British Journal of Pharmacology

British Journal of Pharmacology (2017) **174** 2422–2424 2422

COMMENTARY

Does endothelial tetrahydrobiopterin control the endothelial NO synthase coupling state in arterial resistance arteries?

Correspondence Professor Dr Thomas Münzel and Professor Dr Andreas Daiber, Center for Cardiology, Cardiology I, University Medical Center Mainz, Geb. 605, Langenbeckstr. 1, 55131 Mainz, Germany. E-mail: tmuenzel@uni-mainz.de; daiber@uni-mainz.de

Thomas Münzel and Andreas Daiber

Center for Cardiology, Cardiology I, University Medical Center Mainz, Mainz, Germany

LINKED ARTICLE

This article is a Commentary on Chuaiphichai S, Crabtree MJ, McNeill E, Hale AB, Trelfa L, Channon KM *et al.* (2017). A key role for tetrahydrobiopterin-dependent endothelial NOS regulation in resistance arteries: studies in endothelial cell tetrahydrobiopterin-deficient mice. Br J Pharmacol 174: 657–671. https://doi.org/10.1111/bph.13728

Abbreviations

BH4, tetrahydrobiopterin; eNOS, endothelial NOS; Gch1$^{fl/fl}$Tie2cre, endothelial-specific GTPCH knockout; GTPCH, GTP-cyclohydrolase-1

Chuaiphichai *et al.* (2017) have investigated whether a genetic endothelial-specific GTP-cyclohydrolase-1 (GTPCH) deficiency in Gch1$^{fl/fl}$Tie2cre mice will lead to endothelial NOS (eNOS) uncoupling and an impaired endothelium-dependent relaxation in arterial resistance arteries. As expected, genetic GTPCH deletion resulted in a significant loss of GTPCH protein in mesenteric arteries accompanied by an almost complete absence of tetrahydrobiopterin (BH4) in the endothelial cell layer. Despite unchanged eNOS protein expression and phosphorylation levels at Ser^{1177} and Thr^{495}, eNOS-derived NO formation was virtually absent. Instead, increased endothelial formation of ROS was evident in vessels of Gch1$^{fl/fl}$Tie2cre mice, supporting the presence of uncoupled eNOS. The mesenteric arteries of Gch1$^{fl/fl}$Tie2cre mice developed a super-sensitivity to vasoconstrictors such as phenylephrine and the thromboxane A_2 agonist U46619 and a shift of vascular tone homeostasis towards prostacyclin- and hydrogen peroxide-dependent vasodilatation. The endothelial dysfunction observed in the vessels of Gch1$^{fl/fl}$Tie2cre mice was reversed by exogenous delivery of sepiapterin, a BH4 precursor. These findings mirror the results obtained with cultured endothelial cells (sEND.1 mouse endothelial cell line) upon genetic deletion of GTPCH by treatment with specific siRNA. In these studies, BH4 levels were substantially reduced and, as another indication of eNOS uncoupling (Zou *et al.*, 2002), the eNOS dimer/monomer ratio was decreased by approximately 50% despite unchanged total eNOS protein levels. In addition, the observations made in resistance arteries strikingly resemble those obtained in arterial conductance vessels (aorta) of endothelial GTPCH-deficient mice (Chuaiphichai *et al.*, 2014).

A controversial discussion has continued for many years concerning the relevance of BH4 levels for the eNOS coupling state and implications for the development of endothelial dysfunction. BH4 levels have been shown to be up- rather than down-regulated in whole aortic tissue and plasma despite obvious endothelial dysfunction (Antoniades *et al.*, 2007; Kossmann *et al.*, 2014). The inherent problem is that BH4 synthesis is also strongly linked to inflammation in vascular tissue and, thus, BH4 levels are typically increased along with an increase in the expression of the inducible NOS (iNOS) since BH4 is required for iNOS activity (Kossmann *et al.*, 2014).

DOI:10.1111/bph.13827

GTPCH is constitutively active in macrophages and is an enzyme that can be induced by pro-inflammatory cytokines, suggesting that vascular BH4 levels, upon infiltration of macrophages, will not simply reflect endothelial BH4 content. Indeed, using a model of angiotensin II-induced hypertension, our group recently established an essential role of vascular inflammation for the underlying pathogenesis (Wenzel *et al.*, 2011). In a subsequent study, increased vascular oxidative stress, eNOS uncoupling, impaired aortic NO formation and endothelial dysfunction were observed despite a significant up-regulation of aortic GTPCH protein and accordingly higher vascular levels of BH4 (Kossmann *et al.*, 2014). The eNOS uncoupling, despite more vascular BH4, was demonstrated by two independent methods, L-NAME-inhibitable endothelial ROS formation and increased eNOS S-glutathionylation respectively. The latter parameter can be viewed as an established marker for eNOS uncoupling (Chen *et al.*, 2010).

Channon and co-workers have stressed for a long time the concept that endothelial GTPCH and BH4 levels are the essential determinants of eNOS functionality and coupling state. Indeed, as shown previously in a clinical study, vascular but not plasma BH4 is an important determinant of eNOS coupling state, endothelium-dependent vasodilatation and superoxide production in human vessels, whereas plasma BH4 instead reflects a systemic inflammatory response (Antoniades *et al.*, 2007). With the present studies, Chuaiphichai *et al.* again provide molecular proof of their postulate and clearly demonstrate that an endothelial deficiency of BH4 is sufficient to cause endothelial dysfunction and eNOS uncoupling in arterial resistance arteries as measured by endothelium-specific, L-NAME-inhibitable ROS formation and diminished eNOS dimer/monomer ratio.

Besides BH4, many other 'redox switches' within the enzyme eNOS were identified and discussed with respect to their relevance to render the enzyme inactive or even uncoupled [among them zinc–sulfur complex oxidation (Zou *et al.*, 2002), S-glutathionylation (Chen *et al.*, 2010), phosphorylation at Tyr^{657} and Thr^{495} and asymmetric dimethylarginine (ADMA) synthesis and degradation, reviewed in Daiber *et al.* (2017)], challenging the unique role of BH4 deficiency in this process, especially in the presence of different cardiovascular risk factors. As a potential link between these different 'redox switches' in eNOS, a close interaction between BH4 availability and eNOS S-glutathionylation was reported previously (Crabtree *et al.*, 2013). However, this link was not confirmed in the present work nor was a correlation found between BH4 levels and activating/inactivating phosphorylations (Ser^{1177}, Thr^{495}) of eNOS.

The results by Chuaiphichai *et al.* substantially contribute to our understanding of the mechanism underlying the development of endothelial dysfunction in resistance arteries, which can be regarded as an early correlate of atherosclerosis and a major trigger for the development of future cardiovascular disease (Panza *et al.*, 1990; Vita *et al.*, 1990) and prognosis (Schachinger *et al.*, 2000; Gokce *et al.*, 2002) (for a review, see Daiber *et al.*, 2017). Their results may also explain our previous clinical data that BH4 infusion corrected endothelial dysfunction in chronic smokers and therefore reversed eNOS uncoupling, whereas the structural analogue tetrahydroneopterin (NH4), which cannot bind to eNOS, had no effect on endothelial dysfunction (Heitzer *et al.*, 2000).

Arterial hypertension is the most important cardiovascular risk factor in industrialized countries because of its very high prevalence (Lim *et al.*, 2012; Murray *et al.*, 2012). In 1991, Nakazono and co-workers demonstrated that the administration of heparin-binding superoxide dismutase was able to significantly lower arterial blood pressure in spontaneously hypertensive rats, suggesting that increased production of ROS at least in part mediates arterial hypertension (Nakazono *et al.*, 1991). Further research established the vascular NADPH oxidase as a significant superoxide source (Rajagopalan *et al.*, 1996). In 2002, Mollnau *et al.* were the first to demonstrate the presence of an uncoupled eNOS in the setting of angiotensin II-induced hypertension (Mollnau *et al.*, 2002). It is known that in the setting of increased oxidative stress within endothelial cells (e.g. induced by the NADPH oxidase), BH4 may be oxidized to the $^{\bullet}$BH3 radical (Kuzkaya *et al.*, 2003), all of which can result in eNOS uncoupling (Forstermann and Munzel, 2006). The important implication is that a combination of antioxidant therapy and BH4 supplementation may be required in order to successfully treat cardiovascular diseases (Munzel *et al.*, 2010).

This pathophysiology may be one of the reasons why, in large-scale clinical trials, non-selective antioxidant drugs have failed to show any health benefits for the treatment of cardiovascular disease (Munzel *et al.*, 2010).

Acknowledgements

The authors acknowledge the continuous support by the Foundation Heart of Mainz and the DZHK (German Center for Cardiovascular Research), Partner Site Rhine-Main, Mainz, Germany.

Conflict of interest

The authors declare no conflicts of interest.

References

Antoniades C, Shirodaria C, Crabtree M, Rinze R, Alp N, Cunnington C *et al.* (2007). Altered plasma versus vascular biopterins in human atherosclerosis reveal relationships between endothelial nitric oxide synthase coupling, endothelial function, and inflammation. Circulation 116: 2851–2859.

Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA *et al.* (2010). S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. Nature 468: 1115–1118.

Chuaiphichai S, McNeill E, Douglas G, Crabtree MJ, Bendall JK, Hale AB *et al.* (2014). Cell-autonomous role of endothelial GTP cyclohydrolase 1 and tetrahydrobiopterin in blood pressure regulation. Hypertension 64: 530–540.

Chuaiphichai S, Crabtree MJ, McNeill E, Hale AB, Trelfa L, Channon KM *et al.* (2017). A key role for tetrahydrobiopterin-dependent endothelial NOS regulation in resistance arteries: studies in endothelial cell tetrahydrobiopterin-deficient mice. Br J Pharmacol 174: 657–671.

Crabtree MJ, Brixey R, Batchelor H, Hale AB, Channon KM (2013). Integrated redox sensor and effector functions for tetrahydrobiopterin- and glutathionylation-dependent endothelial nitric-oxide synthase uncoupling. J Biol Chem 288: 561–569.

Daiber A, Di Lisa F, Oelze M, Kroller-Schon S, Steven S, Schulz E *et al.* (2017). Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function. Br J Pharmacol 174: 1670–1689.

Daiber A, Steven S, Weber A, Shuvaev VV, Muzykantov VR, Laher I *et al.* (2017). Targeting vascular (endothelial) dysfunction. Br J Pharmacol 174: 1591–1619.

Forstermann U, Munzel T (2006). Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 113: 1708–1714.

Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Menzoian JO, Vita JA (2002). Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 105: 1567–1572.

Heitzer T, Brockhoff C, Mayer B, Warnholtz A, Mollnau H, Henne S *et al.* (2000). Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers: evidence for a dysfunctional nitric oxide synthase. Circ Res 86: E36–E41.

Kossmann S, Hu H, Steven S, Schonfelder T, Fraccarollo D, Mikhed Y *et al.* (2014). Inflammatory monocytes determine endothelial nitric-oxide synthase uncoupling and nitro-oxidative stress induced by angiotensin II. J Biol Chem 289: 27540–27550.

Kuzkaya N, Weissmann N, Harrison DG, Dikalov S (2003). Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. J Biol Chem 278: 22546–22554.

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H *et al.* (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2224–2260.

Mollnau H, Wendt M, Szocs K, Lassegue B, Schulz E, Oelze M *et al.* (2002). Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. Circ Res 90: E58–E65.

Munzel T, Gori T, Bruno RM, Taddei S (2010). Is oxidative stress a therapeutic target in cardiovascular disease? Eur Heart J 31: 2741–2748.

Murray CJ, Ezzati M, Flaxman AD, Lim S, Lozano R, Michaud C *et al.* (2012). GBD 2010: design, definitions, and metrics. Lancet 380: 2063–2066.

Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M (1991). Does superoxide underlie the pathogenesis of hypertension? Proc Natl Acad Sci U S A 88: 10045–10048.

Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE (1990). Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 323: 22–27.

Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK *et al.* (1996). Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 97: 1916–1923.

Schachinger V, Britten MB, Zeiher AM (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101: 1899–1906.

Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC *et al.* (1990). Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 81: 491–497.

Wenzel P, Knorr M, Kossmann S, Stratmann J, Hausding M, Schuhmacher S *et al.* (2011). Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction. Circulation 124: 1370–1381.

Zou MH, Shi C, Cohen RA (2002). Oxidation of the zinc–thiolate complex and uncoupling of endothelial nitric oxide synthase by peroxynitrite. J Clin Invest 109: 817–826.