Mechanisms whereby rapid RV pacing causes LV dysfunction: perfusion-contraction matching and NO

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ABSTRACT

Incessant tachycardia induces contractile, metabolic, and flow abnormalities reflecting flow-function matching early, but progresses to LV dysfunction late, despite restoration of flow and metabolism. The shift to flow-function mismatch is associated with impaired myocardial NO production.

Both incessant tachycardia in humans (16, 17, 38, 46, 59) and rapid pacing in experimental animal models (6, 55, 62, 69) produce contractile dysfunction and dilated cardiomyopathy (DCM). Although structural and functional myocardial abnormalities such as distortion of contractile elements (34), impaired regional coronary flow reserve (54), abnormal sarcoplasmic calcium handling (47), or enhanced proapoptotic signaling (24) have been described in such experimental models, the precise mechanisms for the contractile dysfunction and these structural changes remain incompletely understood. Despite severe contractile dysfunction after weeks of rapid pacing, functional recovery is observed after cessation of the tachycardia. This suggests that the contractile dysfunction reflects a compensation designed to limit irreversible myocardial injury. Recently, investigation has focused to the role those myocardial energetic imbalances and shifts in metabolic substrate utilization may play in the pathogenesis of human (23, 30, 64) or experimental heart failure (28, 70). Nitric oxide (NO) has been implicated to play a critical role in modulating myocardial contractility (10), as well as myocardial O2 consumption (MV\textsubscript{O2}) and myocardial preferences for metabolic substrates in experimental models of heart failure (49, 50).

We hypothesized that excessive chronotropic demands imposed by fixed rapid right ventricular (RV) pacing require compensatory reductions in myocardial requirements over time. Therefore, the goal of our study was to characterize the left ventricular (LV) systemic hemodynamic, coronary blood flow (CBF), and metabolic alterations associated with continuous rapid RV pacing that results in DCM in conscious dogs. A second goal was to investigate the role of myocardial NO metabolite production in mediating these dynamics over time.

METHODS

Surgical procedure and instrumentation. Twenty-four mongrel dogs of either sex weighing between 16 and 22 kg were sedated with xylazine (10 mg/kg) and anesthetized with halothane (1–1.5 vol%). Through an incision in the fifth intercostal space, Tygon catheters were placed in the descending thoracic aorta and left atrium (LA), and a Silastic

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catheter was placed in the coronary sinus. A solid-state pressure transducer (Konigsberg Instruments) was implanted in the left ventricle through an apical approach that facilitated high-fidelity recordings of LV pressure (LVP). A Transonics flow probe was placed on the proximal portion of the left circumflex coronary artery for continuous measurement of CBF. A similar Transonics flow probe was placed on the ascending aorta to measure aortic blood flow. Piezoelectric ultrasonic dimension crystals were implanted on the anterior and posterior endocardial surfaces of the left ventricle to measure the internal short-axis diameter in end diastole (LVEDD) and end systole (LVESD). Piezoelectric crystals were implanted on the endocardial and epicardial surfaces of the posterior wall to measure regional wall thickening (WTh). A sutureless pacing lead was implanted on the epicardial surface of the right ventricle.

All catheters were tunneled subcutaneously and externalized infrascapularly, after which the thoracotomy was closed in layers, and the thoracic cavity was evacuated of air. All animals received analgesics as needed for the first 72 h following surgery, and cephalixin (1 g iv) was administered daily for 7 days. The dogs were allowed to recover from the surgical procedure for 2 wk, during which time they were trained to lie quietly on the experimental table in a conscious, unrestrained state. All catheters were flushed daily and filled with a 50% heparin solution to maintain patency. Animals used in this study were maintained in accordance with the guidelines of the Committee of Animals of Allegheny General Hospital and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (DHHS Publications No. NIH 85-23, Revised 1985).

Heart failure induction protocol: hemodynamic measurements. Hemodynamic measurements were obtained at the fully conscious state, at baseline (before initiation of pacing), and during rapid RV pacing (240 beats/min), serially at 10, 30, 60, 120, 180, and 360 min to characterize the acute hemodynamic responses. The dogs were returned to their kennels and continued to be paced at the same fixed rate. To investigate the chronic phase of rapid RV pacing, followup hemodynamic measurements were obtained at 1, 3, 7, 14, 21, and 28 days of continuous pacing. All measurements were conducted in the conscious and fasting state and during rapid RV pacing. Measurements consisted of LVP, LV first derivative pressure (dP/dt), aortic systolic, diastolic, and mean pressure, LA and right atrium pressure, cardiac output, CBF, regional myocardial WTh, LVEDD, and LVESD. Mean CBF was measured on the circumflex coronary artery. MVO₂ was calculated as the product of the left circumflex CBF and the myocardial arteriovenous difference of O₂ content. MVO₂ was measured as the sum of measured plasma HCO₃⁻, CO₂ bound to reduced hemoglobin, and freely dissolved CO₂ in the red blood cells or carbaminoco₂. Total myocardial CO₂ production was calculated as the sum of measured plasma HCO₃⁻, CO₂ bound to reduced hemoglobin, and freely dissolved CO₂ in the red blood cells or carbaminoco₂. Total arterial and coronary sinus CO₂ was calculated and converted into volume, as previously described (49). Myocardial RQ was then calculated as the ratio: RQ = (cs - ao) total CO₂/(ao - cs) O₂ content, where cs is coronary sinus and ao is aorta.

In nine dogs, transmural myocardial gradients of nonesterified fatty acids (FFA) and lactate were measured at control and after rapid RV pacing for 21–28 days. Plasma FFA were measured using the NEFA C test kit purchased from Wako Diagnostics (Richmond, VA), which utilizes an in vitro enzymatic colorimetric method. Measurement of plasma lactate was carried out using a test kit purchased from Sigma Diagnostics (St. Louis, MO), utilizing a reaction with lactate dehydrogenase to produce pyruvate and NADH. Myocardial uptake of FFA and lactate was calculated as the product of the respective transmural gradient and the CBF.

Myocardial tissue analysis for endothelial NOS activity. LV samples were obtained at the time of euthanasia in three control dogs and in four dogs with advanced DCM, manifest after 27 ± 4 days of RV pacing. Briefly, LV tissue was rapidly removed and frozen in liquid nitrogen. Cryostat sections (6 μm thick) were immunostained with monoclonal anti-human NOS antibody. Additional frozen LV tissue samples were homogenized for eNOS protein detection by Western blotting (140 kDa). The methods for eNOS myocardial immunohistochemical (IHC) staining and protein analysis by Western blotting have been previously described elsewhere in detail (11, 15, 53).

Myocardial glycogen content. Glycogen content was determined using 4% buffered formalin-fixed tissues, dehydrated in ethanol, embedded in paraffin, and cut at 3-μm sections. We stained the sections with periodic acid Schiff (PAS) stain to detect glycogen and also performed digestion with amylase followed by PAS staining verifying the PAS-positive material being glycogen (68). These histological sections of LV myocardium were evaluated quantitatively for glycogen content using morphological analysis with a Nikon microscope connected to a computer with Metamorph imaging software. Sections stained with PAS were examined by using the green image from a color RGB Spot camera where 10–100 fields of view.
Table 1. Systemic hemodynamic changes during rapid pacing in conscious dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10 min</th>
<th>1 h</th>
<th>6 h</th>
<th>1 day</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP, mmHg</td>
<td>118 ± 2</td>
<td>105 ± 4</td>
<td>104 ± 5</td>
<td>101 ± 4</td>
<td>96 ± 3*</td>
<td>94 ± 2*</td>
<td>93 ± 2*</td>
<td>93 ± 2*</td>
<td>95 ± 4*</td>
<td>94 ± 4*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>12 ± 1</td>
<td>26 ± 2*</td>
<td>28 ± 2*</td>
<td>32 ± 2*</td>
<td>33 ± 2*</td>
<td>36 ± 2*</td>
<td>38 ± 2*</td>
<td>36 ± 2*</td>
<td>40 ± 2*</td>
<td>39 ± 2*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>94 ± 2</td>
<td>94 ± 4</td>
<td>93 ± 4</td>
<td>89 ± 3</td>
<td>84 ± 3</td>
<td>79 ± 3</td>
<td>78 ± 2</td>
<td>80 ± 2</td>
<td>82 ± 4</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>SV, ml</td>
<td>24 ± 2</td>
<td>7 ± 1*</td>
<td>6 ± 1*</td>
<td>6 ± 1*</td>
<td>6 ± 1*</td>
<td>5 ± 1*</td>
<td>5 ± 1*</td>
<td>6 ± 1*</td>
<td>6 ± 1*</td>
<td>7 ± 1*</td>
</tr>
<tr>
<td>LV dP/dt, mmHg/s</td>
<td>2,492 ± 98</td>
<td>1,798 ± 110*</td>
<td>1,677 ± 136*</td>
<td>1,535 ± 110*</td>
<td>1,445 ± 110*</td>
<td>1,362 ± 76*</td>
<td>1,301 ± 87*</td>
<td>1,096 ± 56*</td>
<td>970 ± 74*</td>
<td>764 ± 80*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>38 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>37 ± 1</td>
<td>38 ± 1</td>
<td>39 ± 1</td>
<td>42 ± 1</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>LV dP/dt/LVEDD, mmHg/s·mmHg^{-1}</td>
<td>0.65 ± 4</td>
<td>0.53 ± 3</td>
<td>0.49 ± 4*</td>
<td>0.44 ± 3*</td>
<td>0.42 ± 3*</td>
<td>0.38 ± 2*</td>
<td>0.35 ± 2*</td>
<td>0.28 ± 2*</td>
<td>0.23 ± 2*</td>
<td>0.19 ± 2*</td>
</tr>
<tr>
<td>WTh, mm</td>
<td>3.1 ± 0.3</td>
<td>3.4 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVP, left ventricular (LV) pressure; LVEDP, LV end-diastolic pressure; MAP, mean arterial pressure; SV, stroke volume; LV dP/dt, LV first derivative pressure; LVEDD, LV end-diastolic dimension; WTh, wall thickness. *Statistically significant P values (<0.05) compared with control.

LV (from subendomycocardial to subepimycocardial) were examined by using the ×10 objective of the microscope. Glycogen volume percent per area for each animal was expressed as the average of all fields examined.

Statistical analysis. Hemodynamic and metabolic (MV˙O2 and RQ) parameters were expressed as means ± SE and compared by using repeated-measures ANOVA. Myocardial NO production was expressed as coronary sinus-aortic concentration difference (in μM), as well as temporally related indexes reflecting myocardial NO production “per minute” (in nmol/min) or “per heartbeat” (in nmol/beat). These indexes were derived by multiplying coronary sinus minus aorta concentration times CBF (ml/min) and dividing this product by the heart rate (baseline native sinus rate or 240 beats/min), respectively. All indexes have been previously utilized to investigate myocardial NO production (49) and were also compared over time using repeated measures ANOVA. Myocardial eNOS expression from paced animals was compared with controls from our laboratory, using qualitative analysis of IHC and semiquantitative analysis of optical densitometry units (ODU) in Western blotting protein gel electrophoresis. Myocardial glycogen was expressed as a volume percentage per area of myocardium stained positive as described above. A level of P value <0.05 was accepted to indicate statistical significance.

RESULTS

Induction of DCM-systemic hemodynamics. The temporal changes in systemic hemodynamic parameters during rapid RV pacing are depicted in Table 1. Initiation of rapid RV pacing resulted in immediate (10 min) increases in LV end-diastolic pressure and decreases in LV dP/dt and stroke volume. These changes persisted through the acute phase of RV pacing and intensified during the chronic phase, leading to progressively worsening heart failure. LVEDD demonstrated a trend to decrease during the acute phase of rapid pacing, which was followed by LV dilatation in the chronic phase after 7 days of pacing. To account for the preload dependence of the index of isovolumic contraction, LV dP/dt was normalized by LVEDD for serial comparisons. There was a modest trend to an increase in WTh on initiation of pacing (10 min). Overall, WTh did not change during the acute phase, but there was a significant decrease in WTh by 7 days that persisted throughout the chronic phase.

Myocardial flow-function relationship. Upon initiation of rapid RV pacing, there was a significant decline in LV contractile function ([LV dP/dt]/LVEDD index) from 65 ± 4 to 44 ± 3 mmHg·s^{-1}·mm^{-1} after 6 h of pacing (P < 0.01). This was accompanied by a parallel, significant decline in both CBF and MV˙O2, normalized for the change in heart rate (CBF/beat: from 0.33 ± 0.02 ml to 0.19 ± 0.01 ml, MV˙O2/beat: from 0.031 ± 0.002 to 0.016 ± 0.001 ml O2 at 6 h, respectively; P < 0.001, Table 2). Compared with the values obtained at 10 min after initiation of rapid pacing, there was a progressive decline in both CBF per beat and MV˙O2 per beat over the first 72 h. This pattern of “matched” decline in myocardial blood flow and contractile function characterized the acute phase of rapid RV pacing in our model (Fig. 1A).

During the chronic phase of RV pacing, we observed a significant dissociation between changes in myocardial blood flow and contractile function. Whereas LV contractility continued to decline (to 19 ± 2 mmHg·s^{-1}·mm^{-1} at 28 days, P < 0.001), both CBF per beat and MV˙O2 per beat reached their nadir values at 3 days of pacing (CBF/beat: 0.021 ± 0.001 ml and MV˙O2/beat: 0.013 ± 0.0001 ml O2 at 6 h, respectively; P < 0.001, Table 2). Compared with the values obtained at 10 min after initiation of rapid pacing, there was a significant (Fig. 1B), suggesting “flow-function mismatch” during the chronic phase of rapid RV pacing (3–28 days). Similarly, we observed a pattern of preserved myocardial WTh during the early phase of pacing (3.1 ± 0.3 to 3.2 ± 0.3 mm at 6 and 24 h of pacing), followed by progressive decline during the chronic phase (to 2.2 ± 0.2 mm at 28 days of pacing, P < 0.05), at a time where CBF had already started to increase (Fig. 2), consistent with flow-function mismatch in the later phase of pacing-induced cardiomyopathy.

Myocardial metabolic requirements during tachycardia. During the initial 10 min of rapid RV pacing, there was a significant rise in MV˙O2 (from 2.3 ± 0.1 to 4.6 ± 0.3 ml O2/min, P < 0.001). This was related to the
excessive chronotropic demand imposed by fixed pacing at 240 beats/min, as the MV ˙O2 per beat declined from 0.031 ± 0.002 to 0.019 ± 0.001 ml O2 (P < 0.001). Thereafter, there was a decline in both MV ˙O2 and MV ˙O2 per beat, reaching a nadir at 3 days of rapid RV pacing (Table 2). During the chronic phase, there was a significant increase in both MV ˙O2 (from 3.3 ± 0.2 ml O2/min at 3 days to 4.7 ± 0.2 ml O2/min at 28 days of pacing, P < 0.01) and MV ˙O2 per beat (from 0.013 ± 0.001 ml O2 at 3 days to 0.021 ± 0.001 ml O2 at 28 days, P < 0.001), resulting in a return to levels comparable to those observed immediately after initiation of pacing.

Myocardial SW declined significantly upon initiation of rapid RV pacing (27 ± 4 to 9 ± 1 g·m at 10 min, P < 0.0001) and continued to decline along the time course of acute and chronic rapid ventricular pacing (Table 3). Myocardial efficiency was also significantly impaired during the acute phase (MEI: 11.9 ± 1.7% to 5.3 ± 0.6% at 10 min, P < 0.0001) and declined further on continuation of rapid pacing for 28 days (to 2.9 ± 0.4%, P < 0.0001).

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**Table 2. Changes in coronary blood flow and MV ˙O2 during the course of chronic rapid pacing in conscious dogs**

<table>
<thead>
<tr>
<th></th>
<th>CBF, ml/min</th>
<th>CBF/beat, ml</th>
<th>MV ˙O2, ml O2/min</th>
<th>MV ˙O2/beat, ml O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25 ± 1</td>
<td>0.33 ± 0.02</td>
<td>2.3 ± 0.1</td>
<td>0.031 ± 0.002</td>
</tr>
<tr>
<td>10 min</td>
<td>49 ± 2*</td>
<td>0.20 ± 0.01*</td>
<td>4.6 ± 0.3*</td>
<td>0.019 ± 0.001*</td>
</tr>
<tr>
<td>1 h</td>
<td>44 ± 2*</td>
<td>0.19 ± 0.01*</td>
<td>4.3 ± 0.3*</td>
<td>0.018 ± 0.001*</td>
</tr>
<tr>
<td>6 h</td>
<td>42 ± 2*</td>
<td>0.19 ± 0.01*</td>
<td>3.9 ± 0.3*</td>
<td>0.016 ± 0.001*</td>
</tr>
<tr>
<td>1 day</td>
<td>39 ± 2*</td>
<td>0.16 ± 0.01*</td>
<td>3.4 ± 0.2*</td>
<td>0.014 ± 0.001*</td>
</tr>
<tr>
<td>3 days</td>
<td>36 ± 2*</td>
<td>0.15 ± 0.01*</td>
<td>3.3 ± 0.2*</td>
<td>0.013 ± 0.001*</td>
</tr>
<tr>
<td>7 days</td>
<td>40 ± 2*</td>
<td>0.17 ± 0.01*</td>
<td>4 ± 0.2*†</td>
<td>0.017 ± 0.001*</td>
</tr>
<tr>
<td>14 days</td>
<td>42 ± 2†</td>
<td>0.19 ± 0.01†</td>
<td>4.1 ± 0.2†</td>
<td>0.018 ± 0.001†</td>
</tr>
<tr>
<td>21 days</td>
<td>47 ± 3†</td>
<td>0.20 ± 0.01†</td>
<td>4.2 ± 0.3†</td>
<td>0.019 ± 0.001†</td>
</tr>
<tr>
<td>28 days</td>
<td>54 ± 4†</td>
<td>0.25 ± 0.01†</td>
<td>4.7 ± 0.3†</td>
<td>0.021 ± 0.001†</td>
</tr>
</tbody>
</table>

Values are means ± SE. CBF, coronary blood flow; MV ˙O2, myocardial O2 consumption. All parameters except control values reflect measurements during rapid right ventricular pacing at 240 beats/min. *Statistically significant P values (<0.05) compared with baseline (control); †Statistically significant secondary increases from the nadir values observed at 3 days of pacing.

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Fig. 1. Matched “flow-function” decline during acute stage of rapid right ventricular (RV) pacing (0–6 h, A). A comparable decline in left ventricular (LV) first derivative of pressure (dP/dt)/LV end-diastolic diameter (EDD) (P < 0.01), coronary blood flow (CBF)/beat (P < 0.001), and myocardial O2 consumption (MV ˙O2)/beat (P < 0.001) from control values was observed as early as 10 min and persisted throughout the first 6 h of pacing (n = 18). During the chronic stage of rapid RV pacing (7–28 days, B), there was a “flow-function” mismatch pattern. (LV dP/dt)/LV EDD continued to decline (P < 0.0001 at 28 days vs. control), whereas CBF/beat and MV ˙O2/beat increased from their nadir values observed at 3 days of pacing (P < 0.01, n = 18).

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Fig. 2. Changes in regional myocardial wall thickening (WTh) in relation to CBF/beat. During the acute stage of RV pacing, regional WTh was preserved (A). In contrast, during chronic stage of pacing, regional WTh significantly decreased at 7 days (P < 0.03 vs. control, n = 14) and remained depressed, despite an increase in CBF/beat (B).
Myocardial metabolic substrate utilization. Myocardial RQ increased progressively from a baseline value of 0.72 ± 0.06 to an end-stage value of 0.91 ± 0.07 (P, 0.05), suggesting of a shift in myocardial metabolic substrate utilization from FFA to glucose. Notably, the RQ increased substantially by 7 days of rapid RV pacing, associated with increasing MV\textsubscript{O\textsubscript{2}} and decreased myocardial work efficiency.

These changes in myocardial RQ were correlated with measurements of FFA and lactate uptake in a subset of nine animals studied in control and following chronic pacing. Corroborating the observed increases in RQ, transmyocardial FFA extraction and uptake (control: 5.2 ± 0.4 vs. chronic pacing: −0.4 ± 1.9 μmol/min, P < 0.03) was significantly impaired in chronic pacing, whereas the lactate extraction and uptake (control: 1.7 ± 1.9 vs. chronic pacing: 9.8 ± 5.8 μeq/min, P ~ 0.07) were significantly increased in chronic pacing (Fig. 3).

Myocardial glycogen expression. Figure 4 depicts PAS staining for myocardial glycogen in control (n = 5), early (n = 3), and late (n = 8) phases of rapid RV pacing. Early rapid pacing (3 days) was associated with decreased glycogen content. In contrast, chronic pacing resulted in increased myocardial glycogen content (control: 0.9 ± 0.2 vol% vs. chronic pacing: 5.3 ± 1.3 vol%, P < 0.05) to values greater than those observed in controls.

Table 3. Myocardial respiratory quotient, myocardial stroke work, and myocardial efficiency index during rapid pacing in conscious dogs

<table>
<thead>
<tr>
<th></th>
<th>RQ</th>
<th>SW, g·m</th>
<th>MEI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.72 ± 0.06</td>
<td>27 ± 4</td>
<td>11.9 ± 1.7</td>
</tr>
<tr>
<td>10 min</td>
<td>0.70 ± 0.06</td>
<td>9 ± 1*</td>
<td>5.3 ± 0.6*</td>
</tr>
<tr>
<td>1 h</td>
<td>0.71 ± 0.08</td>
<td>8 ± 1*</td>
<td>4 ± 0.6*</td>
</tr>
<tr>
<td>6 h</td>
<td>0.75 ± 0.04</td>
<td>7 ± 1*</td>
<td>3.7 ± 0.5*</td>
</tr>
<tr>
<td>1 day</td>
<td>0.73 ± 0.08</td>
<td>6 ± 1*</td>
<td>3.4 ± 0.4*</td>
</tr>
<tr>
<td>3 days</td>
<td>0.76 ± 0.03</td>
<td>5 ± 1*</td>
<td>2.9 ± 0.4*</td>
</tr>
<tr>
<td>7 days</td>
<td>0.86 ± 0.08†</td>
<td>5 ± 1*</td>
<td>2.8 ± 0.3*</td>
</tr>
<tr>
<td>14 days</td>
<td>0.88 ± 0.09†</td>
<td>5 ± 1*</td>
<td>2.8 ± 0.3*</td>
</tr>
<tr>
<td>21 days</td>
<td>0.85 ± 0.08†</td>
<td>5 ± 1*</td>
<td>2.8 ± 0.3*</td>
</tr>
<tr>
<td>28 days</td>
<td>0.91 ± 0.07†</td>
<td>6 ± 1*</td>
<td>2.9 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. RQ, respiratory quotient; SW, stroke work; MEI, myocardial efficiency index. A temporal relationship between higher RQ values (shift to glucose utilization) and decreased energetic efficiency was observed along the course of rapid pacing, as the dogs progressively developed tachycardia-induced cardiomyopathy. (*P < 0.001, †P < 0.05 from baseline control values).

Myocardial NO production. Myocardial NO metabolite production remained essentially unchanged during the acute phase of rapid RV pacing (−2 ± 17 to −18 ± 19 nmol NO/min at 6 h, not significant). Myocardial
NO metabolite production increased between 1 and 7 days of pacing, but the difference did not reach statistical significance. During the chronic phase of rapid RV pacing, NO metabolite production decreased significantly to $2174 \pm 639$ nmol NO/min at 28 days ($P < 0.05$, $n = 8$) in myocardial tissue obtained from chronically paced dogs ($C$, animal tissue obtained after 30 days of pacing). All images were acquired at the same magnification ($\times40$). Difference in glycogen content in volume (%) is quantitatively depicted in $D$.

Myocardial eNOS expression. The decline in myocardial NO metabolite production in chronic pacing was accompanied by decreased expression of eNOS, compared with control animals, as assessed by IHC staining. In addition, myocardial eNOS protein by Western blotting was substantially reduced in chronic pacing compared with controls (Fig. 6). Semiquantitative analysis of the Western blot gels from control and cardiomyopathic dogs with the use of ODU confirmed a significant decrease in eNOS expression in the chronic pacing (control: $2,412 \pm 361$ vs. chronic pacing: $1,129 \pm 165$ ODU, $P < 0.05$).

Competitive NOS inhibition protocol. To determine the role of NO in flow-function match observed during acute pacing, we studied six dogs during acute pacing in the presence and absence of NOS inhibition. The mean arterial pressure, CBF, and $\text{MV}_2$ responses were accentuated during acute rapid pacing (6 h) in dogs following L-NNA, whereas the normalized LV contractile response ($\text{LV dP/dt}/\text{EDD}$) was depressed to a greater extent compared with controls (Fig. 7). Importantly, the difference was not attributable to the increase in afterload imposed by NOS inhibition, because the response to pacing was similar between control and during phenylephrine infusion (2–5
DISCUSSION

In normal dogs, continuous rapid pacing imposes an excessive myocardial metabolic demand that eventually results in LV dysfunction, ventricular dilatation, and heart failure.

In the present study, we observed the hemodynamic, CBF and metabolic dynamics throughout the 28 days of rapid pacing. We observed that the acute onset of rapid pacing was associated with a progressive decline in CBF per beat and MVO2 per beat and parallel declines in LV contractility over the first 24–72 h. This was associated with preservation of myocardial NO metabolites and depletion of myocardial glycogen stores. Thereafter, during the chronic phase of rapid pacing there was progressive recovery of the CBF and

\[ \mu \text{g.kg}^{-1}.\text{min}^{-1} \text{over 6 h, } n = 3 \], designed to match the increase in mean arterial pressure associated with L-NNA administration.

**Fig. 5.** Relationship between myocardial nitric oxide (NO) production and metabolic demand (MVO2) during acute (A) and chronic (B) phase of rapid RV pacing in conscious dogs. Note relatively stable time course of both parameters during the acute phase, followed by a progressive decline in myocardial NO metabolite production (\( P < 0.05 \) vs. control, \( n = 11 \)) during the chronic phase (7–28 days) of pacing-induced cardiomyopathy.

![Myocardial eNOS Control](image1)

![Myocardial eNOS Chronic Pacing](image2)

**Fig. 6A**

**Fig. 6B**

**Fig. 6C**

![Western blot gel electrophoresis for eNOS protein](image3)

**Fig. 6**. Myocardial immunohistochemical (IHC) staining for endothelial NO synthase (eNOS) from dogs before initiation of pacing and after development of advanced pacing-induced cardiomyopathy (A: top, control; bottom: chronically paced animal for 28 days). Both images were acquired at the same magnification (×20). LV tissue Western blot gel electrophoresis for eNOS protein (B) in control dogs (lanes 1, 2, and 4) and dogs with chronic rapid pacing-induced cardiomyopathy (lanes 3, 5, and 6). Bar graph (C) illustrates difference in optical densitometry units (\( P < 0.05 \)).
The MV\textsubscript{O}2 responses, yet the LV contractile response continued to deteriorate. This pattern was accompanied by a progressive decrease in myocardial eNOS protein and myocardial NO metabolites, a shift to glycolysis as the preferred metabolic substrate, and increases in myocardial glycogen stores. Taken together, these data suggest that normal myocardium subjected to the continuous stress of rapid pacing develops a profile of energy sparing as opposed to contractile efficiency, as a compensatory strategy. The early phase is transient and accompanied by flow-function matching and ultimately replaced by a pattern of reduced metabolic and contractile function observed in acute myocardial hibernation (9, 39, 40, 66). The depletion of myocardial glycogen content during the early period of pacing stress is consistent with these observations. Depletion in myocardial glycogen stores has been associated with increased myocardial NO production (7) through a cGMP-dependent mechanism (7, 42). NO has also been shown to stimulate glycogen phosphorylase (65), contributing to glycogen breakdown. In contrast, during the more chronic phase of rapid pacing, there is perfusion-contraction uncoupling associated with progressive depletion in eNOS and consequently, myocardial NO metabolites. These changes are associated with a metabolic phenotype of increased myocardial glycogen storage and glycolytic flux. This is consistent with the well-characterized regional postischemic contractile dysfunction in patients with collateral-dependent circulation (40). Under these circumstances, limited coronary flow reserve predisposes to brief periods of myocardial ischemia leading to an uncoupling of perfusion and contraction. In the model of pacing-induced heart failure in conscious dogs, our laboratory (54) has shown previously that advanced heart failure (4 wk) is associated with impaired flow reserve despite normal resting CBF per beat might predispose to brief repetitive myocardial dysfunction in the face of persistent tachycardia. Under this scenario, we cannot determine whether the decline in eNOS

Fig. 7. Hemodynamic and MV\textsubscript{O}2 alterations associated with pharmacologic NOS inhibition with N\textsuperscript{\textendash}nitro-L-arginine (L-NNA) during the acute (0–24 h) phase of rapid RV pacing. A: experimental design to control for the systemic vasoconstrictive effects of L-NNA, using phenylephrine (PHE) to attain a similar mean arterial pressure (MAP) response. B: myocardial contractility (LV dP/dt/LVEDD) associated with rapid pacing was further attenuated by L-NNA \((P < 0.01, n = 6)\), but not by PHEN \((P \approx 0.27, \text{NS}, n = 3)\). C and D: accentuation of CBF \((P < 0.001)\) and MV\textsubscript{O}2 requirements \((P < 0.001)\) in the L-NNA-treated dogs, respectively, throughout the acute phase of rapid RV pacing. In contrast, PHE had no measurable effect on either CBF \((P > 0.23, \text{not significant (NS)})\) or MV\textsubscript{O}2 \((P \sim 0.12, \text{NS})\) during pacing, despite similar effects on systemic hemodynamics (A).
protein and activity is a cause or a consequence of brief bouts of repetitive demand-related ischemia.

Most evidence suggests that NO maintains myocardial contractation following recovery of ischemia, i.e., stunning (13, 36, 58), and that inhibition of NOS is associated with prolonged, more intense postischemic contractile dysfunction. (3, 4, 19, 21, 26, 33, 35, 67). In contrast, myocardial hibernation has been associated with NO upregulation, which is generally regarded an adaptive mechanism (51), minimizing metabolic requirements (MV\(\dot{O}_{2}\)) (26, 57, 71).

The role of NO in models of cardiomyopathy not associated with epicardial coronary ischemia is less well understood (10, 27, 31, 72). Studies have reported either increased (14, 18, 41, 44, 45) or decreased (1, 32, 43, 61) myocardial NO in different clinical or experimental settings of heart failure. The issue is further complicated by different temporal, spatial, and preferential isoform expression of NO synthase (eNOS vs. inducible NOS) during the evolution of LV dysfunction from a compensated to a decompensated state (11, 15, 20, 53, 55).

In a similar model, Recchia et al. (49) reported decreased myocardial NO production in relatively late (7–21 days of rapid RV pacing) stages of pacing-induced cardiomyopathy in conscious dogs. They also correlated NO inhibition with increased resting MV\(\dot{O}_{2}\), implying a role of NO in modulating metabolic requirements in this model. These observations are similar to what has been described by Heusch et al. (25, 26) in myocardial hibernation. Furthermore, Recchia et al. (49) demonstrated an association between a decline in myocardial NO production and a shift in metabolic substrate preference from FFA to carbohydrate utilization.

Our study extends the observation of Recchia et al. (49, 50) in two ways. We investigated myocardial NO production and its associations with LV contractile function, CBF, myocardial metabolic requirements (MV\(\dot{O}_{2}\)), and substrate utilization (RQ) serially, during the entire rapid pacing protocol. We have not limited our experiments to the late stages of rapid RV pacing when advanced phenotypic changes of cardiomyopathy have already ensued. Instead, we observed dynamic modifications in flow, metabolism, and function from the onset of rapid RV pacing and by recording events serially and at frequent intervals. We compared these data with those obtained at a more chronic stage, similar to that described by previous investigators (49, 50). In addition, all measurements in our study were conducted while the dogs were actively paced at the fixed, rapid rate (240 beats/min) and not in their native sinus rhythm. Our study was designed to investigate the impact of excessive chronotropic demands on metabolic requirements and myocardial function. To normalize for the inevitable influence of heart rate differences between baseline and paced status on CBF and MV\(\dot{O}_{2}\), we also reported rate-adjusted parameters (CBF per beat and MV\(\dot{O}_{2}\) per beat). Similarly, to normalize for preload changes associated with progressive ventricular dilatation, we calculated and compared [(LV dP/dt)/LVEDD] as index of myocardial contractility. Importantly, we extend the observations regarding the role of NO in metabolic substrate preference to a role in mediating the dynamics of flow-function coupling. Eventually, during the stress of the chronic rapid pacing, declines in the myocardial production of NO were associated with flow-function mismatch. Finally, we observed that the decline in myocardial NO production was associated with alterations in myocardial eNOS protein.

Using this experimental design, we were able to define two discrete temporal stages during the process of development of rapid pacing-induced DCM in conscious dogs. The first stage encompassed the entire acute pacing phase (0–6 h) and the subsequent first 3–7 days of pacing, where a parallel decline of both contractile function [LV dP/dt, (LV dP/dt)/LVEDD] and metabolic requirements (MV\(\dot{O}_{2}\) per beat, CBF per beat) was observed. Myocardial NO production was preserved during this stage. The protocol in which rapid pacing was conducted in the presence of NOS inhibition supports the mechanistic role of NO in mediating perfusion-contractation matching during the early phase of rapid pacing. Importantly, the difference in response was not attributable to increased load imposed by rapid pacing, because there was no difference between responses in control and during phenylephrine infusion. These findings suggest that excessive chronotropic demand also leads to a state of myocardial hibernation, where inotropy is reduced, to balance energy requirements. Myocardial NO during this initial adaptive phase played a mechanistic role in maintaining this compensatory strategy.

During the evolution of the late or chronic phase (7–28 days) of rapid pacing, when DCM developed, a gradual increase in O\(_2\) consumption (MV\(\dot{O}_{2}\) per beat) was observed while contractile function continued to decline. This was accompanied by a sharp decline in myocardial NO production, which was corroborated by downregulation of myocardial eNOS (NOS-3) protein expression. Although myocardial RQ demonstrated a modest increase during the early phase, the RQ increased significantly in the chronic phase, suggestive of metabolic substrate shift from FFA toward glucose and lactate utilization. This pattern of increased MV\(\dot{O}_{2}\) and substrate shift is also in agreement with prior investigations that have focused on the advanced stage of heart failure (52, 60), although these studies were conducted after termination of pacing. The association between defective myocardial NO production and eNOS expression supports the hypothesis that once the regulatory function of NO is lost, the fine balance between myocardial function and metabolic demands is compromised. This is associated with the development of flow-function mismatch. This sequence suggests that early hibernation is an adaptive process (5, 51), modulated at least in part by NO, and late stunning is a sequel to the loss of the “protective” NO-mediated downregulation in O\(_2\) consumption and energy requirements. Similar MV\(\dot{O}_{2}\)-sparing effects of NO donors (and, conversely, deleterious effect of NO inhib-
itors) have been previously established in other settings in vitro (37) or in vivo, with regard to attenuating the excessive metabolic demands associated with exercise in normal conscious dogs (2), or patients subjected to either atrial pacing (29) or pharmacological dobutamine stress (60). The application of the physiology of myocardial stunning to the condition of rapid pacing is further supported by the reversible nature of the chronic pacing-induced hemodynamic changes.

Our assessment of RQ is a well-validated surrogate method for investigating metabolic substrate utilization (49, 50), and it was corroborated by biochemical data indicative of decreased FFA uptake, although these measurements were limited to the control and advanced heart failure stages (where the RQ differences were found to be the greatest). However, either method can be considered rather semiquantitative compared with more accurate radiolabeled substrate infusion techniques described by other investigators (63), which might have examined myocardial metabolic substrate utilization in more detailed terms.

In conclusion, our study demonstrated that rapid RV pacing induces a dynamic state of progressive contractile dysfunction, characterized by an early phase of acute hibernation (0–3 days of pacing) followed by a later phase of progressive myocardial stunning (7–28 days). Our study also confirmed that NO plays a pivotal role in mediating these dynamics, because the switch to myocardial stunning physiology with a disproportionate increase in $MVO_2$ and increased preference for glycolytic substrate utilization were associated with significant declines in myocardial NO production and eNOS expression.

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