Role of Nitric Oxide in the Control of Cardiac Oxygen Consumption in B2-Kinin Receptor Knockout Mice


Abstract—The aim of this study was to determine whether bradykinin, the angiotensin-converting enzyme inhibitor ramiprilat, and the calcium-channel antagonist amlodipine reduce myocardial oxygen consumption (MV\(\dot{O}_2\)) via a B2-kinin receptor/nitric oxide–dependent mechanism. Left ventricular free wall and septum were isolated from normal and B2-kinin receptor knockout (B2 \(-/-\)) mice. Myocardial tissue oxygen consumption was measured in an airtight chamber with a Clark-type oxygen electrode. Baseline MV\(\dot{O}_2\) was not significantly different between normal (239±13 nmol of O\(_2\) \cdot min\(^{-1}\) \cdot g\(^{-1}\)) and B2 \(-/-\) (263±24 nmol of O\(_2\) \cdot min\(^{-1}\) \cdot g\(^{-1}\)) mice. S-nitroso-N-acetyl-penicillamine (10\(^{-7}\) to 10\(^{-4}\) mol/L) reduced oxygen consumption in a concentration-dependent manner in both normal (maximum, 36±3%) and B2 \(-/-\) mice (28±3%). This was also true for the endothelium-dependent vasodilator substance P (10\(^{-10}\) to 10\(^{-7}\) mol/L; 22±7% in normal mice and 20±4% in B2 \(-/-\) mice). Bradykinin (10\(^{-7}\) to 10\(^{-4}\) mol/L), ramiprilat (10\(^{-7}\) to 10\(^{-4}\) mol/L), and amlodipine (10\(^{-7}\) to 10\(^{-3}\) mol/L) all caused concentration-dependent decreases in MV\(\dot{O}_2\) in normal mice. At the highest concentration, tissue O\(_2\) consumption was decreased by 18±3%, 20±5%, and 28±3%, respectively. The reduction in MV\(\dot{O}_2\) to all 3 drugs was attenuated in the presence of NG-nitro-L-arginine-methyl ester. However, in the B2 \(-/-\) mice, bradykinin, ramiprilat, and amlodipine had virtually no effect on MV\(\dot{O}_2\). Therefore, nitric oxide, through a bradykinin-receptor–dependent mechanism, regulates cardiac oxygen consumption. This physiological mechanism is absent in B2 \(-/-\) mice and may be evidence of an important therapeutic mechanism of action of angiotensin-converting enzyme inhibitors and amlodipine. (Hypertension. 1999;34:563-567.)

Key Words: heart \textbullet} oxygen \textbullet} angiotensin-converting enzyme inhibitors \textbullet} amlodipine

Nitric oxide synthase (NOS) metabolizes L-arginine into nitric oxide (NO) and citrulline. NOS is a Ca\(^{2+}\)/calmodulin-dependent enzyme present in endothelial cells that is inhibited by analogs of L-arginine such as N\(^{G}\)-nitro-L-arginine-methyl ester (L-NAME). NO is involved in numerous physiological mechanisms, including the inhibition of platelet aggregation and neuronal communication and the regulation of vascular tone. We and others have shown that NO can modulate mitochondrial respiration in vivo and in vitro. NO attenuates mitochondrial respiration by inhibiting complexes I and II of the electron transport chain and by interactions with cytochrome oxidase.

Bradykinin, an endogenous vasodilator, activates B2-kinin receptors, which are primarily on endothelial cells to augment the release of NO. The angiotensin-converting enzyme (ACE) converts bradykinin into an inactive form; hence, ACE inhibitors, such as ramiprilat, are vasodilators, inhibit the inactivation of bradykinin, and augment the effects of bradykinin on NO release.

Recent studies using the calcium-channel antagonist amlodipine, a dihydropyridine, have shown improvement in myocardial function and mortality and mortality of patients with severe, chronic, nonischemic heart failure. We have also shown that amlodipine decreases oxygen consumption in the normal and failing heart through a bradykinin-dependent mechanism subsequent to the release of NO in coronary microvessels. In the present study, we further elucidated the role of the B2-kinin receptor in the control of cardiac O\(_2\) consumption and its potential therapeutic mechanisms (ie, we determined if ACE inhibitors and amlodipine work through a kinin-dependent mechanism) by regulating oxygen consumption in hearts from mice deficient in the B2-kinin receptor.

Methods

The use of mice was approved by the Institutional Animal Care and Use Committee of New York Medical College, and it conformed to the Guiding Principals for the Use and Care of Laboratory Animals, published by the National Institutes of Health. Female B2 \(-/-\) mice were acquired from the Henry Ford Hospital in Detroit, Mich. Female normal mice (+/+ ) were purchased from Jackson Laboratories (Bar Harbor, Me). We used all these methods previously.

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Experimental Preparations and Measurement of 
O\(_2\) Consumption

All animals were euthanized by cervical dislocation after anesthesia. The heart was removed immediately, and the left ventricle was bisected to give equal parts of left ventricular free wall and septum in each piece of tissue. Myocardial tissues (20 to 40 mg) were then incubated in Krebs bicarbonate solution containing (in mmol/L): NaCl 118, KCl 4.7, CaCl\(_2\) 1.5, NaHCO\(_3\) 25, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.1, and glucose 5.6 at 37°C; they were then bubbled with a solution of 21% O\(_2\)/5% CO\(_2\)/74% N\(_2\) (pH 7.4) to equilibrate for 2 hours. After the incubation period, oxygen consumption was measured polarographically with a YSI 5300 Biological Oxygen Monitor with Clark-type oxygen electrodes (Yellow Springs Instrument Co.). Tissues were placed in a stirred bath containing 2.5 mL of Krebs buffer solution with 10 mmol/L HEPES at 37°C (pH 7.4), and the tissue bath was sealed with the oxygen electrode; hence, the rate of oxygen consumed by the tissue was recorded on a strip chart. Concentration-response curves for the effect of various drugs on cardiac oxygen uptake were examined. Only a single drug was studied in each tissue slice, and the duration for each concentration of the agonist was 5 minutes. Succinate (10\(^{-3}\) mol/L) and then sodium cyanide (10\(^{-2}\) mol/L) were administered at the end of each experiment to ensure changes in oxygen consumption originated from mitochondria. The following experiments were performed in several mouse hearts before and after the administration of L-NAME and in hearts from B\(_2\)\(-/-\) mice.

**Bradykinin and Substance P**

Bradykinin at concentrations of 10\(^{-7}\) to 10\(^{-4}\) mol/L and substance P at concentrations of 10\(^{-8}\) to 10\(^{-3}\) mol/L were added in a cumulative concentration-dependent manner. Bradykinin and substance P were used to measure the effects of the stimulation of endogenous NO production on tissue O\(_2\) uptake. The response to these drugs was examined after preincubation with L-NAME (10\(^{-3}\) mol/L) to determine the role of NO in the regulation of MV\(\Delta\)O\(_2\). NO Donor

S-nitroso-N-acetylpenicillamine (SNAP), at concentrations of 10\(^{-3}\) to 10\(^{-4}\) mol/L, was added in a cumulative concentration-dependent manner to assess the effects of exogenous NO on cardiac O\(_2\) uptake. The response to SNAP was examined after preincubation with L-NAME (10\(^{-3}\) mol/L).

**Ramiprilat and Amlodipine**

The ACE inhibitor ramiprilat (10\(^{-3}\) to 10\(^{-4}\) mol/L) and the calcium-channel antagonist amlodipine (10\(^{-3}\) to 10\(^{-4}\) mol/L) were added in a cumulative concentration-dependent manner to assess the potential role of the B\(_2\)-kinin receptor as a mediator of cardiac O\(_2\) uptake. The response to these drugs was examined after preincubation with L-NAME (10\(^{-4}\) mol/L).

**Drugs**

SNAP, bradykinin, substance P, L-NAME, and sodium cyanide were purchased from Sigma. The ramiprilat was a gift from Hoechst Marion Roussel (New Brunswick, NJ), and the amlodipine was a gift from Pfizer (Groton, Conn).

**Statistical Analysis**

All data are expressed as mean±SEM. The rate of decrease in baseline tissue O\(_2\) consumption was performed using ANOVA, and the changes in O\(_2\) consumption caused by various drug treatments were analyzed using 2-way ANOVA followed by multiple comparisons between different treatment groups using the Tukey test. Statistical significance was achieved at P<0.05.

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**Results**

**Baseline Cardiac O\(_2\) Consumption in Normal and B\(_2\)\(-/-\) Mice**

Baseline MV\(\Delta\)O\(_2\) was not different between normal and B\(_2\)\(-/-\) mice (normal, 239±13 nmol of O\(_2\) \cdot min\(^{-1}\) \cdot g\(^{-1}\); n=52, versus B\(_2\)\(-/-\) 263±24 nmol of O\(_2\) \cdot min\(^{-1}\) \cdot g\(^{-1}\); n=40, respectively, P>0.05). Inhibition of NOS with L-NAME had no effect on baseline tissue O\(_2\) consumption in either normal (L-NAME-treated, 206±14 nmol of O\(_2\) \cdot min\(^{-1}\) \cdot g\(^{-1}\); n=50, P>0.05 versus normal) or B\(_2\)\(-/-\) mouse hearts (L-NAME-treated, 203±29 nmol of O\(_2\) \cdot min\(^{-1}\) \cdot g\(^{-1}\); n=28, P>0.05 versus B\(_2\)\(-/-\) alone).

**Bradykinin and Substance P**

Cumulative concentrations of bradykinin (10\(^{-7}\) to 10\(^{-4}\) mol/L) in tissues taken from normal mouse hearts caused concentration-dependent decreases in MV\(\Delta\)O\(_2\) (Figure 1A). In contrast, in myocardial tissues taken from the B\(_2\)\(-/-\) mice or from the normal +/+ mice treated with L-NAME, bradykinin caused virtually no change in MV\(\Delta\)O\(_2\) (Figure 1A). Cumulative concentrations of substance P (10\(^{-10}\) to 10\(^{-7}\) mol/L) in tissues taken from normal mice also caused concentration-dependent decreases in MV\(\Delta\)O\(_2\) with a maximum reduction of 22±7% at 10\(^{-7}\) mol/L (Figure 1B). Responses to substance P were attenuated in the presence of L-NAME (10\(^{-7}\) mol/L: −10±5%). Substance P also caused concentration-dependent
decreases in MV $\dot{O}_2$ in B$_2$ mice that were attenuated in the presence of L-NAME (Figure 1B).

**Amlodipine and Ramiprilat**

In normal mouse hearts, the ACE inhibitor ramiprilat and the calcium channel antagonist amlodipine caused concentration-dependent decreases in MV$\dot{O}_2$ (Figure 2). Responses to both ramiprilat (at $10^{-5}$ and $10^{-4}$ mol/L, control: $-23\pm4\%$ and $-20\pm5\%$ versus L-NAME: $-1.1\pm6\%$ and $-8\pm6\%; P<0.05$) and amlodipine (at $10^{-6}$ and $10^{-5}$ mol/L, control: $-26\pm3\%$ and $-28\pm3\%$ versus L-NAME: $-16\pm3\%$ and $-18\pm3\%; P<0.05$) were attenuated in the presence of L-NAME. In addition, such ramiprilat- or amlodipine-induced reductions in MV$\dot{O}_2$ were not observed in cardiac tissues taken from B$_2$ mice (Figure 2).

**NO Donor SNAP**

In both the normal and B$_2$ mice hearts, the NO donor SNAP caused concentration-dependent decreases in MV$\dot{O}_2$ (Figure 3). SNAP ($10^{-4}$ mol/L) reduced $O_2$ consumption by $36\pm3\%$ in the normal mice and by $28\pm3\%$ in the B$_2$ mice. These responses were not affected by L-NAME (normal mice, $-32\pm4\%;$ B$_2$ mice, $-35\pm5\%).$

**Discussion**

The major findings of this study are as follows: (1) bradykinin and substance P can regulate cardiac oxygen consumption in the mouse heart, and this is, at least in part, NO-dependent; (2) the actions of bradykinin are mediated by the B$_2$ kinin receptor; and (3) the ACE inhibitor ramiprilat and the calcium-channel blocker amlodipine stimulate a B$_2$-kinin receptor–dependent mechanism and NO release to modulate oxygen consumption. These conclusions are supported by an earlier study from our laboratory in which icatibant (HOE 140) was used to block the B$_2$-kinin receptor and to block the decrease in cardiac oxygen consumption in the canine left ventricle in vitro in response to 3 different ACE inhibitors. Bradykinin activates the B$_2$-kinin receptor and stimulates the release of NO in cardiac tissue from normal mice to decrease oxygen consumption. This decrease in MV$\dot{O}_2$ was attenuated by L-NAME. These results agree with previous work indicating that NOS inhibitors block the effects of bradykinin; more recently, we demonstrated that the bradykinin-induced reduction in MV$\dot{O}_2$ was mediated by endothelial NOS-derived NO. The present study also showed that the effects of bradykinin on cardiac oxygen consumption were virtually absent in the B$_2$ mice. Similar results have been shown for vascular relaxation and the loss of the cardioprotective effect of preconditioning by kinins on myocardial ischemia and reperfusion injury. These studies confirm the absence of the bradykinin B$_2$ receptor in those mice and also suggest that the B$_1$ kinin receptor does not regulate MV$\dot{O}_2$ to any significant degree in response to bradykinin.

Substance P is an endothelium-dependent vasodilator that primarily activates the neurokinin (NK)-1 receptor. Substance P does have a slight affinity for the NK-2 and NK-3 receptors, both of which are present on smooth muscle or endothelial cells. Furthermore, Tagawa et al showed...
that substance P–induced coronary vasodilation is mediated by NO. Substance P decreased MVO₂ in hearts from both normal and B₂-kinin receptor knockout mice. L-NAME significantly attenuated the decrease in O₂ consumption in response to substance P in cardiac tissue from both normal and B₂-kinin receptor knockout mice. These data indicate that the NK-1 receptor is still able to release NO to modulate cardiac oxygen consumption in the B₂-kinin receptor knock-out mouse heart, indicating that there is no global defect in the ability of receptors to stimulate NO production.

Our data indicate a concentration-dependent decrease in MVO₂ in cardiac tissue from normal mice after addition of the ACE inhibitor ramiprilat. This is NO-dependent; the effects of ramiprilat were attenuated by pretreatment of the tissue with L-NAME. This NO-mediated decrease in MVO₂ supports other studies that show ACE inhibitors release NO to decrease cardiac hypertrophy and afterload. In addition, this property of ramiprilat may contribute to the efficacy of the drug in the reduction of mortality in patients with clinical symptoms of heart failure. In the present study, ramiprilat had virtually no effect on oxygen consumption in cardiac tissue from the B₂ −/− mice. This supports other studies from this laboratory suggesting that the effects of ACE inhibitors on cardiac oxygen consumption in vitro are dependent on activation of the B₂-kinin receptor and activation of NOS. This effect of ACE inhibitors also supports the presence of a system generating endogenous kinins, because no exogenous bradykinin was added in these studies.

Amlodipine significantly reduced MVO₂ in tissue from normal mice. The decrease in MVO₂ was significantly attenuated after treatment with L-NAME. These data support our previous studies indicating that amlodipine modulates MVO₂ via the release of NO. Furthermore, the current study supports the conclusion that the mechanism of action of amlodipine is through activation of the B₂-kinin receptor. Amlodipine had no effect on oxygen consumption in cardiac tissue from B₂ −/− mice, suggesting that the B₂-kinin receptor has an important role in controlling tissue oxygen consumption. In addition, because no kinins were added, it seems that amlodipine modulates local kinin production, which subsequently activates the B₂-kinin receptor in the mouse heart. One discrepancy exists in our data: L-NAME was not entirely effective in blocking NO-mediated reduction in O₂ consumption. L-NAME partially but significantly attenuated the response to amlodipine, whereas the response was abolished in the B₂ −/− mouse heart. In previous studies in the canine heart, we found that only a portion of the response to amlodipine was blocked by nitro-L-arginine, and we concluded that the remaining portion of the reduction in O₂ consumption was dependent on the calcium-channel blocking activity of amlodipine. Although unresolved, the current study calls that conclusion into question and suggests that (1) L-NAME blocked only a portion of the NO release induced by amlodipine or (2) the B₂ receptor may have an additional action that involves calcium-channel activation.

Most likely, NO directly decreases mitochondrial tissue respiration via an interaction with cytochrome oxidase, and the addition of an NO donor such as SNAP would decrease oxygen consumption independent of endogenous NO synthetase and independent of the presence of the B₂-kinin receptor. In this regard, SNAP caused a concentration-dependent decrease in oxygen consumption in cardiac tissue from both normal and B₂-kinin receptor knockout mice. Pretreatment of the tissue with L-NAME had no effect on the reduction in tissue oxygen consumption caused by SNAP in the hearts from either normal mice or the B₂-kinin receptor knockout mice because this is not dependent on local NO production.

In conclusion, bradykinin and substance P reduce oxygen consumption in the mouse heart in vitro, and this reduction is NO-dependent. The effects of bradykinin, but not substance P, are eliminated in hearts from B₂−/− mice, indicating a potentially important role for bradykinin and the B₂ receptor in the control of cardiac oxygen consumption. Finally, the calcium-channel antagonist amlodipine and the ACE inhibitor ramiprilat both activated a B₂-kinin/NO–dependent mechanism to modulate MVO₂. This is due to modification of the production of kinins locally in the mouse heart; the control of tissue oxygen consumption by amlodipine and ramiprilat is entirely absent in hearts from B₂−/− mice. Furthermore, the control of tissue oxygen consumption by kinins and NO may be part of the basis for the therapeutic uses of amlodipine and ACE inhibitors in the treatment of cardiovascular disease.

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References


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