NO and Central Cardiovascular Control
A Simple Molecule With a Complex Story

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The 1998 Nobel Prize in Physiology or Medicine recognized the great contributions of Robert Furchgott, Louis Ignarro, and Ferid Murad through “their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system.” Furchgott’s earlier work describing a vasodilatory factor that was activated by acetylcholine acting on an intact endothelium not only set the stage for subsequent recognition that NO was the endothelium-derived relaxation factor but also acted as a harbinger of much subsequent study of interactions between classic neurotransmitters and NO. Three isoforms of NO synthase (NOS), the biosynthetic enzyme for synthesis of NO, have been identified. Two are constitutive enzymes, endothelial NOS (eNOS) and neuronal NO (nNOS). Each has been identified in the nucleus tractus solitarii (NTS), the primary site of termination of baroreceptor afferent nerves, but the role of each, or for that matter the role of NO itself, in baroreflex transmission through the NTS remains very much in question. The presence of iNOS in the NTS could indicate that there had been stress in animals in which it was found.

In keeping with the potential that NO may link transmitter mechanisms and may integrate vascular and neuronal functions, the work by Waki et al in this issue of Hypertension provides further evidence for complex integration through NO mechanisms in the NTS and for contributions of NO in disorders of cardiovascular homeostasis. That work is the latest from the Paton/Kasparov laboratories at the University of Bristol and further supports there being a cascade of transduction events that involve NO derived from eNOS, as well as several additional putative transmitters in NTS regulation of blood pressure. One such potential transmitter is angiotensin. Particularly since the work of Casto and Phillips, it has been recognized that angiotensin may act at the level of the NTS to inhibit the baroreflex. The cellular mechanisms of sympathetically mediated hypertension seen when angiotensin was introduced into the NTS have not been fully clarified, but the Bristol laboratory has begun to provide a compelling argument supported by highly innovative methods to approach the question. Using viral vectors to upregulate eNOS in the NTS, they showed previously that the enzyme played a critical role in the attenuation of the baroreflex by angiotensin, a fascinating link given the presence of both angiotensin-converting enzyme and eNOS in caveolar endothelial membranes. Their work also suggested that an inhibitory mechanism for that attenuation may lie in integration between eNOS-supported nitrooxidergic mechanisms and GABAergic mechanisms in the NTS. Thus, they provided further support for the interesting possibility that there is a link not only between signaling molecules in the brain but also between events initiated in local blood vessels and neuronal signal transduction in the NTS. Their current study associates those links with hypertension in one experimental model in which baroreflex function is attenuated. Although their work is both stimulating and compelling, work from other laboratories suggests that much of the NO story still remains to be told.

The Waki study concentrated on eNOS, but the isoform nNOS has also been found both in NTS neurons and in vagal afferent nerve fibers. Its location and juxtaposition to baroreceptor synapses in the NTS would be consistent with an interaction between NO and baroreflex transmitters in the NTS or, alternatively, a primary role for NO as a transmitter itself. Indeed, considerable data have suggested that NO released in the NTS may modulate release of the baroreflex transmitter glutamate and, through that release, may modulate blood pressure and heart rate. Other studies provided further support for integration between NO and glutamate actions. For example, both nNOS and vesicular glutamate transporters were found within the same neuronal elements in the NTS, and nerve fibers that contained nNOS have often been found in immediate apposition to NTS neurons that expressed glutamate receptors. The occurrence of nNOS both in presynaptic and in postsynaptic elements in the NTS suggested that integration between NO and glutamate was going to be complex. That suggestion has been correct at many levels. In addition to the possibility that NO may modulate release of glutamate, as well as responses to glutamate, accumulating evidence has also shown that glutamate receptors may themselves be critical for full physiological expression of the actions of NO. For example, both in the NTS and in other central sites, physiological studies have shown that effects of NO or nitrosyl factors acting through soluble guanylate cyclase may depend on inotropic glutamate receptors. Conversely, responses to glutamate receptor activation may depend on activation of synthesis and release of NO, which might then act on soluble guanylate cyclase. These findings supported a role for NO in the regulation of blood pressure by the NTS and suggested that perturbations in those nitrooxidergic mechanisms would lead to disturbed blood pressure regulation as is seen with baroreflex dys-
function. The dichotomy between such studies and the current ones that focus on NO derived from eNOS raises the possibility that different results were simply explained by different enzymatic sources of NO. However, different implications for actions of NO have also come from studies of eNOS.

Indeed, some recent studies have shown that upregulation of eNOS in the NTS leads to prolonged reductions in arterial blood pressure.\(^{14}\) That, when considered with the observation that inhibition of neuronal NO synthesis leads to hypertension,\(^{15}\) raised the possibility that NO derived from eNOS served an excitatory role both in baroreflex transmission, as well as in cardiopulmonary afferent transmission in the NTS. As is clear from the studies out of the Bristol laboratories, even that conclusion is overly simplistic.

It is very clear that data from different laboratories support quite different actions of NO in the NTS. Such divergence of conclusions could lead to skepticism about findings from any one laboratory, but such a negative outlook is hardly necessary in this case. It would behoove us to accept the possibility that data from each laboratory are sound and that, in some circumstances, NO may serve an excitatory role, whereas in other circumstances, it may serve an inhibitory role in the NTS. It would seem unlikely that these differing responses were related to different locations of the respective biosynthetic enzymes in NTS in that eNOS and nNOS immunoreactivity in the NTS can be found in structures that lie adjacent to, and as little as 5 \(\mu\)m from, each other.\(^{16}\) Thus, neuronal (through nNOS) and vascular (through eNOS) sources of NO lie well within the projected biologically relevant 100-\(\mu\)m diffusion\(^{17}\) of NO. Within reach of an NO molecule, once released from a single source, could be several million synapses that could be affected by the active agent.\(^{17}\) Further complicating the interpretation is the general acknowledgement that NO is freely diffusible through cell membranes. Accepting, at least, that functions of NO may be quite different depending on circumstances could promote our asking a number of critical questions, including those below.

How could such a diffusible agent manifest specificity of actions? How could responses to NO from 1 source differ so greatly from responses when the molecule was generated from another nearby source? Could NO be incorporated into different biologically active molecules as a result of its synthesis by different cell types (eg, endothelium versus neuron)?

Some have considered the possibility that the action of NO on a neuron may depend on the underlying activity of that neuron when NO affects it.\(^{17}\) However, that seems an unlikely explanation for such specificity here given that the divergent actions of NO on baroreflex function within the NTS are manifest in a single reflex pathway and in a single locus of that pathway. It seems equally unlikely that the preponderance of neurons affected by NO in one study favored excitatory synapses, whereas in another study it favored inhibitory synapses given the large number of synapses likely affected on release of the diffusible agent. Clearly both glutamate and GABA synapses are found in abundance in the NTS, and, more importantly, the 2 synaptic types lie in immediate apposition to each other.\(^{18}\) One possible explanation that has received little study would require our rethinking mechanisms of action of NO and considering the possibility that the compound may have biological activity, not as a free-standing molecule, but instead as part of a more directed compound with affinity for specific membrane sites. Our own studies did provide some support for stereoselectivity of nitrosoyl compounds in the NTS and suggested that it was s-nitrosylated thiols that may be biologically active instead of, or in addition to, NO itself.\(^{11}\) Others have arrived at the same conclusion when studying ventilatory control through NTS,\(^ {19} \) and still others have suggested that similar molecules are responsible for vascular actions of NO.\(^ {20} \) With the studies provided by the Bristol group contrasted with those provided by other laboratories, the need is clear for greater exploration of the molecular mechanisms involved in transduction of signals now attributed to NO alone.

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None.

**References**

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