Invited Review: point-counterpoint

Nitric oxide’s role in the heart: control of beating or breathing?

Walter J. Paulus and Jean G. F. Bronzwaer

Institute for Cardiovascular Research, Vrije Universiteit, 1081 BT Amsterdam, The Netherlands

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Paulus, Walter J., and Jean G. F. Bronzwaer. Nitric oxide’s role in the heart: control of beating or breathing? Am J Physiol Heart Circ Physiol 287: H8–H13, 2004; 10.1152/ajpheart.01147.2003.—Beneficial actions of nitric oxide (NO) in failing myocardium have frequently been overshadowed by poorly documented negative inotropic effects mainly derived from in vitro cardiac preparations. NO’s beneficial actions include control of myocardial energetics and improvement of left ventricular (LV) diastolic distensibility. In isolated cardiomyocytes, administration of NO increases their diastolic cell length consistent with a rightward shift of the passive length-tension relation. This shift is explained by cGMP-induced phosphorylation of troponin I, which prevents calcium-independent diastolic cross-bridge cycling and concomitant diastolic stiffening of the myocardium. Similar improvements in diastolic stiffness have been observed in isolated guinea pig hearts, in pacing-induced heart failure dogs, and in patients with dilated cardiomyopathy or aortic stenosis and have been shown to result in higher LV preload reserve and stroke work. NO also controls myocardial energetics through its effects on mitochondrial respiration, oxygen consumption, and substrate utilization. The effects of NO on diastolic LV performance appear to be synergistic with its effects on myocardial energetics through prevention of myocardial energy wastage induced by LV contraction against late-systolic reflected arterial pressure waves and through prevention of diastolic LV stiffening, which is essential for the maintenance of adequate subendocardial coronary perfusion. A drop in these concerted actions of NO on diastolic LV distensibility and on myocardial energetics could well be instrumental for the relentless deterioration of failing myocardium.

myocardium; diastole; mitochondria; energetics

NO is universally accepted as an important regulator of vascular tone, capillary permeability, and platelet adhesion. NO’s myocardial actions are unfortunately less well understood despite the growing clinical awareness that progressive dysfunction of the failing heart could well result from an imbalance between myocardial NO and oxidative stress induced by excess neurohormonal and inflammatory mediators (32). The appreciation of a beneficial role of NO in failing myocardium was a recent turnaround (8, 11, 15, 33) and resulted mainly from an appraisal of NO’s favorable effects on cardiac energetics (52) and on left ventricular (LV) diastolic distensibility (35) clearly outweighing the negative inotropy of NO reported in the very initial studies looking at its contractile effects. These studies had observed a reduction of extent and velocity of shortening of isolated cardiomyocytes after administration of exogenous NO (3) and an increase in extent and velocity of shortening of adrenergically stimulated cardiomyocytes after inhibition of endogenous NO production (1). At that time, these experiments were thought to provide an explanation for simultaneously published clinical observations that reported inducible NO synthase (NOS2) activity in patients with nonischemic dilated cardiomyopathy (10). These initial experimental studies and the potential link to myocardial dysfunction of nonischemic dilated cardiomyopathy were, however, rapidly rebutted by several investigators reporting positive inotropic effects of low doses of exogenous NO or of cGMP (23, 29) and by clinical studies reporting myocardial NOS2 expression in ischemic cardiomyopathy, in valvular heart disease, and even in the athlete’s heart (5, 16). These clinical studies also found higher NOS2 expression in heart failure patients with lower functional class, larger stroke work, and preserved LV diastolic distensibility. Nevertheless, the idea of NO being deleterious because of a negative inotropic effect gained widespread acceptance and subsequently hindered a correct appreciation of NO’s favorable effects on failing myocardium.

NO-INDUCED MYOCARDIAL CONTRACTILE DEPRESSION: TIME FOR ACQUITTAL!

Detailed analysis of the time course of isometric contraction of isolated cat papillary muscle strips revealed endogenous NO, released from the endothelium, to affect cardiac muscle contraction in a unique way (29, 48): NO induced an earlier onset of isometric tension decay, which reduced peak isometric tension without an effect on the rate of rise of tension (Fig. 1A). This effect was attributed to a NO-induced reduction in myofilamentary calcium sensitivity because of phosphorylation of troponin I by cGMP-dependent protein kinase as evident from simultaneous recordings in isolated cardiomyocytes of cell lengthening and of the calcium transient (45). In these cardiomyocytes, diastolic cell length was not clamped, and, after the administration of NO or of cGMP, diastolic cell length con-
systolic, end-systolic, and end-diastolic pressures, and rightward displacement of the diastolic LV pressure-volume relation (Fig. 1C). In patients with a hypertrophied LV of aortic stenosis, the NO-induced rightward displacement of the diastolic LV pressure-volume relation was larger than in controls (27). In patients with dilated cardiomyopathy, the rightward displacement of the diastolic LV pressure-volume relation was accompanied by a significant increase in LV stroke volume because of improved recruitment of the LV preload reserve (18). The lower LV end-systolic pressure at unaltered LV end-systolic volume observed during intracoronary infusions of NO donors or of substance P implied a downward shift of the LV end-systolic pressure-volume relation and was therefore consistent with a negative inotropic effect of NO. The unaltered LV \( dp/dt_{\text{max}} \) at larger LV end-diastolic volume was also theoretically consistent with a lower myocardial inotropic state. The simultaneous fall in LV end-diastolic pressure and the rise in LV stroke volume or stroke work, however, argue against significant cardiac contractile depression as a result of these NO-induced effects. Finally, direct positive inotropic effects of NO were recently demonstrated in normal control patients (7), in whom an intracoronary infusion of \( N^2\)-monomethyl-L-arginine (L-NMMA) induced a modest (14%) drop in LV \( dp/dt_{\text{max}} \). In the same study, intracoronary \( L\)-NMMA failed to alter LV \( dp/dt_{\text{max}} \) in dilated cardiomyopathy patients despite myocardial expression of NOS2 in simultaneously procured endomyocardial biopsies.

After \( \beta\)-adrenoreceptor stimulation of isolated cardiac muscle strips, the NO-induced relaxation-hastening effect was larger probably because of simultaneous phosphorylation of troponin I by cAMP-dependent and cGMP-dependent protein kinases (29). In dilated cardiomyopathy patients, similar cooperative effects of NO and of \( \beta\)-adrenoreceptor stimulation were reported: during concomitant intravenous infusion of dobutamine, intracoronary infusion of substance P caused a larger drop in LV end-systolic pressure (\( \pm 30 \) mmHg) and a small (6%) reduction in LV \( dp/dt_{\text{max}} \) (34). Both effects were again accompanied by a fall in LV end-diastolic pressure implying absence of hemodynamic deterioration. Similar findings have been observed during concomitant intravenous infusion of dobutamine and intracoronary infusion of the NO synthase inhibitor \( L\)-NMMA (14, 47) or of the angiotensin-converting enzyme (ACE) inhibitor enalaprilat (51). Intracoronary \( L\)-NMMA infusion raised LV \( dp/dt_{\text{max}} \) and increased the slope of the LV end-systolic pressure-volume relation without a change in LV end-diastolic pressure, again implying no substantial change in overall hemodynamic status. Intracoronary enalaprilat in the presence of angiotensin II receptor blockade caused bradykinin-induced coronary endothelial release of NO, and this also resulted in a small reduction of LV \( dp/dt_{\text{max}} \) accompanied by a fall in LV end-diastolic pressure and no change in LV stroke volume. Hence, from these observations in dilated cardiomyopathy patients, it can be concluded that in terms of overall LV performance, improvement in diastolic LV function also overrode the NO-induced attenuation of the LV contractile response to \( \beta\)-adrenoreceptor stimulation.

In transgenic mice with cardioselective overexpression of endothelial NOS (NOS3) (6) and a 60-fold increase in myocardial NOS3 activity (\( L\)-[\(^3\)H]citrulline production), there was only a small reduction in peak LV developed pressure without hemodynamic consequence mainly because of myofilamentary
desensitization. A similar small reduction in peak LV developed pressure without signs of cardiac dysfunction was also observed in transgenic mice with cardioselective overexpression of NOS2 and a 20-fold increase in myocardial l-[3H]citrulline production (17). In these mice, the addition of l-arginine to the perfusion augmented the drop in LV developed pressure to 20% of the basal value again without hemodynamic consequence. In experimental volume overload, basal isometric twitch characteristics were more depressed in papillary muscles retrieved from decompensated than from compensated rats despite similar myocardial NOS2 activity (12).

MAINTENANCE OF PRELOAD RESERVE: AN IMPORTANT TASK FOR NO IN THE STRESSED HEART!

In isolated ejecting guinea pig hearts, a perfusate containing l-NMMA raised LV end-diastolic pressure and reduced preload recruitable LV stroke work because of an acute left and upward shift of the diastolic LV pressure-volume relation (40). In this preparation, use of the LV preload reserve also induced a rise in the NO concentration of the coronary effluent. This preload-triggered enhancement of myocardial NO production confirmed earlier observations using porphyrinic sensors inserted in the wall of the beating rabbit heart (38). In rats receiving 8 wk of treatment with a NOS inhibitor, the diastolic LV pressure-volume relation shifted upward with reduced LV unstressed volume and no increase in LV mass despite the elevated blood pressure (26). In chronically instrumented dogs, oral administration of a NOS inhibitor resulted in a left and upward shift of the diastolic LV pressure-volume relation and a drop in LV stroke work despite a simultaneous small upward displacement of the LV end-systolic pressure-volume relation (39). NO-related modulation of diastolic LV distensibility was also observed in the pacing-induced heart failure dog model (41), in which a fall in myocardial NO production occurred after 4 wk of pacing. This fall was accompanied by a drop in LV stroke volume and a steep rise in LV end-diastolic pressure, probably because of reduced diastolic LV distensibility.

A NO-induced diastolic LV distensibility increasing effect was observed not only in experimental models but also in the normal human heart (35), in the cardiac allograft (36), in the hypertrophied LV of aortic stenosis (27), and in the failing LV of dilated cardiomyopathy (18). In dilated cardiomyopathy patients with elevated LV filling pressures (18), enhanced myocardial NOS3 activity during intracoronary substance P infusion increased LV stroke volume and LV stroke work. This acute increase in LV stroke work resulted from a simultaneous NO-induced increase in diastolic LV distensibility and LV preload reserve (5).

In patients with dilated cardiomyopathy, limited LV preload reserve (19) corresponds with a restrictive LV filling pattern on the Doppler echocardiogram (37). This phenotype of dilated cardiomyopathy is characterized by a worse symptomatic course and a worse prognosis. Low intensity of LV endomyocardial NOS2 and NOS3 gene expression was recently demonstrated to coincide with this hemodynamic phenotype (5). In contrast, dilated cardiomyopathy patients with maintained LV preload reserve, normal Doppler echocardiographic LV filling dynamics, and low LV diastolic stiffness had a high intensity of LV endomyocardial NOS2 and NOS3 gene expression, comparable to the intensity observed in the athlete’s heart (5).

Low LV diastolic stiffness and high LV preload reserve are also typical features of the athlete’s heart and could also be NO mediated because of the well-documented upregulation of NOS3 activity and expression by intense physical exercise (2, 43). Further evidence for a beneficial effect of high endomyocardial NO activity on prognosis of dilated cardiomyopathy was recently provided by studies looking at NOS3 gene polymorphism in humans. In these studies, dilated cardiomyopathy patients of a genotype with high NOS3 activity had a more benign course of their disease than patients of a genotype with low NOS3 activity (28). These findings also resemble the superior long-term outcome of LV remodeling after myocardial infarction in wild-type mice compared with NOS3 knockout mice (42). Finally, the improved prognosis of dilated cardiomyopathy patients treated with ACE inhibitors or β-blockers is paralleled by an upregulation of their endomyocardial NOS3 activity (18).

A beneficial effect of high endomyocardial NO activity on diastolic LV distensibility of the cardiomyopathic heart could result not only from NO-induced phosphorylation of troponin I and a concomitant reduction of diastolic cross-bridge cycling but also from prevention of endomyocardial fibrosis. Chronic inhibition of NO synthesis has indeed been demonstrated to induce progressive myocardial fibrosis through a signaling cascade involving endothelin, angiotensin II, aldosterone, and transforming growth factor-β. A recent study looked at the interaction between endomyocardial NOS gene expression and fibrosis in patients with dilated cardiomyopathy (4). This study found no correlation between endomyocardial NOS mRNA and collagen volume fraction and observed additive but opposite effects of intensity of NOS gene expression and of fibrosis on diastolic LV stiffness (Fig. 2). The lack of correlation between NOS expression and collagen does not exclude involvement of NOS in the development of myocardial fibrosis in dilated cardiomyopathy but suggests excessive deposition of collagen in cardiomyopathic hearts to result from upregulation of stimulatory pathways such as endothelin, angiotensin II, or aldosterone rather than from downregulation of inhibitory pathways such as NO or natriuretic peptides.

NO’S CONTROL OF DIASTOLE AND ENERGETICS: TWO OF A KIND?

The altered energetics of failing myocardium are characterized by 1) reduced creatine kinase activity and phosphocreatine

![Diagram](image)

**Fig. 2.** Additive and opposite effects of intensity of inducible NO synthase (NOS2) gene expression and of myocardial fibrosis on diastolic LV stiffness in the failing human heart. The relation between the LV stiffness modulus (Stiffness-Mod) and NOS2 gene expression is shifted downward in patients with low level myocardial fibrosis (collagen volume fraction < 10%).

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levels, 2) loss of control of myocardial mitochondrial respiration leading to excessive oxygen consumption, and 3) a switch in preferential myocardial substrate utilization from free fatty acids to glucose. Apart from creatine kinase activity (13), NO appears to correct all these derangements of myocardial energetics (50). NO can bind to heme moieties of proteins involved in mitochondrial respiration. Reduced inhibition of these enzymes explains the higher myocardial oxygen consumption in conscious dogs during NOS inhibition (46) and in terminal stages of experimental (52) and clinical heart failure (25), which are characterized by low cardiac NO production (18, 41). In failing human myocardium, this excessive myocardial oxygen consumption reacted favorably to ACE inhibitors, amlodipine, or a neutral endopeptidase inhibitor (25), all of which are known to raise myocardial NO content through inhibition of kinin degradation. In pacing-induced heart failure, at the time of transition to decompensation, a drop in myocardial NO production is observed, which coincides with a switch in myocardial substrate utilization from free fatty acids to glucose (41). A similar switch was also observed in transgenic mice with absent NOS gene expression (49). The altered energetics of failing myocardium are paralleled by a shift of myofilamentary gene expression toward isofoms with higher calcium sensitivity and lower ATPase activity. This shift in gene expression enhances contractile efficiency of failing myocardium even in the presence of a deranged oxygen consumption (21). In the human cardiomyopathic heart, administration of l-NMMA does not affect its efficiency (47) despite the augmented LV contractile response to β-adrenergic stimulation.

NO’s effects on LV contractile performance appear to be synergistic with its effects on energetics through prevention of inappropriate contractile augmentation in the setting of reperfusion, through prevention of myocardial energy wastage induced by LV contraction against late-systolic reflected arterial pressure waves and through prevention of diastolic LV stiffening, which preserves adequate subendocardial coronary perfusion.

In NOS3 knockout mice subjected to 30 min of global ischemia, followed by reperfusion, a paradoxical increase in NO production was observed because of superinduction of NOS2 (22). The accompanying increase in NO’s myofilamentary desensitizing action reduced the hyperdynamic myocardial contractile response characteristic of early reperfusion. This protected the reperfused heart because of reduced myocardial energy demand at a time of low energy availability. Similar NO-mediated flow-metabolism-function matching was also observed in the high oxygen demand model of pacing-induced heart failure (30). In the early pacing period, when myocardial NO production and NOS2 gene expression were high, there were parallel reductions in coronary blood flow, myocardial oxygen consumption, and LV dP/dt max. In a later phase, when myocardial NO production and NOS2 gene expression declined, flow-metabolism-function matching was disrupted because of a continuing fall in LV dP/dt max despite higher coronary blood flow and myocardial oxygen consumption. Using the same model, other investigators had previously observed a reduction in diastolic LV distensibility as evident from a steep rise in LV end-diastolic pressure, when myocardial NO production started to decline (41). Taken together, these three studies suggest NO to be responsible for myocardial flow-metabolism-function matching through modulation of LV preload reserve. NO-induced prevention of reperfusion-related LV dysfunction was also evident in vitro from the NO-conferred protection of isolated cardiomyocytes against reoxygenation contracture (44).

In the human heart, intracoronary infusions of NO donors or of substance P revealed a NO-induced reduction in late systolic LV pressure generation because of earlier onset of LV isometric relaxation (Fig. 1B) (35, 36). This reduction in late systolic LV pressure generation corresponded with a reduced LV contractile effort against reflected arterial pressure waves. This reduced LV contractile effort prevented myocardial energy wastage induced by late systolic afterload augmentation. These beneficial effects of NO on myocardial contractile performance and energetics are in concert with NO’s well-established effects on the vasculature. A high vascular NO activity induces arteriolar vasodilation and improves arterial distensibility, both of which reduce magnitude and traveling speed of reflected arterial pressure waves. A high myocardial NO activity provides appropriate timing of isometric LV relaxation, thereby preventing an amplifying effect elicited by late systolic LV contraction against the reflected arterial pressure wave (31).

Myocardial mechanical deformation during systole is an important stimulus for NO release by cardiac endothelial cells (24). With the use of an intramyocardial porphyrinic NO sensor, beat-to-beat release of NO was recorded in the rabbit heart with a brisk rise at end systole to optimally hasten myocardial relaxation and to lower LV filling pressures (38). The latter effects are beneficial for diastolic coronary perfusion especially at higher heart rates when there is a disproportionately larger reduction in diastolic coronary perfusion period. In the same preparation, an increase in LV preload was accompanied by a higher beat-to-beat release of NO allowing for a larger desensitizing effect of NO counteracting the preload-induced augmentation of myofilamentary calcium sensitivity. When preload rises in the setting of contractile dysfunction, as occurs in failing myocardium, systolic mechanical deformation and the NO transient will fail to increase leaving preload-induced augmentation of myofilamentary calcium sensitivity unopposed. Because of induction of the fetal gene program, failing myocardium already expresses myofilamentary isoforms with increased calcium sensitivity. This isoform shift, a high LV preload, and a low NO transient will all cause additive augmentation of myofilamentary calcium sensitivity and can therefore predispose failing myocardium to slow relaxation kinetics and diastolic crossbridge cycling, both of which compromise diastolic coronary perfusion and myocardial oxygen supply. In such a setting, the expression of NOS2, which produces NO independently of mechanical deformation, could be beneficial because it would interrupt the vicious circle of reduced mechanical deformation, reduced NOS3-dependent NO production and deranged diastolic LV function (18) and energetics (9).

In conclusion, in heart failure patients, beneficial effects of NO on diastolic LV function always overrule a small NO-induced attenuation of LV developed pressure in terms of overall hemodynamic status, either at baseline or after β-adrenergic stimulation. The absence of hemodynamic deterioration in transgenic mice overexpressing either myocardial NOS2 or NOS3 confirms these clinical observations. In failing myocardium, NO’s correction of diastolic LV dysfunction
reinforces NO’s energy sparing effects and the concerted action of NO on both diastolic LV dysfunction and deranged energetics could well be instrumental for preventing relentless deterioration of failing myocardium.

REFERENCES


