Nitric Oxide and Cardiac Contractility in Human Heart Failure
Time for Reappraisal

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The free radical gas nitric oxide (NO) is produced by 3 isoforms of nitric oxide synthase (NOS). All of them are present in the heart: NOS1 (nNOS, “neuronal”) NOS has been detected in cardiac conduction tissue and intracardiac neurons; NOS2 (iNOS, “cytokine-inducible”) NOS can be expressed by virtually all cells in the heart, often in conjunction with the expression of inflammatory cytokines; and finally, NOS3 (eNOS, “endothelial-constitutive”) NOS is expressed in coronary endothelium, endocardium, and cardiac myocytes. NOS3 regulates the tone of vascular smooth muscle cells; the permeability and platelet adhesion of endothelial cells; and the receptor-effector coupling, energetics, contractility, and apoptosis of cardiomyocytes. Since the original demonstration of myocardial NOS2 activity in idiopathic dilated cardiomyopathy, a negative inotropic effect of NO frequently was hypothesized to contribute to the depressed contractile function of failing myocardium. This hypothesis was inspired by the simultaneous publication of experimental results that showed a depressed contractile response of isolated cardiomyocytes to β-adrenergic agonists after NOS2 induction by lipopolysaccharides.

To further explore this depressant action of NO on myocardial contractility, numerous experimental and clinical studies were performed, but they yielded apparently conflicting results on the inotropic effect of NO from either endogenous or pharmacological sources. As reported in this issue of Circulation, Cotton et al tackled the question of the inotropic action of NO by measuring left ventricular (LV) performance in normal control subjects and in patients with dilated cardiomyopathy during intracoronary infusion of the NOS inhibitor Nω-monomethyl-L-arginine (L-NMMA). During L-NMMA infusion, Cotton et al observed a modest drop (14%) in LV dp/dt max in the control group and no change in LV dp/dt max in the cardiomyopathy group, despite myocardial expression of NOS2 in their patients. They concluded that the small baseline positive inotropic effect of NO in control patients disappeared during development of heart failure. These observations raise several important questions: (1) How does the positive inotropic effect of NO observed in the present study relate to previous clinical reports demonstrating a negative inotropic effect of NO under baseline conditions or after β-adrenoceptor stimulation? (2) What are the underlying mechanisms for a positive inotropic effect of NO? (3) What are the implications for heart failure management?

Relation to Previous Clinical Studies
Bicoronal infusions of the NO donor sodium nitroprusside had no effect on LV dp/dt max but caused a 10-mm Hg drop in LV end-systolic pressure (LVESP) at unchanged LV end-systolic volume (LVESV) consistent with a fall in LV end-systolic elastance (Ees), another index of LV contractility. Similar results were obtained in dilated cardiomyopathy, after cardiac transplantation, and during infusion of substance P, which causes receptor-mediated release of NO from the coronary endothelium. After pretreatment with dobutamine, the fall in LV Ees during intracoronary administration of substance P was twice as large and accompanied by a 6% fall in LV dp/dt max. A similar augmentation of NO-related negative inotropic effects by dobutamine was observed during intracoronary infusion of L-NMMA, which potentiated the dobutamine-induced rise in LV dp/dt max by 51% in cardiomyopathy patients but had no effect in normal control subjects.

A biphasic dose-dependent response of myocardial contractility to NO explains the apparent discrepancy between the positive inotropic effect of NO in the study by Cotton et al and the negative inotropic effect of NO in previous clinical studies. Two experimental studies on isolated cardiomyocytes and papillary muscles almost simultaneously reported this biphasic dose-response pattern, with low doses of NO resulting in a positive inotropic effect and high doses of NO resulting in a negative inotropic effect. Pretreatment with β-adrenoceptors shifted the descending limb of the dose-response curve to the left with a negative inotropic effect appearing at lower concentrations. This biphasic pattern resulted from multiple sites of action of NO in cardiomyocytes, some of them direct (through nitrosylation of proteins), some of them indirect (through cyclic guanosine monophosphate [cGMP]). The summation of effects determined the resultant change in contractile performance. If the normal heart is operating at the top of the biphasic dose-response curve, both a reduction in NO content during L-NMMA administration, as performed by Cotton et al, and administration of an NO donor or of substance P, as per-
formed in previous studies, would result in a negative inotropic effect. Because of its higher NO content, failing myocardium could be operating on the descending limb of the biphasic dose-response curve. This could blunt the negative inotropic effect of L-NMMA, as observed by Cotton et al, or could result in a positive inotropic effect of L-NMMA, as observed by Hare et al after prior β-adrenoreceptor stimulation.

**Mechanisms of In Vivo Inotropic Effects of NO**

Mechanisms accounting for in vivo inotropic effects of NO include parasympathetic and sympathetic outflow to the heart, myocardial cholinergic and adrenergic receptor-effector coupling, myocardial sarcolemmal or sarcoplasmic protein nitrosylation, and myocardial cGMP-dependent myofilamentary desensitization. The overall effects of NO on central and peripheral neuronal pathways responsible for autonomic cardiovascular activity are parasympathetic activation and sympathetic withdrawal. Because of unaltered heart rate during intracoronary L-NMMA infusion in the study by Cotton et al, altered autonomic outflow to the heart seemed an unlikely explanation for their findings. Through its effects on myocardial cholinergic and adrenergic receptor-effector coupling, NO can modulate intracellular cGMP and cyclic adenosine monophosphate (cAMP) levels. A positive inotropic effect can result from cGMP-induced inhibition of phosphodiesterase 3 and a concomitant rise in cAMP content.

Recent evidence on the competing effects of caveolin on translocation of receptor-agonist complexes and on NOS3 activity supports an important role of NO in cholinergic or adrenergic receptor-effector coupling. Uprogelation of caveolin 3 in failing myocardium indeed explained the larger increase in heart failure patients than in normal subjects in the dobutamine-induced rise in LV dp/dt max during intracoronary L-NMMA infusion. Direct nitrosylation of proteins enhances sarcoplasmic reticular calcium cycling by activation of sarcolemmal and sarcoplasmic proteins and affects the myocardial force-frequency relation. In rat cardiomyocytes, frequency dependence of NO production, which presumably is due to more intense activation of NOS2 as a result of larger calcium/calmodulin availability, has also been reported. In the study by Cotton et al, intracoronary L-NMMA, however, failed to influence the pacing-induced rise of LV dp/dt max in normal control subjects or to restore the blunted force-frequency response in cardiomyopathy patients. The latter therefore appears to result from the decline in sarcoplasmic reticular calcium cycling characteristic of failing myocardium regardless of the extent of activation of myocardial NOS. Phosphorylation of troponin I by cGMP-dependent protein kinase reduces myofilamentary calcium sensitivity. This decreases myocardial contractile performance mainly through earlier onset of relaxation and concomitant abbreviation of contraction period. Phosphorylation of troponin I also increases myocardial diastolic distensibility through prevention of calcium-independent diastolic crossbridge cycling. The small decrease in LV Ees observed in clinical studies during intracoronary infusion of NO donors or of substance P—most likely was induced by cGMP-dependent phosphorylation of troponin I because of the simultaneous occurrence of earlier onset of LV relaxation and improvement of diastolic LV distensibility.

**Implications for Heart Failure Management**

In the study by Cotton et al, intracoronary infusion of L-NMMA left contractility of the cardiomyopathic LV unaltered, even in the presence of NOS2 gene expression in simultaneously procured cardiac biopsies. This confirmed a previous in vitro study on muscle strips of explanted cardiomyopathic human hearts, which also failed to observe a change in muscle force development after L-NMMA administration, irrespective of the presence or absence of NOS2 gene expression. In dilated cardiomyopathy patients with elevated LV filling pressures, intracoronary infusion of substance P enhanced myocardial NOS2 activity and slightly reduced LV Ees, but it improved overall hemodynamic status because of a simultaneous increase in diastolic LV distensibility resulting in higher LV stroke volume and LV stroke work. In the same patients, simultaneous improvement of diastolic LV distensibility also overrode the NO-induced attenuation of the LV contractile response to β-adrenoreceptor stimulation in terms of overall LV performance. In 2 studies using prior intravenous administration of dobutamine, agonist-induced coronary endothelial release of NO resulted in a fall in LV contractility indices such as LV dp/dt max and LV Ees. However, the fall in LV contractility indices caused no hemodynamic deterioration, as was evident from an unaltered LVESV and a significant drop in LVEDP. This drop in LVEDP occurred at unaltered LVEDV and therefore must have resulted from a simultaneous NO-induced increase in diastolic LV distensibility. A beneficial effect of high myocardial NO content on diastolic LV distensibility was also observed in a pacing-induced heart failure dog model. In this model, there was only minimal deterioration of LV function in the time interval from 1 to 4 weeks despite increased cardiac NO production. After 4 weeks of pacing, a fall in cardiac NO production occurred, which was accompanied by a steep rise in LVEDV and a fall in LVESV. From the foregoing observations, it can be concluded that there is little clinical evidence for a physiologically important role for myocardial NO, whether derived from NOS2 or NOS3, to account for the deteriorating hemodynamic status of heart failure patients, either at baseline or after β-adrenoreceptor stimulation.

The absence of acute hemodynamic deterioration related to high myocardial NO content does not preclude the possibility of deleterious effects of high levels of myocardial NO content on myocyte cell loss, especially in the presence of increased myocardial oxidative stress, as recently observed in studies comparing post-infarction LV remodeling in wild-type and NOS2 knockout (NOS2−/−) mice. In the early post-infarction period, LV contractile performance in wild-type and NOS2−/− animals was similar, but 4 months after infarction, NOS2−/− mice had superior hemodynamics and better survival because of reduced myocyte loss in the noninfarcted zone. Although similar findings were reported by Feng et al, these authors did note an increase in LV end-diastolic diameter and decreased fractional shortening somewhat ear-
lier, at 1 month post-infarction, in wild-type mice when compared with NOS2−/−. One important distinction between these 2 reports is that Feng et al18 used C57BL/6 controls, whereas Sam et al17 used backcrossed animals from the F6 generation, a more robust control.

Conclusions

Cotton et al,5 by demonstrating that an intracoronary L-NMMA infusion increased LV dP/dt max in normal subjects and induced no change in LV dP/dt max in cardiomyopathy patients, have added further evidence to the growing amount of clinical data that have failed to show an important adverse hemodynamic effect of myocardial NOS2-derived NO in patients with heart failure. Altered cAMP/cGMP balance or direct nitrosylation of sarcolemmal/sarcoplasmic proteins could explain the NO-induced positive inotropic effects noted in normal subjects. Finally, the absence of important hemodynamic sequelae after a rise in myocardial NOS2 content in heart failure patients does not preclude a physiologically important role for NO in mediating myocyte loss, perhaps induced by oxidative stress, peroxynitrite generation, and induction of apoptosis.

References


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