Nitric Oxide Modulates Mitochondrial Respiration in Failing Human Heart

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Background—Our objective for this study was to investigate whether nitric oxide (NO) modulates tissue respiration in the failing human myocardium.

Methods and Results—Left ventricular free wall and right ventricular tissue samples were taken from 14 failing explanted human hearts at the time of transplantation. Tissue oxygen consumption was measured with a Clark-type oxygen electrode in an airtight stirred bath containing Krebs solution buffered with HEPES at 37°C (pH 7.4). Rate of decrease in oxygen concentration was expressed as a percentage of the baseline, and results of the highest dose are indicated. Bradykinin (10^{-4} mol/L, −21±5%), amlodipine (10^{-3} mol/L, −14±5%), the ACE inhibitor ramiprilat (10^{-4} mol/L, −21±2%), and the neutral endopeptidase inhibitor thiorphan (10^{-4} mol/L, −16±5%) all caused concentration-dependent decreases in tissue oxygen consumption. Responses to bradykinin (−2±6%), amlodipine (−2±4%), ramiprilat (−5±6%), and thiorphan (−4±7%) were significantly attenuated after NO synthase blockade with N-nitro-l-arginine methyl ester (10^{-4} mol/L; all P<0.05). NO-releasing compounds S-nitroso-N-acetyl-penicillamine (10^{-4} mol/L, −34±5%) and nitroglycerin (10^{-4} mol/L, −21±5%), also decreased tissue oxygen consumption in a concentration-dependent manner. However, the reduction in tissue oxygen consumption in response to S-nitroso-N-acetyl-penicillamine (−35±7%) or nitroglycerin (−16±5%) was not significantly affected by N-nitro-l-arginine methyl ester.

Conclusions—These results indicate that the modulation of oxygen consumption by both endogenous and exogenous NO is preserved in the failing human myocardium and that the inhibition of kinin degradation plays an important role in the regulation of mitochondrial respiration. (Circulation. 1999;100:1291-1297.)

Key Words: nitric oxide ■ oxygen ■ heart failure

The vast amount of ATP produced by the cardiac mitochondria is used mainly for cardiac muscle contraction. Abnormalities of mitochondria in cardiomyocytes in heart failure have been well documented in both animals^1–4 and human studies,^5–7 which provided structural and metabolic evidence of mitochondrial dysfunction. Mitochondrial DNA damage with increased mitochondrial DNA deletion in patients with heart failure has also been reported,^8,9 a defect associated with the impairment of oxidative phosphorylation.10 On the other hand, normal mitochondrial metabolism has also been documented in chronic heart failure.11,12 However, the role of nitric oxide (NO) in the control of mitochondrial metabolism is not well established. Our laboratory and others have demonstrated that attenuation of NO production increases whole-body or organ oxygen consumption.13–16 The initial observation of the interaction between NO and mitochondrial enzymes was reported in cell culture studies of macrophage-induced cytotoxicity of neoplastic cells,17,18 The activated macrophage induced reduction of electron transfer by inactivating iron-sulfur–containing complexes I and II of the respiratory chain and aconitase in the Krebs cycle. This effect was shown to be l-arginine dependent, inhibited by NO synthase blockers, and subsequently shown to be dependent on NO.19,20 These concepts are consistent with some earlier observations explaining the antimicrobial properties of nitrite on Clostridium botulinum by binding to iron-sulfur enzymes forming iron-NO complexes.21 More recent evidence has supported a physiological interaction of NO with another respiratory enzyme.22 In that study, nanomolar concentrations of NO were found to reversibly compete with oxygen for the common binding site on cytochrome c oxidase, inhibiting electron transfer to oxygen.

Endothelial dysfunction has been described in many cardiovascular diseases including heart failure, and we have previously shown that in a canine model of pacing-induced dilated cardiomyopathy, basal cardiac NO release is de-
increased. Therefore alteration of NO production in pathological conditions may alter tissue oxygen consumption. ACE inhibitors and nitrates are commonly used in the treatment of heart failure. In a recent clinical trial, amiodipine, a long-acting, dihydropyridine-derivative calcium channel blocker, unlike other calcium channel blockers, has been shown to reduce morbidity and mortality rates in patients with nonischemic dilated cardiomyopathy. Our laboratory has recently demonstrated that amiodipine but not nifedipine or diltiazem releases NO.

On the basis of these findings, our aim was to determine the role of NO in the modulation of mitochondrial respiration in the failing human myocardium with the use of agents that release NO either spontaneously or through the activation of NO synthase. We hypothesized that agents that (1) directly stimulate the release of endogenous NO production through endothelial cell surface receptor activation, (2) indirectly stimulate the release of NO through the interaction with and/or increased concentrations of local kinins, or (3) spontaneously release of NO will decrease tissue oxygen consumption in the failing human myocardium.

Methods

Preparation of Failing Human Myocardial Tissue Slices and Measurement of Tissue Oxygen Consumption

Left and right ventricular tissues were obtained from 14 failing explanted human hearts at the time of transplantation. The myocardium was cleared of the epicardium, endocardium, large arteries, fat, and fibrotic and connective tissues and cut into 30- to 50-mg slices. Myocardial tissues were then incubated in Krebs bicarbonate solution containing (mmol/L): NaCl 118, KCl 4.7, CaCl₂ 1.5, NaHCO₃ 25, KH₂PO₄ 1.2, MgSO₄ 1.1, and glucose 5.6 at 37°C, bubbled with 21%O₂/5%CO₂/74%N₂, pH 7.4 to equilibrate for at least 2 hours. At the end of the incubation period, each piece of tissue was placed in a stirred bath with 3 mL of air-saturated Krebs bicarbonate solution containing 10 mmol/L HEPES, pH 7.4. The bath was sealed with a Clark-type platinum oxygen electrode (Yellow Springs Instruments) that was connected to an oxygen monitor (model YSI 5331) to record the uptake of oxygen by the tissue. Succinate (5×10⁻⁴ mol/L, Sigma), a substrate for complex II, and sodium cyanide (10⁻⁴ mol/L, Sigma), an inhibitor of complex IV of the electron transport chain, were added after the completion of the concentration response curve to each agonist to confirm that changes in myocardial oxygen consumption were of mitochondrial sources. For instance, the 200% increase in oxygen consumption to succinate suggests that oxygen is not lacking, and the complete abolition of oxygen consumption by sodium cyanide suggests that the chief intracellular organ consuming oxygen is the mitochondria.

Effect of Bradykinin and Amlodipine on Tissue Oxygen Consumption

The effect of endogenous NO agonists on myocardial oxygen consumption was tested with the use of bradykinin (10⁻⁷ to 10⁻⁴ mol/L, Sigma) and the calcium channel antagonist amlodipine (10⁻⁷ to 10⁻³ mol/L, Pfizer), added at increasing cumulative concentrations. These agents were studied in the presence of the NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME, 10⁻⁴ mol/L, Sigma) to examine the role of NO in the modulation of tissue oxygen consumption. In addition, the effect of bradykinin-induced change in oxygen consumption was examined in the presence of the B₂-kinin receptor antagonist HOE-140 (10⁻⁵ mol/L).

Effect of ACE and Neutral Endopeptidase Inhibitors on Tissue Oxygen Consumption

The effect of endogenous NO agonists on myocardial oxygen consumption was also tested with the use of the ACE inhibitor ramiprilat (10⁻⁵ to 10⁻⁴ mol/L) and the neutral endopeptidase (NEP) inhibitor thiorphan (10⁻⁷ to 10⁻⁴ mol/L). Increasing cumulative concentrations of these agents were studied in the absence or presence of L-NAME (10⁻⁴ mol/L).

Effect of NO Donors on Tissue Oxygen Consumption

The effect of exogenous sources of NO production on myocardial oxygen consumption was tested with the use of the NO donors S-nitroso-N-acetylpenicillamine (SNAP, 10⁻⁴ to 10⁻⁴ mol/L, Sigma) and nitroglycerin (10⁻⁷ to 10⁻⁴ mol/L, Parke Davis). Increasing cumulative concentrations of these agents were studied in the presence or absence of L-NAME (10⁻⁴ mol/L).

Results

Patients

Myocardial tissues from 14 patients (7 male and 7 female; age range 2 to 64 years old) with end-stage heart failure were collected at surgery for heart transplantation. Five patients had idiopathic dilated cardiomyopathy, 6 patients had complex congenital heart disease with severe ventricular dysfunction, 2 had ischemic cardiomyopathy, and 1 patient had pulmonary hypertension. Average pulmonary capillary wedge pressure, mixed venous saturation, and cardiac index were 27±2 mm Hg, 50±8%, and 2.2±0.3 L·min⁻¹·m⁻², respectively. All patients had severe congestive heart failure classified as New York Heart Association class IV. Patients (n=13) were treated with a combination of drugs including dobutamine, milrinone, digoxin, ACE inhibitors, dopamine, antiarrhythmics, warfarin, and heparin until the time for transplantation. One patient had a left ventricular assist device implanted for 5 months before transplantation and therefore did not receive long-term medications.

Basal Oxygen Consumption in Failing Human Myocardium

Baseline myocardial oxygen consumption in failing heart (HF) was not statistically different from that observed in the...
presence of NO synthase blockade with L-NAME (10^{-4} \text{ mol}\cdot\text{L}^{-1}) \ [HF: 168 \pm 9 \text{ nmol oxygen/min per gram (n=62)} \ vs \ HF+L-NAME-treated: 144 \pm 10 \text{ nmol oxygen/min per gram (n=57)}, P=\text{NS}].

Effect of Bradykinin and Amlodipine on Tissue Oxygen Consumption
Cumulative doses of bradykinin (10^{-7} to 10^{-4} \text{ mol/L}, n=13) and amlodipine (10^{-7} to 10^{-5} \text{ mol/L}, n=11) caused concentration-dependent decreases in oxygen consumption in tissues from the failing human myocardium (bradykinin from −10 \pm 3\% to −21 \pm 5\%; amlodipine from −5 \pm 3\% to −14 \pm 5\%) (Figure 1). The responses to bradykinin (n=12) and amlodipine (n=10) were attenuated in the presence of L-NAME (bradykinin from −2 \pm 2\% to −2 \pm 6\%; amlodipine from −2 \pm 3\% to −2 \pm 4\%) (Figure 1). In addition, the reduction in tissue oxygen consumption induced by bradykinin (10^{-5} \text{ mol/L}) was abolished by HOE-140 (10^{-5} \text{ mol/L}) [HF (n=13) −15 \pm 3\% vs HF+HOE-140–treated (n=11): 2 \pm 5\%, P<0.05].

Effect of ACE and NEP Inhibitors on Tissue Oxygen Consumption
Cumulative doses of the ACE inhibitor ramiprilat (10^{-7} to 10^{-4} \text{ mol/L}, n=13) and the NEP inhibitor thiorphan (10^{-7} to 10^{-4} \text{ mol/L}, n=11) also caused concentration-dependent decreases in oxygen consumption in tissues from the failing human myocardium (ramiprilat from −6 \pm 4\% to −21 \pm 2\%; thiorphan from −3 \pm 2\% to −16 \pm 5\%) (Figure 2). The responses to ramiprilat (n=11) and thiorphan (n=10) were attenuated in the presence of L-NAME (ramiprilat from 0.2 \pm 2\% to −5 \pm 6\%; thiorphan from 5 \pm 7\% to −4 \pm 7\%) (Figure 2).

Effect of NO Donors on Tissue Oxygen Consumption
Cumulative doses of the NO-releasing agents SNAP (10^{-7} to 10^{-4} \text{ mol/L}, n=7) and nitroglycerin (10^{-6} to 10^{-3} \text{ mol/L}, n=7) caused concentration-dependent decreases in oxygen consumption in tissues from the failing human myocardium (SNAP from −5 \pm 3\% to −34 \pm 5\%; nitroglycerin from −7 \pm 5\% to −21 \pm 5\%) (Figure 3). In this instance, responses to both SNAP (n=7) and nitroglycerin (n=7) were not affected by the treatment with L-NAME (SNAP from −4 \pm 3\% to −35 \pm 7\%; nitroglycerin from −8 \pm 4\% to −16 \pm 5\%) (Figure 3).
The presence of a dense capillary network surrounding myocytes in the heart allows for a short diffusion distance of capillary endothelium-derived NO to nearby cardiac myocytes. Previous studies have demonstrated that coronary microvessels of <80 μm in diameter isolated from the failing human myocardium are able to release NO when stimulated by agonists such as bradykinin and acetylcholine.

ACE catalyzes the removal of the carboxy terminal Phe-Arg of kinins, resulting in their inactivation. Attenuating ACE activity with ACE inhibitors leads to the accumulation of endogenous kinins, thereby increasing the stimulus for NO production. ACE inhibitors release NO from microvessels from the human heart, thus the effect of ACE inhibition in the regulation of tissue oxygen consumption was directly addressed previously by us in the normal canine heart. Zhang et al showed that the ACE inhibitors captopril, enalaprilat, and ramiprilat all reduced oxygen consumption in a manner that was blocked by the NO synthase inhibitor nitro-L-arginine and the B2-kinin receptor antagonist HOE-140. Those observations support the present findings that bradykinin and ramiprilat caused significant decreases in oxygen consumption that were blocked by L-NAME. In addition to ACE, NEP also plays a role in the metabolism of kinins. The effect of inhibition of NEP with the use of phosphoramidon and thiorphan has been shown to increase cardiac NO production through a kinin-dependent mechanism. Other investigators have also confirmed the presence of functional NEP activity in endothelial cells. In patients with heart failure, the effect of NEP inhibition on cardiac function and its efficacy in the treatment of heart failure have been studied; however, there is no report on the effect of NEP inhibition on mitochondrial metabolism. In the present study, thiorphan at 10–4 mol/L caused a 16% reduction in oxygen consumption in the failing human myocardium that was entirely blocked by L-NAME. Our data are consistent with our previous findings that preventing the breakdown of endogenous kinins with either ACE or NEP inhibitors increases stimulation of NO production and decreases mitochondrial respiration. Given that the combined treatment of ACE and NEP inhibitors in experimental heart failure significantly improved cardiac function and its efficacy in the treatment of heart failure have been studied, however, there is no report on the effect of NEP inhibition on mitochondrial metabolism. In the present study, the magnitude of NO release induced by amlodipine was comparable to that caused by ACE inhibitors, and the combination of low-dose ACE and NEP inhibitors enhanced cardiac NO production, it is likely that inhibiting both ACE and NEP simultaneously results in a synergistic effect, producing an even more pronounced reduction in oxygen consumption in the failing human myocardium.

Amlodipine, in addition to its calcium channel–blocking effect, also releases NO from blood vessels. In this previous study, the magnitude of NO release induced by amlodipine was comparable to that caused by ACE inhibitors, and the effect of amlodipine was attenuated by L-NAME, HOE 140, and dichloroisocoumarin, a serine protease inhibitor that blocks the action of endogenous kinin-forming enzymes. This supports our conclusions that amlodipine decreases myocardial oxygen consumption by an NO-dependent mechanism after the activation of local kinin production. Further support for the role of kinin in this mechanism comes from the evidence that amlodipine–induced reduction of oxygen consumption was abolished in tissues taken from B2-kinin receptor knockout mice. This unique property of amlodipine is
likely to have contributed to its potential therapeutic benefit for long-term use in the treatment of chronic heart failure.24

Organic nitrates are another class of drugs most widely used in the treatment of heart failure. Nitrates induce peripheral venous, arterial, and arteriolar dilation, resulting in decreased venous return and reduced ventricular systolic and diastolic pressures. Lowering myocardial oxygen consumption is helpful in improving the imbalance between myocardial oxygen supply and demand in patients with heart failure.22 In our study, the NO donors SNAP and nitroglycerin decreased oxygen consumption in isolated human myocardium, an effect independent of the action of NO synthase. The direct inhibitory effect of nitroglycerin on mitochondrial respiration probably contributes toward its high efficacy in the treatment of heart failure. In 1951, Krantz et al43 examined the effect of nitroglycerin on mitochondrial respiration probably contributes toward its high efficacy in the treatment of heart failure. In 1951, Krantz et al43 examined the effect of nitroglycerin on myocardial oxygen consumption in rat isolated aortic tissue and found that nitroglycerin significantly decreased arterial tissue oxygen consumption. Such a decrease in arterial tissue oxygen consumption was interpreted as a result of the inhibitory action of nitroglycerin on arterial ATPase, thereby attenuating the breakdown of ATP and reducing arterial tone. However, this mechanism is unlikely to account for the effect we observed because all changes in tissue oxygen consumption were blocked by sodium cyanide, an inhibitor of complex IV of the respiratory chain. In a more recent study44 performed with the use of a methodology similar to that used by Krantz et al, nitroglycerin was reported to inhibit oxygen consumption in rat isolated myocardium. Although the mechanism of action in that study was not addressed, it is possible that oxygen consumption was inhibited through the action of NO.

In our study, baseline tissue oxygen consumption in the control human failing myocardium (168±9 nmol oxygen/min per gram) was not different from the rate after addition of an NO synthase inhibitor (144±10 nmol oxygen/min per gram). The lack of an effect by NO synthase blockade on basal oxygen consumption was consistent with our previous in vitro studies to suggest that basal NO release, in the absence of a chemical stimulus or flow, had only negligible effects on isolated tissue oxygen consumption.16,27–29 Xie et al28 demonstrated that baseline oxygen consumption in isolated failing canine myocardial tissue was 54% greater than that in normal healthy canine hearts, whereas the bradykinin-induced reduction in oxygen consumption was attenuated in failing canine hearts compared with normal hearts. These findings suggest that the impairment of NO biosynthesis during the development of heart failure contributes to the elevation of basal tissue oxygen consumption and to the reduced ability of bradykinin to decrease tissue oxygen consumption. In our study, it is not known whether basal oxygen consumption is elevated in the failing human myocardium because the appropriate control, normal human myocardial tissues, are difficult to obtain.

In our study, most patients with end-stage heart failure received long-term inotropic support with the combination of the β1-adrenoreceptor agonist dobutamine, the phosphodiesterase inhibitor milrinone, or the sodium-potassium ATPase inhibitor digoxin while awaiting transplantation. One patient had a left ventricular assist device (LVAD) for 5 months. There is evidence of morphological improvement in myocardial mitochondria from patients with heart failure after inotropic support,6 and this may add some variability to our results. Implantation of an LVAD in end-stage heart failure has been documented to decrease myocardial workload and therefore to decrease myocardial oxygen consumption.45,46 Lee et al47 investigated the effect of long-term therapy with an LVAD on mitochondrial function and found that mitochondria isolated from failing ventricles in patients with LVAD implanted significantly improves the efficiency of mitochondrial metabolism when NADH-dependent substrates were used to compare to the non-LVAD heart failure group. In addition, some patients in the current study also received ACE inhibitors in combination with the inotropic support. Long-term treatment with ACE inhibitors has been shown to improve the tightness of coupling of oxidation to phosphorylation in experimental heart failure.48 Whether long-term inotropic support or the use of an LVAD and/or ACE inhibitors in patients has contributed to the preservation of endogenous NO production on stimulation and its ability to modulate mitochondrial respiration in this study is yet to be determined.

Some data in our study are difficult to interpret because of the lack of appropriate control human tissue that is not exposed to disease or long-term medications. Despite our previous studies showing that endothelial NO synthase gene expression and cardiac NO production are reduced in experimental heart failure23,49 and production of NO from coronary microvessels is reduced in the failing compared with the nonfailing human heart,50 no clear conclusions can be drawn on whether the role of endogenous NO in the regulation of oxygen consumption is altered in these failing tissues. In addition, the severity and specific causes of heart failure have been demonstrated to affect myocardial mitochondrial electron transport and hence the rate of oxygen consumption in both experimental and human studies.1,7 It should clearly be pointed out that we are measuring oxygen consumption in noncontracting cardiac muscle, which, by definition, is low. However, we have found in vivo that the regulation of oxygen consumption by NO is maintained at increased cardiac work during exercise in conscious dogs.15 With the small patient group used in this study, it is not possible to evaluate whether the effect of NO on tissue oxygen consumption is different across failing hearts of different pathogeneses. Nevertheless, our data clearly demonstrate that endogenous NO release is in part preserved in the failing human heart to modulate mitochondrial respiration. The ability of NO donors or activation of a local kinin system by amlodipine, an ACE inhibitor, or an NEP inhibitor to release NO to lower myocardial oxygen demand suggests an additional cardioprotective mechanism of NO. This study provides the basis for the further understanding of the role of NO in the regulation of mitochondrial metabolism and suggests that NO may contribute to the beneficial effects of drugs currently used in the treatment of heart failure.

Acknowledgments

This study was supported by grants PO-1-HL-43023, RO-1-HL-50142, and RO-1-HL-53053 from the National Heart, Lung, and
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Circulation. 1999;100:1291-1297
doi: 10.1161/01.CIR.100.12.1291

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