Nitric oxide and cardiac parasympathetic control in human heart failure

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ABSTRACT

Cardiac parasympathetic control has prognostic significance in heart failure, but the control mechanisms of this system remain poorly defined. We have demonstrated previously a facilitatory role for nitric oxide (NO) in the parasympathetic control of heart rate in young healthy human subjects. In view of the complex abnormalities of regional NO activity observed in chronic heart failure, we now aim to establish if this mechanism is active in subjects with this condition. Groups of 12 heart failure patients [NYHA class II–III; mean age 52 years (range 38–67 years)] and 12 age/sex-matched healthy control subjects [mean age 50 years (range 36–62 years)] were studied. Heart rate variability and baroreflex sensitivity were measured during inhibition of endogenous NO production with \( N^G\)-monomethyl-L-arginine (L-NMMA; 3 mg [kg\(^{-1}\)] [h\(^{-1}\)]) and during administration of an equipressor dose of the control vasoconstrictor phenylephrine (12–36 \( \mu\)g [h\(^{-1}\)] [kg\(^{-1}\)]). Basal levels of nitrate + nitrite were measured in the plasma as an indication of systemic NO production. In the heart failure patients, despite an equal rise in blood pressure with both drugs, high-frequency indices of heart rate variability increased less with L-NMMA than with phenylephrine: RMSSD (root mean square of successive RR-interval differences) increased by 4 ± 2 ms compared with 26 ± 8 ms (P < 0.001) and high-frequency power increased by 97 ± 62 ms\(^2\) (P < 0.001). The increases in cross-spectral baroreflex sensitivity were also lower with L-NMMA than with phenylephrine [high-frequency \( \alpha \)-index, 2.2 ± 1.3 and 12.6 ± 3.8 ms/mmHg respectively (P < 0.001); low-frequency \( \alpha \)-index, 1.3 ± 0.9 and 4.3 ± 1.7 ms/mmHg respectively (P < 0.05)]. Healthy subjects showed a similar discrepancy in the response of high-frequency indices of heart rate variability to the two drugs, although baroreflex sensitivity responses were significantly different only for the high-frequency \( \alpha \)-index. Levels of plasma nitrate + nitrite were significantly higher in the heart failure patients compared with controls. These data demonstrate that baroreflex-mediated cardiac parasympathetic activation in human heart failure, as in health, is dependent upon endogenous NO synthesis.

INTRODUCTION

Chronic heart failure is characterized by abnormalities of cardiac autonomic function, including high levels of sympathetic activity, impaired parasympathetic control and subnormal arterial baroreflex gain [1,2]. The degree of impairment of parasympathetic control as measured by heart rate variability (HRV) and baroreflex sensitivity (BRS) has been shown to be a powerful and independent indicator of an adverse prognosis [3]. It seems likely that, as with excess sympathetic activity, impaired parasympathetic control contributes actively to disease pro-
obtained during L-NMMA infusion with those obtained
nomic action was controlled for by comparing the results
consequent baroreflex activation. This confounding auto-
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staff at our institution. Patients were in sinus rhythm,
matched healthy control subjects were recruited from
months) were recruited from outpatient clinics. Age
patients with stable heart failure (no changes in medical
therapy and no hospital admissions within the last 2
months) were recruited from outpatient clinics. Age/sex-
matched healthy control subjects were recruited from
staff at our institution. Patients were in sinus rhythm,
normotensive (blood pressure < 140/90 mmHg), with
normal renal function (serum creatinine < 130 µmol/l) and
had an echocardiographic ejection fraction of < 40% at study entry. No patient was receiving nitrate
therapy, and β-blockers were omitted for 72 h prior to
each study visit. Other medications were held constant
for the duration of the patients’ enrolment. Control
subjects had no evidence of cardiovascular disease on
history, physical examination or ECG. All subjects were
asked to abstain from food and drink for at least 2 h prior
to the study, and from caffeine and alcohol for 24 h. A
dietary history was taken to estimate dietary nitrate
intake at the time of study [16]. The protocol conformed
with the Declaration of Helsinki (1989) of the World
Medical Association, and was approved by the ethics
committee of our institution. All subjects gave written
consent.

Experimental protocol
Protocols were of a single-blind, random-order, cross-
over design. All subjects had a preliminary ac-
climatization visit, where they were trained to breathe
to an audio signal set close to the individual’s resting
respiratory rate. Each patient was then randomly assigned
to receive either an intravenous infusion of L-NMMA
(3 mg h⁻¹ kg⁻¹) or a control infusion of the pressor
agent phenylephrine (12–36 µg h⁻¹ kg⁻¹) during the first
of two studies. The remaining agent was given during a
separate study visit 7–14 days later.

Studies were performed at a uniform time of day and at
ambient temperature (24 ± 1 °C). Subjects rested semi-
supine, and signals from a three-lead ECG, a Portapres
device (TNO Biomedical Instrumentation) recording
continuous arterial pressure and a strain gauge attached
around the subject’s chest quantifying respiratory ex-
cursion were recorded digitally at 500 Hz. A venous
cannula was inserted into an antecubital vein for drug
administration, after which subjects rested for 30 min
before selected periods for all three signals were stored to
disk during breathing at the predetermined frequency.
Two 5 min recordings were acquired at baseline during a
30 min normal saline infusion, and a further two
recordings were acquired during the vasoactive infusion
once a steady mean heart rate and arterial pressure were
achieved. Infusion rates were titrated to achieve a target
increment of 5–10 mmHg in mean arterial pressure, as
assessed from a continuously updated 60 s integrated
mean of the Portapres signal and by automated in-
termittent arm cuff sphygmomanometry.

Data analysis
The investigator performing the analysis was blinded to
both the subject and the vasoactive agent under study. All
ECG series were reviewed manually, and those con-
taining more than 2% ectopic beats were excluded. Less
frequent ectopics were replaced by interpolation from

METHODS
To investigate whether NO contributes to the regulation
of cardiac parasympathetic control in patients with
moderate to severe heart failure, we have investigated
the effects of systemic NOS inhibition with N⁰-
monomethyl-L-arginine (L-NMMA) on non-invasive
markers of parasympathetic heart rate control, namely
HRV and BRS. The inhibition of vascular NO produc-
tion by L-NMMA results in a pressor response and
consequent baroreflex activation. This confounding au-
tonomic action was controlled for by comparing the results
obtained during L-NMMA infusion with those obtained
during an equipressor infusion of a control vasocon-
strictor, phenylephrine, a specific α₁-adrenergic agonist
without direct effects on NO pathway activity.

Subjects
Patients with stable heart failure (no changes in medical
therapy and no hospital admissions within the last 2
months) were recruited from outpatient clinics. Age/sex-
matched healthy control subjects were recruited from
staff at our institution. Patients were in sinus rhythm,
the previous and subsequent sinus intervals. HRV and BRS were analysed off-line using Lab-View 5.0 software.

HRV
Standard time-domain measures of the S.D. of RR-interval values, expressing overall variability, and RMSSD (root mean square of successive RR-interval differences), reflecting high-frequency (‘beat to beat’) variation mediated principally by the vagus nerve [17], were employed. Frequency-domain analysis was performed on stationary RR-interval series using autoregressive modelling (Burg algorithm) to determine spectral powers at low frequency (LF; centred at ~ 0.1 Hz) and at high frequency (HF; corresponding to the observed respiratory frequency), as described previously [11].

BRS
Spontaneous BRS was assessed by fast Fourier transformation cross-spectral analysis of RR interval and systolic blood pressure variability using methods that have been described previously [11]. The α-index was derived as the transfer function of systolic power into RR-interval power for both the HF (α-HF) and LF (α-LF) bands, provided that the squared coherence between the two signals was ≥ 0.5.

Measurement of plasma combined nitrate + nitrite (NO₂⁻) concentration
Plasma concentrations of NO₂⁻, representing stable breakdown products of NO, were measured as a surrogate marker of NO formation. Nitrate was first reduced to nitrite by enzymic conversion with nitrate reductase. Following deproteinization, plasma NO₂⁻ concentrations were measured using the Griess reaction [18]. To minimize intra-subject variability secondary to changes in dietary nitrate consumption, values are quoted as the mean of readings obtained on each of the two study days.

Statistical analysis
Haemodynamic data and indices of HRV and BRS were expressed as the mean of the two recording periods for each infusion. Data for mean arterial pressure, RR interval and plasma NO₂⁻ were compared by a two-tailed paired Student’s t test. Differences between groups for indices of HRV and BRS were determined using the Wilcoxon signed rank test for paired data. Statistical significance was taken as P < 0.05, and values are expressed as means ± S.E.M.

RESULTS
A total of 16 heart failure patients were recruited, of whom four were excluded at the initial training visit due to a high incidence of ventricular ectopy. The remaining 12 patients comprised ten men and two women (mean age 52 years; range 38–67 years) with chronic heart failure (NYHA class II–III) due to coronary artery disease (seven patients) or idiopathic dilated cardiomyopathy (five patients). The mean ejection fraction (assessed by two-dimensional M-mode trans-thoracic echocardiography) at the time of study was 27% (range 15–33%). All patients were taking angiotensin-converting enzyme (ACE) inhibitors and loop diuretics, five were taking digoxin, two amiodarone and one spironolactone. Three patients were taking carvedilol, which was omitted for 72 h before the studies. Twelve healthy controls were also enrolled, comprising ten men and two women (mean age 50 years; range 36–62 years). None were taking any medication.

The frequency of metronomic breathing was within the range 0.18–10.26 Hz for both study groups. When comparing baseline measurements prior to the administration of L-NMMA with those prior to the administration of phenylephrine, no significant differences were seen in mean arterial pressure, RR interval or indices of HRV or BRS within each of the two study groups (Table 1).

In the heart failure group, infusion of L-NMMA and phenylephrine resulted in equal rises in mean arterial pressure (Table 2). However, the increase in mean RR interval was markedly lower with L-NMMA than with phenylephrine. The increases in all indices of HF HRV in both the time and frequency domains (i.e. RMSSD and HF power) were significantly smaller in response to L-NMMA than in response to the equipressor phenylephrine infusion (Table 2, Figure 1). The LF/HF ratio, a proposed (although not universally accepted) indicator of sympathetic relative to parasympathetic influence, was unchanged with L-NMMA, in contrast with the significant fall seen with phenylephrine infusion. Increases in cross-spectral BRS were lower during L-NMMA infusion than during phenylephrine infusion. This was true for both α-HF and α-LF (Table 2).

The healthy control subjects had higher values at baseline for mean arterial pressure, mean RR interval, α-HF and the majority of HRV indices in comparison with the patient group (Table 1). Again, the rise in mean arterial pressure was well matched between the L-NMMA and phenylephrine infusions, whereas the mean RR interval tended to lengthen less with L-NMMA (P = 0.1). The majority of indices of parasympathetic cardiac control showed a significantly lower rise with L-NMMA than with phenylephrine (RMSSD, HF power and α-HF; Table 2).

The patient and control groups did not differ significantly in terms of estimated dietary nitrate intake (57±4 and 72±9 mg/day respectively; P = 0.17). Plasma NO₂⁻ levels were significantly higher in the heart failure patients than in the healthy controls (28.6±13.6 and 19.3±3.3 μmol/l respectively; P < 0.05).
DISCUSSION

These data demonstrate for the first time that endogenous NO synthesis remains an important vagotonic modulator of human cardiac autonomic control, not only in health but also in heart failure. This is revealed by the lack of any significant rise in BRS or in HF indices of HRV – both well validated indicators of cardiac parasympathetic control [17] – during baroreflex loading with a NOS inhibitor in patients with heart failure. In contrast, there was a notable rise in these indices during an equipressor infusion of a control vasoconstrictor. This suggests an impaired ability to increase cardiac parasympathetic activity during baroreflex stimulation when NO synthesis is inhibited. Similar results were seen in age-matched healthy control subjects. These results are in agreement with our previous findings in a population of young healthy volunteers, where we demonstrated a vagotonic action not only for endogenous NO but also for exogenous NO donors [11]. Spieker et al. [19] have also recently demonstrated an important role for NO in the baroreflex control of heart rate in healthy human volunteers.

Animal data also confirm a vagotonic action of NO. Discrete neuronal populations containing NOS are distributed widely throughout the peripheral and central components of the cardiac baroreflex pathway [20]. Functionally, NO has been shown to increase neuronal activity within central sites regulating parasympathetic outflow to the heart [4,9]. Peripherally, NO potentiates the bradycardic effects of parasympathetic stimulation [5,6] and also enhances the ability of the efferent vagus to antagonize sympathetic cardiac responses, with both pre- and post-synaptic mechanisms postulated [10]. Consistent with these data, nNOS knockout mice display reduced cardiac parasympathetic tone [7].

How can our finding of a preserved vagotonic influence for NO in heart failure be interpreted in the...
Nitric oxide and cardiac vagal control

The spectra were obtained for a 38-year-old male with idiopathic dilated cardiomyopathy (NYHA class II; ejection fraction 32%) displaying typical data at baseline (A) and at steady state during equipressor infusions of L-NMMA (B) and phenylephrine (C). The area under the HF peak, an index of cardiac parasympathetic control, is visibly smaller with L-NMMA than with phenylephrine. PSD, power spectral density.

It appears more likely that our results can be explained by changes in iNOS expression. This isoform is not expressed in health, but in human heart failure there is a well documented cytokine-mediated induction of iNOS within the circulation and myocardium, leading to the sustained release of large amounts of NO [12–14]. In agreement with this, we found that the systemic production of NO was not only preserved but actually increased in heart failure, with the demonstration of higher levels of plasma NO	extsubscript{x} in patients compared with healthy controls, despite similar levels of dietary nitrate intake. Since the expression of iNOS in the failing myocardium has been shown to include the right atrium, it is likely that NO bioavailability in the region of the sino–atrial node is increased [13], providing one possible mechanism by which NO generated by iNOS may interact with autonomic signalling to the heart. The hypothesis that preserved cardiac autonomic activity of NO in heart failure is attributable mainly to cardiac iNOS induction, offsetting potential deficiencies in constitutive NOS signalling, appears attractive but further research is needed. For instance, changes in nNOS activity in human heart failure have not been studied comprehensively. It is not known whether effects on central nNOS expression conform to those seen in the brains of rats with heart failure [15], or indeed what changes occur within the peripheral nervous system. There is some evidence that nNOS expression is increased within nerve fibres in the left ventricle of rats with ischaemic heart failure [21]. Furthermore, an nNOS-like isoform has been described in the cardiac sarcoplasmic reticulum, and the expression of this in heart failure has not been studied [22].

The present study has some limitations. First, we used a non-specific NOS inhibitor to examine the influence of endogenous NO on autonomic modulation in conscious subjects with intact baroreflexes. This design does not enable us to actively determine the site of NO generation nor the isoform of NOS responsible for the observed effects. Furthermore, the lack of a specific HRV marker of sympathetic activity makes it difficult to comment on the role that a nitrergic influence on cardiac sympathetic tone would have had on our results.

β-Blockers were withheld in view of their powerful effects on cardiac autonomic control; their profound bradycardic effect might have limited that elicited by baroreflex loading and therefore masked differences in the responses to phenylephrine and L-NMMA. Ethical considerations prohibited us from discontinuing other established medications, some of which may have had an autonomic influence. Both digoxin (five patients) and amiodarone (two patients) were continued, but the dose of these agents was unchanged during the study period and the crossover design of the study ensured that the influence of these drugs on autonomic control was identical in both limbs. However, ACE inhibitor therapy (which was taken by all patients) may have had a complex influence on our results. Chronic ACE inhibition is known to exert a powerful cardiac vagotonic influ-
ence [23]. Furthermore, it has been shown to increase eNOS expression and NO release in the vasculature of rats and the atria of human patients, as well as leading to recruitment of human nNOS, possibly via the accumulation of endogenous kinins [24–26]. Despite the difficulty in predicting the impact of these effects in our study, the results are perhaps more notable for the fact that the observed reliance upon NO generation for cardiac parasympathetic activation in our heart failure population was additional to the vagotonic effects of ACE inhibitors.

In conclusion, the present study provides the first demonstration of a role for NO in the control of cardiac parasympathetic function in human heart failure. The advent of novel methods of manipulation of NO pathway activity may provide a means of correcting the impairment of cardiac parasympathetic control observed in this condition, with potentially beneficial prognostic consequences.

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REFERENCES


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