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A novel role for endothelial tetrahydrobiopterin in mitochondrial redox balance

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ABSTRACT

The redox co-factor tetrahydrobiopterin (BH_4) regulates nitric oxide (NO) and reactive oxygen species (ROS) production by endothelial NOS (eNOS) and is an important redox-dependent signalling molecule in the endothelium. Loss of endothelial BH_4 is observed in cardiovascular disease (CVD) states and results in decreased NO and increased superoxide (O_2^-) generation via eNOS uncoupling. Genetic mouse models of augmented endothelial BH_4 synthesis have shown proof of concept that endothelial BH_4 can alter CVD pathogenesis. However, clinical trials of BH_4 therapy in vascular disease have been limited by systemic oxidation, highlighting the need to explore the wider roles of BH_4 to find novel therapeutic targets. In this study, we aimed to elucidate the effects of BH_4 deficiency on mitochondrial function and bioenergetics using targeted knockdown of the BH_4 synthetic enzyme, GTP Cyclohydrolase I (GTPCH). Knockdown of GTPCH by > 90% led to marked loss of cellular BH_4 and a striking induction of O_2^- generation in the mitochondria of murine endothelial cells. This effect was likewise observed in BH_4-depleted fibroblasts devoid of NOS, indicating a novel NOS-independent role for BH_4 in mitochondrial redox signalling. Moreover, this BH_4-dependent, mitochondria-derived ROS further oxidised mitochondrial BH_4, concomitant with changes in the thioredoxin and glutathione antioxidant pathways. These changes were accompanied by a modest increase in mitochondrial size, mildly attenuated basal respiratory function, and marked changes in the mitochondrial proteome and cellular metabolome, including the accumulation of the TCA intermediate succinate. Taken together, these data reveal a novel NOS-independent role for BH_4 in the regulation of mitochondrial redox signalling and bioenergetic metabolism.

1. Introduction

Tetrahydrobiopterin (BH_4) is a redox-active cofactor and has long been known to be a critical regulator of nitric oxide synthase (NOS) coupling and the production of nitric oxide (NO) [1,2]. Loss of endothelial BH_4, via decreased synthesis or oxidation to 7,8-dihydrobiopterin (BH_2), is associated with endothelial NOS (eNOS) uncoupling, whereby the superoxide radical (O_2^-), rather than NO, is produced [3,4]. Moreover, the reaction of O_2^- and NO forms peroxynitrite ($ONOO^-$), leading to further loss of NO and increased uncoupling due to BH_4 oxidation, thereby profoundly altering the balance between production of NO and reactive species (termed NO-redox balance) and associated signalling pathways [5–7]. The cellular NO-redox state is fundamental to the maintenance of vascular homeostasis and uncoupled eNOS is strongly associated with the development and progression of cardiovascular vascular disease (CVD) [8]. However, BH_4 also has other cofactor and antioxidant roles, throughout a wide range of biological processes and pathological states, including phenylalanine catabolism, synthesis of neurotransmitters, and ether lipid metabolism [8]. Furthermore, BH_4 has other important roles in cellular redox-sensitive signalling pathways [9,10].

GTP cyclohydrolase I (GTPCH), encoded by *Gch1*, is the primary rate-limiting enzyme involved in the synthesis of BH_4. Targeted augmentation of *Gch1* expression in endothelial cells has proven sufficient to normalise endothelial function, eNOS coupling, and reactive oxygen species (ROS) production in models of diabetes and atherosclerosis [11–13]. Similarly, targeted knockout of *Gch1* in endothelial cells has led to elevated blood pressure in mouse models

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in vivo [14]. However, clinical trials of BH_4 therapy in vascular disease have been limited by systemic oxidation and limited endothelial uptake of BH_4, highlighting the need explore the wider roles of BH_4 in order to identify novel therapeutic targets.

Redox signalling is the specific, usually reversible, reduction/oxidation modification of signalling pathway components by reactive