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Review Article

Targeting endothelial and myocardial dysfunction with tetrahydrobiopterin

An L. Moens [a,*], Rinrada Kietadisorn [a], Judy Y. Lin [a], David Kass [b]

[a] Maastricht University Medical Centre, Cardiovascular Research Institute Maastricht, Dept. of Cardiology, Maastricht, The Netherlands
[b] Johns Hopkins Medical Institutions, Dept. of Cardiology, Baltimore, USA

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ABSTRACT

Tetrahydrobiopterin (BH_4) is an essential cofactor for aromatic amino acid hydroxylases and for all three nitric oxide synthase (NOS) isoforms. It also has a protective role in the cell as an antioxidant and scavenger of reactive nitrogen and oxygen species. Experimental studies in humans and animals demonstrate that decreased BH_4-bioavailability, with subsequent uncoupling of endothelial NOS (eNOS) plays an important role in the pathogenesis of endothelial dysfunction, hypertension, ischemia–reperfusion injury, and pathologic cardiac remodeling. Synthetic BH_4 is clinically approved for the treatment of phenylketonuria, and experimental studies support its capacity for ameliorating cardiovascular pathophysiologies. To date, however, the translation of these studies to human patients remains limited, and early results have been mixed. In this review, we discuss the pathophysiologic role of decreased BH_4 bioavailability, molecular mechanisms regulating its metabolism, and its potential therapeutic use as well as pitfalls as an NOS-modulating drug. This article is part of a special issue entitled "Key Signaling Molecules in Hypertrophy and Heart Failure."

Contents

1. Introduction

Tetrahydrobiopterin (BH_4) is an essential cofactor for the three aromatic amino acid hydroxylase enzymes involved in the synthesis of neurotransmitters, as well as the nitric oxide synthase isoforms. In the cardiovascular system, the NOS isozymes have a central role in mediating vascular tone, endothelial function, platelet aggregation, cardiac myocyte contraction and excitation–contraction coupling, and many other properties. NOS activity depends on several cofactors, including flavin adenine dinucleotide, flavin mononucleotide, a heme group, and BH_4, as well as structural features such as a zinc–thiolate cluster. BH_4 facilitates electron transfer from reductase to oxidase NOS domains, coupling this to the conversion of arginine to citrulline and NO generation. It also promotes and stabilizes NOS in its active homodimeric form [1]. Decreased BH_4 bioavailability results in unstable NOS that becomes physically less compact and functionally uncoupled, reducing NO-production and enhancing the generation of superoxide. Though the ratio between "coupled" and "uncoupled" NOS in normal or pathological disease conditions remains unclear, uncoupling can be reversed by exogenous BH_4 administration, igniting interest in BH_4 as a potential cardiovascular therapeutic. Both a reduced bioavailability of the substrate L-arginine and the cofactor BH_4 can lead to uncoupling of eNOS. However, in *in vivo* settings the latter is the most prominent factor. Recoupling of eNOS accounts for the majority of the benefits that result from BH_4

* Corresponding author at: Maastricht University Medical Centre, Cardiovascular Research Institute Maastricht, P. Delbeyelaan 25, 6202 AZ Maastricht, The Netherlands. Tel.: +31 43 387 1587; fax: +31 43 387 2870.
E-mail address: an.moens@mumc.nl (A.L. Moens).

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administration in a model of pressure overload. Moens et al. [2] have demonstrated that administration of the potent antioxidant Tempol (which did not recouple the uncoupled eNOS), did not had any significant effect on remodeling, except on myocyte dimensions (but still less than the effect of BH_4 on myocyte dimensions).

However, despite encouraging experimental data, clinical translation studies remain limited, and those reported (largely preliminary data) have been less than encouraging. The basic science continues to drive efforts to understand the biochemistry better, and ultimately identify a pathway to successfully adjust decreased BH_4-levels. Here, we review key background regarding BH_4 chemistry and physiology, and highlight new insights and controversies in this area of research.

1.1. Regulation of BH_4 synthesis

BH_4 is synthesized by one of the two pathways, *de novo*, or salvaged from its oxidized forms. For *de novo* synthesis, the enzymes GTP cyclohydrolase (GTPCH), 6-pyruvoyltetrahydropterin synthase and sepiapterin reductase convert GTP into BH_4 [3,4]. GTPCH is the rate limiting enzymatic step and primary regulator for new synthesis (overview see Fig. 1). GTPCH colocalizes with eNOS in caveolae, and upregulation of one enzyme requires matching upregulation of the other in order to maintain normal NOS-NO synthesis [5]. For example, eNOS overexpression in the absence of compensating GTPCH levels leads to excessive reactive oxygen species (ROS) generation by NOS rather than enhanced NO production [5]. Salvage of BH_4 from BH_2 is achieved by dihydrofolate reductase (DHFR), or by quinonoid dihydrobiopterin through dihydropteridine reductase (DHPR). DHFR is mainly involved in folate metabolism but also converts inactive BH_2 back to BH_4 and plays an important role in the metabolism of exogenously administered BH_4. A study in bovine aortic endothelial cell (BAECs) culture showed that angiotensin II (Ang II) down-regulates DHFR expression via endothelial NADPH-oxidase-derived H_2O_2, resulting in reduced NO production due to BH_4 deficiency and the uncoupling of eNOS [6]. Overexpression of DHFR restores all of these abnormalities [6]. Seujange et al. demonstrated that renal ischemia/reperfusion injury significantly reduced renal DHFR mRNA and protein levels which were restored by administration of ACE-inhibitor or Ang II receptor type 1 blocker (ARB) [7].

1.2. NOS uncoupling

The stoichiometry of intracellular BH_4/eNOS interactions (or for that matter BH_4 with other NOS isoforms) remains poorly defined, making it unclear whether intracellular BH_4 deficiency alone is sufficient to induce eNOS uncoupling. Crabtree et al. [8] found that the ratio of BH_4:BH_2 and absolute molar concentration of BH_4 are the key determinants of eNOS coupling *in vivo*. Furthermore, they showed that eNOS:BH_4 ratio and biopterin redox status are responsible for determining the degree of eNOS coupling even in the absence of vascular disease or oxidative stress. The degree of BH_4 oxidation, BH_2 accumulation, and superoxide production directly correlated with intracellular eNOS:BH_4 ratio. BH_4 can have substantial effects on the levels of cellular reactive oxygen species production through mechanisms independent of eNOS, such as direct reactive oxygen species scavenging. This suggests that general antioxidant properties of BH_4 act in direct scavenging of superoxide and maintenance of intracellular redox balance. Moreover, they demonstrate that eNOS-dependent superoxide production occurs in addition to basal superoxide formation overwhelming antioxidant defenses. This is surprising as superoxide reacts with BH_4 *in vitro* with a rate constant >10,000-fold slower (3.9×10^5 mol l^{-1} s^{-1}) than its near diffusion limited reaction with NO (6.7×10^9 mol l^{-1} s^{-1}) [9,10].

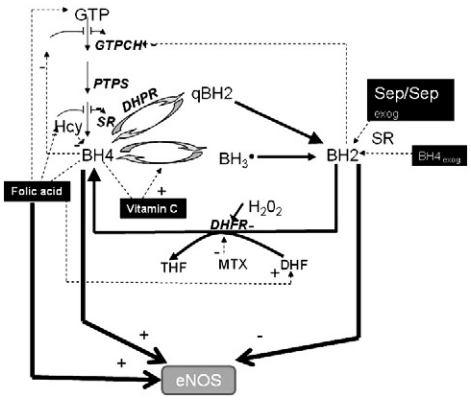

Fig. 1. Biosynthesis of BH_4.

The role of BH_2 in the pathogenesis of eNOS uncoupling has recently been elucidated. Before, BH_2 was described as an inactive and oxidized form of BH_4. Because the K_m of BH_4 and BH_2 for eNOS are similar (~80 nm), earlier studies speculated that BH_2 competed with BH_4 for eNOS binding, thus promoting NOS uncoupling and O_2^- production [11]. This was recently shown, and the fall in BH_4:BH_2 ratio, rather than decline in absolute BH_4, determined NO and O_2^- production by eNOS [12]. Thus, strategies to augment BH_4 while suppressing BH_2 may be required to optimally achieve therapeutic benefit.

Although GTPCH is the key regulator of the total amount of intracellular biopterins, DHFR is critical to eNOS function as it determines BH_4:BH_2 ratio and thus eNOS coupling. In particular, DHFR is important in preventing "self-propagated" eNOS uncoupling where there are low total biopterin levels, and eNOS-dependent oxidation of BH_4 that would further exacerbate this state can be rescued by DHFR [13]. Inhibition of DHFR activity or reduction of DHFR protein (by methotrexate or DHFR-specific siRNA, respectively) resulted in BH_4 oxidation to BH_2, reducing NO generation and increasing eNOS-derived O_2^-. This was particularly effective if BH_4 was reduced. Sugiyama et al. [14] showed in BAECs that while GTPCH knockdown reduced overall biopterin levels, lowering eNOS-NO synthesis, it did not enhance superoxide production. In contrast, DHFR knockdown yielded a marked increase in BH_2, though no substantial effect on total biopterin, reducing NO generation while greatly enhancing ROS production. These data suggest that decreased NO production and increased ROS production are not intrinsically linked by BH_4 depletion. Even if BH_4 levels are lowered to the point of uncoupling NO synthesis, BH_2 appears necessary to observe eNOS-dependent H_2O_2 synthesis. Taken together, these data imply that BH_2 plays a key role in generating ROS from eNOS in cultured endothelial cells, and the ratio of BH_4:BH_2, rather than the absolute concentrations is the critical determinant.

Recently, it was suggested that BH_4 also has a role in mediating cardiac mitochondrial NOS (mtNOS), though the exact identification of a mtNOS species remains controversial. Using permeabilized cat ventricular myocytes, Dedkova et al. showed that BH_4 reduced mitochondrial ROS generation and mitochondrial permeability transition pore opening while increasing mitochondrial NO generation [15].

1.3. BH_4 and vascular pathophysiology

Endothelial dysfunction is defined by inability of endothelium to maintain vascular tone, increased platelet activation, leukocyte adhesion, and smooth muscle proliferation; and all can be linked to inadequate NO generation [5]. Patients with diabetes, heart failure, and hypertension, each conditions with excessive endothelial ROS, exhibit improved endothelium-dependent vasodilation after BH_4 supplementation [16] compared with general antioxidants. In smokers, endothelial dysfunction is partially due to increased lipid peroxidation and the increased formation of oxidized low-density