Nitric oxide and myocardial function in heart failure: friend or foe?

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There is good evidence that nitric oxide has important autocrine/paracrine effects in the myocardium, serving to optimise and fine tune cardiac function

Nitric oxide (NO) has been the subject of intense research since endothelial derived relaxing factor was first described in 1980.¹ Indeed, the 1998 Nobel prize for biology and medicine was awarded for work which characterised the fundamental roles of this simple gas in the cardiovascular system. Whereas the effects of endothelial derived NO in the vasculature are well recognised, only in the last decade has it become evident that NO released from cardiac endothelial cells and/or generated within cardiac myocytes themselves also has important autocrine/paracrine effects on myocardial function.²

All three NO synthase (NOS) isoforms can be expressed in the heart. Endothelial type NOS (eNOS) is expressed constitutively in endothelial and endocardial cells and, at a much lower concentration, in cardiac myocytes. Neuronal NOS (nNOS) is found in nerve fibres as well as within cardiac myocytes. Upon appropriate stimulation, notably by cytokines, the high output inducible NOS (iNOS) can be expressed by many different cell types—for example, infiltrating inflammatory cells, endothelial cells, and cardiac myocytes.^{2,3} In this brief article, we review the main physiological actions of NO on cardiac myocyte function and consider its potential involvement in the myocardial dysfunction that is characteristic of heart failure.

PHYSIOLOGICAL ACTIONS OF NITRIC OXIDE ON THE MYOCARDIUM

Many diverse and often contradictory effects of NO or NO donors on myocardial function have been reported which, until relatively recently, have been difficult to make sense of. However, there is now an emerging consensus that NO generally acts to fine tune and optimise cardiac pump function.

Several experimental studies have shown that low (submicromolar) doses of NO exert small *positive inotropic effects*, which may serve to enhance basal cardiac function.^{4–6} Recently, we demonstrated a similar effect in normal human subjects undergoing cardiac catheterisation, in whom intracoronary NOS inhibition with L-NMMA caused a small reduction in the maximal rate of left ventricular pressure development (LV dp/dt_{max}), independent of changes in cardiac loading.⁷ An

increasing body of data suggests that NO derived both from eNOS located in sarcolemmal caveolae and nNOS located probably in the sarcoplasmic reticulum (SR) of the cardiac myocyte may modulate central events of *excitation–contraction coupling* such as calcium influx through sarcolemmal L type channels and the release and re-uptake of calcium by the SR.^{8,9} The precise physiological role of these effects remains to be better defined, but there is a clear suggestion of a fundamental autoregulatory role for NO in intra-myocyte calcium cycling.

At slightly higher but still “physiological” doses, NO enhances *myocyte relaxation and diastolic function*. Endothelium derived NO accelerates relaxation and reduces diastolic tone in a wide range of experimental preparations and species.^{2,5,6,10,11} The underlying mechanism of these effects is thought to be a cGMP induced reduction in myofilament responsiveness to calcium,^{11,12} an action that is also supported by studies in anaesthetised pigs.¹³ Importantly, these effects have been confirmed in normal human subjects studied invasively in the catheterisation laboratory with intracoronary infusions of the NO donor, sodium nitroprusside, or of substance P, an agonist that releases NO from endothelial cells.^{14,15}

Analogous to the effects on contractile function, NO generated locally within the myocardium also modulates *basal heart rate*. In particular, NO exerts biphasic effects on atrial rate and automaticity through modulation of the hyperpolarisation activated pacemaker current, I_r.¹⁶ Low concentrations of NO donors increase rate whereas higher concentrations are negatively chronotropic. A final important effect of endothelium derived NO on basal function is the reversible inhibition of *myocardial O₂ consumption*, independent of contractile function, which has been demonstrated both in vitro¹⁷ and in large animals in vivo¹³ (although some authors have failed to confirm these data^{18,19}). The underlying mechanism involves effects on the mitochondrial electron transport chain and may be at least partly cGMP independent. This action, like the others previously discussed, may be considered as potentially beneficial for global cardiac function.

An important recent experimental observation is that intracardiac NO generation is cyclical, with

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Abbreviations: DCM, dilated cardiomyopathy; NO, nitric oxide; NOS, NO synthase; eNOS, endothelial NOS; iNOS, inducible NOS; nNOS, neuronal NOS; SR, sarcoplasmic reticulum

a brisk rise around the time of early diastolic filling, and that it is augmented by increased chamber stretch (preload).²⁰ It has therefore been suggested that mechanical stimuli (for example, stretch, shear), whose qualitative and quantitative characteristics are determined largely by cardiac contractile properties and loading conditions, may serve to match contractile (and other) functions of the heart to altered workload on a beat-to-beat basis. As an example, experimental studies have shown that endogenous NO enhances the Frank-Starling response in the isolated heart.²¹ Furthermore, NO has been shown to play an important role in the stretch induced activation of cardiac muscle.²² Such effects may be especially important during periods of increased cardiac workload—for example, exercise.

NO is also reported to modulate inotropic, chronotropic, and dromotropic responses to β adrenoceptor stimulation; low doses enhance and high doses reduce β adrenergic response.² In a physiological context, these effects may be regarded as beneficial by optimising or “damping” responses to β stimulation. The precise source of this NO, and in particular whether sympathetic or cholinergic stimulation might lead to NO generation within the cardiac myocyte, remains a subject of some controversy despite attempts to resolve the issue specifically using gene modified mice lacking eNOS.²³ Interestingly, an effect of endogenous NO to modulate β adrenergic inotropic responses in humans *in vivo* could only be demonstrated in patients with impaired LV function and not in “normal” subjects.²⁴ Likewise, in carefully conducted studies in anaesthetised pigs, there was no demonstrable effect of endogenous NO on β adrenergic inotropic responsiveness.²⁵

IS NITRIC OXIDE INVOLVED IN THE MYOCARDIAL DYSFUNCTION OF HEART FAILURE?

The discovery that the high output isoform iNOS is expressed in the myocardium of patients with heart failure,²⁶ coupled with previous *in vitro* data that iNOS expression can induce contractile dysfunction,^{2,3} has led to considerable interest in the potential role of NO in the pathophysiology of cardiac dysfunction in heart failure. iNOS expression and/or activity have been documented both in end stage failing heart tissue and in endomyocardial biopsies obtained from patients with less severe heart failure of diverse aetiology.^{26–29} Most studies show iNOS expression in inflammatory and vascular cells whereas expression in cardiac myocytes has been more variable. An important point to bear in mind in interpreting such studies is that NOS expression level does not necessarily reflect the amount of bioactive NO produced. With substrate or co-factor deficiency, NOS activity is impaired and the enzyme may even be dysfunctional, resulting in generation of superoxide (O_2^-) rather than NO.³⁰ Additionally, the prevailing redox balance and antioxidant status are of critical importance in determining NO bioactivity. In settings of increased oxidative stress, NO is inactivated by O_2^- in a reaction that can generate peroxynitrite (ONOO⁻). Not only does this reaction reduce NO concentrations, but peroxynitrite itself is a potentially toxic species that can disrupt the function of diverse proteins through nitration and oxidation reactions.

Direct evidence for a deleterious role of iNOS in human heart failure remains limited. The initial speculative suggestions that excessive NO production by iNOS has acute negative inotropic effects are almost certainly too simplistic. Treatment with NOS inhibitors had no effect on basal function either in myocardial strip preparations or isolated myocytes from end stage failing hearts.^{28,31} In patients with dilated cardiomyopathy (DCM) of varying severity, intracoronary L-NMMA infusion had no effect either on basal contractile function or the force–frequency relation.⁷ Likewise, intracoronary L-arginine (the substrate for NOSs) did not alter basal function.³² However, it remains possible that iNOS may have important *chronic* deleterious effects on the myocardium

either caused by increased NO production or, more likely, mediated via peroxynitrite, which are not readily reversible by acute administration of NOS inhibitors. Based on *in vitro* findings, such deleterious effects could include cellular apoptosis, an irreversible impairment of contractile function, an irreversible decrease in myocardial O_2 consumption, and abnormal heart rate and rhythm regulation.² However, it is important to appreciate that good evidence to support such effects of iNOS is currently lacking even in experimental animal models of heart failure.

Until relatively recently, the possibility that alterations in eNOS expression might also be important for myocardial function in heart failure has not been considered, despite the acceptance that coronary endothelial NO bioactivity is reduced both in human and experimental heart failure. In addition, alterations in myocardial eNOS expression have also been found, although the direction of change has been variable.^{29,33}

In part, these differences may relate to the cell types in which eNOS is expressed as well as disease severity. A number of functional correlates of altered eNOS expression and/or coupling are now becoming apparent. Several years ago, it was reported that the contractile response to the β adrenergic agonist dobutamine was augmented by intracoronary L-NMMA in DCM patients.³⁴ Similarly, the concurrent infusion of intracoronary substance P and intravenous dobutamine induced potent negative inotropic effects in DCM patients.³⁴ These effects were difficult to attribute to increased iNOS expression, given the lack of effect of acute NOS inhibition on basal function (see above). However, it now appears likely that these data reflect an enhanced interaction between eNOS and its activation by β adrenergic receptor stimulation in the failing human heart (even in the presence of overall reduced total eNOS concentrations).³⁵ Recent data suggest an important role for the β_3 adrenoceptor subtype in this regard.²

Alterations in eNOS expression may also affect diastolic function. In a recent study in DCM patients, a linear correlation was found between NOS mRNA expression level in endomyocardial biopsies and left ventricular stroke work.³⁶ In the same study, the stimulated release of NO (with intracoronary substance P) caused a rightward shift of the diastolic pressure–volume relation and a concomitant increase in left ventricular stroke work. These findings are consistent with the notion that NO may benefit left ventricular diastolic function in heart failure, particularly in patients with limited reserve who are more dependant on the Frank-Starling response to maintain cardiac output. Other effects of reduced eNOS expression are also possible—for example, reduction in myocardial O_2 consumption and changes in substrate utilisation.

CONCLUSIONS

There is now good evidence that NO has important autocrine/paracrine effects in the myocardium in physiological settings, in general serving to optimise and fine tune cardiac function through actions on inotropic state, excitation–contraction coupling, diastolic function, heart rate, and β adrenergic responsiveness. It is also clear that the myocardial expression and activity of eNOS and iNOS, and the biological activity of NO generated by these isoforms, is altered during human heart failure. However, the spatial and temporal characteristics of these alterations and their relation to disease severity remain poorly characterised. The functional consequences of altered NOS expression and NO bioactivity in the failing human heart are only just beginning to be explored. Studies to date suggest that a reduction in eNOS expression or activity may be detrimental whereas the consequences of increased iNOS expression remain unclear. In addressing this area, a particularly important aspect will be to also study tissue redox state, given that the presence of oxidative stress often

dramatically alters the actions of NO (generally from beneficial to deleterious).³⁰ There is still a long way to go before a better understanding of the NO pathway in heart failure could be translated into clinical therapeutic advances.

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