Intravenous Allopurinol Decreases Myocardial Oxygen Consumption and Increases Mechanical Efficiency in Dogs With Pacing-Induced Heart Failure

Ulf E.G. Ekelund, Robert W. Harrison, Ori Shokek, Rajiv N. Thakkar, Richard S. Tunin, Hideaki Senzaki, David A. Kass, Eduardo Marbán, Joshua M. Hare

Abstract—Allopurinol, an inhibitor of xanthine oxidase, increases myofilament calcium responsiveness and blunts calcium cycling in isolated cardiac muscle. We sought to extend these observations to conscious dogs with and without pacing-induced heart failure and tested the prediction that allopurinol would have a positive inotropic effect without increasing energy expenditure, thereby increasing mechanical efficiency. In control dogs (n=10), allopurinol (200 mg IV) caused a small positive inotropic effect; (dP/dt)max increased from 3103±162 to 3373±225 mm Hg/s (+8.3±3.2%; P=0.01), but preload-recruitable stroke work and ventricular elastance did not change. In heart failure (n=5), this effect was larger; (dP/dt)max rose from 1602±190 to 1988±251 mm Hg/s (+24.4±8.7%; P=0.03), preload-recruitable stroke work increased from 55.8±9.1 to 84.9±12.2 mm Hg (+28.1±5.3%; P=0.02), and ventricular elastance rose from 6.0±1.6 to 10.5±2.2 mm Hg/mm (P=0.03). Allopurinol did not affect myocardial lusitropic properties either in control or heart failure dogs. In heart failure dogs, but not controls, allopurinol decreased myocardial oxygen consumption (~49±4.6%; P=0.002) and substantially increased mechanical efficiency (stroke work/myocardial oxygen consumption; +122±42%; P=0.04). Moreover, xanthine oxidase activity was ~4-fold increased in failing versus control dog hearts (387±125 versus 78±72 pmol/min · mg⁻¹; P=0.04) but was not detectable in plasma. These data indicate that allopurinol possesses unique inotropic properties, increasing myocardial contractility while simultaneously reducing cardiac energy requirements. The resultant boost in myocardial contractile efficiency may prove beneficial in the treatment of congestive heart failure. (Circ Res. 1999;85:437-445.)

Key Words: myocardial contractility ■ xanthine oxidase ■ oxidant stress ■ heart failure

Drugs that reverse neurohormonal activation and/or vasoconstriction have proven successful in the treatment of heart failure.1 On the other hand, drugs that increase myocardial contractility tend to worsen patient survival rates.2 Positive inotropic agents increase contractility and increase cardiac energy consumption and may therefore worsen already decreased mechanical and energetic efficiency (oxygen cost of contractility)3,4 of the failing heart. As reduced energy reserve has been theorized to contribute to progression of heart failure,5,6 a drug that improves the relation between contractility and energy expenditure has the potential to benefit heart failure patients. Agents that sensitize the myofilament apparatus to Ca²⁺ hold promise as a class of drugs that may cause cardiac myocytes to generate more force for a given amount of cytoplasmic free Ca²⁺.6 Abnormalities in myocyte force–Ca²⁺ relationships that occur in heart failure7,8 lend further support for the use of such drugs.

Oxidative stress may contribute to pathological Ca²⁺ handling in heart failure by mechanisms that involve either damage9 or functional modification of proteins.10,11 Oxygen free radicals, including O₂⁻, H₂O₂, OH, and peroxynitrite, damage cardiac myocytes and induce endothelial dysfunction12 by initiating cell membrane lipid peroxidation, amino acid oxidation, and polypeptide degradation.9 Xanthine oxidase (XO), an enzyme that forms O₂⁻ in the catalysis of the terminal steps in purine metabolism, has been implicated as a pathogenic factor in heart failure and reperfusion injury.13,14 XO is a molybdenum-containing enzyme found both in the cytoplasm and bound to the plasma membrane of endothelial cells,15 as well as circulating in the plasma.16,17 In most mammals, including humans, XO activity is found primarily in the liver and intestine, from which it may be released into the circulation and affect remote organs such as the heart.18 Additionally, despite wide species variation, XO is also found in most mammalian myocardium.19–21

The observation of hyperuricemia in patients with decompensated valvular disease22 suggests that XO activity in humans with heart failure may be increased. Moreover, Pérez
et al.\(^2\) have recently demonstrated that the XO inhibitor allopurinol increases responsiveness to Ca\(^{2+}\) in normal and stunned trabeculae from rat heart. During twitch contractions in muscle that had been exposed to allopurinol, Ca\(^{2+}\) transients were smaller, but force greater, than in controls. Both allopurinol and oxyurin, its active metabolite, increased the maximal force that could be generated by the myofilaments. However, the Ca\(^{2+}\) concentration range over which force was activated was not altered. These properties suggested that such compounds have the potential to boost cardiac contractility without increasing oxygen consumption. In addition, the lack of a shift in the range of calcium activation would minimize the risk for increased force at diastolic Ca\(^{2+}\) levels and, hence, for diastolic dysfunction.

The aims of the present study were to determine the hemodynamic and energetic effects of intravenous allopurinol in dogs both at baseline and after induction of heart failure by rapid pacing and to analyze the activity of XO in normal and failing hearts. We tested the following hypotheses: (1) that allopurinol has a positive inotropic effect associated with improved cardiac efficiency, and (2) that XO activity is increased in heart failure.

**Materials and Methods**

**Reagents**

Allopurinol, XO, xanthine, phenylmethylsulfonyl fluoride (PMSF), and DTT were purchased from Sigma.

**Surgical Preparation for Chronic Dog Protocol**

Male mongrel dogs (20 to 30 kg) were anesthetized with 1% to 2% halothane after induction with sodium pentothal. The chest was opened via a lateral thoracotomy, and indwelling catheters (Tygon; Norton Plastics and Synthetic Division) were secured in the right atrium (for drug infusion) and in the descending aorta (for pressure measurement). An indwelling high-fidelity micromanometer (P22, Konigsberg Instruments) was placed in the left ventricle (LV) through an apical stab. Endocardial sonomicrometer crystals (Konigsberg Instruments) was placed in the left ventricle (LV) for measurement of anterior-posterior short-axis dimension (sonomicrometry to short-axis dimension). An indwelling high-fidelity micromanometer (P22, Konigsberg Instruments) was placed in the left ventricle (LV) through an apical stab. Endocardial sonomicrometer crystals were inserted for the measurement of anterior-posterior short-axis dimension, and a pneumatic occluder was placed around the inferior vena cava (IVC) to allow preload reduction so that LV pressure-dimension relationships and the arterial pressure response were recorded in the steady state and during IVC occlusion at baseline, every 10 minutes during infusion, and 10 and 20 minutes after cessation of the infusion. The ECG was continuously monitored.

**Catheterization for the Measurement of Cardiac Oxygen Consumption**

Experiments to analyze the impact of allopurinol on cardiac energetics were performed under isoflurane anesthesia (1.5 to 2.5%), after induction with sodium pentothal (25 mg/kg), in normal and heart failure dogs. Isoflurane was chosen as the anesthetic because of its relatively mild and stable effect on the cardiovascular system.\(^2\)–\(^7\) A Doppler flow velocimeter (0.014, Cardiomediics) and a 6F angiography catheter (AL-1 or JL 3.5, Cordis Laboratories, Inc.) were inserted through an 8F sheath (Cordis) in the right femoral artery and advanced to the left circumflex coronary artery. These catheters permitted measurement of coronary flow and injection of contrast for the measurement of coronary diameter. A catheter (AZ multipurpose, 6F) was advanced from the left external jugular vein via a 7F sheath (Cordis) into the great cardiac vein for withdrawal of mixed coronary venous blood. To measure cardiac oxygen consumption, blood samples from the coronary sinus and the femoral artery were obtained simultaneously. At each time point, blood flow velocity in the left circumflex artery was measured, and coronary angiography was performed.

In 4 of 11 experiments, preexisting indwelling sonomicrometer crystals, Konigsberg micromanometers, and IVC occluders (see above) were used for dimension and pressure measurements and for acute preload reduction. In the other 7 experiments, a combined micromanometer-conductance catheter (Millar) was advanced to the LV and positioned for continuous measurement of LV pressure and volume via a 7F sheath (Cordis) in the femoral artery. A Swan-Ganz catheter (Arrow, 7F) was advanced via a 9F sheath in the femoral vein to the pulmonary artery for measurement of cardiac output and for hypertonic saline wash-in to calibrate the volume signal.\(^8\)–\(^9\) This catheter was then replaced with a balloon occlusion catheter (Cordis) positioned in the IVC for acute preload reduction for pressure-volume analysis.

**Dobutamine Protocol**

In 5 of the 6 dogs undergoing assessment of energetics after heart failure, dobutamine 10 \(\mu\)g/kg \(\times\) min \(^{-1}\) was also infused. This was done to compare the energetic consequences (oxygen cost for increasing myocardial contractility) between allopurinol and a \(\beta\)-adrenergic agonist. This study was performed on a separate day.

**Hemodynamic and Energetic Data Analysis**

The analysis of pressure-dimension relationships allowed the evaluation of variables related to myocardial systolic and diastolic performance. Short-axis dimension has been validated as an index of LV volume.\(^30\) To establish whether heart failure remodeling would invalidate a relationship between short-axis dimension and total chamber volume, we compared LV volume derived from 3 orthogonal axes assessed by sonomicrometry to short-axis dimension (n=5). The correlation obtained by multiple linear regression was excellent both in the control (\(r=0.94, P<0.0001\)) and heart failure (\(r=0.97, P<0.0001\)) states (data kindly provided by Chi-Ping...
Cheng, Wake Forest University School of Medicine, Winston-Salem, NC).

Averaged data from 10 to 20 consecutive beats were used to derive steady-state parameters, and data measured during transient unloading of the heart by occlusion of the IVC was used to assess pressure-dimension or pressure-volume relations. Preload was indexed as the left ventricular end-diastolic anterior-posterior short-axis dimension (LVEDD) or the LV end-diastolic volume from the conductance catheter. Afterload was evaluated as effective arterial elastance (Ea, ratio of LV systolic pressure to stroke dimension).31,32 This parameter is not preload-dependent and has been validated to reflect total afterload, which incorporates systemic vascular resistance, aortic impedance, and the reflected wave properties of the vasculature. Contractility was indexed by peak +dP/dt and the load-independent parameters preload-recruitable stroke work (PRSW; slope of stroke work [SW]/end-diastolic dimension relation), and ventricular elastance (Ees; slope of the end-systolic pressure dimension or volume relation).31,33,34 Diastolic performance was measured by peak –dP/dt, time to peak filling rate (tpf), and time constant of relaxation (tau).35

Myocardial oxygen consumption per unit time (MV O2) in the left circumflex artery territory was calculated from the difference in arteriovenous oxygen saturation (AV O2) in simultaneously sampled coronary sinus and aortic blood, multiplied by left circumflex coronary blood flow. This, in turn, was calculated from flow velocity multiplied by left circumflex diameter. Left circumflex diameter was analyzed from a film projector (CAP 35B, Angiogram Projection System) using quantitative angiography (Cath View, version 1.36, Image Conn Systems). The external useful work of the LV was indexed as SW (area of pressure-volume loops). Both SW and MV O2 were converted to J/beat.36 Cardiac mechanical efficiency was calculated as SW/MV O2.

Hemodynamic pressure-dimension data were digitized at 200 Hz and stored for subsequent analysis on a personal computer using customized software.

Analysis of XO Activity

Myocardial tissue samples were obtained from additional animals (n=12) immediately after euthanization with intravenous KCl. The analysis was also performed in 2 dogs that had received allopurinol on the same day. Samples were immediately frozen in liquid nitrogen and stored at –80°C for analysis of XO activity. The analysis was performed using a modification of the procedure of Xia and Zweier.9 Frozen tissue samples were ground and homogenized in a potassium phosphate buffer, pH 7.8, containing 1 mmol/L PMSF and DTT. The reaction mixture contained 0.1 mL of effluent, in 50 mmol/L phosphate buffer containing PMSF and DTT, and 0.15 mmol/L xanthine in a 1-mL cuvette at room temperature. Similar analyses were performed using plasma from control (n=10) and heart failure (n=5) dogs that had not received allopurinol treatment. The extinction coefficient for the 295-nm absorbance of uric acid is 21,000.

Data Analysis

All results are reported as mean±SEM. Baseline hemodynamic variables before and after the 4-week pacing protocol were compared using the Student t test or the Kruskal-Wallis test, as appropriate. Concentration-effect relationships were analyzed with a 2-way ANOVA using a term for individual experiment. To analyze shifts in slope or position of the PRSW relation (SW versus end-diastolic dimension), we compared SW-dimension data by multiple linear regression with an interaction term for drug effect. Similar analysis was performed for Ees (slope of the end-systolic pressure dimension

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Hemodynamic Data in Control and Heart Failure Dogs</th>
<th>Control (n=10)</th>
<th>Heart Failure (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>138±1.8</td>
<td>141±0.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>124±5</td>
<td>111±7</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>11.2±2.1</td>
<td>23.5±5.2*</td>
</tr>
<tr>
<td>Stroke dimension, mm</td>
<td>7.9±0.3</td>
<td>4.8±0.9†</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>35.2±1.4</td>
<td>36.9±2.6</td>
</tr>
<tr>
<td>(dP/dt)max, mm Hg/s</td>
<td>3103±162</td>
<td>1602±190†</td>
</tr>
<tr>
<td>PRSW, mm Hg</td>
<td>81.3±4.3</td>
<td>55.8±9.1*</td>
</tr>
<tr>
<td>Ea, mm Hg/mm</td>
<td>15.2±0.8</td>
<td>27.7±9.3</td>
</tr>
<tr>
<td>tau, ms</td>
<td>32.2±1.1</td>
<td>63.6±21.4*</td>
</tr>
<tr>
<td>(dP/dt)max, mm Hg/s</td>
<td>−2520±130</td>
<td>−1767±281*</td>
</tr>
<tr>
<td>Ees, mm Hg/mm</td>
<td>10.2±1.2</td>
<td>6.0±1.6*</td>
</tr>
<tr>
<td>Do, mm</td>
<td>16.2±1.6</td>
<td>18.4±3.4</td>
</tr>
</tbody>
</table>

HR indicates heart rate; SBP, systolic blood pressure; (dP/dt)max, peak rate of rise in LV pressure; tau, time constant of LV relaxation; (dP/dt)min, peak rate of decrease in LV pressure; Do, intercept of Ees with the dimension axis.

*P<0.05, † P<0.001 vs normal dogs.

Results

Effects of Allopurinol on Left Ventricular Performance in Conscious Dogs

Table 1 summarizes baseline hemodynamic variables in conscious dogs before and after the induction of heart failure. Chronic pacing for 4 weeks resulted in a syndrome of heart failure characterized by decreased contractility, as evidenced by decreased (dP/dt)max, PRSW, and Ees, and elevated LVEDP. Overall impaired cardiovascular performance was evidenced by a decrease in stroke dimension.

Figure 1 shows the time course of the allopurinol-induced changes in LV contractility in conscious dogs before and after pacing-induced heart failure. In control dogs (n=10), allopurinol increased (dP/dt)max (Figure 1A) from a baseline value of 3103±162 to 3373±225 mm Hg/s (+8.3±3.2%, P=0.01) at the peak response, which occurred 10 minutes after the end of the infusion. The positive inotropic effects of allopurinol persisted and, in some cases, continued to rise, with values not completely returning to baseline in 20 minutes. However, neither PRSW (Figure 1B) nor the slope or position of the end-systolic pressure dimension relation (data not shown) was significantly changed. There were no changes in preload as measured by LVEDP or EDD, afterload as measured by Ea, or vascular-ventricular coupling as assessed by Ea/Ees (Table 2).

After induction of heart failure (n=5), the effect of allopurinol on contractility was much more dramatic than in the control dogs. (dP/dt)max (Figure 1A) increased from 1602±190 to 1988±251 mm Hg/s (+24.4±8.7%; P=0.03 and P=0.05 versus increase in control dogs). Moreover, PRSW (Figure 1B and 1C) increased from 55.8±9.1 to 84.9±12.2 mm Hg (+28.1±5.3%; P=0.02 and P=0.004...
The effects of allopurinol on myocardial contractility in conscious dogs. Allopurinol was infused centrally in conscious dogs before and after induction of heart failure. In the control state, allopurinol did not change the PRSW relation, whereas after induction of heart failure, allopurinol produced a positive inotropic effect, reflected as a leftward shift in the relationship in 3 of the 4 experiments. In control dogs, there were no significant changes (Table 2) in preload (LVEDP, LVEDD), afterload (Ea), or Ea/Ees ratio.

Figure 1C shows the pattern of response to allopurinol with representative SW—end-diastolic dimension data from a conscious dog before and after the induction of heart failure. The agent did not affect the slope of the PRSW relation in the control state but increased the slope and shifted the relation leftward after heart failure, indicating a positive inotropic effect. Taken together, these findings indicate that allopurinol has a positive inotropic effect in chronically instrumented conscious dogs, and that this effect is greater in the failing heart.

Effects of Allopurinol on Diastolic Performance and Heart Rate in Conscious Dogs

The lusitropic effects of allopurinol in the conscious dog LV were evaluated using LVEDP, tau, (dP/dt)$_{max}$ and ttpf (Tables 2 and 3). In both normal and heart failure dogs, there was no change in either LVEDP or lusitropic indices due to allopurinol. To evaluate the heart rate response to allopurinol in the conscious state, the above measurements were also performed in the absence of acute pacing (Table 2). No significant effects on heart rate, either in normal or in heart failure dogs, were observed. Thus, allopurinol does not appear to interfere with myocardial relaxation or chronotropy.

Effects of Allopurinol on Myocardial Energetics

The influence of allopurinol on cardiac energetics was assessed in 5 control and 6 heart failure dogs. The primary energetic abnormality in anesthetized heart failure dogs was a nearly 50% decrease in myocardial efficiency, as assessed by the ratio of SW to MV$\dot{O}_2$ ($P=0.05$). Coronary flow and AV$O_2$ at baseline were comparable in normal and heart failure dogs. The decrease in efficiency was due to a $>50\%$ decrease in SW (2008±450 versus 922±199 mm Hg $\cdot$ mL; $P=0.005$).

The effects of allopurinol (200 mg IV over 30 minutes) on myocardial contractility during anesthesia were similar to those in conscious dogs. In the control state, allopurinol did not increase indices of contractility—(dP/dt)$_{max}$, PRSW, and Ees—or affect preload or afterload. In heart failure, allopurinol produced a positive inotropic effect, reflected as a 12.7±6.7% increase in (dP/dt)$_{max}$ and a leftward shift in Ees (Figure 2). PRSW was unchanged. However, preload was decreased from baseline 10 minutes after the infusion; LVEDP decreased by 13±10% ($P=0.01$) and LVEDV by 5.6±3.5% ($P=0.02$). Ea remained unchanged. Thus, whereas allopurinol stimulated myocardial contractility in anesthe-

### Table 2. Effects of Allopurinol on Loading Conditions in Control and Heart Failure Dogs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (n=10)</th>
<th>Heart Failure (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Peak</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>11.2±2.1</td>
<td>11.8±2.0</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>35.2±1.4</td>
<td>35.7±1.4</td>
</tr>
<tr>
<td>Ea, mm Hg/mm</td>
<td>15.2±0.8</td>
<td>14.6±0.9</td>
</tr>
<tr>
<td>Ea/Ees</td>
<td>1.4±0.1</td>
<td>1.3±0.1</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDP, mm Hg</td>
<td>23.5±5.2</td>
<td>22.4±5.1</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>36.9±2.6</td>
<td>36.2±2.7</td>
</tr>
<tr>
<td>Ea, mm Hg/mm</td>
<td>27.7±9.3</td>
<td>24.0±6.2</td>
</tr>
<tr>
<td>Ea/Ees</td>
<td>2.7±0.4</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

### Table 3. Effects of Allopurinol on Diastolic Performance and Heart Rate

<table>
<thead>
<tr>
<th></th>
<th>Control (n=10)</th>
<th>Heart Failure (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tau, ms</td>
<td>32.2±1.1</td>
<td>63.6±21.4</td>
</tr>
<tr>
<td>(dP/dt)$_{max}$, mm Hg/s</td>
<td>-2520±130</td>
<td>-1767±281</td>
</tr>
<tr>
<td>ttpf, ms</td>
<td>127±12.4</td>
<td>129±26</td>
</tr>
<tr>
<td>Unpaced HR, bpm</td>
<td>113±7</td>
<td>123±8</td>
</tr>
<tr>
<td></td>
<td>33.3±2.5</td>
<td>36.4±4.4</td>
</tr>
<tr>
<td></td>
<td>-2627±160</td>
<td>-1995±244</td>
</tr>
<tr>
<td></td>
<td>121±13.2</td>
<td>112±18</td>
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<tr>
<td></td>
<td>120±5</td>
<td>121±9</td>
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</tbody>
</table>
tized heart failure animals, the hemodynamic effects were blunted relative to those in conscious dogs possibly because of the decreased preload and/or anesthesia itself.

Figure 3 shows the effects of allopurinol on oxygen consumption in the left circumflex area (A) and mechanical efficiency (B) in normal and heart failure dogs. In normal dogs, there was no significant change of oxygen consumption or mechanical efficiency. In heart failure dogs, allopurinol decreased oxygen consumption by $49 \pm 4.6\%$ ($P=0.002$), with an attendant $122\pm42\%$ increase in mechanical efficiency ($\text{SW}/\text{MV} \cdot \text{O}_2$; $P=0.04$) at 10 minutes after the end of the infusion.

Figure 4 depicts an original tracing of left circumflex blood flow velocity during allopurinol infusion. The decline in oxygen consumption was due primarily to a decrease in left circumflex blood flow ($-40\pm6\%$ 10 minutes postinfusion, $P=0.0015$), whereas myocardial arteriovenous oxygen difference ($\text{AV}_2$) was not changed. These results indicate that allopurinol decreases oxygen utilization and increases mechanical efficiency in the failing canine LV.

Given the substantial enhancement of myocardial efficiency by allopurinol, we compared the energetic effects of allopurinol with those of a $\beta$-adrenergic agonist, which is not expected to improve efficiency. Dobutamine (10 $\mu$g/kg $\cdot$ min$^{-1}$) was infused in 5 of the 6 heart failure dogs. In contrast to allopurinol, dobutamine caused a significant decrease in mechanical efficiency (Figure 5). After infusion of dobutamine, oxygen consumption increased by $145\pm53\%$ ($P=0.007$), and $\text{SW}/\text{MV} \cdot \text{O}_2$ decreased by $29.3\pm6.8\%$ ($P=0.05$).

**XO Activity in Normal Versus Failing Canine Hearts**

XO activity was significantly increased in failing hearts ($387\pm125$ pmol/min $\cdot$ mg$^{-1}$; $n=7$) compared with normal controls ($78\pm72$ pmol/ $\cdot$ mg$^{-1}$; $n=5$) ($P=0.04$; Figure 6). This activity was greatly suppressed in heart failure dogs receiving allopurinol at the time of euthanization ($14\pm14$ pmol/ $\cdot$ mg$^{-1}$; $n=2$). XO activity was not detectable in plasma from control (n=10) or heart failure (n=6) dogs. Thus, elevated myocardial XO activity may explain, at least in part, the increased effects of allopurinol on left ventricular performance and mechanical efficiency observed in heart failure.

**Discussion**

In this study we report the novel observation that the XO inhibitor allopurinol increases cardiac mechanical efficiency in dogs with pacing-induced heart failure. Allopurinol had a moderate positive inotropic effect but substantially decreased
myocardial oxygen consumption, leading to a near normalization of the abnormal myocardial energetics in heart failure. In addition, XO activity was 4-fold elevated in the failing dog heart. The present data indicate that allopurinol has unique calcium-sensitizing properties; these findings offer novel pathophysiological insights and have obvious therapeutic implications for the treatment of congestive heart failure.

The results presented here extend previous experiments\textsuperscript{23} that demonstrated an increase in calcium responsiveness in

Figure 4. Representative original tracing of the ECG, LV pressure, and left circumflex blood flow velocity before and 10 minutes after allopurinol infusion in a heart failure dog. Blood flow decreased, whereas coronary oxygen extraction remained unchanged, resulting in a reduction in myocardial oxygen consumption.

Figure 5. Effect of allopurinol and dobutamine on mechanical efficiency in anesthetized dogs with heart failure. Dogs were instrumented as in Figure 3. Shown is the percentage change in mechanical efficiency (SW/MV\textsubscript{O\textsubscript{2}}) relative to baseline due to dobutamine (n=5, △) and allopurinol (n=6, ○). Solid symbols represent mean±SEM. *P<0.05 vs dobutamine.

Figure 6. XO activity in myocardium obtained from control (Normal) and heart failure (HF) dogs. Myocardial tissue samples were obtained from both normal (n=5, □) and heart failure dogs (n=7, ○) immediately after euthanization. Tissue extracts were incubated with xanthine and assayed spectrophotometrically (295 nm) for the production of uric acid. Solid symbols represent mean±SEM. *P<0.05 vs normal myocardium.
isolated right ventricular trabeculae. In rat heart muscle microinjected with the Ca\(^{2+}\) indicator fura-2, Ca\(^{2+}\) transients were smaller, but myocardial force was greater during twitch contractions after exposure to the XO inhibitors allopurinol or its active metabolite oxypurinol. XO inhibitors did not shift the range of calcium activation, so that myocardial force was not activated at Ca\(^{2+}\) concentrations below normal diastolic levels. Moreover, these findings were even more dramatic in trabeculae with a blunted baseline response (caused by ischemia-reperfusion), raising the possibility of an augmented effect in myocardium with reduced force generation.

The present study was designed to confirm and extend these findings from isolated muscle to the in vivo situation. Using conscious control dogs, we demonstrated a small, positive inotropic effect without changes in preload or afterload. In conscious heart failure dogs, there was a significantly greater positive inotropic effect, again without preload or afterload changes. On the basis of our in vitro findings of a calcium-sensitizing property of allopurinol, myocardial energetics were studied in anesthetized dogs in vivo. In the control dogs, allopurinol did not change LV oxygen consumption or mechanical efficiency. In heart failure, however, there was a marked decrease in oxygen consumption associated with a substantial increase in mechanical efficiency in response to allopurinol.

Associated with the hemodynamic and energetic findings was a near 4-fold increase in XO activity in failing myocardium relative to control hearts. Because we used whole-heart extracts, we were unable to determine the precise cellular source of XO activity. Both endothelial cells and myocytes are potential sites of production. As XO may circulate in the plasma and affect distal organs, we assayed plasma for XO activity. The observation that XO activity was not detectable in plasma from either control or heart failure animals suggests that circulating XO is not the primary source.

Although oxidant stress has been implicated in the pathogenesis of heart failure, little is known regarding the genesis of increased free radical production in the failing heart. Studies have demonstrated increases in circulating markers of increased free radical production, and effective heart failure therapies have antioxidant properties. The present observations provide a novel mechanism for increased superoxide generation in the failing heart and suggest that the resultant oxidant stress might contribute to the depressed energetics characteristic of the failing heart. There is additional support for the notion that the hemodynamic and energetic effects of allopurinol are due to inhibition of XO and reduction in O\(_2\)\(^{-}\) production. Exogenous O\(_2\)\(^{-}\) has been reported to depress calcium responsiveness in isolated myofibrillar preparations, suggesting a mechanism by which increased O\(_2\)\(^{-}\) might contribute to decreased myofilament calcium responsiveness in failing myocardium. In addition, O\(_2\)\(^{-}\) serves as a precursor of other damaging oxidants such as hydrogen peroxide, hydroxyl radical, and peroxynitrite. It is attractive to speculate that O\(_2\)\(^{-}\) might constitute a link between energy metabolism and myocyte contraction, and that oxygen free radicals participate in physiological cardiac signaling. In this regard, O\(_2\)\(^{-}\) has been shown to act as a physiological signaling molecule for growth factors in fibroblasts. The findings of lingering effects of allopurinol after washout in the prior muscle studies, and, in the present study after infusion in vivo, imply that allopurinol induces chemical changes that persist for some time after drug removal. Lingering effects would be expected if the mechanism involves XO inhibition, because allopurinol inactivates the enzyme in a prolonged manner by being oxidized to oxypurinol, a noncompetitive XO inhibitor, at the enzymatic active site. Despite these observations, we cannot exclude the possibility that the effect of allopurinol is unrelated to XO inhibition.

In addition to enhancing myofilament calcium responsiveness, allopurinol blunts calcium transients in isolated myocardium: the net result is a modest increase in cardiac contractile force, which reflects the balance of the force-potentiating myofilament effect and the negatively inotropic decrease of [Ca\(^{2+}\)]. The mechanism of the latter effect remains uninvestigated. Nevertheless, from a practical point of view, the decrease of calcium availability would be a desirable property in failing myocardium; less energy would be required for calcium sequestration during each heartbeat. The obvious potential downside to such an energy-sparing effect would be a decrease in contractility, which here is more than offset by the concomitant increase in myofilament calcium responsiveness.

One hypothetical mechanism for heart failure progression is the idea that the heart is energy-depleted and that the dysfunctional LV is forced to work with decreased efficiency in an attempt to compensate for its lack of contractility. Whether or not this is a significant contributor to the development of LV dysfunction, the energy-sparing properties of allopurinol offer a unique advantage over conventional pharmacological therapy.

Currently available Ca\(^{2+}\) sensitizers shift the range of Ca\(^{2+}\) activation, so that force is activated at lower levels of Ca\(^{2+}\) with a consequent risk for diastolic dysfunction. Their effects are comparable in normal and failing hearts, and most of them have phosphodiesterase inhibitor activity, which may contribute to poor patient outcome. In our study, we found that allopurinol had no adverse effect on diastolic function and acted preferentially in dogs with heart failure. With regard to clinical use, allopurinol, a pyrazolo[3,4-d] pyrimidine, has been used clinically for decades, its safety is well established, and it is well tolerated by heart failure patients. More potent XO inhibitors, such as aminoxydopyrazolopyrimidine derivatives, are under development and may also be potentially useful in treating such patients.

Two considerations regarding the energetic observations warrant mention. First, ventricular-vascular coupling can impact energetic efficiency. Allopurinol had no effect on preload and afterload in our experiments with conscious dogs and no effect on afterload in the experiments with anesthetized dogs. The minimal reduction in the coupling ratio (Ea/Ees) observed in heart failure, which was not statistically significant, was due entirely to the positive inotropic effect of allopurinol. Studies by De Tombe et al examining the relation between ventricular-vascular coupling and efficiency in isolated hearts indicate that a decrease in Ea/Ees of the...
magnitude observed would be expected to only account for minimal improvements in myocardial efficiency. Second, our energetic measurements were limited to an assessment of mechanical efficiency. Total mechanoenergetic efficiency (slope between pressure-volume area and myocardial oxygen consumption; PVA-MVO$_2$) was not assessed, as this requires measurements of steady-state MVO$_2$ under different loading conditions. Such a determination was not undertaken given the possibility that any pharmacological means to do so (eg, nitrates) would likely exert independent and confounding effects. A complete assessment of PVA-MVO$_2$ permits separation of the components of O$_2$ consumption for basal metabolism, excitation-contraction coupling, and myocardial contraction. Additional experimental investigation in isolated hearts will be required to dissect the basis of the profound increase in mechanical efficiency observed in this study.

A limitation of this study was the possible effect of anesthesia during evaluation of cardiac energetics in response to allopurinol. Isoflurane is known to dampen myocardial contractility but does not appear to change mechanical efficiency. It is also associated with relatively stable myocardial performance during anesthesia. Therefore, although we cannot exclude a quantitative influence of anesthesia on the energetic effects of allopurinol, it seems reasonable to assume that it did not qualitatively influence the results.

In summary, the present study suggests that allopurinol can act as a novel inotropic agent that simultaneously decreases oxygen consumption and markedly increases myocardial mechanical efficiency in the canine heart in vivo. Moreover, the actions of allopurinol are enhanced in the failing heart. These findings may form the basis for a novel therapeutic approach to human heart failure.

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References


Intravenous Allopurinol Decreases Myocardial Oxygen Consumption and Increases Mechanical Efficiency in Dogs With Pacing-Induced Heart Failure

Ulf E. G. Ekelund, Robert W. Harrison, Ori Shokek, Rajiv N. Thakkar, Richard S. Tunin, Hideaki Senzaki, David A. Kass, Eduardo Marbán and Joshua M. Hare

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