Red Blood Cell-Mediated Hypoxic Vasodilatation

A Balanced Physiological Viewpoint

To the Editor:

We were disappointed to read the editorial presented by Drs Gladwin and Schechter,1 which appeared in the same issue of Circulation Research as our article.2 We believe that the views reflected a selective bias from 1 of 2 camps that have posed diametrically opposite views. This editorial was simply another review on nitrite by these authors. We are not members of 1 “camp” or of the other, but simply wish to present our observations in the hope that more physiological data may provide clarification.

Although Gladwin and Schechter began by stating that the purpose of their editorial was not to discuss our article, they proceeded to criticize our work throughout the editorial. Here, we wish to set the record straight on several important scientific issues that we believe were misrepresented in the Gladwin and Schechter editorial. Specifically,

1. They make a lengthy point of questioning the levels of nitrosyl hemoglobin (HbNO) we describe and how this might be reflected in vivo and advise follow-up studies with electron paramagnetic resonance (EPR). We do not believe that EPR can add to our results, other than by further confirming HbNO production. EPR is capable of selectively measuring accurately only 5 coordinate α HbNO hyperfine signals. With the use of appropriate control spectra from 5 and 6 coordinate α purified HbNO and a poorly resolved βHbNO, complicated spectral simulation and subtraction yields qualitative information on these subunits/species in a human blood sample at best. Importantly, the suggested studies will not influence the correlation we demonstrated in our article between S-nitrosohemoglobin (HbSNO) content and red blood cell (RBC)-induced hypoxic vasodilatation.

2. The authors made selective use of references, omitting our articles and those of others that might counter their arguments. Several groups using totally different methodologies (Funai et al.,3 Stamler et al.,4 and Datta et al5) have found levels of HbNO within the same order of magnitude as ours; yet this point is ignored. We previously published basal and NO-treated EPR detectable levels of HbNO in RBC from controls and patients with insulin-dependent diabetes mellitus.3 We have recently reported cross-pulmonary gradients of HbSNO in human subjects using exactly these techniques.6 Several key articles have discussed the methodologies and techniques in detail, including considerable debate on the value of making measurements of HbSNO by chemiluminescence.4 Several groups have successfully derived very low in vivo values using chemiluminescence, not surprisingly given that these groups used the same chemical treatment of blood. Yet, the published work using chemiluminescence is far from watertight. For example, in a Nature Medicine article6 that apparently confirms nitrite vasodilates the human circulation, the chemiluminescence signals for HbNO and HbSNO depicted from artery and vein could not have been detected using this methodology, which was published by these authors in the online supplement. (The stabilization solution they themselves propose will get rid of HbNO, so, how then was it detected in the sample without mercury treatment?) The devil is indeed in the details.

3. The authors offer strong advice that it is critically important to carefully compare NO-treated RBC with nonallosteric NO donors.1 This is exactly what was reported in our article. We demonstrated that S-nitroso-glutathione, a small molecule incapable of oxygen-linked allosteric structural transitions, shows increasing vasodilation of aortic vessel rings under hypoxic conditions. They fail to discuss that we also showed RBC to have a significantly enhanced hypoxic effect compared with this appropriate control.

4. We agree the “nitrite” theory is at first glance tempting. It may indeed be of interest to the authors to compare our NO-RBC–induced vessel dilatation with NO-RBC made from equimolar additions of nitrite to deoxy RBC. However, these experiments address 2 very distinct theories: (1) that NO metabolites within RBC (devoid of large extracellular nitrite) release a vasoactive agent when placed in a hypoxic environment and, separately, (2) that under certain conditions, deoxy RBC utilize exogenous nitrite to produce a vasoactive agent (as they have already described).6 To clarify, there is no question that nitrite can influence the NO metabolite ratio within RBC in vitro and in vivo. Furthermore, the fact that nitrite can affect vasodilation in humans is well documented and certainly not conceived by Drs Gladwin and Schechter. The idea that deoxyHb is responsible for the exercise vasodilation by nitrite in Cosby et al6 could not be more strongly refuted by their own data. (Deoxy Hb levels decrease with nitrite infusion, and nitrite does not potentiate the increase in flow observed in hypoxic exercise [versus hypoxic exercise alone]). NO from nitrite does not appear to be a valid explanation to the results we present in our article.2

The question remains how RBCs mediate vasodilatation in the absence of large doses of extracellular nitrite—this is far from elucidated by the plethora of recent reviews. We remind the interested reader that extracellular nitrite is not required to mediate oxygen-dependent RBC-mediated vasodilation of aortic vessels, as evidenced by our work and others. Freshly harvested RBCs (without platelets or plasma and essentially devoid of extracellular nitrite) also induce vessel relaxation at low O2.

Let’s reach a balanced scientific consensus on a physiological mechanism with appropriate recognition of pioneering work. Data from pathological conditions may help us achieve this.

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