Decreased Nitric Oxide Availability in Normotensive and Hypertensive Rats With Failing Hearts After Myocardial Infarction

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Abstract—Endothelial NO synthase, being deficient in arginine and/or tetrahydrobiopterin, produces in addition to NO a significant concentration of superoxide (O$_2^-$). We investigated whether such an imbalance between O$_2^-$ and NO production is present in dysfunctional aortas of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) with failing hearts after myocardial infarction. Heart failure was induced by permanent occlusion of the left coronary artery, resulting in a large infarction of the free left ventricular wall. Eight weeks after myocardial infarction, when WKY and SHR had compensated heart failure and congestive heart failure, respectively, calcium ionophore-induced NO release (assessed by a NO-sensitive microsensor) from aortic endothelial cells was significantly reduced from 478±48 to 216±16 nmol/L and 693±131 to 257±53 nmol/L in WKY and SHR, respectively. Concomitantly, significant increases in calcium ionophore-stimulated O$_2^-$ production (assessed by an electrochemical sensor) could be observed in aortic endothelial cells from infarcted WKY rats (22±3.2 versus sham, 10.1±1.2 nmol/L) and SHR (102±8 versus sham, 67±5 nmol/L). A dramatic increase in endothelial peroxynitrite concentration (chemiluminescence method) from 35±4 to 90±3 nmol/L for WKY and from 60±5 to 170±10 nmol/L for SHR also was detected. Thus, the markedly decreased NO availability probably caused by impaired endothelial NO synthase activity with enhanced O$_2^-$ and peroxynitrite production appears to be attributable to endothelial dysfunction in normotensive rats with chronic heart failure and especially in hypertensive rats with severe congestive heart failure. (Hypertension. 2001;38:1367-1371.)

Key Words: heart failure ■ endothelium ■ nitric oxide ■ rats, spontaneously hypertensive ■ rats, WKY

Numerous studies showed that impaired endothelium-dependent vasodilation and cardiac dysfunction in heart failure (HF) appear to be attributable to decreased endothelial NO synthesis and/or possibly to increased NO degradation by enhanced generation of superoxide (O$_2^-$). Large deficiencies of endothelial NO synthase (eNOS) mRNA and protein as well as basal and stimulated NO production were observed in aortic endothelial cells and cardiac microvessels from conscious dogs with pacing-induced overt congestive heart failure (CHF). In the same animal model at the onset of cardiac decompensation, reduced basal cardiac NO production was associated with a fall of cardiac contractility and an elevated left ventricular end-diastolic pressure. Similarly, impaired basal and stimulated NO productions, which were indirectly assessed by vascular cyclic GMP, were demonstrated in aortas and pulmonary arteries from Wistar-Kyoto rats (WKY) with chronic HF after myocardial infarction (MI). In comparison, a normal acetylcholine-induced relaxation with reduction of basal NO release was found in small mesenteric arteries from this rat HF model.

Conflicting data exist with regard to eNOS expression and activity in human beings with chronic HF. Increased cardiac expression of eNOS, however, with no increased eNOS activity, was found in end-stage human heart failure. In contrast, a reduced eNOS expression and decreased basal NO release were observed in the coronary microcirculation in patients with HF. In comparison, basal forearm blood flow was preserved or even increased. Acetylcholine-induced dilation was attenuated in patients with chronic CHF, and acetylcholine-induced nitrite production in isolated coronary microvessels from the human failing heart was depressed in comparison to control vessels. There is evidence that endothelial dysfunction in CHF results from enhanced oxidative stress. Aortic rings from WKY subjected to MI showed endothelial dysfunction caused by an increased vascular O$_2^-$ generation, which rapidly inactivates NO. Also, increased basal O$_2^-$ generation could be observed in myocyte homogenates from patients with CHF, and increased reactive oxygen species, primarily O$_2^-$, were reported to occur in patients with CHF.
To date, NO bioavailability in chronic HF induced by MI was indirectly shown only, for example, by measurement of vasodilator response, vascular nitrite, or cyclic GMP production. Therefore, we directly investigated receptor-independent calcium ionophore (CaI)-stimulated NO release from aortic endothelial cells by using a porphyrinic microsensor placed in close proximity to the cell surface. Second, we simultaneously addressed CaI-stimulated $\text{O}_2^-$ generation in aortic endothelial cells, which by the fast reaction with NO forms peroxynitrite (ONOO$^-$) and cytotoxic radicals, thereby possibly playing a role in endothelial and cardiac dysfunction induced by MI.

**Methods**

**Animals and Surgical Procedures**

The study was carried out with adult male WKY rats and spontaneously hypertensive rats (SHR) weighing 250 to 300 g (4 month old). For induction of HF by MI, the rats were anesthetized with a mixture of ketamine/xylazine (35/2 mg/kg IP). After intubation and ventilation, a left thoracotomy was performed under aseptic conditions through the third intercostal space. The pericardium was opened, the left coronary artery was ligated 2 mm distal to the aortic origin, and the chest was closed. Sham operation was identical but without coronary occlusion. All experiments were performed in accordance with the German animal protection law.

**Final Measurements**

Eight weeks after surgery, the animals were anesthetized again. Mean arterial blood pressure (MAP) and heart rate were measured through the right carotid artery. Thoracic aorta was taken for evaluation of endothelial function and for determination of NO and $\text{O}_2^-$ production.

**Determination of Infarct Size**

The left ventricle was transversely sectioned into 4 slices from the apex to the base. Infarct size was determined by planimetry and cytototoxic radicals, thereby possibly playing a role in endothelial and cardiac dysfunction induced by MI.

**Measurements of NO and $\text{O}_2^-$**

Detection of NO by a porphyrinic microsensor and its preparation were performed as previously described. A microsensor capable of almost instantaneous indirect detection of $\text{O}_2^-$ was prepared according to the general procedure described previously, subsequently modified in our laboratory for single-cell measurements. The sensor operated at a constant potential of 0.68 V versus saturated calomel electrode. A microsensor capable of almost instantaneous indirect detection of $\text{O}_2^-$ was prepared according to the general procedure described previously, subsequently modified in our laboratory for single-cell measurements. The sensor operated at a constant potential of 0.68 V versus saturated calomel electrode.

**Statistical Analysis**

The data are given as mean±SEM. ANOVA was followed by multiple pairwise comparisons according to Tukey. Null hypotheses were rejected at a level of $P<0.05$.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

**Blood Pressure and Heart Rate**

The MAP was slightly reduced in infarcted WKY (72±2 versus sham 82±6 mm Hg) and significantly decreased in infarcted SHR (100±6 versus sham 124±8 mm Hg). Sham-operated SHR showed a significantly greater MAP when compared with sham-operated WKY rats (124±8 versus 82±6 mm Hg). The heart rate remained unaltered in infarcted WKY (351±14 versus sham 344±8 bpm) and was significantly enhanced in infarcted SHR (361±21 versus sham 316±8 bpm).

**Working Heart**

Contractility (dP/dt max) was significantly decreased in infarcted WKY (2471±212 versus sham 3593±240 mm Hg/s) and in infarcted SHR (3674±312 versus sham 4860±164 mm Hg/s). In addition, contractility was significantly greater in sham-operated WKY (240±212 versus sham 359±380 mm Hg/s) and in sham-operated SHR (4863±161 mm Hg/s) when compared with sham-operated WKY (3597±246 mm Hg/s). AFI was measured by a transonic flow probe (Transonic Systems Inc) connected to a flowmeter (Transonic Systems) and related to left ventricular mass. AFI was slightly but not significantly reduced in infarcted WKY (16.4±2.7 versus sham 20.5±2.7 mL · min$^{-1}$ · g heart wet weight$^{-1}$) and significantly reduced in infarcted SHR (13.8±1.9 versus sham 23.3±1.3 mL · min$^{-1}$ · g heart wet weight$^{-1}$).

**Endothelial NO, $\text{O}_2^-$, and ONOO$^-$**

The typical amperograms (current calibrated as a concentration versus time) showing CaI (A23187)-stimulated release of NO from aortic endothelial cells are depicted in Figure 1, a and b. The rate of NO release was much faster for WKY (350±20 nmol/s) than for SHR (180±20 nmol/s). Chronic HF caused a decrease of the rates of NO release to similar ($\approx$60 nmol/s) for both rat strains. Maximally CaI-stimulated NO release from aortic endothelial cells was significantly reduced by 47% (from 478±48 to 216±16 nmol/L) in WKY with compensated HF and by 47% (from 693±131 to 257±53 nmol/L) in SHR with CHF, when compared with respective sham-operated animals (Figure 2a). The amount of diffusible NO released by single endothelial cells in the period of 15 seconds after addition of CaI was similar ($\approx$70 amol) for both WKY and SHR. This amount decreased to $\approx$20 amol for both strains after chronic CHF (Figure 2b).

Chronic HF led to an increase of CaI-stimulated $\text{O}_2^-$ production in aortic endothelial cells of both rat strains. An
increase of 50% (from 10 to 22 nmol/L) was observed in aorta of WKY with MI (Figure 3a), correlating well with the 47% decrease of NO in these animals (Figure 2a). Sham-operated SHR revealed a 6- to 7-fold higher \( \text{O}_2^- \) production (67 nmol/L) than in respective WKY. A further significant (34%) increase of \( \text{O}_2^- \) concentration to the level of 102 nmol/L was observed for SHR with CHF.

The concentration of \( \text{ONOO}^- \) was much higher in endothelial cells of sham-operated SHR than in endothelial cells of the respective WKY (Figure 3b). HF dramatically increased \( \text{ONOO}^- \) concentrations (3-fold increase for WKY as well as for SHR).

**Endothelial Function**

Endothelium-dependent relaxation in response to acetylcholine (0.1 \( \mu \)mol/L) was significantly reduced in norepinephrine-precontracted (0.1 \( \mu \)mol/L) aortic rings from infarcted animals when compared with the respective sham-operated groups (Figure 4). Acetylcholine-induced aortic relaxation of sham-operated WKY was significantly less than in the respective aortas from SHR.

**Discussion**

This study shows that pronounced aortic endothelial and cardiac dysfunction in normotensive WKY with compensated HF, and especially SHR with CHF, are associated with decreased Cal-stimulated NO release from aortic endothelial cells. Concomitantly, increased Cal-stimulated \( \text{O}_2^- \) and \( \text{ONOO}^- \) productions are observed in these cells. MI-induced reduction of bioactive NO reached similar levels in both rat strains, whereas the absolute increases in \( \text{O}_2^- \) and \( \text{ONOO}^- \) production by MI are much higher in SHR. In our study, we also observed a slightly higher peak concentration of Cal-stimulated NO release from aortic endothelial cells of sham-operated SHR than from aortic endothelial cells of the respective sham-operated groups; **P<0.05 vs respective sham-operated WKY.**

**Figure 1.** Amperograms showing differences (\( \Delta \)) in Cal (A23187)-stimulated (8 \( \mu \)mol/L) NO concentrations, released from aortic endothelial cells of (a) WKY and (b) SHR 8 weeks after MI. Upper curves, Sham-operated animals; lower curves, infarcted animals. Basal NO release: 38±6 and 17±5 nmol/L for sham-operated WKY and SHR, respectively; 20±4 and 9±3 nmol/L for infarcted WKY and SHR, respectively.

**Figure 2.** a, Peak concentrations of Cal (A23187)-stimulated (8 \( \mu \)mol/L) release of NO from aortic endothelial cells 8 weeks after MI. Open bars, Sham-operated animals (n=8); solid bars, infarcted animals (n=12). b, Amounts (amol) of NO produced by single endothelial cells (during 15 seconds) after stimulation with Cal (A23187) (8 \( \mu \)mol/L). *P<0.05 vs respective sham-operated animals.

**Figure 3.** a, Peak concentrations of Cal (A23187)-stimulated (8 \( \mu \)mol/L) production of \( \text{O}_2^- \) in aortic endothelial cells 8 weeks after MI. Open bars, Sham-operated animals (n=11); solid bars, infarcted animals (n=12). **P<0.05 vs respective sham-operated groups; ***P<0.05 vs respective infarcted WKY. b, Concentrations of Cal (A23187)-stimulated (8 \( \mu \)mol/L) production of \( \text{ONOO}^- \) in aortic endothelial cells 8 weeks after MI. Open bars, Sham-operated animals (n=7); solid bars, infarcted animals (n=12). *P<0.05 vs respective sham-operated groups; **P<0.05 vs respective sham-operated WKY; ***P<0.05 vs respective infarcted WKY.
Ca²⁺-stimulated O₂⁻ formation was significantly higher (6- to 7-fold) in endothelial cells from SHR compared to sham-operated WKY. This may be explained by a higher eNOS expression in SHR than in WKY. A compensatory enhanced aortic eNOS expression and activity in SHR, when compared with age-matched WKY,25,26 are well documented. Interestingly, enhancement of aortic eNOS expression observed 8 weeks after MI in female WKY is not associated with an improvement of aortic endothelial dysfunction.14 Thus, it can be suggested that the excess of O₂⁻, which leads to a high concentration of ONOO⁻, is an important mechanism for endothelial dysfunction in WKY with MI, and in particular for the severe CHF in infarcted SHR. However, in contrast to the peak concentrations of Ca²⁺-stimulated NO release from aortic endothelial cells, the amounts of NO produced over the time of 15 seconds by single endothelial cells were similar for both WKY and SHR. This indicates that a significant amount of NO produced by SHR is consumed by O₂⁻. As the result of this reaction, the amount of diffusible, bioactive NO does not increase/increase. Of NO produced by SHR is consumed by O₂⁻ despite a normal or even enhanced NO synthesis has been shown in endothelial dysfunction, for example, associated with hypertension,18 hypercholesterolemia,27 and high-salt diet.28 One reason for elevated endothelial O₂⁻ formation may be a dysfunctional activity of eNOS. It has been demonstrated that purified eNOS in the absence of its cofactor tetrahydrobiopterin (BH₄)29,30 or its substrate L-arginine31 generates significant quantities of O₂⁻ rather than NO. Furthermore, exogenous addition of BH₄ to purified eNOS or 5-minute incubation of aortic rings from SHR with BH₄ results in a decreased/increased O₂⁻/NO production.32,33 Several studies with cultured human endothelial cells,33 canine coronary arteries,34 or aortic rings from rats35 indicate the capability of eNOS to produce O₂⁻ under certain pathological conditions. Dysfunctional eNOS also appears to be the main source of the Ca²⁺-stimulated O₂⁻ production in aorta of prehypertensive36 and old37 SHR. Other source(s) of the increased O₂⁻ formation may be vascular smooth muscle cells.14 Furthermore, enhanced angiotensin II generation in HF38 may also be responsible for enhanced vascular O₂⁻ formation through activation of a NAD(P)H-dependent oxidase in rat aortic smooth muscle cells.39,40

Our data indicate that in chronic HF and especially in CHF, increased production of endothelium-derived O₂⁻ and ONOO⁻ appears to be a relevant mechanism for endothelial dysfunction by inactivating vasoprotective NO.

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References


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