Tetrahydrobiopterin (BH$_4$) plays an important role in functional and metabolic cellular homeostasis, with additional effects on proliferation, immune responsiveness, and neuronal activity. Mutations in either de novo biosynthetic or regeneration (salvage) pathways result in BH$_4$ deficiency associated with diminished levels of serotonin and dopamine with progressive neurologic symptoms. The phenotypic presentation of these synthetic mutations can be predicted in large part by the role of BH$_4$ as an obligatory cofactor in phenylalanine hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase, suggesting tight coupling of the cofactor with enzyme. However, the precise function(s) of BH$_4$ in NOS enzymatic activity is not as well defined as in the aromatic amino acid hydroxylases and may vary according to enzyme isoform. From the Department of Anesthesiology and the Center for Free Radical Biology, University of Alabama at Birmingham. Correspondence to Margaret M. Tarpey, Department of Anesthesiology and the Center for Free Radical Biology, University of Alabama at Birmingham, 619 19th St South, Birmingham, AL 35233. E-mail margaret.tarpey@ccc.uab.edu

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In addition to its role in the biosynthesis of monoamine neurotransmitters, BH$_4$ serves as an essential cofactor in all isofoms of nitric oxide synthases (NOS), with the Km for BH$_4$ for NOS several orders of magnitude lower than for the aromatic amino acid hydroxylases: NOS, $\sim$0.3 $\mu$mol/L vs $\sim$3 $\mu$mol/L for phenylalanine hydroxylase, $\sim$30 $\mu$mol/L for tyrosine hydroxylase and tryptophan hydroxylase, suggesting tight coupling of the cofactor with enzyme. However, the precise function(s) of BH$_4$ in NOS enzymatic activity is not as well defined as in the aromatic amino acid hydroxylase enzymes and may vary according to enzyme isoform. For endothelial NOS (eNOS), BH$_4$ has been reported to modulate the heme iron environment and stabilize and increase L-arginine binding, thus resulting in allosteric modulation of enzyme activity. The importance of BH$_4$ in regulating protein dimerization, while critical in iNOS, is diminished for eNOS. Importantly, eNOS has been demonstrated to generate superoxide in a calcium/calmodulin-dependent fashion that is influenced by BH$_4$ levels, as well as the availability of L-arginine substrate. This NADPH-dependent formation of superoxide anion in the absence of NO production has been referred to as uncoupling of NOS activity. Superoxide formation from eNOS is critically controlled by BH$_4$ with increasing production of superoxide occurring at low levels of reduced perin, even in the presence of L-arginine. More recently, partially oxidized analogues of BH$_4$ have been shown to also enhance rates of superoxide formation from purified eNOS in the presence of saturating L-arginine concentrations, suggesting that the ratio of reduced and oxidized biotin may be physiologically important in determining rates of NO production versus uncoupled superoxide formation from eNOS.

The increase in oxidant formation that appears to accompany most, if not all, disease processes associated with endothelial-dependent vascular dysfunction may potentially be a result of inadequate BH$_4$ concentrations within the vasculature, with ensuing uncoupling of eNOS, increased superoxide formation and diminished NO formation. Thus, there is intense interest in the potential for BH$_4$ to modulate eNOS activity. Numerous groups have recently reported beneficial effects of acute (≤60 minutes) administration of BH$_4$ or analogs such as sepiapterin on endothelial function in both clinical and experimental models of hypercholesterolemia, atherosclerosis, hypertension, and cigarette smoking. Additionally, the beneficial effects of vitamin C administration on endothelial function are in part due to stabilization of intracellular BH$_4$ with resultant enhanced NO bioactivity. A similar mechanism of BH$_4$ stabilization may underlie the influence of folate supplementation on eNOS activity. Alterations in BH$_4$-dependent eNOS activity have also been implicated in diabetic vascular dysfunction where sepiapterin has been reported to diminish eNOS-derived superoxide in human vascular segments. Acute administration of a BH$_4$ analog augmented endothelial-dependent relaxation in a streptozotocin model of diabetes. Spontaneously diabetic BB rats have diminished GTP-cyclohydrolase 1 activity and decreased BH$_4$ levels, while sepiapterin treatment for 48 hours normalized BH$_4$ levels and increased NO production from endothelial cells isolated from diabetic BB rats. In an insulin-resistant rat model, chronic oral administration of BH$_4$ also increased vascular BH$_4$ content and improved endothelial-dependent vascular function. Given these positive outcomes after administration of BH$_4$, either longer term in vitro exposures or chronic administration studies are warranted in models of atherosclerosis.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Vasquez-Vivar et al report their interesting findings on the influence of 6 hours sepiapterin incubation on vascular BH$_4$ levels and endothelial function in vessels isolated from rabbits fed a high-cholesterol diet. They demonstrate a marked reduction in vascular BH$_4$ content in hyperlipidemic vessels compared with controls, which could be restored by incubation with sepiapterin. These findings are in agreement with the relatively few other studies reporting on BH$_4$ content in diseases associated with vascular dysfunction. While vascular BH$_4$ levels have been reported to be
normal in spontaneously hypertensive rats (SHR) before the development of hypertension. BH₄ levels are decreased in SHR with established hypertension.¹⁰,¹¹ Several models of diabetes have also reported decreased vascular BH₄ content.²⁷,²⁸

In contrast to the improvement of endothelial function seen in acute studies, however, long-term exposure to sepiapterin resulted in a further derangement in response to either acetylcholine or the calcium ionophore A23187, while endothelial-independent function was not altered. These data are similar to those reported by Tsutsui et al.²² in which canine middle cerebral arteries exhibited diminished relaxation to A23187 after 24-hour incubation with 100 µmol/L sepiapterin. In that study, the authors concluded that the inhibition of endothelial-dependent relaxation was due to enhanced generation of superoxide anion, as exogenous superoxide dismutase (SOD) ameliorated the effects of sepiapterin. It is well-recognized that BH₄ itself can redox cycle and thus generate superoxide.³¹ This effect is likely to be enhanced in the setting of vascular relaxation studies, in which the O₂⁻ concentration is typically 95%. However, in the present report, only the hyperlipidemic vessels demonstrated a sepiapterin-dependent inhibition of endothelial-dependent relaxation. It should be noted that the normal vessels did not demonstrate an increase in BH₄ content after incubation with sepiapterin, while the rise in BH₄ content with sepiapterin supplementation did not exceed levels seen in control vessels.

If the effects on vessel relaxation are secondary to BH₄ autooxidation, this would suggest that there is also at least a relative deficiency of SOD in the hyperlipidemic vessels. Alternatively, in the face of a functionally abnormal eNOS, could sepiapterin supplementation result in sepiapterin-dependent superoxide generation from eNOS itself, as has been described for the purified enzyme?²⁶ The effect of either exogenous SOD or a cell-permeant SOD mimetic would be informative.³³ On the other hand, BH₄ can serve to scavenge superoxide with a rate constant of 10⁶ M⁻¹ · s⁻¹ and reacts with peroxynitrite.¹²,¹³,³⁴ Thus, the effects of sepiapterin are the result of extent of salvage pathway activity and conversion to BH₄, balanced with rates of BH₄ autooxidation and consumption by peroxynitrite, as well as sepiapterin binding to eNOS in competition with BH₄.

The inhibitory effect of 6 hours of sepiapterin treatment also contrasts with 2 studies of 8-week in vivo administration of BH₄ demonstrating improved endothelial function in models of SHR and diabetes.²⁸,³¹ Are these differences due to unrecognized secondary reactions of sepiapterin as opposed to BH₄ itself? The report by Vazquez-Vivar et al.²⁹ opens new avenues of investigation with respect to the role of biopterins in vascular dysfunction.

References


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