Response to Tetrahydrobiopterin and Endothelial Nitric Oxide Synthase Uncoupling

Although we certainly recognize the excellent work done by Tsikas et al.1 using purified endothelial NO synthase (eNOS) and incubation with tetrahydrobiopterin (H4B) at much higher concentrations (see Reference 4 and Figure in Reference 1) than what is found in vivo by many independent groups including us,2–4 we respectfully disagree based on the following grounds. First, accurate assessment of the uncoupling state of eNOS and determination of endogenous H4B content to the level of picomoles per milligram of protein in vivo can be successfully achieved. A large body of published work from many independent groups including ours has confirmed this consistently.2–4 In our case, we used the highly sensitive electron spin resonance method to measure superoxide production and high-performance liquid chromatography to measure aortic H4B content, which many others and we have used for both tissue and cells to characterize uncoupling of eNOS. Hence, we believe that our assessment of eNOS (un)coupling in the present study is convincing.

Furthermore, in the Figure provided by Tsikas et al.,1 the amount of H4B used in the in vitro system to assess eNOS function was ≈1000 times more than what can be detected in the in vivo system.2–4 Although in our study we did not specifically measure the molar amount of eNOS enzyme present in the aorta, one could estimate its level by the production of NO as we did or, for comparison with the data given by Tsikas et al.,1 total nitrite and nitrate (NOx) levels. In the data given, total NOx levels plateau in the range of ≈300 nmol/L, with the lowest H4B level used at 100 nmol/L. This would give a ratio of ≈1:3 of H4B to NOx produced. In the in vivo condition, previous studies report the aortic NOx levels to be on the order of 1 to 3 nmol/L per milligram protein,5 whereas our measured H4B levels in the aorta (Figure 3B in our article4 and others1,2 are on the order of 2–5 pmol/mg of protein). This would give a ratio of ≈1:1000 of H4B to NOx produced. Although total NOx levels is not the most accurate way to assess eNOS function, this rough calculation would seem to suggest that the failure to detect any changes in NO bioavailability in vitro under reduced H4B condition may be because of the extremely high or excessive H4B levels used.

Finally, we would like to point out that there have been tons of literature that have consistently suggested that angiotensin II type 1 receptor reduces reactive oxygen species production to improve NO bioavailability in both experimental models and human patients, including a classic article by Rajagopalan et al.6 In addition, recent work from others has confirmed our observation of angiotensin II uncoupling of eNOS in vitro and in vivo. Consistent with the findings by Lobysheva et al.7 published this year, we previously identified an upstream role of NADPH oxidase in mediating angiotensin II uncoupling of eNOS.8

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Disclosures
None.

Kin Lung Siu
Division of Molecular Medicine
Cardiovascular Research Laboratories
Departments of Anesthesiology and Medicine/Cardiology
David Geffen School of Medicine
University of California Los Angeles
Los Angeles, CA

Ling Gao
Department of Endocrinology
Renmin Hospital
Wuhan University
Wuhan, China

Hua Cai
Division of Molecular Medicine
Cardiovascular Research Laboratories
Departments of Anesthesiology and Medicine/Cardiology
David Geffen School of Medicine
University of California Los Angeles
Los Angeles, CA

References


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