Contrasting Inotropic Effects of Endogenous Endothelin in the Normal and Failing Human Heart

Studies With an Intracoronary ET\textsubscript{A} Receptor Antagonist

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**Background**—Endothelin-1 (ET-1) is a potent positive inotrope in vitro, but its physiological effects on intrinsic myocardial contractile function in humans in vivo are unknown. Plasma ET-1 levels are elevated in heart failure, and ET-1 may be involved in the pathophysiology of this condition. However, its effects on contractile function of the failing human heart are also unknown.

**Methods and Results**—A specific ET\textsubscript{A} receptor antagonist, BQ123, was infused (40 nmol/min, 16 minutes) into the left coronary artery in 8 patients with atypical chest pain (normal left ventricular [LV] function and coronary arteries) and 8 patients with nonischemic dilated cardiomyopathy (DCM) who were undergoing diagnostic catheterization. In normal subjects, BQ123 rapidly induced a significant reduction in LV dP/dt\textsubscript{max} ($-270\pm71$ mm Hg/s after 16 minutes; $P<0.05$) and in LV dP/dt at a developed pressure of 40 mm Hg (LV dP/dt\textsubscript{40}) ($-179\pm54$ mm Hg/s; $P<0.05$). In DCM patients, however, BQ123 caused no reductions in LV dP/dt\textsubscript{max} ($62\pm49$ mm Hg/s after 16 minutes) or LV dP/dt\textsubscript{40} ($83\pm51$ mm Hg/s; $P<0.05$ compared with normal subjects). BQ123 had no effect on heart rate, LV relaxation, LV end-diastolic pressure, right atrial pressure, or pulmonary pressure in either patient group.

**Conclusions**—Endogenous ET-1 has a tonic positive inotropic effect in normal subjects, independent of effects on the peripheral vasculature and unmasked by inhibition of ET\textsubscript{A} receptors. However, the effect of short-term ET\textsubscript{A} blockade in DCM patients was opposite to that in normal subjects, which suggests that ET-1 may cause negative inotropic effects in the failing heart. (*Circulation*. 2000;101:142-147.)

Key Words: endothelin | cardiomyopathy | contractility

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Endothelin-1 (ET-1)\textsuperscript{1} has potent in vitro positive inotropic effects on normal myocardium of several species, including humans.\textsuperscript{2} The effects of ET-1 in the whole heart are more difficult to interpret. Exogenous ET-1 may potentially exert opposing direct positive inotropic effects and indirect negative inotropic effects, secondary to the potent coronary vasoconstriction induced by the peptide and to the increased cardiac load that results from peripheral vasoconstriction. The use of ET receptor antagonists to inhibit the effects of “basal” ET-1 release may be a better approach for addressing this question. Both ET\textsubscript{A} and ET\textsubscript{B} receptors are expressed on cardiac myocytes, although in adult human myocardium, only ET\textsubscript{A} receptors are reported to be functionally important.\textsuperscript{3} We previously reported that a specific ET\textsubscript{A} receptor antagonist, BQ123, induced earlier and faster myocardial relaxation in isolated ferret papillary muscle preparations\textsuperscript{4} and isolated perfused ejecting guinea pig hearts.\textsuperscript{5} Likewise, McClellan et al\textsuperscript{6} reported that BQ123 induced a negative inotropic effect in isolated rat cardiac trabeculae. These in vitro studies suggested that basal release of ET-1 may have positive inotropic effects and may delay myocardial relaxation. Using an analogous approach in normal humans, Webb and colleagues reported that ET antagonists infused either locally into the forearm\textsuperscript{7} or systemically\textsuperscript{8} decreased vascular resistance and reduced blood pressure. Thus, endogenous ET-1 appears to have a role in maintaining basal vascular tone and blood pressure in healthy humans. However, the role of endogenous ET-1 in regulating intrinsic cardiac contractile function in healthy humans is unknown.

Recent experimental and clinical studies indicate a role for ET-1 in the pathophysiology of heart failure. Elevated production of ET-1 is thought to contribute to systemic and pulmonary vasoconstriction, as well as to cardiac hypertrophy, fibrosis, and remodeling.\textsuperscript{9-13} Plasma ET-1 levels are elevated in patients with congestive heart failure (CHF) and correlate positively with New York Heart Association func-
Having a blunted positive inotropic effect or even a negative inotropic effect in failing myocardium, which could contribute to impairment of left ventricular (LV) function. However, the effect of endogenous ET-1 on intrinsic contractile function of the failing human heart, independent of changes in systemic loading, remains unknown.

In this study, we compared the short-term effects of endogenous ET-1 on intrinsic cardiac contractile function in normal subjects and in patients with dilated cardiomyopathy (DCM). To assess the effects of endogenous ET-1 independent of changes in systemic loading, we studied the response to intracoronary infusion of a locally acting dose of the specific ETA receptor antagonist BQ123.

**Methods**

The study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Institutional Research and Ethics Committee. All subjects provided written informed consent.

**Patient Characteristics**

The first group comprised 8 patients (2 men, mean age 47.3 ± 5.4 years) undergoing diagnostic cardiac catheterization for investigation of atypical chest pain, who had normal epicardial coronary arteries and normal LV function. None of these patients were thought to have syndrome X. Three were taking β-blockers, 2 were taking long-acting nitrates, and 2 others were taking calcium antagonists. The second group comprised 8 patients (5 men, mean age 47 ± 3.2 years) with idiopathic DCM, all with LV ejection fraction < 40% and normal coronary arteries. Patient characteristics of this group are shown in the Table. Other than ACE inhibitors, all medication was withheld in both groups for ≥ 24 hours before the study. All subjects abstained from alcohol, caffeine-containing drinks, and smoking for ≥ 24 hours.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>NYHA Class</th>
<th>Ejection Fraction, %</th>
<th>Medication</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>M</td>
<td>II</td>
<td>36</td>
<td>ACEI, frusemide, digoxin</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>M</td>
<td>III</td>
<td>17</td>
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<tr>
<td>3</td>
<td>52</td>
<td>M</td>
<td>III</td>
<td>30</td>
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</tr>
<tr>
<td>4</td>
<td>41</td>
<td>F</td>
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<td>5</td>
<td>54</td>
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<tr>
<td>6</td>
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<td>II</td>
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<td>8</td>
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</table>

ACEI indicates ACE inhibitor.

All pressures were referenced to atmospheric pressure at the level of the mid chest. A Meddars 1300 physiological measurement system was used to record high-fidelity LV pressure; aortic, right atrial, and pulmonary artery pressures; and a bipolar standard lead of the ECG; the results were also fed via a Macintosh analogue-digital converter (AD Instruments) into a Macintosh personal computer with Chart software (version 3.5s, AD Instruments).

At least 20 minutes after coronary angiography and ≥ 10 minutes after insertion of additional catheters, baseline recordings of pressures and ECG were obtained. BQ123 (American Peptide Co) was then infused into the left coronary artery at 40 nmol/min (flow rate 2 mL/min) for 16 minutes with an IVAC P4000 anesthesia syringe pump (Welmed Ltd). Previous studies by Verhaar and colleagues showed that a 10 nmol/min infusion in the human forearm induced maximal vasodilatation yet remained locally active (ie, blood flow was unchanged in the noninfused forearm). We chose a dose of 40 nmol/min to achieve roughly equivalent local concentrations (0.4 μmol/L, assuming a coronary flow rate of ~100 mL/min). This concentration is 10- to 100-fold higher than the reported affinity of binding sites for BQ123 in human LV (0.73 nmol/L) or the K for BQ123 inhibition of ET-1 response in human heart (3.3 nmol/L) yet is still selective for the ETA receptor. Pressures, heart rate, and ECG were monitored during BQ123 infusion and for ≥ 15 minutes after cessation of infusion. At the end of BQ123 infusion, a normal saline infusion at the same flow rate was substituted. LV angiography was performed after completion of the study.

**Data Analysis**

Data were analyzed offline. Maximal and minimal rates of LV pressure development (LV dP/dt max and LV dP/dt min, respectively) and LV dP/dt at a developed pressure of 40 mm Hg (LV dP/dt 40) were obtained from the first derivative of the LV pressure signal. The time constant of isovolumic LV relaxation, t, was calculated as described previously.

Data are expressed as mean ± SEM. Comparison of absolute values and changes in pressure data and heart rate during and after BQ123 infusion in the 2 groups of patients were made by 2-way ANOVA for repeated measures with StatView (version 4.5) software. Post hoc analysis was performed with Tukey’s test. Baseline parameters in the 2 groups were compared with independent Student’s t tests. P < 0.05 was considered significant.

**Protocol**

Diagnostic coronary angiography was performed via the right femoral approach in a quiet cardiac catheterization laboratory (temperature 23°C). Subjects were included for study if the coronary angiogram was normal. Heparin (5000 IU bolus) was administered intra-arterially. A 5F Swan-Ganz catheter was inserted via the right femoral vein and positioned to measure pulmonary artery and right atrial pressure simultaneously. A 6F micromanometer-tipped pigtail catheter (Millar Instruments) was inserted via the left femoral artery and positioned in the LV. A 5F left Judkins catheter was used for intracoronary infusion. The Millar catheter was calibrated externally against a mercury reference and matched against luminal pressure.
Heart rate was significantly higher in the DCM group, whereas mean right atrial pressure, mean pulmonary artery pressure, and mean aortic pressure at baseline were not significantly different (Figure 1). No untoward side effects were encountered during the study in any subject, and there were no changes in the ECG.

Figure 1 shows the effect of BQ123 infusion on hemodynamic parameters in the 2 groups. In subjects with normal LV function, BQ123 caused a significant decrease in LV dP/dt max within 4 minutes, and this became progressively greater with continuing infusion. The reduction in LV dP/dt max was not accompanied by changes in LVEDP, mean aortic pressure, right atrial pressure, heart rate, or pulmonary artery pressure, which suggests that BQ123 did not exert significant systemic effects. In the DCM group, however, there was no significant fall in LV dP/dt max at any time point with intracoronary BQ123. Two-way ANOVA revealed a significant (P<0.001) group-time interaction for LV dP/dt max, which indicates that the changes were significantly different between the 2 groups. Individual patient data for the maximal effect of BQ123 on LV dP/dt max showed that in some DCM patients, BQ123 induced increases in LV dP/dt max that ranged from 13% to 26%, without an associated change in mean aortic pressure, heart rate, or LVEDP.

Figure 2 shows changes in LV dP/dt max, LV dP/dt 40, and peak LV pressure (relative to the baseline preinfusion values) during and after BQ123 infusion in both groups. The mean changes in LV dP/dt max and LV dP/dt 40 differed significantly between the groups at all time points shown, and in peak LV pressure, the mean changes differed at the end of infusion and 12 minutes after the end of infusion. Within-group analyses (ANOVA) revealed significant reductions in LV dP/dt max and LV dP/dt 40 in normal subjects (P<0.05) but no change in DCM patients.

At baseline, LV dP/dt min was significantly lower and t was significantly prolonged in the DCM group compared with normal subjects. No significant changes were observed in these parameters with BQ123 infusion in either group (data not shown). Baseline time to LV dP/dt min was significantly lower in the DCM group (related to the higher heart rate), but there were no changes with BQ123 infusion in either group.

Discussion

A possible role of endogenous ET-1 in modulating intrinsic cardiac contractile function in humans in vivo has not been addressed previously. To investigate this, we studied the effects of local intracoronary infusion of a specific ET A receptor antagonist, BQ123, in patients undergoing cardiac catheterization. Our results indicate that (1) endogenous ET-1 has a tonic positive inotropic effect in normal humans, independent of changes in heart rate, cardiac preload (as assessed by LVEDP), or afterload (as assessed by mean aortic pressure); and (2) the effects of BQ123 are significantly different between normal and DCM patients. The significant changes in LV dP/dt max and LV dP/dt 40 in normal subjects but not in DCM patients suggest that ET-1 may play a role in the regulation of cardiac contractile function in vivo.
pressure), and (2) this effect of ET-1 is lost in the failing human heart. The loss of the tonic inotropic effect of ET-1 may contribute to contractile dysfunction in heart failure.

Physiological Role of Endogenous ET-1 in Regulating Human Heart Contractility

Despite the well-established positive inotropic actions of exogenous ET-1 in vitro, its physiological relevance for cardiac contractility in vivo has remained uncertain. Because ET-1 acts primarily as an autocrine/paracrine factor released by endothelial cells, preferentially to the abluminal surface of these cells, its effects in the intact heart will depend on the sites of release and the local concentrations achieved. The use of specific ET receptor antagonists enables inhibition of ET-1 effects at these sites and has been successfully employed in previous studies in the peripheral human vasculature. In normal humans, systemic administration of an ET A/B antagonist, TAK-044, increased cardiac index, but this was attributed to the concomitant decrease in systemic vascular resistance. In the present study, the use of intracoronary infusion allowed us to assess local (intracardiac) effects of BQ123. The lack of systemic effect of BQ123 was verified by the absence of changes in aortic pressure, pulmonary artery pressure, and right atrial pressure. In this setting, and in the absence of changes in LVEDP, measurement of LV dP/dt max and LV dP/dt 40 can be considered appropriate indices of intrinsic LV “contractility.” The degree of reduction in these indices (≈15%) was relatively modest but probably underestimates the true magnitude of effect, because the duration of invasive monitoring was limited to <30 minutes for ethical reasons. In analogous studies of BQ123 in the forearm, the maximal effect was observed after >60 minutes. Furthermore, under conditions in which ET-1 release is augmented (eg, altered shear, hypoxia, and exposure to cytokines), the magnitude of the inotropic effect may be correspondingly greater.

Acute Effects of Endogenous ET-1 on LV Contractility in Heart Failure

The lack of reduction in LV dP/dt max and LV dP/dt 40 with intracoronary BQ123 in DCM patients indicates that endogenous ET-1 had no positive inotropic effect in this group. Indeed, there was a trend toward increases in these parameters with BQ123 (Figure 2), which suggests that endogenous ET-1 might even exert negative inotropic effects in this group. These findings are consistent with the results of recent experimental studies. Exogenous ET-1 had negative inotropic effects in myocytes isolated from a porcine pacing heart failure model, in contrast to the positive inotropic effect observed in normal myocytes. In a rabbit pacing heart failure model, the same group found that the response to exogenous ET-1 was diminished in isolated cardiac myocytes. A reduced inotropic response to ET-1 was also reported in a rabbit epirubicin cardiomyopathy model. Systemic administration of the nonselective ET A/B receptor antagonist bosentan improved cardiac performance in animals and patients with heart failure, although this was accompanied by concomitant decreases in systemic vascular resistance, which make the results difficult to interpret. A recent article also reported reduced positive inotropic effects of exogenous ET-1 in ventricular muscle strips from human end-stage failing hearts compared with normal hearts. The present study therefore provides additional evidence that ET-1 may exert negative inotropic effects in CHF, in contrast to its positive inotropic effects in the normal state.

Available data from experimental studies suggest that the beneficial effects of ET antagonists on mortality, functional status, LV remodeling, and pulmonary hypertension are attributable at least in part to their favorable hemodynamic effects, which off-load the failing heart in a manner similar to
ACE inhibitors.\textsuperscript{2,11–13,17,21} Inhibition of the mitogenic effects of ET-1 may also be involved.\textsuperscript{2} Recently, it was reported that myocardial ET-1 production was significantly increased in Dahl-sensitive rats only at the stage of CHF and that ET-1 levels correlated with the degree of LV contractile dysfunction.\textsuperscript{18} Long-term treatment with bosentan attenuated the impairment of LV function without affecting the degree of hypertrophy in that study,\textsuperscript{18} independently of changes in afterload. The present clinical study, as well as the above experimental studies, suggests that changes in the direct myocardial actions of ET-1 may also contribute to LV contractile dysfunction in human patients with CHF. In the present study, however, we were unable to correlate changes in LV contractility with ET-1 levels, because no measurements of ET-1 levels were made.

Potential Subcellular Mechanisms Underlying the Contrasting Effects of ET-1
The positive inotropic effects of ET-1 are believed to result from G\textsubscript{i} protein-mediated activation of protein kinase C and a consequent variable combination of elevation of cytosolic calcium levels and myofilament sensitization to calcium.\textsuperscript{2,30,31} The absent (or negative) inotropic effect of ET-1 in CHF could be due to downregulation or desensitization of ET receptors. However, no alterations in ET receptor density or affinity were found in end-stage failing human myocardium.\textsuperscript{3} ET receptors can couple to G\textsubscript{i} proteins and inhibit adenyl cyclase, thereby potentially exerting negative inotropic effects.\textsuperscript{32} Although the functional activity of G\textsubscript{i} proteins is increased in CHF,\textsuperscript{33} this appears an unlikely explanation, because ET-1 is reported to couple to G\textsubscript{i} only in human atrium and not in LV.\textsuperscript{3} An alternative possibility is that the defect in failing myocardium lies distal to activation of protein kinase C. In the human forearm, at least part of the response to BQ123 involved the production of NO, secondary to activation of endothelial ET\textsubscript{B} receptors by ET-1.\textsuperscript{1,22} It is feasible that the myocardial response to ET\textsubscript{A} inhibition could involve a similar release of endothelial NO, via ET\textsubscript{B} receptor activation. Indeed, NO in high concentrations can exert negative inotropic effects.\textsuperscript{31} However, we have previously reported a quite different pattern of effect of intracoronary NO donors or of endothelium-derived NO in healthy human subjects, namely, a reduction in LVEDP and an earlier onset of LV relaxation without change in LV dP/dt\textsubscript{max}.

Study Limitations
First, we used changes in LV dP/dt\textsubscript{max} and LV dP/dt\textsubscript{a0} as indices of contractility. The determination of LV pressure-volume relations would provide a more load-independent index.\textsuperscript{28} Second, the maximal acute response to ET antagonists in vivo may take >60 minutes, so that the changes reported here probably underestimate the true effect. In particular, more prolonged monitoring might have revealed a positive inotropic effect of BQ123 in more DCM patients. However, a response to ET antagonists is apparent within 15 to 20 minutes in most animal and clinical studies.\textsuperscript{7–10,21,22}

Although ET antagonists are unlikely to reverse agonist-receptor binding,\textsuperscript{2} the agonist-receptor complex is rapidly internalized, and ET antagonists probably inhibit unoccupied or newly expressed surface receptors.\textsuperscript{36} Third, because we only used a selective ET\textsubscript{A} antagonist, the possible role of ET\textsubscript{B} receptors remains unknown. Fourth, because ACE inhibitors were continued in our DCM patients for ethical reasons, we cannot exclude the possibility that this may have influenced responses to BQ123. However, the hemodynamic effects of ET antagonists are additive to those of ACE inhibitors in CHF patients.\textsuperscript{10} Finally, although all other medications were omitted for ≥24 hours before the study, it is conceivable that some effects of longer-acting drugs could persist to confound the results.

Summary
This study provides the first evidence that endogenous ET-1 has a tonic positive inotropic effect in the healthy human heart in vivo and that ET-1 may have opposing negative inotropic effects in patients with DCM.

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References


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