

Correspondence

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Endothelial Nitric Oxide Synthase and Heart Rate

To the Editor:

We read with interest the recent study by Brunner et al,¹ which provides compelling evidence that high concentrations of nitric oxide (NO) generated from endothelial nitric oxide synthase (eNOS) overexpression reduces cardiac contraction by decreasing myocardial calcium sensitivity. Their data also suggest that NO has very little effect on the autonomic modulation of cardiac excitability. This latter finding, however, must be viewed with caution. Although there is no convincing functional evidence supporting a role for endogenously produced NO in the postsynaptic autonomic regulation of heart rate, NO donors are capable of increasing heart rate by cyclic guanosine monophosphate (cGMP) dependent activation of the hyperpolarization-activated current (I_f) in pacemaking cells.² Moreover, NO generated from neuronal nitric oxide synthase (nNOS) in intrinsic cardiac ganglia³ during stimulation of the vagus nerve enhances the magnitude of the bradycardia⁴ by facilitating acetylcholine release.⁵ Consistent with this, nNOS knockout mice have an impaired heart rate response to vagal activation, whereas their rate response to acetylcholine itself remains intact.³ Thus, although eNOS-NO has very little effect on the cholinergic regulation of heart rate to bath applied neurotransmitter, nNOS-NO activated physiologically by vagal stimulation clearly affects heart rate control. When these results are taken together with Brunner et al,¹ they further reinforce the notion of the differential site-specific action that various NOS isoforms play in modulating cardiac function.

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1. Brunner F, Andrew P, Wolkart G, et al. Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Circulation*. 2001;104:3097–3102.
2. Musialek P, Lei M, Brown HF, et al. Nitric oxide can increase heart rate by stimulating the hyperpolarization-activated inward current, I_f . *Circ Res*. 1997;81:60–68.
3. Choate JK, Danson EJ, Morris JF, et al. Peripheral vagal control of heart rate is impaired in neuronal NOS knockout mice. *Am J Physiol Heart Circ Physiol*. 2001;281:H2310–H2317.
4. Herring N, Golding S, Paterson DJ. Pre-synaptic NO-cGMP pathway modulates vagal control of heart rate in isolated adult guinea pig atria. *J Mol Cell Cardiol*. 2000;32:1795–1804.
5. Herring N, Paterson DJ. Nitric oxide-cGMP pathway facilitates acetylcholine release and bradycardia during vagal nerve stimulation in the guinea-pig in vitro. *J Physiol*. 2001;535:507–518.

Response

We thank Drs Herring and Paterson for their comments on our recent publication,¹ in which we found no effect of myocyte-

derived nitric oxide (NO) on heart rate in conscious mice, or on beating rate in isolated hearts perfused with acetylcholine. The authors correctly point out recent evidence for a bradycardic role of NO derived from neuronal NO synthase (nNOS). We are aware of the significantly higher mean heart rate and lower heart rate variability in conscious nNOS knockout mice,² as well as the higher baseline heart rate in isolated right atrial preparations from such animals,³ but would like to stress that the relevance of these observations for in vivo control of heart rate in genetically unaltered mice or other species is not known. Also, in the latter report, pharmacological inhibition of nNOS was only marginally effective in attenuating heart rate response to vagus nerve stimulation (difference to control, 15 to 20 beats per min, at a basal heart rate of ~ 320 min⁻¹), and the tachycardia induced by atropine was only marginally less in nNOS knockout atria than normal mouse atria (5.4% versus 8.0%),³ casting doubt on the significance of prejunctional NO in the release of acetylcholine from parasympathetic nerve terminals in this preparation. Finally, although stimulation of the hyperpolarization-activated inward current (I_f) via NO/cGMP may explain the increased beating rate of isolated spontaneously beating guinea-pig right atria,⁴ blockade of this current with Cs⁺ had a similar effect on spontaneous beating rate in wild-type and nNOS knockout mouse atria,³ suggesting that I_f did not likely contribute to the elevated heart rate in the nNOS knockout atria of this species. Thus, further studies are necessary to substantiate a role for nNOS-derived NO in control of heart rate in the baseline state.

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1. Brunner F, Andrew P, Wöslkart G, et al. Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Circulation*. 2001;104:3097–3102.
2. Jumrussirikul P, Dinerman J, Dawson TM, et al. Interaction between neuronal nitric oxide synthase and inhibitory G protein activity in heart rate regulation in conscious mice. *J Clin Invest*. 1998;102:1279–1285.
3. Choate JK, Danson EJ, Morris JF, et al. Peripheral vagal control of heart rate is impaired in neuronal NOS knockout mice. *Am J Physiol*. 2000;281:H2310–H2317.
4. Musialek P, Lei M, Brown HF, et al. Nitric oxide can increase heart rate by stimulating the hyperpolarization-activated inward current, I_f . *Circ Res*. 1997;81:60–68.