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著者(和文)	徐峰
Author(English)	Feng Xu
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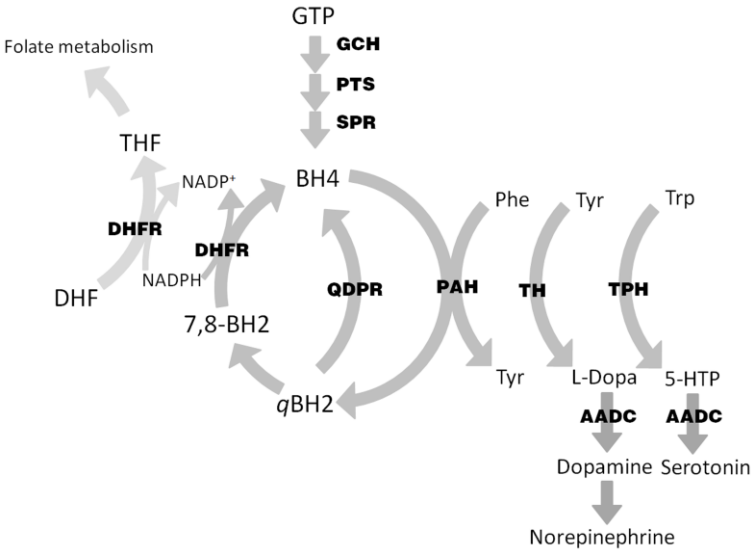
Studies on the disturbed metabolism caused by genetic ablation of *quinonoid dihydropteridine reductase*

Xu Feng

Background

Tetrahydrobiopterin (BH4) is an essential cofactor for the hydroxylation of tyrosine (Tyr) and tryptophan (Trp), which is the rate-limiting step in dopamine and serotonin synthesis, respectively. Dopamine and serotonin are well known to be involved in brain function as neurotransmitters, and consequently, monoamine deficiency due to BH4 depletion causes a variety of neuropsychiatric disorders. Besides, BH4 is also required for phenylalanine hydroxylase (PAH) and all three isoforms of nitric oxide synthases. Therefore, it is of great significance to know how the level of biopterin is maintained and regulated in the cells.

BH4 is *de novo* synthesized from GTP via three enzymatic reactions, which are catalyzed by GTP cyclohydrolase I (GCH), 6-pyruvoyl-tetrahydropterin synthase (PTS) and sepiapterin reductase (SPR). During the hydroxylation reactions, BH4 is oxidized and then dehydrated to *quinonoid* dihydrobiopterin (*q*BH2), which is reduced to BH4 by *quinonoid* dihydropteridine reductase (QDPR). *q*BH2 is unstable and easily converted to 7, 8-dihydrobiopterin (BH2), which can be a substrate for dihydrofolate reductase (DHFR).



The intracellular BH4 contents are regulated at many levels and much investigation has been focused on the regulation of *de novo* synthesis and the rate-limiting enzyme GCH. However, little is known for the roles of recycling pathway. Therefore, in this work, I studied a recently established *Qdpr*-deficient mouse model, which has a total ablation in the *Qdpr* gene, and explored the roles of QDPR and DHFR in the BH4 metabolism.

Outline of the thesis

The thesis is composed of six chapters, and details of each chapter are described as follows.

Chapter 1 presents the research background and purposes; introduces the structure of this dissertation.

Chapter 2 covers the studies related to the alteration in the biopterin metabolism in the *Qdpr*$^{-/-}$ mice. Pteridine quantification revealed a disturbed biopterin metabolism in the transgenic mice, and contents of neopterin, an intermediate metabolite of BH4 *de novo* synthesis were increased as well. However, expression

level or *in vitro* activity of GCH in the transgenic mice was not elevated. These data suggest that BH4 is maintained via an alternative pathway in the absence of QDPR enzyme. Hence, I applied a pharmacological method to prove that DHFR, an enzyme originally associated with folate metabolism, is deeply involved in the BH4 regeneration in the *Qdpr*$^{-/-}$ mice.

Chapter 3 covers the researches on the alteration in the folate metabolism in the *Qdpr*$^{-/-}$ mice. Metabolism of biopterin and folate seems to be independent, but clinical cases and *in vivo* involvement of DHFR in the BH4 regeneration suggest a close connection between them. Measurement of folate derivatives revealed that folates were deficient in the *Qdpr*$^{-/-}$ mouse liver. Metabolome analysis to the *Qdpr*$^{-/-}$ and *Qdpr*$^{+/+}$ mouse tissues and the quantification of plasma homocysteine levels confirmed the wide alterations in the folate-associated metabolism in the *Qdpr*$^{-/-}$ mouse liver. Of note, increase in the oxidative stress biomarkers was observed in the *Qdpr*$^{-/-}$ mice as well, such as glutathione and homocysteine, and most of them were related to folate metabolism.

Chapter 4 intends to uncover the *in vivo* relationship between folate and biopterin metabolism. In order to address this issue, I brought up with several proposals and examine those hypotheses with *in vitro* kinetic assays and *in silico* molecular dynamics simulations. However, the results obtained from these methods indicate that competitive inhibition of dihydrofolate reduction by BH2 or dihydroneopterin is not a plausible reason for the disturbance in the folate metabolism in the transgenic mice. Other possible reasons for the folate deficiency are also discussed in this chapter.

Chapter 5 deals with another remarkable symptom in the *Qdpr*$^{-/-}$ mice, hyperphenylalaninemia. It is surprising that Phe hydroxylation reaction seems to be impaired in the *Qdpr*$^{-/-}$ mice in spite of adequate amount of PAH and sufficient hepatic BH4 supply. Quantification of pteridines in each subcellular fraction suggested decreased BH4 levels in the cytosol of the *Qdpr*$^{-/-}$ mouse hepatocytes, where the hydroxylation of Phe principally occurs.

Chapter 6 concludes this dissertation with a summary of key findings. Some expected future work is also discussed in this chapter.

Conclusions

I characterized a mouse model with a genetic defect in the *Qdpr* gene and revealed that genetic ablation of *Qdpr* caused wide alterations in the biopterin, folate and phenylalanine metabolism. It is the first report revealing the *in vivo* involvement of DHFR in the BH4 metabolism, and proving the genetic defect in the BH4 metabolic process as a possible cause of folate deficiency.