Inhibition of Rat Cardiac Muscle Contraction and Mitochondrial Respiration by Endogenous Peroxynitrite Formation During Posthypoxic Reoxygenation

Yi-Wu Xie, Pawel M. Kaminski, Michael S. Wolin

Abstract—This study was designed to investigate the potential role of endogenous peroxynitrite (ONOO\(^-\)) formation in the inhibition of cardiac muscle contractility and mitochondrial respiration during posthypoxic reoxygenation. Isometric contraction of isolated rat left ventricular posterior papillary muscle was virtually eliminated at the end of an exposure to 15 minutes of hypoxia and remained 40±5% depressed an hour after the reintroduction of O\(_2\). O\(_2\) uptake by rat left ventricular cardiac muscle, measured by a Clark-type O\(_2\) electrode, was also inhibited by 24±2% at 10 minutes after reoxygenation. The inhibition of contractility and respiration during posthypoxic reoxygenation was markedly attenuated by the NO synthase inhibitor nitro-L-arginine, exogenous superoxide dismutase, and the ONOO\(^-\) scavenger urate but not by the hydroxyl radical scavenger mannitol. Generation of ONOO\(^-\) with the NO donor S-nitroso-N-acetylpenicillamine (SNAP) plus the superoxide-releasing agent pyrogallol caused an irreversible inhibition of cardiac contractile and respiratory function. Unlike ONOO\(^-\), exogenous (SNAP) and endogenous (bradykinin) sources of NO inhibited contractility in a reversible manner. Under conditions of comparable amounts of respiratory inhibition in unstimulated incubated muscle, the NO-dependent agents and the mitochondrial antagonist NaCN produced a smaller degree of suppression of contractility compared with ONOO\(^-\) and posthypoxic reoxygenation. These results are consistent with a contributing role for endogenous ONOO\(^-\) formation in the inhibition of cardiac muscle contractility and mitochondrial respiration during posthypoxic reoxygenation. (Circ Res. 1998;82:891-897.)

Key Words: cardiac contractility □ mitochondrial respiration □ hypoxia/reoxygenation □ nitric oxide □ superoxide

Mechanisms leading to tissue injury and organ dysfunction after ischemia/reperfusion or hypoxia/reoxygenation are generally rather poorly understood. Aspects of cardiac contractile performance are markedly depressed during postischemic reperfusion or posthypoxic reoxygenation.\(^1\)–\(^6\) After exposure of the heart to ischemia/reperfusion or hypoxia/reoxygenation, contractile dysfunction is observed together with an elevated production of reactive O\(_2\) and N\(_2\) species\(^4\)–\(^6\) and impaired mitochondrial function.\(^7\)–\(^9\) Endogenous peroxynitrite (ONOO\(^-\)) formation derived from NO and superoxide (O\(_2\)\(^-\)) has been suggested to be responsible for the cardiac contractile dysfunction during postischemic reperfusion.\(^8\)–\(^9\) Infusion of ONOO\(^-\) into isolated rat hearts has been found to impair cardiac contractile performance by decreasing cardiac efficiency.\(^10\) However, the exact mechanisms involved in the mediation of contractile dysfunction remain an open question. The activities of components of the mitochondrial respiratory chain have been reported to be largely reduced after exposure to hypoxia/reoxygenation or ischemia/reperfusion.\(^2\)–\(^3\) Furthermore, we have previously shown that endogenous ONOO\(^-\) formation during posthypoxic reoxygenation contributes to a prolonged suppression of mitochondrial respiration in intact cardiac muscle.\(^11\) Because aerobic metabolism is essential in maintaining cardiac contractile performance, impaired mitochondrial function may contribute to the contractile dysfunction observed during postischemic reperfusion or posthypoxic reoxygenation. Therefore, the present study was focused on examining whether endogenous ONOO\(^-\) formation contributes to cardiac contractile dysfunction and respiratory inhibition after exposure to a short-term model of hypoxia and reoxygenation.

Evidence exists that NO inhibits cardiac myocyte contractility and that this mechanism could be an important process in the control of cardiac function.\(^12\)–\(^14\) We have previously shown that NO and ONOO\(^-\) suppress respiration in bovine cardiac muscle through reversible and irreversible mechanisms, respectively.\(^11\) Therefore, the present study was also designed to investigate the relationship between the effects of NO and ONOO\(^-\) on contractile performance and mitochondrial respiration in isolated rat cardiac muscle.

Materials and Methods

Materials

BK, 8-bromo-cGMP, catalase, mannitol, uric acid, SOD from bovine erythrocytes, HEPES, and lucigenin were purchased from Sigma Chemical Co. L-NA was obtained from Aldrich Chemical Co., and sodium cyanide and pyrogallol were from JT Baker Chemical Co. SNAP was synthesized as described.\(^15\) All materials were dissolved in Krebs’ solution containing (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, pH 7.4.

Received December 5, 1997; accepted February 12, 1998.

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Cardiac muscle was obtained from male Wistar rats at 3 months of age that were anesthetized with pentobarbital sodium (50 mg/kg body wt IP). Hearts were immediately excised and rinsed at least twice in ice-cold Krebs’ solution.

**Respiration Measurement**

Cardiac muscle was isolated from the left ventricle (free wall only) of these rat hearts and then cut into small segments that were 20 to 30 mg in weight and 1 to 2 mm in thickness. Before studies of tissue respiration, these muscle segments were bathed at 37°C for 2 hours in Krebs’ solution, in which 21% O2/5% CO2/balance N2 was continuously bubbled. O2 uptake by cardiac muscle slices was measured polarographically in 3 mL of air-saturated Krebs’ solution buffered with 10 mmol/L HEPES-NaOH (pH 7.4) at 37°C by a Clark-type O2 electrode (Yellow Springs Instrument Co), as previously described.\(^1\)\(^-\)\(^4\) Tissue respiration was calculated as the rate of decrease of O2 concentration after the addition of muscle segments, assuming an initial O2 concentration of 224 mmol/mL, and was expressed as nanomoles consumed per minute per gram tissue (wet weight). O2 uptake by muscle slices was monitored only before one third of the O2 was consumed, and any spontaneous changes in the electrode recording in the absence of tissue were subtracted from each measured rate of tissue O2 consumption. The mitochondrial inhibitor NaCN, the NO donor SNAP, the endogenous NO stimulus BK, the cell-permeable cGMP analogue 8-bromo-cGMP, and ONOO\(^-\)-generating system (0.01 mmol/L SNAP + 0.01 mmol/L pyrogallol) were examined for their acute effects on cardiac muscle respiration. The presence of the ONOO\(^-\)-generating system directly caused a small amount of O2 consumption, and this was subtracted from the amount of respiration observed in the presence of cardiac muscle. Respiration was also examined 15 minutes after the ONOO\(^-\)-generating system was washed out of the tissue. Effects of a short-term model of hypoxia (15 minutes) and reoxygenation (10 minutes) on O2 consumption by cardiac muscle were also studied in the absence or presence of the NO synthase inhibitor L-NA, the O2 scavenger SOD, the ONOO\(^-\) scavenger uric acid, and the hydroxyl radical scavenger mannitol. The typical observation time for respiration measurements was 6 to 8 minutes, and new muscle segments were used for each drug examined. At the end of each measurement, 1 mmol/L of NaCN was added to confirm that changes in O2 consumption were from mitochondrial sources. Data were analyzed as the percent change of the rate of respiration for each muscle slice studied.

**Detection of Superoxide Anion**

Superoxide anion was detected primarily to help identify conditions in which cardiac muscle superoxide production or scavenging was altered by the treatments used. Under the same conditions as the contractility study, lucigenin chemiluminescence was used to detect O2\(^-\) production by rat posterior papillary muscle in a lighttight box with a photon-counting apparatus (Thorn EMI electron tubes) that we have previously described.\(^2\) This photon-counting apparatus includes a cooled Thorn EMI photomultiplier tube (model 9235B), a Thorn EMI amplifier-discriminator (model C604), and a photon counter (model C660). The photon counts were integrated over 5-second periods and shown on a computer monitor; meanwhile, an analog signal of the integrated photon counts was continuously recorded on the same polygraph recorder as was the contractile performance. The photon counts are expressed in the text as counts per minute per gram after subtraction of the background dark current of the photomultiplier. Our previous work\(^20\),\(^21\) examining endogenously produced O2\(^-\) in vascular and cardiac myocyte preparations by lucigenin chemiluminescence is consistent with lucigenin detecting intracellular O2\(^-\) and its scavenging by NO. In the absence of tissue, interventions such as hypoxia and reoxygenation did not cause changes in lucigenin chemiluminescence. In addition, the absolute magnitudes of modifications in cardiac muscle–derived chemiluminescence during hypoxia and reoxygenation need to be interpreted with caution, since they could be affected by processes that potentially alter the sensitivity of the lucigenin method, such as changes in the redox state of lucigenin, pH, and alterations in lipid metabolism (eg, see References 22 to 24).

**Statistical Analysis**

All data in the text are reported as mean±SE, and n in all instances represents the number of different animals studied. Differences in the mean values were analyzed using unpaired and, where appropriate, a paired Student t tests. A Bonferroni correction was applied when multiple groups were compared. Statistical significance was assumed at α<0.05.

**Results**

**Effects of Exogenous NO on Cardiac Muscle Contractile and Respiratory Function**

The NO donor SNAP reduced the developed tension of isolated rat posterior papillary muscle by up to 23±6.5% (n=6) at cumulative doses of 10\(^{-6}\) to 10\(^{-4}\) mol/L (Figure 1), with statistical significance starting at 10\(^{-5}\) mol/L. Under these conditions, O2\(^-\) generation detected by lucigenin chemiluminescence (2.7±1.3×10\(^6\) cm/pmol, n=6) was significantly decreased by 23±8% and 32±13% at the two larger concentrations of SNAP, respectively. Similar doses of SNAP significantly suppressed tissue respiration of rat cardiac

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**Selected Abbreviations and Acronyms**

- BK = bradykinin
- L-NA = \(\text{N}^\ominus\)-nitro-\(\text{L}\)-arginine
- SNAP = S-nitroso-N-acetylpenicillamine
- SOD = superoxide dismutase

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muscle by up to 35±4% (n=5, Figure 1), which is comparable to its previously reported effects on O2 consumption by canine and bovine myocardial muscle.11,16 The inhibition of respiration at each dose of SNAP below 10^{-4} mol/L was significantly greater than the attenuation of developed tension. The average O2 consumption rate of rat cardiac muscle was 299±22 mmol · min^{-1} · g^{-1} (n=10). The effects of SNAP on the attenuation of developed tension were reversed after its removal by washout (2.6±1.0% inhibition).

Effects of Endogenous NO on Contraction and Respiration

The stimulus of endogenous NO biosynthesis, BK, significantly depressed the developed tension of papillary muscle by up to 23±4.4% (n=7) at cumulative doses of 10^{-7} to 10^{-4} mol/L (Figure 2). Meanwhile, none of these doses of BK significantly altered O2 consumption detected by lucigenin chemiluminescence (8.4±1.9×10^4 cpm/g, n=7). Similar concentrations of BK significantly suppressed tissue respiration of rat cardiac muscle by up to 34±1.4% (n=5, Figure 2), which is comparable to its previously reported effects on O2 consumption by canine and bovine myocardial muscle.11,16 The inhibition of respiration at each dose of BK was significantly greater than the attenuation of developed tension. The NO synthase inhibitor L-NA (0.1 mmol/L) did not significantly affect the contractile amplitude of papillary muscle or detection of O2^- generation (not shown), but it did eliminate the suppression of developed tension caused by BK (n=4, Figure 2). Similarly, L-NA markedly attenuated the inhibition of tissue respiration of cardiac muscle caused by BK (n=5, Figure 2). The effects of BK on the attenuation of developed tension were reversed after its removal by washout (5.7±6.0% inhibition).

Effects of a cGMP Analogue on Contraction and Respiration

The cell-permeable and stable cGMP analogue 8-bromo-cGMP reduced the developed tension of papillary muscle by up to 18±4% (n=7) at cumulative doses of 10^{-7} to 10^{-4} mol/L (Figure 3), with statistical significance starting at 10^{-5} mol/L. None of these concentrations of 8-bromo-cGMP significantly affected O2^- generation detected by lucigenin chemiluminescence (11±2.4×10^4 cpm/g, n=7). As previously observed with canine myocardial muscle,16 similar doses of 8-bromo-cGMP suppressed tissue respiration of rat cardiac muscle by up to 18±3% (n=4, Figure 3), with statistical significance starting at 10^{-6} mol/L. The inhibition of respiration was not significantly greater than the attenuation of developed tension at any dose of 8-bromo-cGMP.

Effects of NaCN on Contraction and Respiration

The mitochondrial cytochrome oxidase inhibitor NaCN significantly reduced the developed tension of papillary muscle by up to 76±6.7% (n=9) at increasing doses of 10^{-6} to 10^{-3} mol/L (Figure 4). Meanwhile, O2^- generation measured by lucigenin chemiluminescence (2.1±0.4×10^4 cpm/g, n=9) was significantly increased by 21±12% and 142±22% at the two larger concentrations of NaCN, respectively. Similar doses of NaCN significantly suppressed tissue respiration of the cardiac muscle by up to 73±2.5% (Figure 4). The inhibition of respiration at each dose of NaCN below 10^{-3} mol/L was significantly greater than the attenuation of developed tension.
Effects of ONOO\textsuperscript{−} Generation on Contraction and Respiration

The developed tension of papillary muscle was markedly suppressed by 36 ± 5.3% (n = 5, Figure 5) by exposure to an ONOO\textsuperscript{−}-generating system using 0.01 mmol/L SNAP plus 0.01 mmol/L pyrogallol.\textsuperscript{11} This depression of force generation by ONOO\textsuperscript{−} remained stable for at least 1 hour. The degree of inhibition of force generation by this ONOO\textsuperscript{−}-generating system was significantly greater than the inhibition of 9.9 ± 3.2% caused by 0.01 mmol/L SNAP (in the absence of pyrogallol), and treatment with 0.01 mmol/L pyrogallol alone did not detectably alter force generation. As detected by lucigenin chemiluminescence, O\textsubscript{2}\textsuperscript{−} production by the papillary muscle was not significantly different after exposure to the ONOO\textsuperscript{−}-generating system (7.3 ± 7.4%, n = 5). Similar to observations made in bovine myocardial muscle,\textsuperscript{11} the generation of ONOO\textsuperscript{−} acutely inhibited the tissue respiration of rat cardiac muscle by 30 ± 1.1% (n = 4, Figure 5); cardiac muscle respiration remained suppressed by 29 ± 3.3% (n = 4) when it was reexamined 15 minutes after washout of the ONOO\textsuperscript{−}-generating system.

Effects of Hypoxia/Reoxygenation on Cardiac Muscle Contractile and Respiratory Function

The developed tension of rat papillary muscle was severely depressed 81 ± 4% (n = 6) at the end of a 15-minute period of exposure to severe hypoxia (Figure 6). Reintroduction of O\textsubscript{2} resulted in some recovery of contractile function, but the developed force was still depressed by 40 ± 5.2% for up to 1 hour after reoxygenation (Figure 6). Interestingly, in the presence of 0.1 mmol/L L-NA, the depression of contractile performance during reoxygenation was only 11 ± 1.8% (n = 6, Figure 6). The depression of the developed force during hypoxia was not altered by L-NA (Figure 6). Similar to L-NA, uric acid (0.1 mmol/L), an intracellular scavenger of ONOO\textsuperscript{−},\textsuperscript{11} markedly attenuated the depression of the developed tension of papillary muscle during reoxygenation to 14 ± 2.8% inhibition, without affecting the depression of contractile function by hypoxia (n = 7, Figure 6). The addition of SOD (3 μmol/L) 5 minutes before reoxygenation also largely decreased the suppression of the contractile amplitude of papillary muscle during reoxygenation to 18 ± 7%, without altering the effects of hypoxia (n = 6, Figure 6). However, mannitol (100 mmol/L, added before hypoxia), a scavenger of the hydroxyl radical, did not alter the effects of reoxygenation. These results with L-NA, uric acid, and SOD provide evidence for an involvement of endogenous ONOO\textsuperscript{−} formation from NO and O\textsubscript{2}\textsuperscript{−} in the depression of cardiac contractile function during posthypoxic reoxygenation.

The simultaneous detection of O\textsubscript{2}\textsuperscript{−} with lucigenin chemiluminescence revealed a 91 ± 2% decrease during hypoxia (Figure 7), followed by a transient overshoot (130 ± 27% increase, Figure 7) within the first 2 to 3 minutes of reoxygenation. The steady-state level of detectable O\textsubscript{2}\textsuperscript{−} after the transient increase during reoxygenation was not significantly different from control (n = 6). During reoxygenation, the overshoot of O\textsubscript{2}\textsuperscript{−} levels detected by lucigenin chemiluminescence was significantly attenuated by exogenous SOD (Figure 7). However, SOD did not alter the detection of
superoxide anion when its production returned to the steady-state level seen before exposure to hypoxia. An observation for which we currently do not have an explanation is that the markedly depressed level of lucigenin chemiluminescence observed under hypoxia was slightly greater in the presence of SOD. On the basis of the effects of the addition of SOD on the detection of O$_2^-$ and the protective effects of SOD on contractile function, superoxide anion seems to be released into the extracellular environment only during the first 2 to 3 minutes of reoxygenation.

Tissue respiration of rat cardiac muscle after the 15-minute exposure to hypoxia and 10-minute period of reoxygenation was also significantly depressed by 24±2.4% (n=10, Figure 8), which is similar to the degree of inhibition of O$_2$ consumption produced by a comparable treatment of bovine cardiac papillary muscle. The inhibition of respiration by hypoxia/reoxygenation was significantly less than the attenuation of developed tension. This suppression of tissue respiration by posthypoxic reoxygenation was markedly attenuated (see Figure 8) by the addition of 0.1 mmol/L L-NA (6.7±2.9%, n=6), 0.1 mmol/L uric acid (7.1±3.2%, n=6), and 3 μmol/L SOD (8.8±2.9%, n=5) but not by 100 mmol/L mannitol (27±3.6%, n=5).

### Discussion

The data reported in the present study are consistent with a key role for ONOO$^-$ in the prolonged inhibition of contractility and respiration of rat cardiac muscle observed during posthypoxic reoxygenation. Since L-NA and uric acid did not alter the marked suppression of contractile performance of rat cardiac papillary muscle caused by hypoxia, NO and ONOO$^-$ may not be involved in this response. The reduction in contractile performance during ischemia has been previously suggested to be due to metabolic changes associated with the loss of creatine phosphate, increased intracellular acidosis, and the accumulation of inorganic phosphate and lactate.25–28 During reoxygenation after the 15-minute period of hypoxia, the contractile performance of electrically stimulated rat papillary muscle was markedly depressed by 40% for up to 60 minutes, consistent with the findings of previous studies with ischemia/reperfusion1,2 and hypoxia/reoxygenation. Since L-NA, SOD, and uric acid markedly suppressed the observed depression of contractile function during posthypoxic reoxygenation, NO-derived endogenous ONOO$^-$ formation is mainly responsible for the suppression of force generation during reoxygenation. The absence of an effect of mannitol suggests that hydroxyl radicals do not detectably contribute to this effect of reoxygenation. The transient increase in O$_2^-$ detected by lucigenin on reoxygenation is also consistent with the formation of ONOO$^-$ during this period. Similar to studies with bovine cardiac muscle,11 endogenous ONOO$^-$ appears to form in rat cardiac muscle during posthypoxic reoxygenation in amounts sufficient to inhibit irreversibly tissue respiration. Thus, endogenous ONOO$^-$ formation during posthypoxic reoxygenation is likely to contribute to the observed depression of cardiac contractility and tissue respiration.

In rat cardiac muscle, ONOO$^-$ formation appears to increase the potency and duration of NO as an inhibitor of cardiac muscle contractile performance. Both exogenous and endogenous sources of NO from SNAP and BK, respectively, significantly depressed the contractile amplitude of rat left ventricular papillary muscle. Increased cGMP formation is likely to contribute to these NO-elicted responses, because the cGMP analogue 8-bromo-cGMP also caused an inhibition of contraction. Previous studies indicate that NO has an inhibitory effect on the contractile amplitude of cardiac muscle,12–14 Elevated cGMP levels12,13 and inhibited Ca$^{2+}$ transients, potentially originating from modulation of sarcoplasmic L-type Ca$^{2+}$ channels,19 have been previously suggested to contribute to the effect of NO on contractile function.14 The generation of O$_2^-$ potentiated the inhibitory effect of NO on rat cardiac muscle contractile function, suggesting that the potency of NO was enhanced by conditions that promote ONOO$^-$ formation. Studies on the actions of ONOO$^-$ produced endogenously during posthypoxic reoxygenation indicate that its effects on rat papillary muscle contractility and respiration in rat and bovine cardiac muscle seem to be irreversible. The inhibition of contraction and respiration elicited by BK and SNAP did not appear to involve an interaction of NO with O$_2^-$ and the formation of ONOO$^-$. 

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**Figure 7.** Effects of hypoxia and reoxygenation on papillary muscle O$_2^-$ generation in the absence (control) and presence of SOD. Superoxide production by rat cardiac papillary muscle detected by lucigenin chemiluminescence was markedly depressed by 91% from the basal level (1.6±0.3×10$^4$ cpm/g, n=6) during a 15-minute exposure to hypoxia, and a transient overshoot (Re-O$_2$ peak) to 230±27% of the basal level was seen 2 to 3 minutes into reoxygenation. After 30 minutes of reoxygenation, the steady-state level of O$_2^-$ (Re-O$_2$ SS) was not different from the basal level. The addition of exogenous SOD (3 μmol/L, n=6) 5 minutes before reoxygenation attenuated the transient overproduction of O$_2^-$ observed during reoxygenation. *P<0.05 vs basal level; #P<0.05 for SOD vs control (significant effect of SOD).

**Figure 8.** Effect of hypoxia/reoxygenation on rat cardiac muscle respiration in the absence (control) and presence of probes for NO, O$_2^-$, ONOO$^-$, and hydroxyl radical. Tissue respiration of rat left ventricular cardiac muscle was reduced by 24±2.6% (n=9) after exposure to 15-minute hypoxia and 10-minute reoxygenation. Effects of the NO synthase inhibitor L-NA (0.1 mmol/L, n=5), the ONOO$^-$ scavenger urate (0.1 mmol/L, n=5), exogenous SOD (3 μmol/L, n=4), and the hydroxyl radical scavenger mannitol (Man, 100 mmol/L; n=5) on the hypoxia and reoxygenation-elicted depression of respiration were also examined. *P<0.05 vs control.
since the effects of these agents were reversible. Although BK did not alter the detection of O$_2^-$ by lucigenin chemiluminescence, the two larger doses of SNAP significantly depressed the detection of endogenous O$_2^-$ Thus, if ONOO$^-$ is being produced by these doses of SNAP, the amounts formed do not have appreciable irreversible effects. The inactivation of systems that contribute to energy metabolism (mitochondrial proteins,$^{30}$ glycolytic enzymes,$^{31}$ and possibly creatine kinase$^{32}$), calcium homeostasis (a sarcoplasmic reticulum Ca$^{2+}$-ATPase$^{33}$), and perhaps other processes could potentially contribute to the observed increased potency of ONOO$^-$ over NO as an inhibitor of cardiac muscle contractility. In addition, many of the systems potentially altered by ONOO$^-$, including mitochondrial function,$^{34}$ mitochondrial respiration,$^{35-37}$ and sarcoplasmic reticulum Ca$^{2+}$ handling,$^3$ also seem to be impaired by hypoxia/reoxygenation or ischemia/reperfusion.

The extent to which mitochondrial inhibition contributes to the depression of contractile function after exposure to NO, ONOO$^-$, or posthypoxic reoxygenation is difficult to assess because of the potential complexity of the mechanisms involved and limitations of the present study originating from measuring respiration and contraction in different segments of cardiac muscle under basal and stimulated levels of energy consumption, respectively. The ability of the cytochrome oxidase inhibitor NaCN to depress cardiac contraction and respiration is consistent with the well-established concept that suppression of mitochondrial energy metabolism can lead to depression of cardiac contractile function. However, the effects of the higher doses of NaCN need to be cautiously interpreted, because additional mechanisms are potentially activated by processes, such as an increase in O$_2^-$, from the inhibition of SOD by NaCN. NO is known to be similar to NaCN as a potent reversible inhibitor of cytochrome oxidase.$^{37-39}$ It is difficult to determine the cause-effect relationship between actions of NO on respiration and contractile function, because NO appears to activate mechanisms that alter both of these processes and because force generation and mitochondrial energy metabolism are likely to be dependent on each other in actively contracting cardiac muscle. For example, our previous work involving incubated slices of canine cardiac and skeletal muscle detected evidence that NO suppressed respiration by both a cGMP-derived effect on energy consumption and through a direct inhibitory effect of NO on mitochondrial respiration.$^{16,17}$ The inhibitory effects of NO on O$_2^-$ consumption are also seen in the presence of mitochondrial respiration, which is stimulated in vitro by an uncoupler of electron transport (dinitrophenol)$^{16}$ and in vivo during increased levels of cardiac work,$^{40}$ suggesting that an interaction of NO with cytochrome oxidase is likely to be occurring in actively contracting cardiac muscle. Although the design of the present study does not permit us to evaluate whether the attenuation of contractile function by NO originated from a signaling effect that reduces force generation or from a suppression of respiration, the modest effect observed at large doses of NO suggests that it could have only a minor inhibitory effect on respiration. Several processes may limit the inhibitory effect of large concentrations of NO on mitochondrial function. The inhibition of cytochrome oxidase by NO may reach an equilibrium with a reactivation process, through its ability either to metabolize NO$^{41-43}$ or to cause a rapid dissociation of NO from this enzyme.$^{44}$ This may explain why NO mechanisms produce a maximum of $\approx 40\%$ inhibition of tissue respiration at the very high levels of NO that were examined in the present study and in previous studies with canine$^{16}$ and bovine$^{45}$ cardiac muscle. In addition, the cardiac muscle mitochondrial metabolic state may control the coupling between electron transport and proton pumping by cytochrome oxidase and thermogenesis.$^{45}$ Interestingly, the inhibition of NO biosynthesis in resting conscious dogs increases whole-body O$_2$ consumption associated with an elevation of body temperature.$^{46}$ Thus, an action of NO on cytochrome oxidase may improve the efficiency of O$_2$ utilization for energy metabolism. In the present study, under conditions of equivalent amounts of respiratory inhibition in unstimulated muscle, ONOO$^-$ and posthypoxic reoxygenation elicited a greater suppression of contractile function than did the NO-generating agents or the mitochondrial antagonist NaCN. These observations seem to be consistent with the previously reported loss of cardiac efficiency associated with exposure of isolated rat hearts to an infusion of 40 mU/L ONOO$^-$.$^{10}$ Thus, limitations of the present study, together with the apparent complexity of the interactions that NO and its metabolites have with contractile and respiratory systems, prevent interpretation of the influence of mitochondrial respiration on contractile function.

In summary, an interaction of O$_2^-$ with NO, associated with the formation of ONOO$^-$, appears to be an important process that contributes to the suppression of rat cardiac muscle contractile performance and mitochondrial respiration caused by hypoxia and reoxygenation. The ability of the extracellular scavenger SOD to alleviate contractile dysfunction during posthypoxic reoxygenation observed in the present study or during posts ischemic recovery in isolated rat hearts$^{47}$ is consistent with a potential role for O$_2^-$ production by nearby cells, such as endothelium,$^{48}$ in the depression of cardiac contractile and respiratory function. Development of therapeutic approaches for situations in which cardiac muscle is exposed to hypoxia/reoxygenation, such as coronary bypass surgery, heart transplantation, or thrombolytic therapy, may benefit from considering factors that control the generation and scavenging of ONOO$^-$ and the impact of its formation.

Acknowledgments

This study was funded by US Public Health Service grants HL-43023 and HL-31069. The authors thank Dr. Gabor Kaley for providing sources of Wistar rat hearts and Dr. Francis L. Belloni for providing the Grass S44 stimulator.

References


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Circ Res. 1998;82:891-897
doi: 10.1161/01.RES.82.8.891

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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