Prologue: Nitric oxide–hormones, metabolism, and function

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As we set out to find an interesting new direction as a Special Topic for papers for the *AJP: Heart and Circulatory Physiology*, nitric oxide (NO) was an obvious choice. Since the seminal work of Furchgott and Zawadski, who first defined endothelium-derived relaxing factor (8), to defining the chemical nature of endothelium-derived relaxing factor by Ignarro and colleagues (12), Furchgott (9), and Moncada and Higgs (14), and to defining the signal transduction pathway and the role of guanylate cyclase as a mediator of vasodilation by Murad (15), literally thousands of papers have been written on these subjects. Furthermore, many review papers have appeared describing in great detail the NO-cGMP-vessel relaxation cascade. Therefore, we sought to highlight different aspects of the regulation of cardiac and vascular function by NO. To this end, a number of reports in the literature some dating back to the 1970s have suggested that NO can regulate parenchymal cell function, the action of hormones such as insulin, and even cardiac contractile function. Additionally, these actions may not be related to the stimulation of guanylate cyclase and would also be mediated by unique and novel signaling pathways. Thus the subject for this Special Topic was non-cGMP-mediated signaling by NO with focus on intracellular events, mechanical function, and involvement in cardiovascular disease.

The first five papers in response to this Special Topic deal with actions of NO on mitochondrial function and its subsequent impact on the regulation of cardiac oxygen consumption in the normal and failing heart, which is an interest of ours (17). Bourtaite et al. (2) continue to define the biochemical signaling by which NO controls mitochondrial metabolism, concluding that this is an action on cytochrome oxidase and showing that it is reversible, implying that it is of potential physiological importance. This is an area of historic importance and one both Moncada and colleagues (6) and Brown (3) have pursued previously. The impact of the reversible regulation of mitochondrial function by NO manifests itself in papers by Dai et al. (7) and Nicholaides et al. (16). Dai et al. (7) extend the concept that NO is an important regulator of mitochondrial function to the controversy of which isoform of NO synthase (NOS) is responsible for this regulation in the hypertrophied heart. These authors show that iNOS, the inducible isoform of NOS (NOS2), is most likely involved in the regulation of myocyte energetics and furthermore, in an ex vivo setting, that the production of NO impacts the mechanical function of the hypertrophied heart during acute pacing. Therefore, these studies provide evidence linking NO production in myocytes, or nearby to the contractile function of the heart. Nicholaides et al. (16), using a dog model of dilated cardiac myopathy caused by rapid ventricular pacing, describe a role for NO in the “flow-function mismatch” observed during the development of cardiac compensation and overt heart failure. This is an important example of the pathological consequences of altering NO production on cardiac metabolism. Interestingly, the isoform of NOS studied herein was NOS3 or the constitutive endothelial NOS that also causes relaxation of blood vessels. Whereas, these papers have concentrated on the signaling and functional consequences of NO on mitochondrial function, Carreras et al. (4) address a novel location for the NOS responsible for the control of mitochondrial function, the mitochondrion itself. Thus using isolated rat liver mitochondria to study the function of what is now termed mitochondrial NOS (mtNOS), these authors found an increased mtNOS expression (75%) and reduction in mitochondrial oxygen consumption in mitochondria from hypothyroid rats that correlated with circulating thyroid hormone levels. These actions of NO were on cytochrome oxidase, the site of action defined previously by...
Moncada et al. (6) and Brown (3). These studies further extend the role of NO to the control by circulating hormones such as thyroid hormone and may help to define new mechanisms by which these metabolically active hormones control cellular function. In addition and although still controversial, these data support a novel location for NOS, the mitochondria, and evoke thoughts of local control of tissue metabolism by NO. Having worked out the signaling from NO to the generation of cGMP, Murad and colleagues have now turned to a new and novel signaling pathway by NO, protein nitrosation. Turko, Marconides, and Murad (18) thus examined the relationship between tyrosine nitration of the mitochondrial enzyme succinyl-CoA:3-oxoacid CoA transferase and its activity in normal and diabetic rats. This mitochondrial matrix protein is involved in ketone body metabolism when glucose is not available, as in diabetes. Thus this paper adds two additional concepts to the role of NO in the control of metabolism: 1) that the chemical interaction of NO with proteins resulting in nitration may be important, and 2) that NO may regulate through this and other mechanisms substrate utilization. This second conclusion is also supported by the paper by Nicholaides et al. (16) in which a shift in substrate uptake from fatty acids to lactate occurred in the failing heart as NO production decreased. This group of five papers highlights a growing area of interest, the role of NO in the control of oxygen metabolism, and substrate use in tissues.

The next two papers address the potential role of NO in the control of myocardial contractile state. NO has been reported to be a negative inotrope (1, 10) especially when accompanied by concomitant sympathetic activation. Ziolo et al. (20) examined the interaction of the ryanodine receptor, indirectly by measuring calcium sparks, in isolated myocytes from the rat heart, and NO provided by the decomposition of an NO donor. During exposure to high levels of a β-agonist, NO inhibited spark frequency, and at low levels of β-agonists NO increased spark frequency. This was correlated with force generation but was not altered by the guanylate cyclase inhibitor 1H-(1,2,4)-oxadiazolo-(4,3-a)-quinoxalin-1-one. Ziolo et al. (20) in fact suggest that an alternative mechanism may be through protein nitration, the mechanism discussed by Murad and colleagues (18). Zhang et al. (19) also address the potential role of NO in the regulation of myocardial contractile state, this time focusing on the role of NO in aging. This has been a major focus for this laboratory for some time. In isolated rabbit myocytes the NO donor and S-nitroso-N-acetylpenicillamine reduced contractile state as determined by both velocity of shortening and percent shortening. In myocytes from young hearts, this was partially inhibited by a guanylate cyclase inhibitor, whereas in aged myocytes guanylate cyclase had little role. Because in both young and old myocytes, NO reduced contraction, two different mechanisms must be involved. Perhaps in old myocytes protein nitration plays the major role, and in the young heart, guanylate cyclase-regulated protein phosphorylation plays the dominant role. Thus there appears to be a role for NO in the control of myocyte contractile state that is in part cGMP independent. Although we tend to focus on the local effects of NO in the control of vascular tone, the regulation of mitochondrial metabolism and cardiac contractile state and the integrated cardiovascular response to altered physiological and disease states often is orchestrated by the central nervous system through autonomic outflow. Many investigators have been struck by the profound effects of NOS inhibition on heart rate, leading to bradycardia to the extent often observed during atrioventricular nodal blockade. Whereas this has been attributed to central effects of NOS inhibition, growing evidence suggests that the neuronal isoform of NOS, NOS1, is present in the sinoatrial node (SA) node and may importantly modulate presynaptic vagal influences on the SA node by facilitating ACh release. Choate et al. (5), using neuronal NOS knockout (-/-) mouse hearts, show that the rate of heart rate decline during vagal stimulation was less in nNOS /-- mouse atria, that this was not a postsynaptic action of NO, and that this was guanylate cyclase dependent. Furthermore, they indicate that a selective nNOS inhibitor had the same effects in nNOS +/+ atria. Herring et al. (11) addressed the role of particulate guanylate cyclase in the presynaptic control of vagal control of heart rate using NO and natriuretic peptides. These authors show that stimulation of particulate guanylate cyclase by brain natriuretic peptide or C-type natriuretic peptide has similar effects on vagal control of heart rate in guinea pig atria as reported for NO, supporting an important role for cGMP. Together these papers provide evidence that NO may modulate parasympathetic outflow at the level of presynaptic vagal nerve endings in the heart by an nNOS-guanylate cyclase-dependent mechanism.

The other arm of the autonomic nervous system, sympathetic outflow, may also be controlled by the actions of NO. Li et al. (13) show that administration of glutamate or the glutamate receptor agonist N-methyl-D-aspartic acid into the paraventricular nucleus (PVN) in the brain stem increases systemic arterial pressure, heart rate, and renal sympathetic nerve activity (RSNA), which is enhanced with concomitant local NOS inhibition. This was accompanied by an increase in NO production locally. Inhibition of NO synthesis in the PVN by itself also increased RSNA. The increase in RSNA was eliminated by a glutamate receptor antagonist (RSNA), which is enhanced with concomitant local NOS inhibition. This was accompanied by an increase in NO production locally. Inhibition of NO synthesis in the PVN by itself also increased RSNA. The increase in RSNA was eliminated by a glutamate receptor antagonist, suggesting that under physiological conditions NO ameliorates the influences of N-methyl-D-aspartic acid receptor activation on sympathetic outflow. The isoform of NOS was not identified, but nNOS would be strongly implicated in this regulatory process. Thus the outflow of both arms of the autonomic nervous system may be regulated by NO, occurring at all levels; from the control of transmitter release to the regulation of neuronal cell activation in the cardiovascular centers in the brain stem.

It should become obvious from the breadth of the studies submitted as part of this Special Topic that the
control of cardiovascular function by NO and the processes that NO is involved in are more complicated than simply regulating vascular tone. When picturing the involvement of NO in a disease process in the heart or periphery, it is noteworthy that NO regulates function at all levels that are important in cardiovascular control. Certainly it is important to remember the NO-cGMP vasodilation cascade, but it also important, perhaps increasingly so, to remember other layers of control, including local mitochondrial metabolism, control of calcium concentrations in myocytes, and regulation of integrated neuronal outflow in the autonomic system. These other layers of control may be additive with NO-mediated vasodilation for instance. By reducing the vagal influence on heart rate or sympathetic regulation of peripheral vascular resistance or regulating myocyte oxygen consumption, NO may modify the metabolic feedback control of vascular tone in vivo contributing to the regulation of cardiovascular function in normal and disease states.

REFERENCES