Potential role of eNOS in the therapeutic control of myocardial oxygen consumption by ACE inhibitors and amlodipine

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Abstract

Objectives: Our aim was to investigate the potential therapeutic role of endothelial nitric oxide synthase (eNOS) in the modulation of cardiac O\textsubscript{2} consumption induced by the angiotensin converting enzyme (ACE) inhibitor ramiprilat and amlodipine. Methods: Three different groups of mice were used; wild type, wild type in the presence of N-nitro-L-arginine methyl ester (\textsuperscript{1-NAME}, \(10^{-4}\) mol/l) or genetically altered mice lacking the eNOS gene (eNOS \(-/-\)). Myocardial O\textsubscript{2} consumption was measured using a Clark-type O\textsubscript{2} electrode in an air-tight stirred bath. Concentration-response curves to ramiprilat (RAM), amlodipine (AMLO), diltiazem (DIL), carbachol (CCL), substance P (SP) and S-nitroso-N-acetyl-penicillamine (SNAP) were performed. The rate of decrease in O\textsubscript{2} concentration was expressed as a percentage of the baseline. Results: Baseline O\textsubscript{2} consumption was not different between the three groups of mice. In tissues from wild type mice, RAM (\(10^{-5}\) mol/l), AMLO (\(10^{-5}\) mol/l), DIL (\(10^{-4}\) mol/l), CCL (\(10^{-4}\) mol/l), SP (\(10^{-7}\) mol/l) and SNAP (\(10^{-4}\) mol/l) reduced myocardial O\textsubscript{2} consumption by \(-32\pm4\), \(-27\pm10\), \(-20\pm6\), \(-25\pm2\), \(-22\pm4\) and \(-42\pm4\%), respectively. The responses to RAM, AMLO, CCL and SP were absent in tissues taken from eNOS \(-/-\) mice (\(-7.1\pm4.3\), \(-5.0\pm6.0\), \(-5.2\pm5.1\) and \(-0.4\pm0.2\%) respectively). In addition, \textsuperscript{1-NAME} significantly \((P<0.05)\) inhibited the reduction in O\textsubscript{2} consumption by RAM (\(-9.8\pm4.4\%), AMLO (\(-1.0\pm6.0\%\)), CCL (\(-8.8\pm3.7\%\)) and SP (\(-6.6\pm4.9\%\)) in cardiac tissues from wild type mice. In contrast, NO-independent responses to the calcium channel antagonist, DIL, and responses to the NO donor, SNAP, were not affected in cardiac tissues taken from eNOS \(-/-\) (DIL: \(-20\pm4\%\); SNAP: \(-46\pm6\%\)) or \textsuperscript{1-NAME}-treated (DIL: \(-17\pm2\%\); SNAP: \(-33\pm5\%\)) mice. Conclusions: These results suggest that endogenous endothelial NO synthase derived NO serves an important role in the regulation of myocardial O\textsubscript{2} consumption. This action may contribute to the therapeutic action of ACE inhibitors and amlodipine. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Nitric oxide; Oxygen consumption

1. Introduction

Since the discovery of endothelium-derived relaxing factor in 1980 [1] and its subsequent identification as nitric oxide (NO) in the late 1980s, NO is best known for its role in the local control of vascular tone and in hemostasis [2]. However, the role of NO in the regulation of metabolism is becoming clear. The modulation of cellular respiration by NO is one of the mechanisms that may control organ or tissue oxygen extraction relative to oxygen demand. For instance, Shen et al. [3] showed that blockade of NO synthesis in vivo caused a significant increase in total O\textsubscript{2} extraction and O\textsubscript{2} consumption across the whole body, accompanied by a rise in body temperature. In that study, the increase in O\textsubscript{2} consumption was not attributed to the NO synthase blockade-induced vasoconstriction and a decrease in cardiac output but rather to the inhibitory effect of NO on tissue metabolism [3]. Bernstein et al. [4] found that NO is involved in the control of myocardial O\textsubscript{2} consumption during exercise at any level of cardiac work. More recently, Laycock et al. [5] described the importance of basal NO in the control of renal O\textsubscript{2} consumption and in
maintaining the efficiency of sodium transport in the kidney.

NO released from activated macrophages has previously been shown to interact with mitochondrial enzyme complexes I–IV, inhibiting respiration in a variety of cell types and in isolated mitochondrial preparations [6–10]. There is growing evidence to suggest that the physiological control of mitochondrial respiration by NO is primarily a reversible interaction at cytochrome c oxidase, a common binding site for oxygen at complex IV of the electron transport chain [10–14]. We have previously hypothesized that capillary endothelium is the major source of NO responsible for the regulation of parenchymal cell O2 consumption. This proposal is strongly supported by Clementi et al. [14], who demonstrated that basal and stimulated release of NO from the vascular endothelium can modulate endothelial cell respiration at cytochrome c oxidase. The precise role of NO derived from the constitutive endothelial NO synthase (eNOS) in the regulation of tissue respiration in our previous in vitro studies examining tissue O2 consumption in different vascular beds and in animal species including humans is yet to be determined [15–19]. Therefore, in this study, we investigated therapeutic agents that release NO, such as the angiotensin-converting enzyme (ACE) inhibitor, ramiprilat [20], the calcium channel antagonist, amlodipine [21], and agents classically known to release NO, carbachol and substance P [22], in the control of tissue respiration using tissues taken from mouse hearts deficient in the eNOS gene. This study will lead to a better understanding of the therapeutic mechanism of action of ramiprilat and amlodipine in the control of tissue respiration.

2. Materials and methods

2.1. Animals studied

Heterozygous eNOS +/− mice, originally developed by Shesely et al. [23] were interbred to generate eNOS +/+ , heterozygotes +/− , and homozygous mutant −/− mice. All eNOS mice were genotyped by Southern analysis of DNA as described previously [23]. In the wild type group, eNOS +/+ and healthy strain C57BL/6x129 mice obtained from the Jackson Laboratories were used as controls for the eNOS −/− mice. All protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the current National Institutes of Health and American Physiological Society guidelines for the use and care of laboratory animals.

2.2. Preparation of murine cardiac tissue slices and measurement of tissue O2 consumption

Mice of either sex (16.3±0.7 weeks old) were anesthetized with pentobarbital sodium (65 mg/kg i.p.), and hearts were removed immediately. The atria, large coronary arteries, right ventricle, connective tissues and fat were discarded. The left ventricle was bisected such that each piece of muscle contained the septum, free wall and apex, weighing approximately 25–40 mg. 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 h. 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 using a Clark-type platinum O2 electrode (Yellow Springs Instrument) that was connected to an O2 monitor (model YSI 5331); hence, the uptake of O2 by the tissue was recorded. Succinate (5x10−4 mol/l, Sigma), a substrate for complex II, and sodium cyanide (10−4 mol/l, Sigma) were added at the completion of the concentration–response curve to each agonist. The 100% increase in O2 consumption in the presence of succinate suggests that O2 is not lacking in the bath, and the abolition of O2 consumption after addition of sodium cyanide confirms that changes in myocardial O2 consumption were of mitochondrial sources.

2.3. Effect of carbachol and substance P on tissue O2 consumption

In wild type mice, the effects of increasing cumulative concentrations of the muscarinic agonist carbachol (10−7–10−4 mol/l, Sigma) and the neurokinin receptor agonist substance P (10−10–10−7 mol/l) were examined in the absence or presence of pretreatment with the NO synthase inhibitor, N-nitro-l-arginine methyl ester (l-NAME, 10−4 mol/l, Sigma) to confirm the role of NO in the modulation of tissue O2 consumption. Responses to both carbachol and substance P were examined in tissues taken from eNOS −/− mouse hearts, to investigate the role of eNOS in the modulation of tissue O2 consumption.

2.4. Effect of the ACE inhibitor ramiprilat on tissue O2 consumption

In wild type mouse hearts, the effects of increasing cumulative concentrations of the ACE inhibitor ramiprilat (10−7–10−4 mol/l) on tissue O2 consumption were examined in the absence or presence of pretreatment with l-NAME. Responses to ramiprilat were examined in eNOS −/− mouse hearts.

2.5. Effect of the calcium channel blockers amlodipine and diltiazem on tissue O2 consumption

In wild type mouse hearts, the effects of increasing cumulative concentrations of the calcium channel blocker
that releases NO, amlodipine (10\(^{-7}\)–10\(^{-5}\) mol/l; Pfizer, Groton, CT) and a non-NO releasing calcium channel blocker, diltiazem (10\(^{-7}\)–10\(^{-4}\) mol/l, Sigma), on tissue O\(_2\) consumption were examined in the absence or presence of \(\cdot\text{Name}\). Responses to both amlodipine and diltiazem were examined in eNOS \(-/-\) mouse hearts.

### 2.6. Effect of NO donor S-nitroso-N-acetylpenicillamine on tissue O\(_2\) consumption

The effects of increasing cumulative concentrations of SNAP (10\(^{-7}\)–10\(^{-4}\) mol/l, Sigma) on tissue O\(_2\) consumption were examined in the absence or presence of \(\cdot\text{Name}\) in wild type mouse hearts. Responses to SNAP were examined in eNOS \(-/-\) mouse hearts.

### 2.7. Statistical analysis

All data are presented as mean±S.E.M. The rate of decrease in the bath PO\(_2\) was used as an index of tissue respiration, assuming an initial O\(_2\) concentration of 224 nmol/ml [24] and the results were expressed as nanomoles of O\(_2\) consumed per minute per gram of tissue. The effect of each agonist on tissue O\(_2\) uptake is expressed as a percent change in baseline O\(_2\) consumption. Statistical analysis on baseline O\(_2\) consumption was performed using one-way ANOVA, and the changes in O\(_2\) consumption induced by various agonists were analyzed using two-way ANOVA followed by multiple comparisons between different treatment groups using Tukey’s test. Statistical significance was accepted at \(P<0.05\).

### 3. Results

#### 3.1. Baseline O\(_2\) consumption in eNOS \(+/+\), C57BL/6\(\times\)129 and eNOS \(-/-\) mouse hearts

Baseline O\(_2\) consumption in the eNOS \(+/+\) mouse hearts was not different from that in the C57BL/6\(\times\)129 mouse hearts (eNOS \(+/+\); 202±25 nmol O\(_2\)/min/g, \(n=12\) vs. C57BL/6\(\times\)129: 208±19 nmol/min/g, \(n=27\)). Therefore, results obtained from both the eNOS \(+/+\) and the C57BL/6\(\times\)129 mouse hearts were combined as a wild type group control for eNOS \(-/-\) mouse hearts. There was no significant difference in baseline O\(_2\) consumption between the three different groups of mouse hearts; wild type (206±15 nmol O\(_2\)/min/g, \(n=39\)), normal pretreated with \(\cdot\text{Name}\) (236±13 nmol O\(_2\)/min/g, \(n=39\)) and eNOS \(-/-\) (238±25 nmol O\(_2\)/min/g, \(n=34\)).

#### 3.2. Effect of carbachol and substance P on tissue O\(_2\) consumption

Cumulative concentrations of carbachol (10\(^{-7}\)–10\(^{-4}\) mol/l) in both the eNOS \(+/+\) and C57BL/6\(\times\)129 mouse hearts caused concentration-dependent decreases in O\(_2\) consumption. For instance, in the eNOS \(+/+\) mouse hearts; carbachol decreased O\(_2\) consumption by \(-10\pm2\), \(-22\pm2\), \(-24\pm5\) and \(-28\pm4\%,\) respectively \((n=3)\), and in C57BL/6\(\times\)129 mouse hearts, carbachol decreased O\(_2\) consumption by \(-11\pm4\), \(-19\pm3\), \(-20\pm3\) and \(-25\pm2\%,\) respectively \((n=8)\). Therefore, we have combined the results from the two groups as wild type control for eNOS \(-/-\) mouse hearts. Both carbachol and substance P caused a reduction in O\(_2\) consumption (Fig. 1). These responses were significantly attenuated after pretreatment with \(\cdot\text{Name}\). Importantly, there was no effect in tissues taken from eNOS \(-/-\) mouse hearts (Fig. 1).

#### 3.3. Effect of the ACE inhibitor ramiprilat on tissue O\(_2\) consumption

Cumulative concentrations of ramiprilat (10\(^{-7}\)–10\(^{-4}\) mol/l) caused concentration-dependent decreases in O\(_2\) consumption. For instance, in the eNOS \(+/+\) mouse hearts; ramiprilat decreased O\(_2\) consumption by \(-10\pm2\), \(-22\pm2\), \(-24\pm5\) and \(-28\pm4\%,\) respectively \((n=3)\), and in C57BL/6\(\times\)129 mouse hearts, ramiprilat decreased O\(_2\) consumption by \(-11\pm4\), \(-19\pm3\), \(-20\pm3\) and \(-25\pm2\%,\) respectively \((n=8)\). Therefore, we have combined the results from the two groups as wild type control for eNOS \(-/-\) mouse hearts. Both carbachol and substance P caused a reduction in O\(_2\) consumption (Fig. 1). These responses were significantly attenuated after pretreatment with \(\cdot\text{Name}\). Importantly, there was no effect in tissues taken from eNOS \(-/-\) mouse hearts (Fig. 1).
Fig. 2. Effect of the ACE inhibitor ramiprilat (10^{-7}–10^{-4} mol/l) on tissue \( O_2 \) consumption in wild type (WT), WT in the presence of \( L \)-NAME and alone in eNOS-deficient (eNOS\(-/-\)) mouse hearts. \# \( P < 0.05 \) vs. respective concentrations in WT and \* \( P < 0.05 \) vs. concentration response curve to WT.

Fig. 3. Effect of the calcium channel antagonists (a) amlodipine (10^{-7}–10^{-5} mol/l) and (b) diltiazem (10^{-7}–10^{-4} mol/l) on tissue \( O_2 \) consumption in wild type (WT), WT in the presence of \( L \)-NAME and in eNOS-deficient (eNOS\(-/-\)) mouse hearts. \# \( P < 0.05 \) vs. respective concentrations in WT and \* \( P < 0.05 \) vs. concentration response curve to WT.

Fig. 4. Effect of the NO donor SNAP (10^{-7}–10^{-4} mol/l) on tissue \( O_2 \) consumption in wild type (WT), WT in the presence of \( L \)-NAME and in eNOS-deficient (eNOS\(-/-\)) mouse hearts. There is no significant difference between all treatment groups.

3.4. Effects of the calcium channel blockers amlodipine and diltiazem on tissue \( O_2 \) consumption

Cumulative concentrations of amlodipine (10^{-7}–10^{-5} mol/l) and diltiazem (10^{-7}–10^{-4} mol/l) caused concentration-dependent decreases in \( O_2 \) consumption in wild type mouse hearts (Fig. 3). Responses to amlodipine but not diltiazem were significantly attenuated after pretreatment with \( L \)-NAME. Amlodipine had no effect on tissues taken from eNOS\(-/-\) mouse hearts. In contrast, diltiazem still reduced \( O_2 \) consumption in eNOS\(-/-\) mouse hearts (Fig. 3).

3.5. Effect of the NO donor S-nitroso-N-acetyl-penicillamine on tissue \( O_2 \) consumption

Cumulative concentrations of SNAP (10^{-7}–10^{-4} mol/l) caused concentration-dependent decreases in \( O_2 \) consumption in wild type mouse hearts (Fig. 4). Responses to SNAP were not affected by \( L \)-NAME or in tissues taken from eNOS\(-/-\) mouse hearts (Fig. 4).

4. Discussion

In this study, we demonstrated that eNOS-derived NO is important in the control of cardiac \( O_2 \) consumption. The most significant findings are that the ACE inhibitor, ramiprilat-, and the calcium channel antagonist, amlodipine-induced reductions in cardiac \( O_2 \) consumption were both markedly attenuated in eNOS-deficient mice and...
in wild type mice pretreated with L-NAME. These data are consistent with our earlier studies indicating that both ACE inhibitors and amlodipine release NO from isolated coronary microvessels from the failing human heart [25,26], and the ability of ramiprilat and amlodipine to modulate tissue O₂ consumption in the failing human myocardium is NO-dependent [19]. Those studies demonstrated that despite the development of congestive heart failure, ramiprilat and amlodipine are still able to release NO and modulate mitochondrial respiration in the failing human myocardium. The present study demonstrates the obligatory role of eNOS in the regulation of O₂ consumption and contributes to a further understanding of the therapeutic actions of ramiprilat and amlodipine through the control of tissue respiration. In contrast, the endothelium-independent NO donor SNAP and the calcium channel antagonist that does not release NO, diltiazem [21], both significantly decreased O₂ consumption in wild type mouse hearts. SNAP- and diltiazem-induced reductions in O₂ consumption were not significantly decreased in hearts taken from eNOS−/− or wild type mice pretreated with L-NAME, indicating that tissues taken from eNOS-deficient mouse hearts can still regulate O₂ consumption through either NO-dependent or independent mechanisms. In addition, we found that carbachol and substance P significantly decreased cardiac O₂ consumption in wild type mouse hearts. However, these responses were attenuated in eNOS−/− mouse hearts and after treatment with L-NAME in wild type mice, indicating the control of tissue respiration by carbachol and substance P is both eNOS- and NO-dependent.

The ACE inhibitor ramiprilat is traditionally known for its action in preventing the formation of the potent vasoconstrictor angiotensin II and in inhibiting the degradation of vasodilator kinins. Evidence reported previously by our laboratory as well as in the present study suggests that the mechanism of action of ramiprilat in the control of tissue O₂ consumption is most likely due to the production of NO. The ability of ramiprilat to release NO is blocked by L-NAME, the B₂ kinin receptor antagonist HOE 140, a serine protease inhibitor dichloroisoumarin, and a kinin antibody [20]. In addition ramiprilat did not induce a reduction in O₂ consumption in tissues obtained from mouse hearts deficient in B₂ kinin receptors [27]. Those findings support the presence of an endogenous source of kinins in the coronary vascular bed, and the hypothesis that kinins contribute to the release of NO from capillary endothelial NOS to control tissue respiration. The vasodilation in response to bradykinin is due to release of not only NO but also prostacyclin (PGI₂) from endothelial cells, which is also mediated through the activation of the B₂ kinin receptor [28,29]. However, the effect of ramiprilat in this study is not likely to be due to the action of PGI₂ since we have previously demonstrated that administration of PGI₂ (10⁻¹⁰–10⁻⁶ mol/l) had no effect on skeletal muscle O₂ consumption in normal dogs [16]. Furthermore, there is evidence to suggest that the angiotensin-converting enzyme potentiation of bradykinin responses is due to a direct effect on B₂ kinin receptors [29,30]. More recently, Benzing et al. [31] found that ramiprilat inhibits the bradykinin-stimulated translocation of B₂ kinin receptors to caveolin-rich membranes, which leads to desensitization and thereby allows reactivation of B₂ kinin receptors, an event that is independent of ACE enzyme inhibition. The ramiprilat-induced decrease in O₂ consumption in both canine and human myocardium is attenuated in the presence of dichloroisoumarin, suggesting that local kinin synthesis is important at a step prior to the activation of B₂ kinin receptors [27,32]. Recent clinical studies have demonstrated that ACE inhibitors improve endothelial dysfunction in patients with coronary artery disease and congestive heart failure by increasing the availability of NO [33–35]. However, none of these studies examined the effect of ACE inhibitors on oxygen consumption. An earlier study reported that when the ACE inhibitor enalaprilat was given intracoronary to humans with heart failure, cardiac work and myocardial oxygen consumption were unaffected, despite a clear reduction in coronary vascular resistance [36], suggesting that the effects of ACE inhibitors may still very well be NO-independent, although the role of NO was not investigated in that study.

Amlodipine and diltiazem belong to the dihydropyridine and benzothiazepine families of calcium channel antagonists, respectively. They are both known to inhibit calcium entry through the L-type calcium channel. Amlodipine and diltiazem both induced concentration-dependent decreases in O₂ consumption in wild type mouse hearts. However, the effect of amlodipine, but not that of diltiazem, was attenuated in hearts without functional eNOS or in wild type mouse hearts pretreated with L-NAME. These data suggest that the mechanism of action of amlodipine is at least partially eNOS-dependent. These data support our previous studies indicating that amlodipine but not diltiazem or nifedipine releases NO in isolated canine coronary microvessels [21]. This mechanism of action of amlodipine is L-NAME-sensitive, involves an endogenous source of kinins and the activation of B₂ kinin receptor. In contrast, the inhibition by diltiazem of tissue respiration in wild type and eNOS−/− mouse hearts suggests that diltiazem can regulate cardiac O₂ consumption through a NO-independent pathway. Rather, diltiazem decreases myocardial O₂ consumption by inhibiting calcium entry, thereby preventing contraction that could influence oxidative phosphorylation-mediated control of mitochondrial respiration through a change in energy consumption. We have previously found that decomposition of rapid-pacing-induced heart failure in conscious dogs reduces eNOS gene expression and protein in aortic endothelial cells and is also associated with a fall in NO production across the heart [37,38]. Therefore, we speculate that the additional NO-releasing effect of amlodipine may contribute towards the therapeutic action of amlodipine in the treatment of
patients with congestive heart failure of non-ischemic etiology [39].

In addition, we tested different agonists that are known to stimulate the release of endogenous NO in the control of oxygen consumption in wild type mouse hearts and hearts deficient in eNOS. Both carbachol and substance P induced reductions in tissue O2 consumption require the presence of functional eNOS in the release of endogenous NO. Our study supports our recent findings suggesting that the absence of both eNOS alleles results in the loss of control of O2 consumption in mouse hearts when stimulated by bradykinin [17]. In addition, our data strongly suggest that both the B2 kinin receptor and eNOS-derived NO stimulated through other endothelial receptors, such as muscarinic-2 and neurokinin-1 receptors, are important in the regulation of tissue O2 consumption. Carbachol- and substance-P-induced reductions in O2 consumption are probably due to the activation of muscarinic receptors [16] and predominantly neurokinin-1 receptors [40], respectively. These responses were abolished in eNOS-/− mouse hearts, indicating an eNOS-derived, NO-dependent mechanism in the control of tissue respiration. Furthermore, this study and our previous studies indicate that the role of inducible NOS or neuronal NOS is not likely to be an important physiological regulator of tissue respiration in the heart [16,17]. We have previously demonstrated that carbachol at the same concentration used in the present study (10−5 mol/l) releases NO from isolated coronary microvessels and this production of NO by carbachol can be blocked by L-NAME [20]. Recently, Tsutsui et al. [41] demonstrated that the expression of recombinant endothelial NOS gene in adventitial fibroblasts restores the production of endogenous NO induced by bradykinin and substance P, causing relaxations in isolated cerebral arteries without endothelium. These responses were blocked in the presence of L-NAME [41]. All these findings further support the role of endothelial NOS in the mechanism of action of carbachol and substance P in the release of endogenous NO.

The major limitations in this study are the difficulty in obtaining eNOS+/− mice; we have therefore combined the eNOS+/− mice and the commercially available C57BL/6×129 mice as our wild type group. We have previously used the C57BL/6×129 strain as normal control in vitro to examine cardiac tissue O2 consumption [17]. We have found that there is no significant difference between the eNOS+/− strain and the C57BL/6×129 strain in baseline cardiac O2 consumption or in the ability of their respective cardiac tissues to respond to carbachol. Despite the evidence supporting the role of endothelial-cell-derived NO in the control of tissue O2 consumption, this study has yet to address the origin of eNOS-derived NO, which could be released from any cell type, including endothelial cells [14], myocytes [42,43] or even mitochondria [44–47]. For instance, the short distance for NO diffusion between capillary endothelial cells and myocytes indicates that NO is readily available to modulate respiration of the surrounding myocytes. In support of this proposal, Clementi et al. [14] clearly demonstrated that endogenous NO generated by either bradykinin or ATP in porcine thoracic aortic endothelial cells can decrease endothelial cell respiration. In that study, bradykinin- and ATP-induced inhibition of endothelial cell O2 consumption was dependent on calcium influx from the extracellular space and the level of oxygen at cytochrome c oxidase. Although this study did not demonstrate that eNOS is physiologically and patho-physiologically important in the regulation of myocardial O2 consumption, it showed that eNOS is the major contributor to NO generation in the myocardium. If NO regulation of myocardial O2 consumption is of major physiological importance, then eNOS is the important contributor.

There were some differences in the dose–response curve to agonists in this study compared to a previous study of ours [27]. This difference may simply be due to the number of mitochondria that would be genetically determined or to the sensitivity of the receptors to the actions of amlodipine or ramiprilat in the two animal groups. Despite these variations, we have shown in both studies that the responses to ramiprilat and amlodipine were significantly attenuated after administration of L-NAME and were therefore NO-dependent.

In conclusion, this study demonstrates that eNOS-derived NO is an essential modulator of cardiac O2 consumption. This study also provides a further understanding of the mechanism of the beneficial effect of ACE inhibitors and amlodipine in the treatment of congestive heart failure. In other disease conditions where endothelial injury is common, the loss of endogenous eNOS-derived NO and its control of organ or tissue respiration may contribute towards the development of various pathologies.

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