Effects of Nitric Oxide Synthase Inhibition on Basal Function and the Force-Frequency Relationship in the Normal and Failing Human Heart In Vivo

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Background—Nitric oxide (NO) exerts autocrine/paracrine effects on cardiac function, including alterations of the inotropic state. In vitro studies suggest that NO modulates the myocardial force-frequency relationship. Basal left ventricular (LV) contractility is depressed and the force-frequency relationship is blunted in human heart failure, and it is speculated that an increase in NO production is involved.

Methods and Results—We compared the effects of intracoronary NO synthase inhibition with N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA; 25 \(\mu\)mol/min) on basal LV function and the response to incremental atrial pacing in patients with dilated cardiomyopathy (n=11; mean age, 51 years) and in control subjects with atypical chest pain and normal cardiac function (n=7; mean age, 54 years). In controls, L-NMMA significantly reduced basal LV dP/dt\textsubscript{max} (from 1826 to 1578 mm Hg/s; \(P<0.002\)), but had no effect on heart rate, mean aortic pressure, or right atrial pressure. Pacing-induced increases in LV dP/dt\textsubscript{max} were unaltered by L-NMMA. In patients with dilated cardiomyopathy, L-NMMA had no effect on baseline LV dP/dt\textsubscript{max} (from 1313 to 1337 mm Hg/s; \(P=NS\)). The blunted pacing-induced rise in LV dP/dt\textsubscript{max} in these patients was unaltered by L-NMMA.

Conclusion—Endogenous NO has a small baseline positive inotropic effect in the normal human heart, which is lost in heart failure patients. NO does not significantly influence the force-frequency relationship in either the normal or failing human heart in vivo. Because this study was performed in patients with moderate heart failure, whether the findings apply to subjects with more severe heart failure requires further investigation. (Circulation. 2001;104:2318-2323.)

Key Words: contractility | heart failure | myocardial contraction | nitric oxide | nitric oxide synthase

Nitric oxide (NO), released from coronary microvascular endothelial cells and/or generated within cardiomyocytes, exerts several specific effects on normal myocardial function. These include modulating inotropic and chronotropic states, modulating sarcolemmal Ca\textsuperscript{2+} influx and sarcoplasmic reticular Ca\textsuperscript{2+} cycling, inhibiting ß-adrenergic responses, enhancing diastolic function, and reducing O\textsubscript{2} consumption.\textsuperscript{1,2} Endogenous NO reportedly blunts the force-frequency relationship (FFR) in normal myocardium, as based on studies with NO synthase (NOS) inhibitors in hamster papillary muscle\textsuperscript{3} and rat cardiomyocytes.\textsuperscript{4} However, it is also feasible that NO enhances the FFR, as based on studies demonstrating a role for NO in modulating sarcoplasmic reticular Ca\textsuperscript{2+} cycling.\textsuperscript{1,5}

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The extension of these data to the normal human heart in vivo has been limited. In subjects with normal left ventricular (LV) function and angiographically normal coronary arteries, intracoronary administration of an exogenous NO donor or an agonist (substance P) that stimulates the release of NO enhanced LV relaxation and shifted the LV diastolic pressure-volume relationship downward.\textsuperscript{6,7} Hare et al\textsuperscript{8} studied the effects of concomitant intracoronary N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) infusion on the LV dP/dt\textsubscript{max} response to systemic dobutamine in 7 subjects with normal LV function and found no effect of the NOS inhibitor in this group. However, the effects of L-NMMA treatment per se on basal LV contractile function were not addressed. The effect of endogenous NO on the FFR of the normal human heart in vivo has not been studied. The potential role of NO in the pathophysiology of LV dysfunction in heart failure is controversial.\textsuperscript{1,2} Many clinical studies have documented increased intracardiac expression of inductible NOS, and it has been speculated that increased NO production contributes to the depressed basal LV contractility.
and the blunted FFR that characterizes human heart failure.9–12 However, there is little evidence, even from experimental studies, that inhibition of basal NO production improves LV contractility in this setting. Hare et al8,13 showed that the inotropic response to β-adrenergic stimulation in patients with severe dilated cardiomyopathy (DCM) was potentiated by concurrent intracoronary L-NMMA infusion, suggesting that endogenous NO depressed β-adrenergic responses. Other clinical studies have reported findings consistent with this.12,14,15 However, these studies did not specifically address the effects of endogenous NO on LV function in the absence of β-adrenergic stimulation. Likewise, the effects of NOS inhibition on the FFR in heart failure patients has not been studied. In contradistinction to the hypothesis of deleterious effects of increased NO in heart failure, many studies report that endothelial NOS expression and/or NO bioactivity are decreased in heart failure.11,12,16–18 This may contribute to decreased NO-dependent coronary vasodilatation, worsening of diastolic LV function, and an increase in myocardial O2 consumption.16–18

Thus, the functional effects of basal NO in the normal or failing human heart in vivo remain unclear. The aim of this study was to use intracoronary NOS inhibition to evaluate the contribution of endogenous NO to basal LV performance and the FFR in normal subjects and in heart failure patients.

Methods

Study Population
We studied 24 subjects undergoing diagnostic cardiac catheterization. All subjects provided informed written consent and proceeded to the study if initial angiography demonstrated coronary arteries free of significant disease. The study received institutional ethics committee approval. The control group comprised 10 subjects (3 women; aged 49 to 58 years) with atypical chest pain, LV ejection fraction >55%, and normal hemodynamics. Cardiovascular medications comprised calcium antagonists (3 patients) and nitrates (1 patient). The DCM group comprised 14 subjects (5 women; aged 32 to 70 years) undergoing evaluation of heart failure. All were in sinus rhythm and had an LV ejection fraction <40%. New York Heart Association functional class was II (n=9) or III (n=5). Cardiovascular medications comprised diuretics (n=10), angiotensin-converting enzyme inhibitors (n=9), digoxin (n=1), an angiotensin II receptor antagonist (n=1), and nitrates (n=1). Medications were withheld for >12 hours before the study.

Cardiac Catheterization
Catheterization was performed by the femoral approach. A 3000-U IV bolus of heparin was administered. A 6F micromanometer-tipped pigtail catheter (Millar Instruments) was positioned in the LV cavity through the left femoral artery; was calibrated externally against a mercury reference, and was matched against luminal pressure. A bipolar pacing wire and a multipurpose catheter were positioned in the right atrium. A 5F left Judkins catheter was positioned in the left main coronary artery ostium for intra coronary infusion. Pressures were referenced to atmospheric pressure at mid-chest level. LV pressure, aortic pressure, right atrial pressure, and the ECG were recorded using a Maclab analogue-digital converter (ADJ) and a Macintosh personal computer.

Study Protocol
At least 20 minutes after angiography and 10 minutes after the insertion of additional catheters, baseline recordings of pressures and ECG were obtained. Incremental right atrial pacing was performed up to a rate of 140 bpm or the development of Wenkebach block.

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Values are expressed as mean±SD. LVEF indicates LV ejection fraction; LVSP, LV peak systolic pressure; LVEDP, LV end-diastolic pressure; RAP, mean right atrial pressure; and MAP, mean arterial pressure.

After a 5-minute period without pacing, intracoronary L-NMMA infusion commenced in 7 control subjects and 11 DCM patients. L-NMMA (Cilinafla) was infused at a rate of 25 μmol/min (2 mL/min) to achieve a calculated steady-state coronary artery concentration of ~200 μmol/L (assuming a left coronary artery flow of 125 mL/min).8,15,20 After 8 minutes, incremental atrial pacing was repeated during continuing L-NMMA infusion.

In the control group, atrial pacing was undertaken at 100 bpm for 2 minutes followed by 140 bpm for 2 minutes. In the DCM patients, whose basal heart rates were generally higher than controls, a slightly different incremental regime was used. These subjects underwent pacing for 2 minutes at either 100 or 110 bpm, depending on baseline heart rate; followed by increments of 10 bpm for 2 minutes each, up to a maximum rate of at least 140 bpm. At each rate, measurements of pressures and ECG were made at steady-state, after at least 60 s at the higher rate.

In 3 additional controls and 3 DCM patients, the pacing protocol was undertaken twice, 13 minutes apart, in the absence of L-NMMA infusion to ensure that repeated pacing per se had reproducible effects on LV function.

Data Analysis
Maximal and minimal rates of LV pressure development (LV dP/dt max and LV dP/dt min, respectively) were obtained from the first derivative of LV pressure. The time constant of isovolumic LV relaxation (τ), was calculated as described previously.6 Data are expressed as mean±SD. Baseline parameters between groups were compared using unpaired Student’s t tests. Within-group comparisons were made by paired Student’s t test. Responses before and after L-NMMA were compared by 2-way ANOVA for repeated measures. P<0.05 was considered significant. Because baseline heart rates and, therefore, the pacing increments that were studied varied among the patients, these data were analyzed in 2 ways. First, data were categorized into baseline, 10 to 30 bpm above baseline heart rate, and 40 to 70 bpm above baseline rate, and these categories were compared. As an alternative approach, we also analyzed maximal pacing-induced changes in LV dP/dt max, regardless of rate.

Results

Baseline Hemodynamics
The Table shows patient demographics and baseline hemodynamics. DCM patients had a lower LV peak systolic pressure, LV dP/dt max, LV ejection fraction, and LV dP/dt min than controls. LV end-diastolic pressure (LVEDP), isovolumic relaxation time-constant, mean right atrial pressure, and
heart rate were all significantly higher in DCM patients than in controls.

Effect of NOS Inhibition on Basal LV Function
L-NMMA infusion induced a significant reduction in LV dP/dt max in the control subjects, from 1826±227 to 1578±264 mm Hg/s (P<0.002; Figure 1). There were no significant changes in LVEDP, right atrial pressure, or mean arterial pressure in controls, suggesting that there was no significant change in cardiac loading. LV dP/dt min and τ were unaffected by intracoronary L-NMMA infusion. Mean heart rate was 74±13.5 bpm before and 69±9.4 bpm after L-NMMA infusion (P=NS).

In DCM patients, intracoronary L-NMMA had no effect on LV dP/dt max (1313±214 mm Hg/s before L-NMMA and 1337±238 mm Hg/s after L-NMMA; P=NS; Figure 2). L-NMMA infusion increased τ from 66.2±10.2 ms to 71.4±10.8 ms (P<0.04), LVEDP from 14.1±8.5 mm Hg to 16.4±8.8 mm Hg (P<0.002), and mean arterial pressure from 93.1±16 mm Hg to 98.9±17 mm Hg (P<0.01), but it did not alter right atrial pressure or LV dP/dt min (Figure 2). Heart rate was 84±15.2 bpm before and 88±12.1 bpm after L-NMMA (P=NS). There was no correlation between L-NMMA-induced changes in LVEDP and LV dP/dt max, making it unlikely that the lack of change in LV dP/dt max was due to the confounding effects of altered LVEDP.

Effect of NOS Inhibition on FFR
In the control group, right atrial pacing before L-NMMA increased LV dP/dt max from 1826±227 to 2388±342 mm Hg/s (P<0.001; Figure 3A). LV dP/dt min did not change significantly. After L-NMMA infusion, despite the reduction in baseline LV dP/dt max, the pacing-induced increase in LV dP/dt max was unaltered (Figure 3A). Thus, the heart rate–LV dP/dt max relation after

Figure 1. Effect of intracoronary L-NMMA on baseline hemodynamics and LV function in subjects with atypical chest pain. Both individual and mean data are shown. *P<0.05. Right atrial pressure (RAP) data are shown for 6 of 7 subjects; 1 subject had unanalyzable recordings. MAP indicates mean arterial pressure.

Figure 2. Effect of intracoronary L-NMMA on baseline hemodynamics and LV function in DCM patients. Both individual and mean data are shown. *P<0.05. Right atrial pressure (RAP) data are shown for 9 of 11 subjects; 2 patients had unanalyzable recordings. MAP indicates mean arterial pressure.
L-NMMA was parallel to that before L-NMMA. The pacing-induced effects on LV dP/dt max and τ were also similar before and after L-NMMA.

In DCM patients, pacing-induced changes in LV dP/dt max were significantly blunted compared with those in the controls (Figure 3B). After L-NMMA, the effects of pacing on LV dP/dt max, dP/dt min, and τ were similar to those before L-NMMA (Figure 3B). Figure 4A shows the maximal pacing-induced changes in LV dP/dt max (regardless of heart rate) in each DCM patient before and after L-NMMA. It is evident that L-NMMA had no consistent effect on the FFR assessed in this manner. Figure 4B shows maximal pacing-induced changes in LV dP/dt max in DCM patients, displayed according to the baseline LV dP/dt max of individual subjects. This shows that there was no systematic difference in response to L-NMMA between subjects with varying degrees of LV impairment, as assessed by baseline LV dP/dt max.

In the 3 controls and 3 DCM patients who underwent 2 successive periods of incremental pacing 13 minutes apart, without intervening L-NMMA infusion, maximal changes in LV dP/dt max were similar for both pacing periods (data not shown).

Discussion
The main new findings of this study are the following: (1) inhibition of endogenous NOS in patients with normal LV function leads to a reduction in LV contractility, suggesting that NO has a small physiological positive inotropic effect in the normal human heart in vivo; (2) this positive inotropic effect of endogenous NO is not apparent in the failing human heart; (3) there is no evidence of an acute negative inotropic effect of NO in the unstimulated failing human heart in vivo; and (4) endogenous NO does not seem to alter the myocardial FFR significantly in either the normal or failing human heart in vivo.

NO and Basal Myocardial Contractility in the Normal Human Heart
The effects of endogenous NO on basal myocardial function (ie, in the absence of agonist stimulation) in the experimental setting have been contradictory.1 Several in vitro studies suggest that NO in submicromolar concentrations exerts modest positive inotropic effects.21–24 In human atrial myocytes, both an NO donor and a cGMP phosphodiesterase

![Figure 3. Effect of intracoronary L-NMMA on pacing-induced changes in LV dP/dt max, LV dP/dt min, and τ in subjects with normal LV function (A) and in DCM patients (B).](image)

![Figure 4. Maximal pacing-induced changes in LV dP/dt max in DCM patients. A, Absolute changes in LV dP/dt max in individual subjects before and after L-NMMA infusion. B, Changes in LV dP/dt max in individual subjects displayed according to the baseline LV dP/dt max of subjects.](image)
inhibitor were positively inotropic.24 However, not all authors found similar results.1 Also, in the majority of isolated cardiomyocyte studies, NOS inhibition had no effect on basal function. Explanations for these contradictory data include species differences and alterations in the basal level of β-adrenergic stimulation. Potential mechanisms for a positive inotropic effect of NO include cGMP-mediated inhibition of cAMP phosphodiesterase and consequent rises in intracellular cAMP, direct activation of adenylcyclase, or cGMP-independent alterations in excitation-contraction coupling.1,2,5

The present results in subjects with normal LV function suggest that the predominant effect on basal myocardial function in humans in vivo is a small positive inotropic effect. The lack of significant associated changes in heart rate, LVEDP, right atrial pressure, or mean arterial pressure suggests that L-NMMA did not alter cardiac loading.

NO and Basal Myocardial Contractility in the Failing Human Heart
Although it has been suggested that excessive NO production (especially inducible NOS-derived) contributes acutely to contractile depression in the failing human heart, direct evidence to support this is weak. In particular, whether NO acutely depresses contractile function in the failing human heart in the absence of β-adrenergic stimulation has not been addressed. The present study found no evidence for an acute negative inotropic effect of NO, in that L-NMMA did not improve contractile function. The doses of L-NMMA used were previously shown to inhibit endothelial NO-dependent responses in the human coronary circulation and to be effective in acutely modulating β-adrenergic responses,8,15,20 suggesting that we should have inhibited both endothelial and cardiac myocyte NOS. Although we found no evidence for a negative inotropic effect of endogenous NO, the small positive inotropic effect evident in normal subjects was lost, which could contribute at least in part to the reduced LV contractility in heart failure. The loss of positive inotropic effect could reflect a decrease in the level of bioactive NO, either due to reduced production or increased inactivation by reactive oxygen species, or it may represent a reduction in myocardial response to NO per se. In this regard, the prevailing redox milieu may be particularly important because myocardial responses to NO may be significantly altered in the presence of oxidative stress.1,2,5

L-NMMA infusion also resulted in slightly increased LVEDP and a rise in τ in DCM patients. Interestingly, these selective effects on “diastolic” indices contrasted with the predominantly “systolic” effects observed in the controls, in whom there were no changes in LVEDP or τ. The mechanisms underlying these contrasting effects are speculative, but it is possible that the changes in heart failure subjects reflect an inhibition of the effects of NO on relaxation and diastolic properties, as suggested in previous studies.1,6,7 It may be that the failing heart is more sensitive to the loss of NO-mediated lusitropic effects, given that LV relaxation and diastolic compliance are already impaired in this condition. A definitive answer to this question requires further studies, including a direct assessment of diastolic properties with concomitant measurements of LV volume.

Effect of NO on Basal Function Versus β-Adrenergic Response
The lack of effect of NOS inhibition on basal LV function in DCM patients in this study may seem to conflict with previous reports that NOS inhibitors potentiate β-adrenergic responsiveness.8,13 The explanation for these seemingly divergent findings may be provided by a recent study in canine heart failure.23 Hare et al25 reported that the expression of caveolin-3, a caveolar scaffolding protein important in regulating NOS activity, was increased in heart failure, whereas endothelial NOS expression was unaltered and there was no evidence of inducible NOS expression. The interaction of caveolin with NOS brings NOS into close proximity with sarcolemmal agonists/receptors (eg, the β-adrenergic receptor), while at the same time inhibiting basal NOS activity.26 The increased caveolin-3 may contribute to enhanced NO signaling (ie, depressed β-adrenergic responses) via a compartmentation effect, whereas basal unstimulated NO activity might be depressed.25 Indeed, Hare et al25 observed that NOS inhibition reduced basal myocardial contractility in control dogs but had no effect in heart failure, similar to our results in humans in the present study. Such a mechanism is also consistent with previous data that the interaction between β-adrenergic stimulation and endothelial NO-mediated effects is augmented in heart failure patients.27

NO and the Myocardial FFR in the Human Heart
An increase in heart rate augments myocardial contractility, an effect known as the FFR. This is an important homeostatic mechanism that contributes to the augmentation of cardiac output (eg, during exercise). A fundamental feature of human heart failure is a blunting of the myocardial FFR.28–30 Although abnormalities of excitation-contraction coupling (such as altered expression of sarcoplasmic reticulum proteins) are generally thought to be responsible for the blunted FFR,30 it has also been suggested that an increase in NO production may be a contributory factor. The present study, however, found no evidence for a significant effect of L-NMMA on the response to increased heart rate in either normal subjects or DCM patients. These data are consistent with the lack of effect of NOS inhibition on the response of isolated failing human cardiomyocytes to pacing.31

Study Limitations
Several potential limitations should be mentioned. First, LV dP/dtmax is not a perfect index of myocardial contractility; a full description of contractile state and diastolic properties requires simultaneous measurements of LV pressure and volume. Second, definitive demonstration of a direct action of NO on the cardiomyocyte would require an evaluation of coronary flow. However, it has been shown previously that intracoronary infusion of the dose of L-NMMA we used has only minor effects on basal coronary flow, at least in the normal heart.20 Third, although the protocol for L-NMMA infusion was based on previous studies, it is possible that a significantly longer duration of NOS inhibition might have caused different effects. Fourth, it is feasible that the effects of L-NMMA might be different in patients with very severe, decompensated heart failure. Finally, an in vivo study is...
limited in terms of the extent of mechanistic assessment possible. Thus, we cannot comment on the relative roles of different NOS isofoms nor the mechanisms involved in the effects of endogenous NO (eg, cGMP-dependent or direct protein nitrosylation).

Conclusions

We report the first evidence for a positive inotropic effect of endogenous NO in the normal human heart in vivo. This study does not support a significant acute negative inotropic effect of endogenous NO in the failing human heart in vivo, although it is feasible that altered myocardial NO production may have chronic effects that are not acutely reversible with NOS inhibition. Loss of the small positive inotropic effect of endogenous NO observed in the normal heart could contribute, at least in part, to the impairment of basal LV function in heart failure. Finally, the present data do not support a clinically relevant effect of endogenous NO on myocardial FFR in vivo.

Acknowledgments

Supported by the British Heart Foundation (BHF) and the UK Medical Research Council. Dr Cotton was a BHF Junior Fellow. Dr Shah holds the BHF Chair of Cardiology in King’s College London. We thank all the staff of the Cardiac Catheterization Laboratories of our institution, especially Samantha Chapman and Mark Passey, for their unstinting help.

References

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_Circulation_. 2001;104:2318-2323
doi: 10.1161/hc4401.098515

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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