The Janus Faces of iNOS

To the Editor:

Recently, two groups have reported on the phenotype of transgenic mice with cardiac-specific overexpression of the inducible nitric oxide synthase (iNOS). Although both used the α-MHC promoter to target iNOS expression, our model used the tetracycline-regulated system (αMTRA/iNOS), whereas the other used a nonconditional approach. Intriguingly, the phenotypes reported varied dramatically, from lethal arrhythmias in our case, to no identifiable abnormality in the second. Additionally, a recent publication from the second group1 includes criticisms of our work that are misleading.

The statement that “iNOS activity in [the nonconditional] model is approximately 100–120-fold higher than...” (page 1357) is unfounded. A direct comparison between cardiac iNOS activities in the two models is not possible. Heger et al2 presented S-ethylisothiourea-sensitive conversion of arginine to citrulline (control: 1.7 ± 3.1 versus transgenic: 697 ± 238 pmol/min/mg), whereas we measured L-NMMA-sensitive Ca2+ independent activity (1 μmol/L EGTA) (0.056 ± 0.05 versus 4.57 ± 1.36 pmol/min/mg).1 While both provide measures of iNOS activity, absolute values varied considerably. Although baseline values in the Heger mice were ostensibly ~30-fold higher than ours, it is apparent from these1,2 and other reports that basal cardiac iNOS activity in mice is not different from zero. Thus, it is inappropriate to compare directly the reported iNOS activities in these models. Normalizing iNOS activity using control values is invalid, since fold increases based on a denominator that approaches zero is prone to overestimation.

In our view, a more reliable comparison could be made between total NOS activity, for which accurate basal measurements can be made. Unfortunately, total NOS activity has not been reported for the Heger mice. Indeed, based on the published data, we believe that our model exhibited higher (not lower) NOS activity, as evidenced by Heger mice. Indeed, based on the published data, we believe that our model exhibited higher (not lower) NOS activity, as evidenced by Heger et al.2 Unfortunately, total NOS activity has not been reported for the Heger mice. Indeed, based on the published data, we believe that our model exhibited higher (not lower) NOS activity, as evidenced by Heger et al. Unfortunately, total NOS activity has not been reported for the Heger mice. Indeed, based on the published data, we believe that our model exhibited higher (not lower) NOS activity, as evidenced by Heger et al.2

Additionally, our model is accompanied by enhanced cardiac ONOO− and O2− , suggesting iNOS “uncoupling,” as would be expected in the setting of its marked and sustained overexpression. By contrast, the Heger mice exhibited only ~2- to 3-fold increase in NO production2 and no evidence of uncoupled activity.

Thus, their recent conclusion that “Mungrue et al... failed to provide biochemical evidence of iNOS activity in vivo” (page 1357) chooses to ignore other biologically important products of iNOS activity, namely ONOO− and O2− , suggesting iNOS “uncoupling” as would be expected in the setting of its marked and sustained overexpression. By contrast, the Heger mice exhibited only ~2- to 3-fold increase in NO production2 and no evidence of uncoupled activity.

In conclusion, while the differences between the two models of enhanced cardiac iNOS remain to be fully explained, it is misleading to dismiss cardiac toxicity as an artifact of the conditional system. Rather, it is more reasonable to suggest that the conditional approach is essential to overcome a lethal phenotype or adaptations that result from high levels of cardiac iNOS overexpression, likely related to uncoupled activity and increased free radical generation. Instead, Wunderlich et al3 have suggested that nonspecific effects of the tTA or LacZ coexpression could account for the phenotype of our conditional model. This speculation is particularly misleading. First, age- and sex-matched littermate αMTA/iNOS controls, which express TTA (but not iNOS) in a cardiac-restricted manner, did not display any evidence of cardiac dysfunction,1 consistent with many other reports.4-6 Second, while doxycycline administration would have suppressed both the expression of iNOS and β-gal, the latter is unlikely to have caused our phenotype. β-Gal is a widely used reporter gene, and its expression has not previously been reported to generate a cardiac phenotype.6 Moreover, in our study, line 365IC, which had comparable β-gal activity but no iNOS expression (possibly due to transgene truncation/deletion), did not display any incidence of premature mortality. Finally, preliminary experiments using an iNOS-specific inhibitor (1400W), completely rescued the lethal phenotype of αMTA/iNOS mice (authors’ unpublished data, 2003), providing further confirmation that the cardiac manifestations are a result of increased iNOS activity in this model.

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