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A novel role for endothelial tetrahydrobiopterin in mitochondrial redox balance

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Jade Bailey^a, Andrew Shaw^a, Roman Fischer^b, Brent J. Ryan^c, Benedikt M. Kessler^b, James McCullagh^d, Richard Wade-Martins^c, Keith M. Channon^{a,*}, Mark J. Crabtree^a

^a BHF Centre of Research Excellence, Division of Cardiovascular Medicine, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, United Kingdom

^b Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7FZ, United Kingdom

^c Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics, South Parks Road, Oxford OX1 3QX, United Kingdom

^d Department of Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QR, United Kingdom

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ABSTRACT

The redox co-factor tetrahydrobiopterin (BH₄) 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 BH₄ is observed in cardiovascular disease (CVD) states and results in decreased NO and increased superoxide (O2) generation via eNOS uncoupling. Genetic mouse models of augmented endothelial BH₄ synthesis have shown proof of concept that endothelial BH₄ can alter CVD pathogenesis. However, clinical trials of BH₄ therapy in vascular disease have been limited by systemic oxidation, highlighting the need to explore the wider roles of BH₄ to find novel therapeutic targets. In this study, we aimed to elucidate the effects of BH₄ deficiency on mitochondrial function and bioenergetics using targeted knockdown of the BH₄ synthetic enzyme, GTP Cyclohydrolase I (GTPCH). Knockdown of GTPCH by > 90% led to marked loss of cellular BH₄ and a striking induction of O_2^- generation in the mitochondria of murine endothelial cells. This effect was likewise observed in BH4-depleted fibroblasts devoid of NOS, indicating a novel NOS-independent role for BH4 in mitochondrial redox signalling. Moreover, this BH4-dependent, mitochondria-derived ROS further oxidised mitochondrial BH4, 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 NOSindependent role for BH_4 in the regulation of mitochondrial redox signalling and bioenergetic metabolism.

1. Introduction

Tetrahydrobiopterin (BH₄) 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 BH₄, via decreased synthesis or oxidation to 7,8-dihydrobiopterin (BH₂), is associated with endothelial NOS (eNOS) uncoupling, whereby the superoxide radical (O₂⁻), rather than NO, is produced [3,4]. Moreover, the reaction of O₂⁻ and NO forms peroxynitrite (ONOO⁻), leading to further loss of NO and increased uncoupling due to BH₄ 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, BH₄ 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, BH₄ 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 BH₄. 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

* Corresponding author. E-mail address: keith.channon@cardiov.ox.ac.uk (K.M. Channon).

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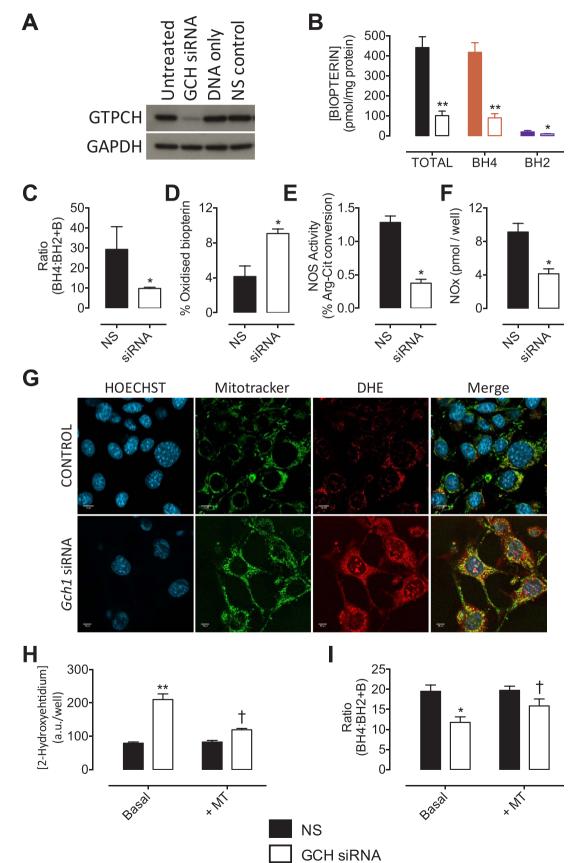
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in vivo [14]. However, clinical trials of BH₄ therapy in vascular disease have been limited by systemic oxidation and limited endothelial uptake of BH₄, highlighting the need explore the wider roles of BH₄ in order to

identify novel therapeutic targets.

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



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