Tetrahydrobiopterin (BH$_4$) 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 BH$_4$ availability impairs this process, and the free radical superoxide anion (O$_2^-$) is released rather than NO, a condition termed “eNOS uncoupling.” BH$_4$, 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 BH$_4$ through a casein kinase 2–dependent phosphorylation of GTP cyclohydrolase-1, the enzyme catalyzing the first step in BH$_4$ 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 BH$_4$ deficiency, leading to excessive endothelial formation of O$_2^-$, which reacts rapidly to form peroxynitrite. Because BH$_4$ is highly sensitive to oxidation by the particular aggressive reactive oxygen species (ROS) peroxynitrite, in most cardiovascular disease states, BH$_4$ 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 BH$_4$ in this regard. Takimoto et al reported that BH$_4$ depletion by BH$_4$ deficiency and subsequent left ventricular hypertrophy in mice subjected to pressure overload. Exogenous BH$_4$ was able to recouple eNOS and reverse pre-established advanced hypertrophy. In addition, depletion of BH$_4$ and subsequent uncoupling of cardiac NO synthase appear to contribute to the development of diastolic dysfunction.

The present issue of Hypertension, Ceylan-Isik et al report that eNOS uncoupling by BH$_4$ depletion through treatment with the GTP cyclohydrolase-1 inhibitor DAHP impaired myocyte and mitochondrial function in the heart (Figure). Their data clearly show that BH$_4$ depletion and subsequent O$_2^-$ 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 O$_2^-$ formation and preserved cardiomyocyte calcium handling and cardiac function. Because elevated blood pressure during DAHP treatment was not normalized by metallothionein overexpression, BH$_4$ depletion appears to impair cardiac structure and function independent of blood pressure changes. Metallothionein overexpression also prevented eNOS phosphorylation at threonine$^{497}$ associated with eNOS uncoupling during BH$_4$ deficiency. An open question remains the relative contribution of other NO synthase isoforms affected by BH$_4$ deficiency. Interestingly, uncoupling of a putative mitochondrial NO synthase in cardiomyocytes was described recently to stimulate mitochondrial ROS production.

In summary, by BH$_4$ depletion using GTP cyclohydrolase-1 blockade, Ceylan-Isik et al clearly demonstrate that BH$_4$ 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 O$_2^-$ through cardiomyocyte-restricted overexpression of metallothionein prevented most sequelae of BH$_4$ deficiency.

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Disclosures
None.
Figure. DAHP inhibits GTP cyclohydrolase-1 (GTPCH-1), the first step enzyme in BH₄ synthesis, causing eNOS to uncouple and produce O₂⁻ rather than NO. Increased O₂⁻ formation triggers cardiomyocyte and mitochondrial dysfunction. The mitochondria are stimulated to produce O₂⁻ themselves, further contributing to cardiomyocyte dysfunction. By scavenging O₂⁻, cardiomyocyte-specific metallothionein overexpression preserves mitochondrial and cardiomyocyte function.

References

Tetrahydrobiopterin, Endothelial Nitric Oxide Synthase, and Mitochondrial Function in the Heart

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