Imbalance Between Xanthine Oxidase and Nitric Oxide Synthase Signaling Pathways Underlies Mechanoenergetic Uncoupling in the Failing Heart

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Abstract—Inhibition of xanthine oxidase (XO) in failing hearts improves cardiac efficiency by an unknown mechanism. We hypothesized that this energetic effect is due to reduced oxidative stress and critically depends on nitric oxide synthase (NOS) activity, reflecting a balance between generation of nitric oxide (NO) and reactive oxygen species. In dogs with pacing-induced heart failure (HF), ascorbate (1000 mg) mimicked the beneficial energetic effects of allopurinol, increasing both contractility and efficiency, suggesting an antioxidant mechanism. Allopurinol had no additive effect beyond that of ascorbate. Crosstalk between XO and NOS signaling was assessed. NOS inhibition with Nω-monomethyl-L-arginine (L-NMMA; 20 mg/kg) had no effect on basal contractility or efficiency in HF, but prevented the +26.2±3.5% and +66.5±17% enhancements of contractility and efficiency, respectively, observed with allopurinol alone. Similarly, improvements in contractility and energetics due to ascorbate were also inhibited by L-NMMA. Because of the observed NOS-XO crosstalk, we predicted that in normal hearts NOS inhibition would uncover a depression of energetics caused by XO activity. In normal conscious dogs, L-NMMA increased myocardial oxygen consumption (MVO₂) while lowering left ventricular external work, reducing efficiency by 31.1±3.8% (P<0.005). Lowered efficiency was reversed by XO inhibition (allopurinol, 200 mg) or by ascorbate without affecting cardiac load or systemic hemodynamics. Single-cell immunofluorescence detected XO protein in cardiac myocytes that was enhanced in HF, consistent with autocrine signaling. These data show that both NOS and XO signaling systems participate in the regulation of myocardial mechanical efficiency and that upregulation of XO relative to NOS contributes to mechanoenergetic uncoupling in heart failure. (Circ Res. 2002;90:297-304.)

Key Words: xanthine oxidase ■ oxidative stress ■ nitric oxide ■ heart failure ■ ascorbate

The failing heart displays substantial energetic inefficiency in both isolated muscle and intact chambers. This phenomenon can be best described as “mechanoenergetic uncoupling,” given that the depression of contractile force is not matched by a concomitant depression of energy consumption. Among the proposed mechanisms is enhanced oxidative stress stemming from mitochondrial and cytosolic free radical generating systems. Xanthine oxidase (XO) is prominent among these enzymes, because it produces superoxide as a byproduct of the terminal two steps of purine metabolism. XO is upregulated in failing myocardium of experimental animals and humans, and its inhibition by allopurinol improves the mechanical efficiency (the ratio of ventricular work performed and oxygen consumed) of intact failing hearts; such an effect was predicted by the initial observations that allopurinol and oxypurinol augment calcium-activated force without increasing activator Ca²⁺ in isolated cardiac muscle. To date, however, the signal transduction mechanisms of the salutary effects of XO inhibitors remain unclear.

Among the potential mechanisms to explain an energy-sparing effect of allopurinol is an interaction between oxidative stress and nitric oxide (NO). NO synthases (NOSs) are constitutively expressed in the sarcolemma and sarcoplasmic reticulum, and mitochondria of myocytes where they regulate autonomic signal transduction, calcium cy-

Original received December 28, 2000; revision received October 25, 2001; accepted December 20, 2001.

From the Department of Medicine, Cardiology Division and Institute of Molecular Cardiobiology, Johns Hopkins Medical Institutions, Baltimore, Md. E.M. owns equity in and has a consulting relationship with Paralex Inc, a company that seeks to develop xanthine oxidase inhibitors for a variety of clinical applications. The present work was not supported by Paralex, nor was E.M. a consultant for Paralex while the work was in review. The Johns Hopkins University also owns equity in Paralex Inc.

This manuscript was sent to Francois M. Abboud, Consulting Editor, for review by expert referees, editorial decision, and final disposition. Presented in part at the 73rd Scientific Sessions of the American Heart Association, New Orleans, La, November 12–15, 2000, and published in abstract form (Circulation. 2000;102[suppl II]:II-125).

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Circulation Research is available at http://www.circresaha.org DOI: 10.1161/hh0302.104531
Materials and Methods

Animals

Dilated cardiomyopathy was induced in adult mongrel dogs (n=21; Bruce Rotz, Shippensburg, Pa) weighing 25 to 30 kg with a 4-week rapid pacing protocol (210 bpm for 3 weeks and 250 bpm for 1 week), as described previously. Animals were monitored weekly to assess progression of left ventricular (LV) failure.

Instrumentation of Animals

Animals were instrumented via a left lateral thoracotomy with indwelling catheters in the descending aorta, right atrial appendage, and great cardiac vein. Endocardial sonomicrometers were inserted in the LV apex for high-fidelity recordings of LV pressure. A miniature pressure transducer (P22, Konisberg Instruments) was placed around the mid-circumflex artery (LCX artery) to measure coronary volume flow. A solid-state flow probe (4 to 5 mm) was placed around the inferior vena cava (IVC) for graded preload occlusion at baseline, every 10 minutes for protocols 1 and 2, every 15 minutes for protocol 3, and also 15 and 20 minutes after cessation of allopurinol infusion for all protocols. At the same time, blood from the coronary sinus and artery was drawn to calculate myocardial oxygen consumption (MV02) as described next.

Experimental Protocol

Studies were performed in conscious animals standing quietly in a sling. Atrial pacing (140 bpm) maintained the heart rate constant during the experiment. Three different protocols were performed on different days. Protocols were performed in both control and heart failure (HF) animals.

Protocol 1

To test the effect of allopurinol on myocardial mechanoenergetics, allopurinol (200 mg) was infused intravenously at the rate of 3.3 mL/min over 30 minutes. Hemodynamic and energetic measures were obtained every 10 minutes during the infusion followed by a 30-minute washout period.

Protocol 2

To test the effect of the antioxidant ascorbic acid (vitamin C) on myocardial mechanoenergetics and to evaluate whether vitamin C was additive or synergistic with allopurinol, vitamin C (1000 mg) was infused intravenously at the rate 3.3 mL/min over a 30-minute period, followed by allopurinol at the same infusion rate and concentration as protocol 1 for an additional 30 minutes.

Protocol 3

To test for an interaction between XO and NOS signaling pathways, L-NMMA (20 mg/kg) was infused (1.5 mL/min over 60 minutes), followed by allopurinol (200 mg, 3.3 mL/min for 30 minutes).

In all protocols, pressure-dimension relationships and arterial pressure response were recorded in the steady state and during IVC occlusion at baseline, every 10 minutes for protocols 1 and 2, every 15 minutes for protocol 3, and also 15 and 20 minutes after cessation of allopurinol infusion for all protocols. At the same time, blood from the coronary sinus and artery was drawn to calculate myocardial oxygen consumption (MV02) as described next.

Hemodynamic and Energetic Measurements

Pressure-dimension data were recorded at steady state and during transient IVC occlusion as previously described. Myocardial contractility and/or work was indexed by maximal +dP/dt, SW (stroke work), Ees (ventricular elastance, slope of the end-systolic pressure-dimension relationship), and PRSW (preload recruitable stroke work). Preload was analyzed as end-diastolic dimension and pressure, and afterload was evaluated as effective arterial elastance (Ea), the ratio of LV systolic pressure to stroke dimension. Hemodynamic pressure-dimension data were digitized at 200 Hz and stored for subsequent analysis on a personal computer using custom software. MV02 per cardiac cycle was calculated from the arteriovenous difference of oxygen saturation (AV02) in simultaneously sampled coronary sinus and aortic blood multiplied by left circumflex coronary flow and divided by heart rate. Cardiac mechanical efficiency was calculated as the SW/MV02 ratio.

Immunofluorescent Microscopy

Isolated myocytes obtained from control and HF dogs (n=2 each) were attached to coverslips with laminin, fixed in 50% methanol/50% acetone, and incubated overnight with a monoclonal antibody to XO (Neo-Markers Laboratory). Secondary incubation using anti-rabbit Alexa 488 (Molecular Probes) and imaging with a Nikon E600FN upright physiological fluorescence microscope with a Bio-Rad MRC-1024/2-P multiphoton imaging system was performed as described.

Data Analysis

All results are reported as mean±SEM. Baseline hemodynamic and energetic variables before and after heart failure were compared using paired Student’s t tests. For statistical analysis of hemodynamic concentration-effect relationships and mechanoenergetics data, we used 2-way ANOVA with an identification term for individual experiment followed by the Student-Neuman-Keuls post hoc test. Differences were considered significant at P<0.05.

Results

Effect of Sustained Pacing-Induced HF on Myocardial Mechanoenergetics

Figure 1 depicts the course of reduced mechanical efficiency in HF, which resulted almost entirely from decreased SW (Figure 1A). MV02 (Figure 1B) was essentially unchanged during the evolution of HF, with a nonsignificant downward trend late in the pacing protocol. SW/MV02 progressively decreased by a maximum of 64.5±7.6% (P<0.001) after 4 weeks of pacing (Figure 1C).

Effect of Vitamin C and Allopurinol on Contractility and Energetics in HF

We have previously shown that XO inhibition with allopurinol improves myocardial mechanical efficiency in dogs with pacing-induced HF. To test whether the response to allopurinol...
nol could be mimicked by an alternative antioxidant, implicating oxidative stress as a mechanism of action, we administered the free radical scavenger ascorbic acid to HF dogs. This agent stimulated myocardial contractility and efficiency in a manner similar to allopurinol (Figure 2). Moreover, when administered after the infusion of vitamin C, allopurinol was unable to further augment contractility or efficiency, suggesting that these agents worked by a shared mechanism (Figure 2).

**Effects of L-NMMA, Allopurinol, and Ascorbate on Myocardial Contractility and Energetics in HF**

We assessed the interaction between L-NMMA, ascorbate, and allopurinol after the induction of HF. To test whether the favorable effect of ascorbate required intact NOS signaling, we infused L-NMMA before ascorbate in a separate series of animals (n=5). L-NMMA alone had no effect on SW or MV\textsuperscript{O}_2 but prevented improvement in both contractility and energetics as a result of ascorbate (Figure 2). In addition, allopurinol coinfused with ascorbate failed to increase SW/MV\textsuperscript{O}_2 in the presence of L-NMMA (data not shown).

We next examined whether the energetic effect of allopurinol in HF reflected crosstalk with NO signaling. Consistent with our previous findings,\textsuperscript{3} allopurinol stimulated myocardial contractility, as manifested by a leftward shift in Ees (Figure 3A and Table 1), and augmented SW/MV\textsuperscript{O}_2 by 66.5±17% (P<0.001 versus baseline). Pretreatment with L-NMMA before allopurinol coinfusion prevented both the positive inotropic effect (Figure 3B and Table 1) and the reduction in MV\textsuperscript{O}_2 associated with subsequent allopurinol infusion. Thus, improvement of chamber efficiency due to allopurinol was completely abrogated by L-NMMA pretreatment and coinfusion (Figure 3C).

**Effect of L-NMMA and Allopurinol on Contractility and Energetics in Normal Hearts**

As previously shown, allopurinol does not enhance cardiac efficiency in normal hearts.\textsuperscript{3} To further explore NOS-XO crosstalk in myocardium, we tested the prediction that NOS...
inhibition in normal hearts might sufficiently reduce NOS activity relative to that of XO to uncover an XO-dependent depression of energetics. Figure 4A and Table 2 depict the response to L-NMMA (20 mg/kg over 1 hour, n=11) and allopurinol coinfusion on myocardial performance in control animals. As previously demonstrated,18,25 L-NMMA had minimal effects on myocardial contractility (not influencing Ees while slightly decreasing peak +dP/dt) but increased cardiac afterload (Ea) by 44.4±6.5% (P<0.001). L-NMMA increased MVO₂ (24.6±8.8%; P<0.05 versus baseline) and decreased SW, thereby reducing SW/MVO₂ by 31.1±4% (P<0.001 versus baseline). Thus, NOS inhibition by L-NMMA caused changes in myocardial efficiency that partially resembled those observed with the development of HF (Table 2).

Unlike basal conditions, allopurinol coadministered with L-NMMA now increased SW and reduced MVO₂ to baseline (6.57±0.84 to 5.84±0.65 µL O₂/beat, −11±2%, P<0.05), restoring myocardial efficiency toward normal (Figure 4B). This response was not due to changes in ventricular load, because allopurinol did not affect indices of preload or afterload (Table 2). In additional studies (n=5), ascorbate was infused after L-NMMA. As depicted in Table 2, ascorbate restored SW and MVO₂ to baseline in a manner similar to allopurinol—the net result being a reversal of the reduction in SW/MVO₂ due to L-NMMA (Figure 4C). After vitamin C infusion, allopurinol had no additional effect on myocardial efficiency. Thus, in control animals, NOS inhibition depressed myocardial efficiency in a manner reversible by allopurinol, suggesting that, in the absence of physiological NO signaling, XO is capable of depressing cardiac efficiency. Thus, there are important interactions between these two signaling enzymes in regulating cardiac energy metabolism.

| TABLE 1. Effect of Allopurinol Alone and During Coinfusion With L-NMMA in Heart Failure |
|----------------------------------------|--------------------------------|
|                                       | Allopurinol Alone | Allopurinol+L-NMMA |
| Ea, mm Hg/mm                          | −10±5.2          | 1.7±4.2           |
| EDD, mm                               | 1.9±0.8          | 1±0.5             |
| Ees, mm Hg/mm                         | 26.2±3.5†        | −6.8±7.5          |
| SW, mm Hg · mm                       | 29.5±7.5†        | −10±5.3           |
| SW/MVO₂                               | 66.5±17†         | −14.4±5.5         |

Ea indicates arterial elastance; EDD, end-diastolic dimension; Ees, ventricular elastance; SW, stroke work; and SW/MVO₂, myocardial efficiency. Data are expressed as percent change from baseline; †SEM.

P<0.001 vs baseline; †P<0.05 vs allopurinol+L-NMMA.

**Immunofluorescent Microscopy**

XO activity has been previously detected in the heart,3 but this has yet to be established to reside within cardiac myocytes. Accordingly, we imaged isolated cardiac myocytes using laser fluorescence microscopy. Figure 5 shows images of normal and failing canine myocytes labeled with anti–XO

![Figure 3](image_url) **Figure 3.** Contractile and energetic response to NOS and XO inhibition in HF. Representative pressure-dimension data from animals receiving allopurinol alone (A) or allopurinol after L-NMMA (B). Steady-state and IVC occlusion data depicted as in Figure 2. Allopurinol increased Ees, but coinfusion of L-NMMA prevented this response (B). Allopurinol (solid circles) increased myocardial efficiency (SW/MVO₂), and this response was prevented by L-NMMA pretreatment (open circles) (C). *P<0.05 vs baseline.

![Figure 4](image_url) **Figure 4.** Energetic response to NOS inhibition and allopurinol or ascorbate in control dogs. Representative pressure-dimension loop depicting the effect of L-NMMA and allopurinol/L-NMMA coinfusion (A). Shown are baseline steady-state pressure-dimension data under each condition and end-systolic pressure-dimension relationship (Ees) obtained from graded IVC occlusion. L-NMMA did not affect ventricular elastance (Ees) but increased arterial elastance (Ea). Subsequent coinfusion of allopurinol did not reduce Ea. B and C, Myocardial mechanical efficiency (SW/MVO₂) after infusion of L-NMMA and allopurinol coinfusion (B) and after vitamin C and vitamin C plus allopurinol (†) coinfusion (C). L-NMMA decreased SW/MVO₂, and both allopurinol and vitamin C coinfusion restored this toward baseline. *P<0.05 vs baseline; †P<0.05 vs L-NMMA.
antibody. In normal cells (Figures 5A and 5B), XO localized principally to the nucleus, with lesser amounts in the cytosol. In contrast, failing myocytes (Figures 5C and 5D) exhibited substantially larger quantities of XO, and the signal was distributed evenly throughout the cytoplasm. These data support the idea that XO abundance is elevated in HF, providing a mechanism for disease-specific augmentation of XO-derived oxygen free radical production.

**Discussion**

Inhibition of XO enhances myocardial chamber efficiency in a load-independent manner in failing hearts. The present study made three novel observations regarding the mechanism of this effect: (1) ascorbic acid mimics the ability of allopurinol to improve HF mechanical efficiency; (2) the improvements of myocardial efficiency resulting from XO inhibition and the free radical scavenger ascorbate in HF depend on intact cardiac NO signaling; and (3) NOS inhibition in normal animals depresses mechanical efficiency in a manner reversible by XO inhibition and ascorbate. Taken together, these findings demonstrate crosstalk between NOS and XO pathways in the regulation of myocardial energetics and demonstrate that optimization of cardiac energy metabolism (and/or its coupling to force production) may be disrupted by either increased XO pathway activity or decreased NOS pathway activity. The increase in XO activity seems to be the predominant factor operative in the pathophysiology of heart failure.

**TABLE 2. Effect of L-NMMA, Allopurinol/L-NMMA, and Ascorbate/L-NMMA on Myocardial Mechanoenergetics in Control Dogs**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>L-NMMA (% Change)</th>
<th>L-NMMA+ Allopurinol (% Change)</th>
<th>L-NMMA+ Ascorbate (% Change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ea, mm Hg/mm</td>
<td>20.6±2.1</td>
<td>44.4±6.5*</td>
<td>39.7±5.8*</td>
<td>34.9±6.2*</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>33.2±1.2</td>
<td>−2.9±2.1</td>
<td>−0.7±1.0</td>
<td>2.4±1.0</td>
</tr>
<tr>
<td>Ees, mm Hg/mm</td>
<td>11.9±1.4</td>
<td>19.1±13.0</td>
<td>17.4±2.9*</td>
<td>12.1±28.3</td>
</tr>
<tr>
<td>D0, mm</td>
<td>13.9±1.4</td>
<td>9.3±8.1</td>
<td>3.8±2.6</td>
<td>11.5±18.0</td>
</tr>
<tr>
<td>SW, mm Hg · mm</td>
<td>955±112</td>
<td>−18.0±3.3*</td>
<td>−4.3±2.5†</td>
<td>−1.6±4.4†</td>
</tr>
<tr>
<td>SW/MVO₂</td>
<td>1.23±0.09</td>
<td>−31.1±3.8*</td>
<td>−7.4±6.7†</td>
<td>−11.2±5.3†</td>
</tr>
<tr>
<td>(dP/dt)_{max}, mm Hg/s</td>
<td>3220±188</td>
<td>−12.3±3.3*</td>
<td>−3.3±2.7†</td>
<td>−6.0±8.8†</td>
</tr>
</tbody>
</table>

Data for baseline are in absolute values and for interventions in percent change. Abbreviations are as in Table 1. D₀ is the x-axis intercept of Ees. (dP/dt)_{max} is the maximum rate of LV pressure generation during systole.

*P<0.005 vs baseline; †P<0.05 vs L-NMMA alone.

**Figure 5.** XO immunofluorescence of isolated myocytes. Depicted are images of myocytes with (B and D) and without (A and C) laser fluorescence from normal and HF dogs. In normal myocytes, immunofluorescence localizes principally to cell nucleus with minimal cytoplasmic staining (B). HF myocytes exhibit substantially larger immunofluorescence in the cytoplasm, consistent with XO upregulation (D).
Energetic Effects of Allopurinol

We and others have previously demonstrated that allopurinol improves cardiac mechanical efficiency and β-adrenergic inotropy in dogs with pacing-induced HF and that these animals exhibit a 4-fold increase in myocardial XO activity. Because XO generates superoxide as a byproduct of purine metabolism, we sought to implicate reduced oxidative stress as a mechanism of action for allopurinol. Accordingly, we administered ascorbic acid, an agent that quenches ·O2−, and the finding that ascorbic acid mimicked the effect of allopurinol and that subsequent coinfusion of allopurinol and ascorbic acid exerted no additional effect is consistent with the idea that allopurinol is acting as an antioxidant agent.

Oxidative stress has been implicated in the pathogenesis of HF largely through demonstrations of elevated markers of oxidative stress in peripheral blood, myocardium, or pericardial fluid of experimental animals or patients. The implications of an imbalance between free radical production and endogenous antioxidant systems are only now being recognized and comprise two general mechanisms: tissue damage or disruption of signaling pathways. The present findings suggest that interference with signaling cascades may be acutely reversible by administration of antioxidant agents and lend additional support to the notion that XO inhibitors may have therapeutic potential in HF.

Role of NO in Myocardial Mechanoenergetics

To explore mechanisms for the favorable energetic response to allopurinol in HF, we hypothesized that there may be a crosstalk between XO and NOS signaling in myocardial energy metabolism. Our finding that NOS inhibition blocked the energetic and inotropic effects of allopurinol strongly supports this notion. This scenario is consistent with an increase in XO relative to NOS activity in HF, an observation that is supported by our present and previously reported biochemical studies.

An interaction between NOS and XO activity was apparent in normal myocardium as well. In control animals, NOS inhibition increased MVO2 and reduced mechanical efficiency. This effect was reversed by subsequent XO inhibition or ascorbate, both of which restored MVO2 to normal and increased efficiency without any changes in ventricular load. Thus, under physiological conditions in the normal heart, inhibition of NOS unleashes an impairment in mechanoenergetic coupling that is likely due to free radical production by XO within myocardium. Optimal efficiency in the myocardium may depend on an appropriate balance between NO and ·O2− and either decreasing NO (using NOS inhibition in control animals) or increasing XO (observed in HF) can depress efficiency.

NO is produced in the heart by NO synthases in at least three subcellular locations: sarcolemmal membranes, mitochondria, and sarcoplasmic reticulum (SR). NO likely acts as a modulator of myocardial energetics, influencing both excitation-contraction coupling and mitochondrial energy production. In myocardium, there are at least two well-established NO-related signaling pathways: guanylyl cyclase–dependent cGMP production and redox-influenced nitrosylation reactions. With regard to the latter, nitrosylation of thiol residues of the ryanodine receptor regulates SR calcium release in a manner that is disrupted by oxidative stress.

NO may influence energetics both at the SR and the mitochondria. In the former, NOS activity has been shown to augment SR calcium-release channel activity via protein nitrosylation in a manner regulated by oxidative stress. Oxidative stress likely promotes maximal channel activity, reducing the ability of NO to exert feedback regulation of SR calcium release. Such a situation is likely to lead to “futile” calcium cycling, which increases ATP expenditure. NO may additionally influence SR Ca2+–ATPase activity; however, this remains controversial with studies demonstrating contradictory results. NO also exerts physiological inhibition of mitochondrial respiratory complex function that can become pathophysiological in situations of excess NO production or oxidative stress. It is also conceivable that oxidant signaling may directly regulate crossbridge cycling kinetics, thereby modulating the efficiency of contraction; however, this possibility has yet to be tested. To the extent that NO and oxidative modulation of proteins via thiol nitrosylation/oxidation is a general phenomenon, many potential proteins involved in cardiac energetics have the potential to be influenced by NOS and/or XO signaling.

Whether NO production is elevated or reduced in HF remains controversial with studies demonstrating increased influences of NO on β-adrenergic inotropy on the one hand and reduced regulation of oxygen consumption on the other hand. We and others have previously demonstrated that NOS abundance and in vitro enzymatic activity (arginine-to-citrulline conversion assay) are not changed in...
ventricular tissue from dogs with pacing-induced HF. Accordingly, it is unlikely that XO activity directly modulates the activity of NOS per se but influences NO signaling at more downstream sites of action. Our present study further supports intact NO signaling in failing dog myocardium and implicates oxidative stress as a primary cause of downstream NO inactivation.

This study is limited by the lack of direct measurement of myocardial NO and ROS production. Thus, the precise interaction of ROS and NO in myocardium operative in the regulation of energetics remains to be elucidated, and different potential scenarios must be considered (Figure 6). First, O$_2^-$ has the potential to inhibit NO signaling either by reacting with NO to produce other oxidant species such as peroxynitrite or by directly interfering with NOS enzyme activity per se. Second, excess ROS may directly interfere with proteins involved in cardiac efficiency (eg, mitochondrial respiration or SR Ca$^{2+}$ cycling). Under this scenario, NO may modulate -O$_2^-$ actions by exerting an antioxidant effect. In either case, the present findings are consistent with the theory that there is a continuum of biological activity spanning physiological signaling and toxicity that is based, at least in part, on relative rates of NO and -O$_2^-$ production.$^{27,49}$

**XO: Function, Localization, and Distribution in HF**

To assess whether XO upregulation occurs within cardiac myocytes, we performed confocal staining of isolated myocytes. These studies demonstrated diffuse cytoplasmic upregulation of XO in HF. The presence of XO within cardiac myocytes, as previously shown for NOS1 and NOS3, provides for the possibility of autocrine regulation of key processes of these cells. Xanthine oxidoreductase exists in two forms, XDH and XO, that are the product of a single gene,$^7$ both of which have potential to produce O$_2^-$. XDH uses NAD as its preferred election acceptor under physiological conditions, whereas XO uses molecular oxygen and is a more potent source of superoxide.$^{27,50}$

In summary, the present study was undertaken to elucidate the nature of the profound and unique ability of XO inhibitors to improve myocardial energetics in HF. Vitamin C mimicked the response to allopurinol, strongly supporting the predicted antioxidant mechanism of action. NOS-XO crosstalk important to the regulation of myocardial energetics was demonstrated in both normal and failing myocardium. In normal hearts, XO effects on energetics could be elicited by NOS inhibition. In HF, inhibition of XO (which is upregulated relative to NOS) only improves energetics when NOS signaling is intact. These findings have biological implications for the role of oxidative stress in myocardial energetic regulation; equally important are the clinical implications for the use of XO inhibitors as a novel therapeutic strategy to reverse the mechanoenergetic uncoupling of HF.

**Acknowledgments**

This work was supported by NIH grants K08 HL-03238 and RO1 HL-65455 (J.M.H.) and by a Specialized Center of Research Grant (P50 HL52307; principal investigator, E.M.) from the National Heart, Lung, and Blood Institute. J.M.H. is the recipient of a Paul Beeson Physician Faculty Scholars in Aging Research Award. E.M. holds the Michel Mirowski, MD Professorship in Cardiology of the Johns Hopkins University.

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Circ Res. 2002;90:297-304; originally published online January 3, 2002;
doi: 10.1161/hh0302.104531

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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