Transcribing the Cross-Talk of Cytokine-Induced Tetrhydrobiopterin Synthesis in Endothelial Cells

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Tetrahydrobiopterin (BH4) is an essential cofactor required for the production of nitric oxide by each of the nitric oxide synthase (NOS) isoforms (see review). Synthesis of BH4 involves a multistep process in which GTP cyclohydrolase I (GTPCH I) is the rate-limiting enzyme required for the initial step in conversion of guanosine-5’-triphosphate (GTP) to BH4. The importance of BH4 has been even more appreciated ever since it was discovered that in its absence, NOS uncoupling could occur, resulting in the production of superoxide anion and hydrogen peroxide rather than nitric oxide. Loss of endothelial nitric oxide attributable to impairment of BH4 metabolism appears to be an important mechanism in pathogenesis of vascular endothelial dysfunction. Indeed, existing evidence indicate that pharmacological or genetic supplementation of BH4 protects against endothelial dysfunction induced by hypercholesterolemia, diabetes, hypertension, and smoking. Based on these observations, it has been suggested that BH4 metabolism could be an attractive therapeutic target for prevention and treatment of vascular disease.

More recent studies have provided insights into the molecular mechanisms underlying regulation of GTPCH I. X-ray crystallography has revealed that GTPCH I exists as a dimer of pentamers (thereby creating a decamer), composed of 25 to 30 kDa subunits. GTPCH I activity is controlled at the posttranslational level by interaction with GTPCH I Feedback Regulatory Protein (GFRP) which, in the presence of excess BH4, has been shown to induce a conformational change in the protein resulting in feedback inhibition of enzymatic activity. This inhibition of GTPCH I activity can be reversed by phenylalanine, which binds to the GFRP/GTPCH I complex and changes its conformation back to an active state. Additional modifiers of GTPCH I activity include GTP substrate availability as well as phosphorylation of the enzyme, which has been shown to correlate with increased BH4 production.

Transcriptional regulation of GTPCH I is of particular importance in control of BH4 metabolism. In the vasculature, coordinated induction of GTPCH I and inducible NOS (iNOS) gene expression has been observed in the presence of inflammatory cytokines, suggesting that basal levels of GTPCH I and BH4 are not adequate for optimal iNOS function. Individual cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), or interleukin-1β (IL-1β) have been shown to induce little GTPCH I mRNA or protein expression. However, the combination of these cytokines have been shown to induce a robust activation of GTPCH I mRNA, protein, and BH4 production both in vivo and in vitro. It is important to note that these observations were made on vascular smooth muscle cells whereas in endothelium, induction of GTPCH I was not associated with induction of iNOS. Whether high production of BH4 in endothelium activated by cytokines is designed to provide additional support for iNOS enzymatic activity in smooth muscle cells or it has some other functions remains to be determined.

In this issue of Circulation Research, Huang and colleagues have significantly increased our understanding of how the coordinated actions of TNF-α and INF-γ induce GTPCH I expression and BH4 production in endothelial cells. In a methodical series of experiments, they convincingly demonstrate that GTPCH I induction requires the activation of both NF-κB and STAT1, cytosolic transcription factors that mediate gene transcription (Figure). By dissecting out the individual signaling pathways of both TNF-α and INF-γ, it was revealed that TNF-α alone was responsible for the degradation of IκB and the nuclear translocation of NF-κB. This observation was further verified by the demonstration that overexpression of a dominant-negative IκB, which is resistant to proteasomal degradation, inhibited the cytokine-induction of GTPCH I mRNA and protein expression.

Because INF-γ is a known activator of the Jak/STAT family of kinases, Huang and colleagues looked at the contribution of this signaling pathway on GTPCH I expression in endothelial cells. In the presence of the Jak2 inhibitor, AG490, they were able to show that the combination of TNF-α and INF-γ was unable to activate STAT1, which resulted in the inhibition of GTPCH I activation. Additionally, using a Jak2 gene silencing approach with small interfering RNA (siRNA), they demonstrated that GTPCH I protein expression after cytokine treatment was silenced as well. These findings as well as studies using STAT1-null fibroblasts verified that GTPCH I induction requires the activation of Jak2 and STAT1 nuclear translocation and that these signals were coming exclusively from INF-γ.

Importantly, the authors were able to uncover another level of complexity influencing cytokine induction of GTPCH I. In an attempt to mimic INF-γ activation of STAT1, oncostatin M, a known activator of STAT1 phosphorylation and nuclear translocation, failed to induce GTPCH I expression when
used in combination with TNF-α. This suggested that STAT1 activation alone is insufficient for the induction of GTPCH I. However, additional experiments revealed that oncostatin M treatment also resulted in the phosphorylation of STAT3, which did not occur when cells were treated with the combination of TNF-α and INF-γ. To further investigate these findings, the authors used siRNA to inhibit STAT3 expression. They found that when STAT3 induction was inhibited, the combination of TNF-α and oncostatin M was able to induce GTPCH I expression similar to that of TNF-α and INF-γ. This data demonstrates that the induction of GTPCH I in endothelial cells requires the combined activation of NF-κB and STAT1 along with the absence of STAT3 activation.

Although these findings give us important insight into the activation of GTPCH I in endothelial cells, several pieces of the puzzle regarding the regulation of GTPCH I transcription remain unsolved: (1) is STAT3 a bona fide repressor of GTPCH I gene activation; (2) taking into consideration the robust production of BH4 after cytokine treatment, is BH4 more than just a cofactor for iNOS (eg, additional antioxidant defense system against reactive oxygen and reactive nitrogen species, which are abundant during inflammation); and (3) are there other cytokines or circulating substances (and corresponding signal transduction pathways) that can activate transcription of GTPCH I in vascular endothelium?

Current findings by Huang and colleagues are consistent with the concept that increased synthesis of BH4 in vascular endothelium is an adaptive response to inflammation. In vivo, high local concentrations of BH4 in the blood vessel wall has consistently been associated with vascular protection. Both supplementation with BH4 or endothelial-specific over-expression of GTPCH I prevent endothelial dysfunction. This protective effect is manifested by preservation of endothelium-dependent relaxations mediated by nitric oxide. The importance of BH4 in control of both nitric oxide production and superoxide anion production demands efficient homeostatic mechanisms designed to preserve intracellular concentration of BH4. Because BH4 is one of the most potent naturally occurring reducing agents, inflammation-induced oxidative stress could oxidize BH4. Indeed, BH4 can be oxidized by reactive oxygen and nitrogen species. Therefore, it appears likely that cytokine-induced upregulation of GTPCH I expression and enzymatic activity is designed to counteract potential loss of BH4 during inflammation and provide optimal concentration of cofactor required for production of nitric oxide. Understanding signal transduction pathways responsible for transcriptional regulation of GTPCH I in vascular endothelium is of fundamental importance in our attempts to harness BH4 metabolism for development of novel strategies in therapy of vascular disease.

References


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