Endothelin receptor blockade has an oxygen-saving effect in Dahl salt-sensitive rats with heart failure

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Noguchi, Teruo, Zengyi Chen, Stephen P. Bell, Lori Nyland, and Martin M. LeWinter. Endothelin receptor blockade has an oxygen-saving effect in Dahl salt-sensitive rats with heart failure. Am J Physiol Heart Circ Physiol 285:H1428–H1434, 2003. First published June 5, 2003; 10.1152/ajpheart.00731.2002.—The effects of endothelin (ET) receptor blockade on oxygen utilization in heart failure (HF) are unknown. We administered ET type A (ETA), ET type B (ETB), and ET\textsubscript{A}/ET\textsubscript{B} antagonists to isolated hearts from Dahl salt-sensitive (DS) rats with HF and controls. Contractile efficiency was assessed as slope of myocardial O2 consumption (V\textsubscript{O2})-pressure-volume area relation. In HF, ETA and ET\textsubscript{A}/ET\textsubscript{B} but not ET\textsubscript{B} blockade decreased the contractility index (E\textsubscript{max}) (−15 ± 3% and −17 ± 2%, P < 0.05), excitation-contraction (E-C) coupling V\textsubscript{O2} (−39 ± 4% and −37 ± 5%, P < 0.01), and efficiency (−15 ± 4% and −17 ± 2%, P < 0.05). Despite decreased efficiency, ETA and ET\textsubscript{A}/ET\textsubscript{B} blockade decreased total V\textsubscript{O2} (−24 ± 3% and −22 ± 2%, P < 0.05). Na\textsuperscript{+}/H\textsuperscript{+} exchanger inhibition decreased E\textsubscript{max} and E-C coupling V\textsubscript{O2} similar to ETA and ET\textsubscript{A}/ET\textsubscript{B} blockade, but did not alter efficiency. In HF, endogenous ET-1 maintains contractility at expense of increased V\textsubscript{O2} through ETA receptor activation, likely mediated by Na\textsuperscript{+}/H\textsuperscript{+} exchange.

METHODS

Animal Model

All protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Sixty 6-wk-old male DS rats (Taconic Farms, Germantown, NY) were divided into groups receiving a HS (8% NaCl) or low-salt (LS) diet (0.3% NaCl). Compensated hypertrophy occurs after 5–6 wk of HS, with progression to HF after 9–12 wk (12, 15). Transthoracic echocardiography was performed on the rats (1.5% isoflurane anesthesia) beginning at 15 wk of age (9 wk HS) with the use of a 15-MHz transducer (Sequoia C-256, Acuson). M-mode echocardiograms were recorded at the LV papillary muscle level to measure end-diastolic and end-systolic dimension, fractional shortening, and end-diastolic posterior wall thickness. HS rats without LV dilatation and decreased shortening after 9–12 wk of HS were excluded.

Each group was divided into subgroups receiving the dual ET\textsubscript{A}/ET\textsubscript{B} antagonist bosentan, the selective ETA antagonist BQ-123, the selective ETB antagonist BQ-788, or dimethylamyloride (DMA), a Na\textsuperscript{+}/H\textsuperscript{+} exchanger inhibitor, during isolated heart studies (see Isolated Heart Preparation). Thus there were eight subgroups, according to the presence or absence of HF and drug interventions: HF + bosentan (n = 8), BQ-123 (n = 8), BQ-788 (n = 8), or DMA (n = 5) and age-matched controls + bosentan (n = 5), BQ-123 (n = 5), BQ-788 (n = 5), or DMA (n = 3). In five additional HF hearts, BQ-123 and DMA were coadministered to determine whether the effects of coadministration differed quantitatively from each drug administered separately.

Isolated Heart Preparation

Our isolated, isovolumically contracting preparation has been described elsewhere (15). Hearts were perfused with Krebs-Henseleit buffer (15). Perfusate was filtered, equilibrated with 95% O\textsubscript{2} + 5% CO\textsubscript{2}, warmed to 37°C, and adjusted.
to pH 7.4. Perfusion pressure was maintained constant at ~90 mmHg in controls and ~120 mmHg in HF rats. These pressures simulate in vivo conditions, and, based on our previous experience (15), were expected to result in similar coronary flows per gram LV in control and failing hearts. A balloon containing a 2.5-French micromanometer was placed in the LV through the mitral orifice (15). A pacing electrode was attached to the LV and used to pace at 240 beats/min (15).

**Mechanoenergetic Parameters**

Coronary arteriovenous O₂ content difference (AVO₂Δ) was measured with an Instech monitor (Instech Laboratories; Plymouth, PA). Perfusion pressure and AVO₂Δ were stored for offline analyses. VO₂/beat was calculated as coronary flow (ml/min) × AVO₂Δ (vol%)/heart rate, and normalized per gram LV to yield total VO₂/beat−1·g−1 (in ml). LV volume was determined as volume of water within the balloon plus its wall and connector volume. LV developed pressure (DP) was taken as peak minus minimum value and end-diastolic pressure (EDP) when rate of pressure development over time (+dP/dt) reached 10% of maximum. Rate of isovolumic pressure decay (τ) was calculated using a nonzero asymptote (7).

**Experimental Protocol**

The first series was designed to delineate the role of endogenous ET-1. The following were administered to HF and controls via the coronary perfusate: 1) bosentan (10 μM); 2) BQ-123 (1 μM); 3) BQ-788 (100 nM); 4) DMA (5 μM); and 5) BQ-123 (1 μM) + DMA (5 μM). Concentrations were determined from previously published studies (14, 24). Hearts were allowed to stabilize for 20 min, following which LV pressure, coronary flow, and AVO₂Δ were measured at various volumes (volume run) during steady-state contractions. Volume was varied between that where peak pressure was zero and a maximal volume (0.15 ml or diastolic pressure >20 mmHg). After a control run, one of the above agents was added to the perfusate for 20 min, and the volume run was repeated. To assess the effects on basal metabolism, three hearts in each group were arrested with intracoronary KCl at zero balloon volume after the first volume run. When coronary flow and AVO₂Δ stabilized, basal VO₂ was measured for 1 min. Hearts were recovered by reinitiating normal perfusate and drug(s) then added to the perfusate. There were no significant differences in baseline hemodynamic measurements before and after recovery from KCl arrest. At the end of the study, KCl was readministered and basal VO₂ measured after drug intervention. In a second series, dose-response relationships of ETₐ receptor blockade were examined with the use of a 10-fold greater (10 μM, n = 5) or a one-tenth lower (0.1 μM, n = 3) concentration of BQ-123.

**Data Analysis**

**Systolic and diastolic function.** For volume runs, EDP and peak-systolic pressure were plotted against LV volume (pressure-volume diagram). The end-systolic pressure-volume relation (ESPVR) was fitted to a nonlinear equation (2, 8, 15)

\[
P = E_{\text{max}}(V - V_o) + \alpha(V - V_o)^2
\]

where P and V are pressure and volume, \(V_o\) is the volume axis intercept, \(\alpha\) is a constant, and \(E_{\text{max}}\) is a contractility index. Contractility was also assessed as DP at a common volume (0.11 ml) in each heart. Diastolic function was quantified as EDP and τ at this same volume.

**Mechanoenergetic parameters.** Total mechanical energy output was quantified as PVA, the area circumscribed by the ESPVR, end-diastolic pressure-volume relation (EDPVR), and the systolic portion of the pressure-volume trajectory (34). PVA was normalized per gram LV (in mmHg·ml·beat−1·g−1). VO₂ was plotted against PVA at the differing LV volumes and a linear regression (VO₂ = aPVA + b) performed. Slope \(a = \frac{\text{O}_2\text{cost of PVA, and intercept } b = \text{VO}_2\text{ at 0 PVA (unloaded or PVA-independent VO}_2\text{). PVA-independent VO}_2\text{ consists of energy for E-C coupling and basal metabolism (34). Slope } a^{-1} = \text{conversion efficiency of VO}_2\text{ to PVA (contractile efficiency) after conversion of PVA and VO}_2\text{ to joules (2). KCl arrest (basal metabolic) VO}_2\text{ was expressed as ml O}_2\text{-min}^{-1}·g^{-1}. E-C coupling VO}_2\text{ (ml O}_2\text{-beat}^{-1}·g^{-1} )\) was estimated as PVA-independent VO₂/min divided by heart rate.

**Chemicals**

BQ-123 was obtained from American Peptide (Sunnyvale, CA), bosentan from Acterion (Allschwil, Switzerland), and BQ-788 and DMA from Sigma (St. Louis, MO).

**Statistical Analysis**

Data are reported as means ± SD. Differences between control and drug conditions were detected by Student’s t-test. Differences in VO₂-PVA regression lines between conditions were detected by analysis of covariance. Comparisons of variables among groups were made by one-way ANOVA. \(P < 0.05\) was taken as significant.

**RESULTS**

**Baseline Echocardiographic, Postmortem, and Mechanoenergetic Parameters**

Table 1 summarizes echocardiographic and pathological parameters. HF rats had increased chamber diameter, decreased fractional shortening, and increased LV and lung-to-body weight ratio.

As summarized in Tables 2 and 3, under baseline conditions, \(E_{\text{max}}\) was lower in HF than controls (average of all animals: 1,976 ± 300 vs. 3,410 ± 331 mmHg·g·ml−1; \(P < 0.05\)). DP was lower (66 ± 5 vs. 105 ± 10 mmHg; \(P < 0.05\)), and LVEDP (12.5 ± 3.4 vs. 4.3 ± 1.8 mmHg; \(P < 0.05\), \(\tau (31 ± 0.5 \text{ vs. } 21.0 \pm 0.6 \text{ ms}; \ P < 0.05)\), and contractile efficiency (66 ± 7 vs. 45 ± 5%; \(P < 0.05)\) were higher in HF. Total VO₂ was

Table 1. Echocardiographic and pathological data

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 18)</th>
<th>Heart Failure (n = 42)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDD, mm</td>
<td>7.1 ± 0.3</td>
<td>9.8 ± 0.3*</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>3.9 ± 0.2</td>
<td>7.5 ± 0.2*</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>1.6 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>FS, %</td>
<td>45 ± 2</td>
<td>23 ± 2*</td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>455 ± 30</td>
<td>360 ± 45*</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>3.2 ± 0.4</td>
<td>5.2 ± 0.7*</td>
</tr>
<tr>
<td>Lung/BW, g/kg</td>
<td>4.8 ± 1.2</td>
<td>7.8 ± 4.3*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats; EDD, end-diastolic diameter; ESD, end-systolic diameter; PWT, posterior wall thickness; FS, fractional shortening; BW, body weight; LV/BW, left ventricular/body weight ratio; lung/BW, lung-to-body weight ratio. *\(P < 0.05\) compared with controls.
reduced in HF rats (673 ± 60 vs. 765 ± 74 ml O₂·beat⁻¹·g⁻¹; *P < 0.05). PVA-independent V₀₂ was also reduced in HF (544 ± 60 vs. 664 ± 81 ml O₂·beat⁻¹·g⁻¹; *P < 0.05). Basal metabolic V₀₂ was very similar in controls and HF. Consequently, the lower PVA-independent V₀₂ reflects lower E-C coupling V₀₂ in HF (322 ± 54 vs. 443 ± 66, ml O₂·beat⁻¹·g⁻¹; *P < 0.05).

**ET-1 Blockade and Mechanics**

As summarized in Table 2, ET-1 blockade did not affect DP, EDP, τ, or Emax in controls. Coronary blood flow was also unchanged. Table 3 summarizes the mechanoenergetics before and after drug interventions in HF. BQ-123 reduced DP (64 ± 7 vs. 50 ± 6 mmHg, *P < 0.05), EDP (12.9 ± 3.0 vs. 7.7 ± 3.1 mmHg, *P < 0.05), and τ (31 ± 12 vs. 14 ± 9 ms, *P < 0.05). BQ-788 had no effect. Bosentan decreased DP, EDP, and τ similarly to BQ-123. BQ-123 modestly increased coronary blood flow (average 5.6%, *P < 0.05), whereas bosentan and BQ-788 had no significant effect. Figure 1A shows effects of BQ-123 on pressure-volume relations in a representative failing heart. BQ-123 shifted ESPVR and EDPVR downward. Figure 1B shows group data for BQ-123, BQ-788, bosentan, and DMA in HF and controls. BQ-123 and bosentan decreased Emax similarly (−16 ± 3% and −18 ± 2%, *P < 0.05, Fig 2A). BQ-788 had no effect. Thus, in HF, ETA, and ETA/ETB

### Table 2. Mechanoenergetics and drug interventions in controls

<table>
<thead>
<tr>
<th>treated group</th>
<th>BQ-123 (n = 5)</th>
<th>BQ-788 (n = 5)</th>
<th>Bosentan (n = 5)</th>
<th>DMA (n = 5)</th>
<th>DMA (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed pressure, mmHg</td>
<td>101.0 ± 9.1</td>
<td>100.5 ± 13.1</td>
<td>108.1 ± 13.5</td>
<td>105.2 ± 11.7</td>
<td>109.8 ± 9.8</td>
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<tr>
<td>EDP, mmHg</td>
<td>4.4 ± 1.8</td>
<td>4.3 ± 1.5</td>
<td>4.4 ± 2.0</td>
<td>4.3 ± 1.7</td>
<td>4.3 ± 1.5</td>
</tr>
<tr>
<td>τ, ms</td>
<td>20.8 ± 0.4</td>
<td>19.8 ± 0.4</td>
<td>20.2 ± 0.7</td>
<td>19.6 ± 0.4</td>
<td>20.9 ± 1.0</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>15.2 ± 1.1</td>
<td>15.9 ± 1.3</td>
<td>14.9 ± 1.3</td>
<td>15.2 ± 1.4</td>
<td>15.5 ± 1.2</td>
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<tr>
<td>Emax, mmHg·g·ml⁻¹</td>
<td>3,445 ± 331</td>
<td>3,373 ± 373</td>
<td>3,415 ± 389</td>
<td>3,340 ± 395</td>
<td>3,356 ± 311</td>
</tr>
<tr>
<td>Intercept, ml O₂·beat⁻¹·g⁻¹</td>
<td>652 ± 72</td>
<td>637 ± 90</td>
<td>670 ± 88</td>
<td>641 ± 76</td>
<td>673 ± 81</td>
</tr>
<tr>
<td>BM, ml O₂·min⁻¹·g⁻¹</td>
<td>54 ± 3</td>
<td>59 ± 3</td>
<td>53 ± 7</td>
<td>54 ± 2</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>E-C, ml O₂·beat⁻¹·g⁻¹</td>
<td>427 ± 62</td>
<td>416 ± 73</td>
<td>449 ± 66</td>
<td>416 ± 59</td>
<td>456 ± 70</td>
</tr>
<tr>
<td>Total V₀₂, ml O₂·beat⁻¹·g⁻¹</td>
<td>788 ± 78</td>
<td>770 ± 83</td>
<td>765 ± 66</td>
<td>746 ± 61</td>
<td>763 ± 80</td>
</tr>
<tr>
<td>Efficiency, %</td>
<td>45.6 ± 5.8</td>
<td>46.8 ± 6.7</td>
<td>44.7 ± 5.1</td>
<td>46.0 ± 6.7</td>
<td>44.3 ± 4.6</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. V₀₂, myocardial O₂ consumption; EDP, end-diastolic pressure; Emax, ESPVR slope; intercept, V₀₂·PVA intercept; BM, basal metabolism; E-C, excitation-contraction coupling V₀₂; efficiency, contractile efficiency. There were no significant differences among “before” conditions.

### Table 3. Mechanoenergetics and drug interventions in HF group

<table>
<thead>
<tr>
<th>treated group</th>
<th>BQ-123 (1 μmol, n = 8)</th>
<th>BQ-788 (n = 8)</th>
<th>Bosentan (n = 8)</th>
<th>DMA (n = 5)</th>
<th>DMA (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed pressure, mmHg</td>
<td>64.4 ± 7.2</td>
<td>50.2 ± 6.4</td>
<td>64.9 ± 11.5</td>
<td>63.9 ± 9.4</td>
<td>67.1 ± 9.7</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>12.9 ± 3.0</td>
<td>7.7 ± 3.1</td>
<td>12.4 ± 2.9</td>
<td>12.0 ± 3.8</td>
<td>12.1 ± 2.9</td>
</tr>
<tr>
<td>τ, ms</td>
<td>31.3 ± 1.2</td>
<td>24.0 ± 0.9</td>
<td>31.0 ± 0.5</td>
<td>31.1 ± 0.6</td>
<td>31.1 ± 0.7</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>18.3 ± 1.1</td>
<td>19.5 ± 0.9</td>
<td>18.4 ± 1.5</td>
<td>18.6 ± 1.2</td>
<td>18.5 ± 1.3</td>
</tr>
<tr>
<td>Emax, mmHg·g·ml⁻¹</td>
<td>1,986 ± 220</td>
<td>1,671 ± 260</td>
<td>1,998 ± 278</td>
<td>1,925 ± 433</td>
<td>2,003 ± 310</td>
</tr>
<tr>
<td>Intercept, ml O₂·beat⁻¹·g⁻¹</td>
<td>540 ± 71</td>
<td>405 ± 81</td>
<td>542 ± 68</td>
<td>531 ± 51</td>
<td>550 ± 69</td>
</tr>
<tr>
<td>BM, ml O₂·min⁻¹·g⁻¹</td>
<td>52 ± 4</td>
<td>51 ± 8</td>
<td>53 ± 5</td>
<td>51 ± 7</td>
<td>54 ± 7</td>
</tr>
<tr>
<td>E-C, ml O₂·beat⁻¹·g⁻¹</td>
<td>323 ± 63</td>
<td>203 ± 50</td>
<td>303 ± 48</td>
<td>297 ± 56</td>
<td>323 ± 56</td>
</tr>
<tr>
<td>Total V₀₂, ml O₂·beat⁻¹·g⁻¹</td>
<td>688 ± 45</td>
<td>515 ± 31</td>
<td>650 ± 50</td>
<td>635 ± 62</td>
<td>670 ± 47</td>
</tr>
<tr>
<td>Efficiency, %</td>
<td>65.3 ± 5.9</td>
<td>54.2 ± 8.1</td>
<td>67.0 ± 6.2</td>
<td>69.1 ± 8.0</td>
<td>67.1 ± 5.7</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with “before” conditions. There were no significant differences among “before” conditions.

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but not ET\textsubscript{B} blockade decreased contractility and improved relaxation and end-diastolic distensibility.

ET\textsubscript{1} Blockade and Energetics

ET\textsubscript{1} blockade did not affect energy consumption in controls (Table 2). Figure 2A shows VO\textsubscript{2}-PVA relations before and after BQ-123 in a representative failing heart. VO\textsubscript{2} was tightly correlated with PVA before and after BQ-123 ($r > 0.95$). In HF, BQ-123 increased the slope of the VO\textsubscript{2}-PVA relation and consequently decreased efficiency (average 17%, $P < 0.05$, Fig. 2B, Table 3). Similarly, bosentan decreased efficiency ($-17 \pm 2\%$, $P < 0.05$), whereas BQ-788 had no effect (Fig. 2B). BQ-123 shifted the VO\textsubscript{2}-intercept of the VO\textsubscript{2}-PVA relation downward (average 25%, $P < 0.01$, Fig. 2A, Table 3). Because basal metabolic VO\textsubscript{2} was unchanged after BQ-123, bosentan, or BQ-788, the decreased intercept after BQ-123 and bosentan was due to decreased E-C coupling VO\textsubscript{2} (Table 3), amounting to $37 \pm 3\%$ and $35 \pm 3\%$, respectively (Fig. 2C). As shown in Table 3 and Fig. 2D, despite decreased efficiency total VO\textsubscript{2} decreased after BQ-123 and bosentan ($-25 \pm 3\%$ and $-24 \pm 3\%$, respectively, $P < 0.05$). BQ-788 did not affect total VO\textsubscript{2}. Thus in HF ET\textsubscript{A} and ET\textsubscript{A}/ET\textsubscript{B} but not ET\textsubscript{B} blockade reduced total VO\textsubscript{2} by effects on E-C coupling despite decreased efficiency.

BQ-123 (10 $\mu$M) decreased mechanoenergetic parameters to the same extent as 1 $\mu$M BQ-123 (Table 3). Treatment with 0.1 $\mu$M BQ-123 had no significant effects (data not shown).

DMA and Mechanoenergetics

To explore the mechanism by which ET\textsubscript{1} blockade decreases contractility and VO\textsubscript{2} in HF, we examined the effects of Na"/H" exchange inhibition. DMA did not alter any of the mechanoenergetic parameters in controls (Table 2). As shown in Table 3 and Figs. 1 and 2, in HF DMA decreased $E_{\text{max}}$ and PVA-independent, E-C coupling, and total VO\textsubscript{2} similarly to bosentan and BQ-123 but had no effect on efficiency, EDP, or $\tau$.

Coadministration of BQ-123 and DMA to HF rats resulted in decreased $E_{\text{max}}$ (−15 ± 3%, $P < 0.05$), E-C coupling VO\textsubscript{2} (−35 ± 3%, $P < 0.05$), and total VO\textsubscript{2} (−22 ± 2%, $P < 0.05$). The percent decrease in $E_{\text{max}}$ was similar to BQ-123 and DMA administered individually. Decreases in E-C coupling VO\textsubscript{2} and total VO\textsubscript{2} were virtually identical to BQ-123 alone and somewhat larger than those produced by DMA alone. Thus coadministration does not produce effects larger than those produced by the agent (BQ-123) with the greatest effects on E-C coupling VO\textsubscript{2} and total VO\textsubscript{2}. This suggests that BQ-123 and DMA do not have separate, additive effects on $E_{\text{max}}$, E-C coupling, VO\textsubscript{2} or total VO\textsubscript{2}.

DISCUSSION

The present study in DS rats with heart failure demonstrates that 1) endogenous ET-1 has a positive inotropic effect, slows relaxation, and decreases end-diastolic distensibility; 2) despite decreasing contractile efficiency, ET\textsubscript{A} and ET\textsubscript{A}/ET\textsubscript{B} but not ET\textsubscript{B} blockade reduce total VO\textsubscript{2} by virtue of energy saving effects on E-C coupling; and 3) inhibition of Na"/H" exchange causes negative inotropic effects and decreases in VO\textsubscript{2} that are quantitatively similar to those induced by ET\textsubscript{A} and ET\textsubscript{A}/ET\textsubscript{B} blockade, without altering efficiency or relaxation.

Endogenous ET-1 and Function in Failing Heart

Our findings that acute ET-1 blockade results in a negative inotropic effect and improved relaxation via the ET\textsubscript{A} receptor in HF are consistent with Sakai et al. (30), who reported similar effects of BQ-123 in the rat coronary ligation model. In contrast, data from other models suggest that ET-1 may have a blunted positive or even a negative inotropic effect (14, 32, 33, 36). These inconsistencies may result from effects of antagonist-induced changes in loading conditions (decreased vascular resistance) on conventional measures of LV performance as well as variable anesthesia, species, HF severity, and experimental preparation.

Data from patients are also apparently discrepant. MacCarthy et al. (19) reported that BQ-123 had no effect on +dP/dt in patients with dilated cardiomyopathy but decreased +dP/dt in nonfailing controls. In
Contrast, Serneri et al. (31) reported upregulation of ET-1 and a positive correlation of ETA receptor density with ejection fraction in patients with ischemic cardiomyopathy, suggesting that ET-1 maintains cardiac function. In agreement with the latter, ET-1 production and receptor density are increased in DS and coronary ligation rats (14, 17). Thus, whereas there is substantial evidence that ET-1 upregulation supports cardiac function in HF, there is divergence with respect to acute effects of antagonists. Although we have specified potential reasons for these inconsistencies, we cannot reconcile them.

We did not specifically investigate the mechanism of enhanced relaxation by ETA and ET/ETB blockade. These results are similar to those thought to be caused by ETB-mediated nitric oxide (NO) release with subsequent cGMP activation and decreased myofilament calcium responsiveness (37). Because we observed that ETB blockade did not alter relaxation, some other mechanism must explain this effect of ET blockade. We (21) previously reported that Dahl rats with HF have increased half-maximal myofilament calcium sensitivity for tension production. Furthermore, we (25) recently documented decreased half-maximal calcium sensitivity of in vitro velocity of intact thin filaments isolated from Dahl rats with HF (25). This thin filament defect was normalized by chronic bosentan treatment. Thus whereas a NO-mediated response is unlikely some other myofilament tension desensitizing effect may be responsible for enhanced relaxation produced by ET blockade, for example decreased intracellular pH due to reduced Na+/H+ exchange (see below).

Endogenous ET-1 and PVA-independent VO2, Contractile Efficiency, and Total VO2

Under basal conditions both PVA-independent VO2 and E-C coupling VO2 were reduced in HF rats. This is similar to our previous report (15) and is best explained by reduced basal Ca2+ cycling in HF. This conclusion is supported by Yoneda et al. (38), who reported reduced intracellular Ca2+ transients in Dahl rats with heart failure. ETA and ET/ETB but not ETB blockade shifted the VO2-PVA intercept downward in HF. This is attributable to decreased E-C coupling VO2 and likely reflects additional depression of Ca2+ cycling induced by ETA receptor inhibition (34). Na+/H+ exchanger inhibition decreased Emax and E-C coupling VO2 to a similar extent as ETA and ET/ETB blockade, without altering efficiency or relaxation. Coadministration of BQ-123 and DMA resulted in changes in Emax and E-C coupling VO2 similar to those resulting from separate administration, suggesting a common mechanism for their effects on these parameters. ET-1 augments myofilament calcium responsiveness, at least in part through alkalization caused by activation of Na+/H+ exchange (37). Activation of Na+/H+ exchange would also be expected to increase activator Ca2+ via secondary effects on Na+/Ca2+ exchange, which should increase E-C coupling VO2.

Because coronary perfusion pressure was higher in HF rats, a factor that could have influenced baseline levels of PVA-independent VO2 in our studies is the Gregg effect or increased contractility occurring in conjunction with increased coronary perfusion pressure and/or flow. We (9) previously reported that the Gregg effect is associated with increases in PVA-independent VO2 without changes in the slope of the VO2-PVA relation. Dijkstra et al. (5) have shown that capillary pressure is in fact the main determinant of the Gregg effect. Therefore, to the extent that the higher perfu-
sion pressure in HF rats resulted in higher capillary pressure this would have tended to improve contractility and increase PVA-independent \( \dot{V}O_2 \) in HF rats, i.e., it would act as a bias against the results we found.

Recently, Takeuchi et al. (35) showed that exogenous ET-1 exerts a positive inotropic effect and increases efficiency through ETA activation. Positive inotropy is also due, at least in part, to increased Na\(^+/\)H\(^+\) exchange. However, ETA-induced increased efficiency is independent of Na\(^+/\)H\(^+\) exchange (35). Ito et al. (13) reported that ET-1 stimulation of Na\(^+/\)H\(^+\) exchange was impaired in rat cardiac hypertrophy. This may indicate that ET-1 stimulation of Na\(^+/\)H\(^+\) exchange via activation of protein kinase C differs in normal and hypertrophied myocytes. However, these authors did not investigate effects of endogenous ET-1 in failing myocardium or compare nonfailing and failing myocardium. Pieske et al. (27) reported that ET-1 exerts inotropic effects through ETA receptor-mediated increased myofibrillar Ca\(^{2+}\) responsiveness even in failing myocardium. They suggested that although the functional effects of ET-1 are attenuated, local ET is activated, implying impaired postreceptor signaling. Taken together, these observations suggest that inotropic effects and increased E-C coupling and total \( \dot{V}O_2 \) produced by endogenous ET-1 in HF are mediated by a combination of increased activator Ca\(^{2+}\) and increased myofilament Ca\(^{2+}\) responsiveness resulting from activation of Na\(^+/\)H\(^+\) exchange.

Contractile efficiency was markedly increased in HF and decreased after ETA and ET\(_A/\)ET\(_B\) antagonism. The former is consistent with our report of reduced myofibrillar ATPase activity in HF in association with increased efficiency (15). In hyperthyroidism increased ATPase caused by increased \( \alpha \)-myosin heavy chain isoform results in reduced efficiency (8). Thus changes in ATPase cause directionally opposite changes in efficiency. McClellan et al. (20) reported that ET-1 decreases aotmyosin ATPase activity in rat hearts and predicted that ET-1 increases efficiency, later confirmed by Takeuchi et al. (35). ET-1 effects on crossbridge cycling are thought to be caused by protein kinase C-mediated phosphorylation of troponins I and T. It is also reported that myocardial ET-1 production is increased in failing DS rats (14). Thus these studies and ours suggest that in HF ET-1 blockade reverses an increase in efficiency caused by endogenous ET-1-mediated alterations in troponin I and/or T phosphorylation. The lack of effect of DMA on efficiency in failing rats underscores the fact that ET-1 influences efficiency by a mechanism distinct from the Na\(^+/\)H\(^+\) exchange, i.e., its effect on myofibrillar ATPase activity.

Our most important finding is that ETA blockade caused a decrease in total \( \dot{V}O_2 \) via ETA receptor inhibition as a result of E-C coupling energy saving effects being larger than contractile machinery energy wasting effects. To the extent that energy depletion occurs in HF (11), increased efficiency caused by ET-1 may be cardioprotective. With respect to ET blockade, however, the net effect of decreased total \( \dot{V}O_2 \) despite decreased efficiency should be favorable.

**ET\(_A\) versus ET\(_B\) Receptors and Energy Consumption**

ET\(_B\) receptors mediate vasodilatation through NO and prostacyclin release from vascular endothelium (10). Although our results demonstrate that ET\(_B\) is not activated in failing DS rats, ETA blockade could result in secondary ET\(_B\) receptor activation, with NO release. In isolated rat heart, Poderoso et al. (28) showed dose-dependent decreases in total \( \dot{V}O_2 \) with increasing NO concentration. In contrast, we reported that inhibition of NO synthase with L-NNA causes a small decrease in E-C coupling \( \dot{V}O_2 \), with no change in basal metabolic \( \dot{V}O_2 \) or efficiency (24). Thus increased NO in our preparation would be expected to slightly increase \( \dot{V}O_2 \). Therefore, a change in NO activity does not appear to play an important role in ET-1-mediated alterations in \( \dot{V}O_2 \) in failing DS rats.

**Clinical Implications**

In conclusion, if energy depletion is important in HF, our results suggest that reduced \( \dot{V}O_2 \) induced by ETA blockade should improve energy supply/demand and favorably affect outcomes via this mechanism.

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**DISCLOSURES**

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**REFERENCES**


