Nitric Oxide in Vascular Endothelial Growth Factor Synthesis and Signaling

To the Editor:

Recently, Matsunaga et al.1 discussed the role of nitric oxide (NO) and vascular endothelial growth factor (VEGF) in coronary collateral growth. They have convincingly demonstrated that multiple occlusion of the left anterior descending coronary artery induced collateralization and was accompanied by VEGF protein production. In control dogs, VEGF peaked at day 3 of the repetitive occlusions but waned thereafter (Matsunaga et al, Figure 3).1 In contrast, in animals treated with Nω-nitro-L-arginine-methyl ester (L-NAME), the VEGF expression was elevated throughout the entire 21-day experiment. The authors concluded that inhibition of NO synthase (NOS) prevents collateral growth but augments expression of VEGF. In our opinion, however, the results concerning the regulation of VEGF by NO can be interpreted in a different way.

Apparently, the sensitivity of detection of blotted proteins differed in the 2 experiments described by Matsunaga et al. The authors made a densitometric analysis of the expression of endogenous VEGF in comparison with the external standard, and they concluded that VEGF protein was elevated throughout the whole experiment in L-NAME-treated animals but not in controls. We suggest, however, that the external standard is not reliable for validation of the level of expression. The better option would be to use an internal protein standard. Matsunaga et al. used the same amount of total protein for analysis (400 μL of myocardial interstitial fluid), and thus, the weaker band of VEGF protein at day 1 after L-NAME treatment may show the inhibition of VEGF synthesis when NOS activity is attenuated. Interestingly, the VEGF expression after 21 days’ treatment with L-NAME seems to be elevated, both at the protein level and at the mRNA level (Matsunaga et al, Figures 3 and 4). Thus, the chronic inhibition of NOS in repetitive ischemia may have a different effect on VEGF synthesis than does short-term treatment with L-NAME.

Our recent data2-3 and studies by Kimura et al.4 and Frank et al.5 indicate that NO induces VEGF synthesis in vascular smooth muscle cells, tumor cells, and keratinocytes, respectively. Thus, NO is not only a downstream mediator of VEGF signaling in endothelial cells but also can operate in an upstream direction, inducing VEGF synthesis.

We showed that in nonischemic conditions, L-NAME2-3 as well as asymmetric dimethylarginine,6 an endogenous inhibitor of NOS activity, decreased VEGF synthesis. Such an effect can be also observed in Figure 4 of Matsunaga et al., in which the expression of VEGF mRNA in nonischemic myocardium seems to be significantly lower in L-NAME–treated animals than in controls. In contrast, L-NAME strongly enhanced VEGF expression in ischemia. Thus, the effect of NO on VEGF synthesis might be different in normoxic and in hypoxic conditions.

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Response

We thank Drs Dulak and Jozkowicz for their interest in our paper, but we disagree with some of their arguments and interpretations. Drs Dulak and Jozkowicz stated that the sensitivity of detection for vascular endothelial growth factor (VEGF) differed in our 2 experiments, but it did not. They further state that we should have used an internal protein standard for normalization of the VEGF signals. The use of an internal protein standard assumes that the concentration of protein in the control and Nω-nitro-L-arginine-methyl ester (L-NAME) groups was the same—an incorrect assumption because the total amount of protein in the interstitial fluid in the control group was different from that in the L-NAME group. To accurately estimate the concentration of VEGF in interstitial fluid, we normalized to volume in both groups, which should reflect the actual concentration in the myocardial interstitial fluid.

We also appreciate the information about the induction of VEGF expression by NO in cultured smooth muscle cells and in keratinocytes.2-4 We do not dispute the results but remind Drs Dulak and Jozkowicz that extrapolation of results from experiments on cultured cells, which likely have a phenotype far different than those in vivo, is not a trivial matter. Moreover, the factors that drive VEGF expression in culture or in a skin-wound healing model may also be quite different from those involved in myocardial ischemia.

Finally, Drs Dulak and Jozkowicz stated that our results support the idea that the effects of NO on expression of VEGF may be different in the presence or absence of ischemia. We emphasize that in our model, repetitive coronary occlusions produce repeated episodes of myocardial ischemia, which induce the expression of VEGF. Basal expression of VEGF (in the absence of ischemia) is low. As coronary collaterals develop during the repetitive occlusions, the ischemic signal wanes, as does the expression of VEGF. When collateral growth is prevented by inhibition of NO synthesis, the level of ischemia is maintained during the occlusions, and the expression of VEGF remains elevated compared with basal levels. Importantly, during early periods of occlusion when the level of ischemia in the control and L-NAME groups is equivalent, the expression of VEGF is also equivalent. Thus, the expression of VEGF in the intact myocardium appears to be related to the presence of ischemia and not to the production of NO.

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