Redox Biology 5 (2015) 409–412

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Editors' Forum

Discussion of controversies in the measurement of nitric oxide metabolites in biological matrices

Santiago Lamas (Associate Editor)

We incorporate a new section in Redox Biology, the Editors' forum. The main objective is to attract short contributions related to previously published articles that may help to clarify concepts, open controversies or constructively criticize conclusions from a specific article. The contribution will show a link to the related article and may be accompanied by the appropriate response of the authors to the commentary on their article.

The article by Drs. Cortese-Krott and Kelm [1] promoted an extensive analysis by Dr. Tsikas about the optimal methodology to characterize eNOS activity in red blood cells and its relevance for the concepts put forward by Cortese-Krott and Kelm. The authors were given the opportunity to respond to this commentary and their answer is shown below the commentary. Indeed this is an important question as the jury appears to be still out concerning the existence of a NOS isoform in human red blood cells. Dr. Tsikas makes a strong case on the validity of the gas chromatography-mass spectrometry (GC-MS) NOS activity assay for a large set of samples, including erythrocytes, and delves into the complex chemistry involved. Drs. Cortese-Krott and Kelm lucidly respond to Dr.Tsikas with equally powerful arguments. We are proud to host this illuminating discussion on a complex chemical topic and we invite all readers to follow this example and submit commentaries that may contribute to enhance progress in the understanding of challenging notions in the field of redox biology.

http://dx.doi.org/10.1016/j.redox.2015.01.018

Can nitric oxide synthase activity be unequivocally measured in red blood cells and platelets? if yes, by which assay?

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To the Editor

I read with interest the article by Cortese-Krott and Kelm [1] who reviewed and discussed the at first glance paradoxical, yet potentially important and formidably challenging issue of nitric oxide (NO) synthesis from L-arginine by endothelial NO synthase (eNOS) in red blood cells (RBCs), a possibly new erythrocrine function. In consideration of the high concentration of hemoglobin in erythrocytes (about 10 mM) and the high affinity of O_2, NO and carbon monoxide (CO) and presumably of other gases to the heme group of hemoglobin, the majority of scientists believes that RBCs behave toward NO as do super-massive black holes behave against matter and even light, with other words as "NO sinks". Yet, in practice we see that RBCs are able to export NO and other NO species that can finally exert cGMP-dependent and cGMP-independent NO-related activities in the circulation. With respect to this issue I fully agree with the statements, arguments and conclusions by Cortese-Krott and Kelm in their review article [1]. However, I do not agree with the authors on their argument concerning the alleged chemistry of NOS-derived NO in RBCs and that this particular NO chemistry may explain our failure to measure NOS activity in human RBCs by means of a previously reported, fully-validated [2] and cross-validated [3] gas chromatography-mass spectrometry (GC–MS) NOS activity assay.

The principle of our GC–MS NOS activity assay [2] used in vivo and in vitro in blood cells including human RBCs [4] and platelets [3,5] and the major reactions of NO and its major metabolites/reaction products, nitrate and nitrite, are illustrated in Fig. 1. We use L-[*guanidino*-$^{15}N_2$]-arginine as the substrate for NOS which oxidizes one of the two ^{15}N-labelled atoms of L-[*guanidino*-$^{15}N_2$]-arginine to ^{15}NO, with L-[*ureido*-^{15}N]-citrulline being the second reaction product. Because authentic ^{15}NO is not detectable at low NOS activity values as in the case of endothelial NOS (eNOS), analytically accessible metabolites/reaction products of ^{15}NO are analyzed instead. It must be emphasized that all eNOS activity assays are exclusively based on the measurement of NO metabolites/reaction products because of the pretty low NO formation rate. In aqueous solutions including blood ^{15}NO is autoxidized to ^{15}N-nitrite ($^{15}NO_2^-$), albeit to a minor extent [6]. NOS isozymes are able to oxidize its own ^{15}NO to ^{15}N-nitrate ($O^{15}NO_2^-$) [3]. In aqueous solutions of recombinant eNOS solutions and in washed human platelets suspensions, the $^{15}NO_2^-$-to-$O^{15}NO_2^-$ molar ratio is about 1:1 [5]. In human erythrocytes suspensions in regular buffers of neutral pH value or in whole blood [6], ^{15}NO-derived $^{15}NO_2^-$ is practically undetectable, because ^{15}NO is rapidly oxidized by oxyhemoglobin ($HbFe^{II}O_2$) to $O^{15}NO_2^-$. Thus, in RBCs eNOS would convert L-[*guanidino*-$^{15}N_2$]-arginine to ^{15}NO which would be oxidized to $O^{15}NO_2^-$ largely by $HbFe^{II}O_2$ and to a minor extent by eNOS (Fig. 1). In theory, a very small fraction of L-[*guanidino*-$^{15}N_2$]-arginine/eNOS-derived ^{15}NO would autoxidize to $^{15}NO_2^-$ which would almost uniformly partition between plasma and RBCs. Erythrocytic $^{15}NO_2^-$ would finally be oxidized to $O^{15}NO_2^-$ by $HbFe^{II}O_2$. This is supported by our observation that synthetic ^{15}NO gas is oxidized in human whole blood to $O^{15}NO_2^-$ and $^{15}NO_2^-$ with a molar ratio of about 250:1 after 15 min of continuous ^{15}NO bubbling at