Tetrahydrobiopterin and Cardiovascular Disease

An L. Moens, David A. Kass

Abstract—Tetrahydrobiopterin (BH4) is an essential cofactor for the aromatic amino acid hydroxylases, which are essential in the formation of neurotransmitters, and for nitric oxide synthase. It is presently used clinically to treat some forms of phenylketonuria (PKU) that can be ameliorated by BH4 supplementation. Recent evidence supports potential cardiovascular benefits from BH4 replacement for the treatment of hypertension, ischemia-reperfusion injury, and cardiac hypertrophy with chamber remodeling. Such disorders exhibit BH4 depletion because of its oxidation and/or reduced synthesis, which can result in functional uncoupling of nitric oxide synthase (NOS). Uncoupled NOS generates more oxygen free radicals and less nitric oxide, shifting the nitroso-redox balance and having adverse consequences on the cardiovascular system. While previously difficult to use as a treatment because of chemical instability and cost, newer methods to synthesize stable BH4 suggest its novel potential as a therapeutic agent. This review discusses the biochemistry, physiology, and evolving therapeutic potential of BH4 for cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2006;26:2439-2444.)

Key Words: tetrahydrobiopterin | nitric oxide synthase | atherosclerosis | inflammation

In 1963, a naturally occurring coenzyme for phenylalanine hydroxylase (PAH) was discovered to be the unconjugated pterin 5,6,7,8-tetrahydrobiopterin (BH4).1 BH4 was subsequently found to be an essential cofactor for several other aromatic amino acid hydroxylases (tyrosine2 and tryptophane3) involved with neurotransmitter biosynthesis, glycercyl-ether mono-oxygenase, and nitric oxide synthase (NOS). To be functional, BH4 must be in its fully reduced form, and depletion and/or BH4 oxidation to BH2 and BH3 reduces its activity. For the cardiovascular system, the role of BH4 in NOS activity is particularly relevant. Reduced BH4 was first shown to contribute to vascular pathophysiology and hypertension, whereas more recent studies have found important roles in cardiac hypertrophy and remodeling, and ischemia/reperfusion physiology. Development of genetic mouse models that modulate BH4 synthesis have greatly advanced understanding of its role to normal NOS and vascular function. Here we briefly review the pharmacology, physiology, and therapeutic potential of BH4.

BH4 Biosynthesis

BH4 is formed by either a de novo or salvage pathway (Figure 2). De novo synthesis starts with guanidine triphosphate cyclohydrolase (GTPCH) in a magnesium, zinc, and NADPH-dependent reaction, and continues through 2 intermediates (7,8-dihydUTURE 2 intermediate) and 6-pyruvoyl-1,5,6,7,8-tetrahydropterin) mediated by 6-pyruvoyl-tetrahydropterin synthase and sepiapterin reductase.4 GTPCH is the rate limiting enzyme and is under negative feedback regulation by GTPCH feedback regulatory protein (GFRP) and BH4 itself, and positive feedback by phenylalanine.5 GTPCH is also regulated at the expression level, being increased by calcium6 and 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibition,7 and by cytokines such as interferon-γ, tumor necrosis factor-α, and interleukin-1β. Cytokine activation may involve coordinated activation of NF-κB and the Jak2/Stat pathway,8 and can increase BH4 levels by increasing GTPCH-1 expression,9–12 reducing GFRP expression,5 and increasing PTPS expression.12 BH4 synthesis is also stimulated by insulin via a phosphatidylinositol-3-kinase–dependent activation of GTPCH-1,13 whereas insulin-resistant states impair this mechanism.14–17 Suppressors of GTPCH-1 activity include glucocorticoids18,19 and cyclic GMP, the latter generated by short-term treatment with NO donors or sodium nitroprusside20 and high levels of 7,8 BH3.21 These and other factors are summarized in the Table.

The salvage pathway generates BH4 from oxidized forms via sepiapterin and sepiapterin reductase22 but cannot compensate for defects in biosynthesis or recycling.22–25 Two other enzymes are also involved with regenerating reduced BH4 from oxidized forms, dihydrofolate reductase and dihydopterine reductase. Dihydrofolate reductase is mainly involved in folate metabolism and converts inactive 7,8-BH2 back to BH4, and plays an important role in the metabolism of exogenously administered BH4. Recently, Chalupsky et al26 demonstrated the role of dihydrofolate reductase in the regulation of BH4 and NO bioavailability in the endothelium. Endothelial NAD(P)H oxidase-derived H2O2 downregulated dihydrofolate reductase expression in response to angiotensin II, resulting in BH4 deficiency and uncoupling of eNOS.
Dihydropteridine reductase catalyzes BH$_4$ regeneration from qBH$_2$ formed under oxidative stress.

**BH$_4$ and NOS Function**

BH$_4$ is an essential cofactor for all 3 NOS isoforms, and basal enzyme activity correlates with the amount of BH$_4$ bound tightly to the protein. NOS is a homodimeric oxidoreductase containing iron protoporphyrin IX (heme), flavin adenine dinucleotide, flavin mononucleotide, and BH$_4$. The flavin-containing reductase domain and a heme-containing oxygenase domain are connected by a regulatory calmodulin-binding domain. Binding of Ca$^{2+}$/calmodulin orients the other domains to allow NADPH-derived electrons generated in the reductase domain to flow to the oxygenase domain, ultimately resulting in the conversion of L-arginine to NO and L-citrulline. This occurs if BH$_4$ is bound in the dimer interface, where it interacts with amino acid residues from both monomers to stabilize NOS dimerization and participate in arginine oxidation through the N-hydroxyl-L-arginine intermediate and the subsequent generation of NO.

The functional influence of BH$_4$ on NOS occurs at several levels. BH$_4$ can shift the NOS heme iron to a high spin state, increasing arginine binding and stabilizing the active dimeric form. NOS-bound BH$_4$ may act as a redox-active cofactor via an unknown mechanism. BH$_4$ increases substrate affinity of NOS and participates in the electron transfer process, being converted to BH$_3$ during the NOS catalytic cycle and then restored to BH$_4$. The best-characterized structural effect of BH$_4$ is its stabilization of NOS dimers, particularly striking for inducible NOS (iNOS). Under certain conditions iNOS dimerization strictly depends on BH$_4$. However, dimeric forms of all 3 isoforms can be obtained in the absence of BH$_4$. Functional dimerization is thought to be a general requirement for normal NOS activity by biophysical alignment of the 2 oxidase domains linked to the opposing monomer reductase domain, thus this influence is thought to impact on enzyme function. Reduction of the ferric iron of endothelial NOS (eNOS) results in formation of an FeII-dioxygen complex, which would yield superoxide. However, BH$_4$ donates an electron to form an iron-oxy species (FeII-O) that in turn participates in arginine hydroxylation and NO generation. BH$_4$ also critical effects on the heme including the shift of the ferric iron spin state equilibrium toward a high spin state, altering the stability of the Fe(II)O$_2$ complex and stabilizing 6-coordinate forms of NOS-ferrous-CO and ferrous-NO complexes. Lastly, BH$_4$ has some modest anti-

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**Influencing Factors of GTPCH**

<table>
<thead>
<tr>
<th>Inhibiting factors</th>
<th>Stimulating factors</th>
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<tr>
<td>NO donors/cyclic GMP</td>
<td>TNF-α/Interferon-γ/IL-1β</td>
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<tr>
<td>IL-4/IL-10/TGF-β</td>
<td>Insulin</td>
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<tr>
<td>Melatonin</td>
<td>Statins</td>
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<tr>
<td>Glucocorticoids</td>
<td>Ca influx</td>
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<tr>
<td>2,4-diamino-6-hydroxypyrimidine</td>
<td>Follicle stimulating hormone</td>
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<td></td>
<td>Epidermal growth factor</td>
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<td>Platelet-derived growth factor</td>
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<td>Vasoactive intestinal peptide</td>
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</tbody>
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**Figure 1.** Biochemical structure of 5,6,7,8-tetrahydrobiopterin.

**Figure 2.** BH$_4$ biosynthesis and metabolism of BH$_4$. BH$_4$ can be formed by both a de novo pathway and a salvage pathway. The de novo pathway starts from guanidine triphosphate (GTP) and is regulated by the enzymes GTP cyclohydrolase (GTPCH), 6-pyruvoyl tetrahydropterin synthase (PTPS) and sepiapterin reductase (SR). The salvage pathway starts from sepiapterin (Sep) and is mediated by the enzymes SR and dihydrofolate reductase (DHFR).
BH4 Bioavailability: Role of Oxidant Stress

BH4 bioavailability is potently influenced by oxidative stress, by decreasing expression of GTPCH, depleting NADPH, which is required for de novo synthesis and is involved with BH4 recycling, and by oxidation to inactive BH2. Oxi-
dized BH4 further augments superoxide anion synthesis from NO3, increasing the synthesis of peroxynitrite (ONOO-), which is a potent oxidizer of BH4. Angiotensin II reduces BH4 by endothelial NAD(P)H oxidase-derived H2O2-dependent downregulation of DHFR, an enzyme involved with reduction of BH3 back to BH4. This response is associated with a significant increase in endothelial O2- production and impaired endothelial function and homeostasis. BH4 oxidation is observed in a number of vascular diseases, and although it cannot act as an NO cofactor, it can exacerbate BH4 availability by competitive binding to NO3.

BH4 Bioavailability and Inflammation/Atherosclerosis

Unlike hypertension, hypertrophy, and oxidant stress stimulation, other stimuli such as inflammatory cytokines have been found to increase BH4 biosynthesis, and this may play a role in atherosclerosis. For example, d’Uscio et al detected elevated BH4 in atherosclerotic aortas of apolipoprotein E-deficient mice caused by increased expression and enzyme activity of GTPCH. Upregulation of GTPCH and BH4 synthesis has been linked to stimulation by certain inflammatory cytokines such as tumor necrosis factor-α, interferon-γ, and IL-1β, and may in this setting serve as a counter response to enhance NO production. In atherosclerotic vessels, total NOS activity is three times higher than in control arteries, caused mostly by increased expression and activity of iNOS. Additional support for upregulated BH4 synthesis in the setting of inflammation comes from studies showing increased neopterin, a side-product of GTPCH-1 activity. Intrinsic upregulation of BH4 biosynthesis per se still does not rule out potential utility of exogenous BH4 supplementation, because uncoupling is often still observed.

BH4 Bioavailability: Role of Homocysteine, Folate, and Ascorbate

Increased vascular homocysteine is a potent risk factor for atherosclerosis and endothelial dysfunction, and some of this effect maybe mediated by its influence on BH4. Homocysteine reduces intracellular BH4 accompanied by apparent inhibition of de novo synthesis, likely by blunting sepiapterin reductase. BH4 administration has beneficial effects on homocysteine-induced impairment of endothelial function, increased superoxide production, and impaired agonist-stimulated NO release.

Folic acid (folate) enhances the binding-affinity of BH4 to NOS by a pteridine-binding domain serving as a locus through which the active form 5-methyltetrahydrofolate (5MTHF) facilitates the electron transfer by BH4 from the NOS reductase domain to the heme. Folate also enhances regeneration of BH4 from inactive BH2 by stimulating DHFR, and it chemically stabilizes BH4.

Ascorbic acid (Vitamin C) assists in BH4 stabilization primarily through antioxidant and other effects. Vitamin C also prevents formation of BH2 from the BH4 radical by facilitating the recycling to BH4. This may explain some of the benefits of ascorbate on endothelial function independent of superoxide scavenging.

BH4 Supplementation: Vascular Effects

Clinical data supporting vascular benefits of exogenous BH4 are largely based on acute or subacute studies examining endothelium-dependent vasodilation by agonists or flow stimuli. BH4 improves endothelial function in those who smoke, diabetic subjects, hypertensive subjects, patients with hypercholesterolemia, and those with coronary artery disease. More recently, Setoguchi et al showed BH4 improves endothelial function in patients with systolic heart failure. Intracoronary administration of BH4 to patients with cardiovascular risks but without flow-limiting coronary artery stenoses (<75%), enhanced endothelial-dependent vasodilation to acetylcholine. Some studies contrasting acute BH4 infusion versus more chronic treatment found beneficial effects on endothelial function only with the latter. This supports changes in NO3 coupling rather than a less specific antioxidant effect likely explain the response. Preliminary results of chronic treatment with BH4 (400 mg twice daily, 4 weeks; Schirks Laboratories, Zurich, Switzerland) revealed
benefits on endothelial dysfunction measured by acetylcholine response in forearm venous occlusion plethysmography in subjects with hypercholesterolemia.  

BH₄ and the Heart  
Reduced BH₄ likely represents an important cellular defect involved with both endothelial and myocyte dysfunction in hearts exposed to ischemia/reperfusion. BH₄ prevents ischemia/reperfusion cardiac dysfunction in vitro, attenuating the normally observed rise in malondialdehyde levels, a marker of lipid peroxidation, and improving endothelial-dependent vasorelaxation. These changes appear independent of the intrinsic radical scavenging action of BH₄. Takimoto et al. recently revealed the importance of BH₄ depletion and consequent NOS3 uncoupling in mice subjected to sustained pressure overload. In this model, myocardial and myocyte hypertrophy, interstitial fibrosis, and eventual cardiac dilation and dysfunction were linked to increased oxidant stress generated by uncoupled NOS3. Mice lacking NOS3 and exposed to the same pressure load developed more compensated concentric hypertrophy with preserved function, whereas control animals displayed marked dilation and dysfunction after 9 weeks of pressure stress. BH₄ tissue levels declined ≥50%, and BH₄ replacement therapy was able to reduce oxidative stress and inhibit cardiac dilation and depressed function in nonmutant controls. These data support potential benefits of BH₄ to the heart under conditions of stress, such as postinfarction remodeling, dilated myopathic remodeling, and hypertrophy.

Clinical Pharmacology  
Exogenous BH₄ or its precursor sepiapterin first increases systemic BH₄ (Figure 2) that is subsequently reduced to BH₂ by DHFR. Oral sepiapterin hydrochloride, the synthetic form of 6R-BH₄, at 2 mg/kg causes a 3-fold increase in BH₄ after 3 hours, returning to baseline at 24 hours. Intracoronary infusion of 1 mg/min results in a rapid increase within 2 minutes raising coronary sinus BH₄ levels nearly 100-fold. These doses are high and unlikely to be used as chronic therapy. They may also have amplified nonspecific antioxidants effects of BH₄ independent of its role to NOS coupling and NO synthesis. Unfortunately, measurement of systemic (plasma) BH₄ has not been particularly useful for assessing local tissue levels and abnormal bioavailability. This has been shown to be true for coronary artery disease in which no significant differences were demonstrated compared with control population. Shinozaki et al. demonstrated that patients with insulin resistance have lower ratios of plasma BH₄/BH₂ and plasma BH₄:total bipterin, whereas BH₄ levels remained unchanged in patients with insulin resistance versus controls.

A potential disadvantage of BH₄ is that it might stimulate neuronal and inducible NOS activity, leading to excessive NO production and toxicity, particularly in inflammatory disorders. This remains controversial. There are also some reports of elevated catecholamines with BH₄ induced by IL-2 treatment in cancer patients, although studies in PKU patients receiving BH₄ have not reported this effect.

To date, the major factor limiting clinical BH₄ use has been its pharmacological preparation. BH₄ tablets have been large with an acidic taste and unstable as BH₄ is hygroscopic and easily oxidized. Thus, the medication had to be maintained frozen at −20°C to maintain long-term stability. However, BH₄ has recently been developed in the form of a thermostable and photostable tablet, with stability at room temperature of nearly 2 years (Biomarin, San Francisco, Calif). This development has opened up broader potential use for cardiovascular indications.

Conclusion  
BH₄ plays a central role to normal NOS3 activity, yet remarkably it appears vulnerable to depletion, thereby providing a key mechanism underlying a number of cardiovascular disorders. This also opens up intriguing potential for replacement therapy, and new developments in BH₄ pharmaceutical preparation should facilitate larger scale testing of such efficacy. Such studies are being initiated now and we can anticipate new information regarding the therapeutic potential for BH₄ treatment of hypertension, vascular dysfunction, and cardiac remodeling in the relatively near future.

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Disclosures  
None.

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