The Janus Faces of NO?

To the Editor:

The functional effects of myocardial iNOS expression are still a matter of controversy.1 To directly address the role of iNOS on myocardial function, two transgenic animal models with cardiac-specific overexpression of iNOS have been generated.2–3 Mungrue et al describe the generation and analysis of a binary transgenic mouse model for a tetracycline-inducible, cardiac-specific expression of iNOS (tTA-iNOS mice). The authors find higher mortality after induction of iNOS expression and link this phenotype to disturbances of the cardiac conductance. The results are in contrast with another report on the effects of chronic overexpression of iNOS in the heart of transgenic mice under the control of the α-MHC promoter (tg-iNOS mice), which was published in this journal.2 These latter mice show only minimal alterations of cardiac function measured in vivo and ex vivo and no signs of higher mortality or sudden death.

Mungrue and coworkers addressed the differences between both models already in an earlier publication and concluded that Heger et al did not analyze myocardial overexpression of iNOS but rather selected for mice with inactive iNOS. To support this view, Mungrue et al claim that Heger et al did not conclusively show that iNOS was active in transgenic hearts in vivo and suspected that the iNOS activity in their conditional model was higher than in the constitutive model.3 This unproven assumption was extended by further unassigned speculations in a Letter to the Editor published in Circulation Research,4 in which Mungrue et al respond to a recent publication by Wunderlich et al in the same journal.5 In this study, Wunderlich et al demonstrate that the cardioprotective function of myoglobin is the reason that tg-iNOS mice, despite high levels of cardiac NO formation, do not develop heart failure.

To make the available data for iNOS activity in the inducible (tTA-iNOS) and constitutive (tg-iNOS) iNOS expression models transparent, a direct comparison of the published data is revealing. Both groups analyzed iNOS activity by the citrulline assay. The basal levels measured are in the same range (tTA-iNOS by Mungrue et al: 0.5 versus tg-iNOS by Heger et al: 1.7 pmol/min per g protein). However, iNOS activity in tg-iNOS (697 pmol/min per g protein) is 150-fold higher than in the conditionally induced tTA-iNOS mice (4.7 pmol/min per g protein). In this context, it should be noted that Mungrue et al in their Letter to the Editor report basal values for NOx activity that were 10-fold lower than in their original study published in the Journal of Clinical Investigation.6 Thus, the authors now claim a 100-fold increase in cardiac NO activity over basal in the conditional model whereas a 10-fold increase was explicitly stated in their previous publication.

Concerning iNOS activity in vivo, conclusive evidence for an active iNOS was only presented by Heger et al showing that both cardiac and plasma NOx levels (WT: 13.7±3.8; tg-iNOS: 28.9±7.8 nmol/mL) increased in tg-iNOS mice. Although measured, Mungrue et al failed to detect elevated plasma NOx levels. This difference is consistent with the low expression level of iNOS in the inducible system. Since Mungrue et al reported that under inducing conditions urinary NOx levels were not elevated, we have now determined in vivo NOx activity in tg-iNOS mice by measuring urinary excretion of NOx. We found that NOx release by tg-iNOS mice was 2.5-fold higher than by WT mice. These data indicate that the NO production rate by the hearts of tg-iNOS mice was at least 2.5-fold higher than by the sum of all nitric oxide synthases of a whole WT mouse. The relative increase is prone to underestimation because basal urinary NOx excretion not only reflects NOX activity but is also due to dietary uptake and/or microbial NOx formation. The only evidence for iNOS activity in vivo presented by Mungrue et al so far is based on an intensity score for nitrotyrosine formation on histological slices that, according to the classification chosen, was found to be moderately elevated.

Mungrue et al further suspect that we might have selected against an active iNOS by our constitutive approach. They state “our attempts to produce nonconditional α-MHC-iNOS mice were not successful after screening ~50 founders” (page e74) and asked how many founders we had to screen to obtain transgenic animals. We found ~15% of the born offspring to be transgenic and 10 of the 17 transgenic lines obtained from 120 born pups were shown to express iNOS to a varying extent. Thus, the failure of Mungrue et al to generate mice expressing iNOS constitutively does certainly not reflect a biological, but rather a technical, problem.

As to the functional role of NO formed by the heart of tg-iNOS mice, we recently provided a mechanistic explanation for the benign phenotype. As shown by Wunderlich et al and by Gödecke et al, it is myoglobin’s ability to inactivate high levels of NO within the heart that accounts for the benign phenotype of tg-iNOS mice. Importantly, generation of tg-iNOS mice devoid of myoglobin by breeding tg-iNOS with myoglobin knockout mice resulted in cardiac hypertrophy, ventricular dilatation, and interstitial fibrosis. Should we have selected against an active iNOS, what would be the cause for this pathological phenotype? Although this study appeared prior to the publication of Wunderlich et al,7 it was not cited by Mungrue et al in their Letter to the Editor.

In view of the discrepant finding, the question remains why mice expressing only low levels of iNOS under control of the tetracycline-regulatable tTA transactivator show such a pronounced cardiac phenotype. Since our experiments clearly show that NO in the presence of myoglobin can be ruled out as a causative agent, it must be considered that in the conditional model, secondary changes due to high-level expression of the tTA transactivator in the heart are induced. In fact, it was reported that the tTA protein inhibited expression of transcription factors such as C/EBP, glucocorticoid receptor, and SP1.7 Together, we believe that it is critical for the interpretation of functional data in transgenic mice that the experiments be properly controlled at the genomic, biochemical, and functional levels. The general conclusion by Mungrue et al that the conditional system is reliable, whereas the constitutive system has fundamental weaknesses, is misleading.

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