Germany. E-mail j.bauersachs@medizin.uni-wuerzburg.de (Hypertension. 2009;53:907-908.)

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Figure. DAHP inhibits GTP cyclohydrolase-1 (GTPCH-1), the first step enzyme in BH4 synthesis, causing eNOS to uncouple and produce $O_2^-$ rather than NO. Increased $O_2^-$ formation triggers cardiomyocyte and mitochondrial dysfunction. The mitochondria are stimulated to produce $O_2^-$ themselves, further contributing to cardiomyocyte dysfunction. By scavenging $O_2^-$, cardiomyocyte-specific metallothionein overexpression preserves mitochondrial and cardiomyocyte function.

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Tetrahydrobiopterin, Endothelial Nitric Oxide Synthase, and Mitochondrial Function in the Heart
Johann Bauersachs and Julian D. Widder

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