Bucindolol Displays Intrinsic Sympathomimetic Activity in Human Myocardium

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Background—Most clinical studies have shown that β-adrenergic receptor antagonists improve long-term survival in heart failure patients. Bucindolol, a nonselective β-receptor blocker, however, failed to reduce heart failure mortality in a recent large clinical trial. The reasons for this failure are not known. Bucindolol has partial agonist properties in rat myocardium, but whether it has agonist activity in human heart is controversial. To address this, we measured the ability of bucindolol to increase cAMP accumulation in human myocardium.

Methods and Results—Myocardial strips (≈1 mm³) obtained from rat and nonfailing human hearts were confirmed to be viable for ≥48 hours in normoxic tissue culture by MTT assay and histology. Freshly isolated strips were exposed to β-adrenergic antagonists and agonists and assayed for cAMP. In both rat and human strips, the full β-adrenergic agonist isoproterenol raised cAMP levels by >2.5-fold at 15 minutes. Carvedilol and propranolol had no effect on basal cAMP levels, whereas metoprolol reduced basal cAMP by ≈25%. In contrast, bucindolol and xamoterol increased cAMP levels in a concentration-dependent manner in both rat and human myocardium (maximum 1.64±0.25-fold and 2.00±0.27-fold over control, respectively, P<0.01 for human tissue).

Conclusions—Bucindolol exhibits ≈60% of the β-adrenergic agonist activity of xamoterol in normal human myocardial tissue. (Circulation. 2002;105:2429-2434.)

Key Words: heart failure β pharmacology β receptors β bucindolol β xamoterol

Neurohormonal activation is an early and harmful compensatory response to heart failure, marked by increased blood levels of norepinephrine, angiotensin II, vasopressin, endothelin-1, and other vasoconstrictor factors. As a consequence of chronic catecholamine excess, β₁-adrenoceptors are phosphorylated and downregulated, 1,2 both β₁- and β₂-adrenoceptors become uncoupled, 1,2 inhibitory Gᵢ proteins are upregulated, 1,3 β-adrenoceptor phosphorylation is increased, 2 and myocardial catecholamine uptake and release are altered. Although sympathetic activation maintains cardiac output in the short term, it is now recognized as a major factor in CHF progression and mortality. 3 Blockade of sympathetic drive has thus become a major goal of current therapy.

The benefits of β-adrenergic blockade in heart failure were first demonstrated in 1975 5 and have since been confirmed in several large randomized, double-blind, placebo-controlled trials. Both β₁-selective and nonselective β-blockers, including metoprolol, bisoprolol, and carvedilol, have produced striking reductions in mortality, together with improvements in clinical status and hemodynamics. 6-11

Despite the abundant evidence in favor of β-blockers, their deployment remains incomplete. Negative inotropic therapy for heart failure is counterintuitive, and concerns persist about withdrawal of adrenergic support, especially in the sickest patients. Adding to these concerns, the nonselective β₁- and β₂-adrenoceptor antagonist bucindolol recently failed to decrease long-term mortality compared with placebo in a large multicenter trial, the Beta-Blocker Evaluation of Survival Trial (BEST). 12 It is unclear whether the BEST trial failed because of population differences in the response to β-blockers (eg, NYHA functional class III versus class IV heart failure, black versus nonblack) or whether bucindolol itself has unique properties that might make it less useful in this clinical setting.

Bucindolol is a nonselective β-adrenergic antagonist with vasodilatory and sympatholytic properties. 13-15 Studies in a variety of animal models have indicated that bucindolol, like xamoterol, can behave as a partial agonist at the β-adrenergic receptor (βAR) and therefore exhibits intrinsic sympathomimetic activity (ISA). 13,16-18 To date, however, there has been...
TABLE 1. Donor Characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
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<tr>
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<td>F</td>
<td>56</td>
<td>Subarachnoid hemorrhage</td>
</tr>
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<td>3</td>
<td>F</td>
<td>62</td>
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</tr>
<tr>
<td>4</td>
<td>M</td>
<td>46</td>
<td>Hanging</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>67</td>
<td>Subarachnoid hemorrhage</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>41</td>
<td>Head injury</td>
</tr>
</tbody>
</table>

scant and conflicting evidence that bucindolol has ISA in human myocardium. 19–24 The question is significant, because partial agonist activity could have negated some of the potential benefits of bucindolol in the BEST study. Here, we describe a short-term tissue culture system in which the ISA of bucindolol and other adrenergic agents can be measured directly via accumulation of intracellular cAMP levels in myocardial tissue strips. Using this method, we find that bucindolol has significant concentration-dependent ISA in both rat and normal human myocardium.

Methods

Adult Rat and Human Myocardial Strip Culture

Adult rats (Simonson, Gilroy, Calif) were anesthetized with pentobarbital according to a protocol approved by the University of Miami Animal Care and Use Committee. The hearts were rapidly removed and placed into sterile, ice-cold, oxygenated, Ca2+-free Tyrode’s solution (mmol/L: 140 NaCl, 4 KCl, 0.5 MgCl2, 0.33 NaH2PO4, 5.5 glucose, 5.5 HEPES). Under a laminar flow hood, hearts were perfused with the same solution to remove blood from the chambers and coronary system.

Nonfailing human myocardium was obtained from donors whose hearts were not used for transplantation (Table 1). Written informed consent for organ donation for research purposes was obtained from the next of kin by one of us (L.O.). The tissue was used within 2 to 3 hours of cross-clamping of the aorta.

Freshly harvested myocardial tissue was carefully trimmed with fine scissors into very small (1- to 2-mm3) pieces in the above solution in ice. The strips were then washed into sterile, ice-cold, oxygenated, Ca2+-free Tyrode’s solution and placed into sterile, ice-cold, oxygenated, Ca2+-free Tyrode’s solution in a 37°C shaking water bath for 10 minutes. IBMX (0.1 mmol/L) was then added to the buffer, and the strips were shaken at 37°C for 10 more minutes. Finally, xamoterol (0.5 to 5 μmol/L), bucindolol, forskolin, isoproterenol, carvedilol (each 0.01 to 10 μmol/L), metoprolol (10 μmol/L), or vehicle was added, and incubation was continued at 37°C for a further 15 minutes. Reactions were stopped by addition of 300 μL of 10% ice-cold trichloroacetic acid. cAMP levels were determined by radioimmunoassay according to the manufacturer’s protocol (Perceptive Diagnostics).

Statistical Analysis

Results are expressed as mean±SEM. Differences between columns were evaluated by 2-tailed Mann-Whitney test. ANOVA was carried out with InStat 2.0 for Macintosh.

Results

Myocardial Strip Viability

The MTT assay was used to characterize the viability of myocardial tissue cultures over a period of 0 to 8 days. In living cells, mitochondrial reductases convert the water-soluble, yellow tetrazolium salt (MTT) into a water-insoluble formazan precipitate. 25 The production of formazan was linearly related to the amount of myocardial tissue in each assay (Figure 1A). As shown in Figure 1B, formazan production was stable over the first 24 hours and remained >90% of starting levels between days 0 and 3 of culture, indicating preserved metabolic activity and viability for ≥72 hours. Identical results were obtained with rat myocardial strips (not shown).

Histological analysis of human myocardial strips after short-term (6 hours) culture and exposure to adrenergic agents revealed normal myocardial morphology with occasional lipofuscin pigment, consistent with the age of the donors (Figure 1C).

On the basis of these observations, we concluded that myocardial strips in short-term culture would serve as a valid model system for testing myocardial cellular responses to exogenous agents. Subsequent assays were performed on the first day of tissue culture to maximize viability.

cAMP Stimulation in Adult Rat Myocardial Strips

In rat myocardium, both forskolin and isoproterenol increased myocardial cAMP levels by ~2.6- and 2.2-fold, respectively, indicating intact receptor and postreceptor coupling to adenylate cyclase (Figure 2). Xamoterol, a β-adrenergic antagonist with known partial agonist properties, also increased myocardial cAMP levels in a concentration-dependent manner, with maximum ISA at 5.0 μmol/L (1.92±0.27-fold over control, P<0.01). Bucindolol also produced a concentration-dependent increase in tissue cAMP, with a maximum effect at 10 μmol/L (2.46±0.42-fold over control, P<0.01), consistent with results in neonatal rat myocyte cultures. 18 Three other β-adrenergic antagonists, carvedilol, propranolol, and metoprolol, however, had no effect on cAMP levels (Figure 2).

cAMP Stimulation in Normal Human Myocardial Strips

We then studied cAMP accumulation in normal human myocardium in response to treatment with the above β-adrenergic agonists and antagonists and with forskolin, which acts downstream of the receptor. Both isoproterenol...
and forskolin induced significant increases in myocardial cAMP (maximum 3.26- and 2.85-fold over control, respectively, *P* < 0.01; see Table 2). In this system, 10 μmol/L forskolin was roughly equipotent to 4 μmol/L isoproterenol. There was a much wider range of responses to isoproterenol compared with forskolin, however, suggesting that states of receptor sensitization varied significantly among samples. These results confirm that the human myocardial tissue

![Figure 1](image1.png)

**Figure 1.** Myocardial strip viability. A, Formazan production in relation to mass of viable myocardium. Small (~1-mm³) strips of myocardium were freshly harvested from adult rat hearts and placed in tissue culture as described in Methods. Samples of ~20 to 200 mg freshly harvested tissue (10 to 100 strips) were incubated with MTT for 2.5 hours before harvesting. Formazan production was quantified by measurement of optical density at 560 nm as described in Methods. A linear correlation is seen between OD₅₆₀ and amount of viable myocardium. Data shown are means of 4 experiments. B, Formazan production by human myocardial tissue in culture over 7 days. Individual samples of 100 myocardial strips from human left ventricular free wall were cultured for indicated periods of time before MTT assay. Ordinate displays percentage of day 0 formazan production. Data shown are means of 3 separate experiments. C, Histology of human myocardial strips. Human myocardial strips were fixed in 5% paraformaldehyde immediately after treatment with β-adrenergic compounds and embedded in paraffin. Sections 6 μm thick were stained with hematoxylin-eosin and analyzed by light microscopy. Representative strips shown were exposed to bucindolol 10 μmol/L; other samples were identical in appearance (not shown). Left, Four representative human cardiac muscle strips (magnification ×1). Right, High-magnification view of cardiac muscle strip in cross section (magnification ×400).

![Figure 2](image2.png)

**Figure 2.** cAMP induction in adult rat myocardial strips. Adult rat myocardial strips were exposed to indicated β-adrenergic compounds on day 0, and cAMP levels were determined by radioimmunoassay (see Methods). Shown is ratio of cAMP levels in treated samples to cAMP levels of vehicle-treated strips (means of 3 independent experiments). Values are mean ± SEM. *P* < 0.05, **P** < 0.01 vs vehicle-treated tissue.

<table>
<thead>
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<th>Agent</th>
<th>cAMP Level, pmol/mg Protein</th>
<th>±SEM</th>
<th><em>P</em></th>
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<td>16.5</td>
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*P* < 0.01, †*P* < 0.01 vs control.
samples used in these assays have intact agonist-dependent β-adrenoeceptor coupling to cAMP generation.

A summary of the results is displayed in Figure 3. These results reflect values obtained in 6 donor hearts. Equal numbers of samples from left and right ventricles and interventricular septa were used in each condition. Both xamoterol and bucindolol displayed partial agonist activity, as reflected by significantly increased tissue cAMP levels. Consistent with earlier reports, the ISA of both xamoterol and bucindolol was most evident at lower concentrations.13 Xamoterol 0.5 µmol/L increased cAMP levels by 2.00±0.27-fold over control (P<0.01), whereas 5.0 µmol/L xamoterol had only a minimal effect (1.29±0.12-fold, P=NS) Similarly, the effects of bucindolol were less prominent at 10 µmol/L (1.48±0.13-fold over control, P<0.05) than at 0.1 or 1.0 µmol/L (1.64±0.26- and 1.52±0.16-fold, P=NS).

Figure 3. cAMP induction in human myocardial strips. A. Bucindolol and xamoterol exhibit partial agonist activity. Human myocardial strips from left ventricle were treated with different β-blockers on day 0 as described in Methods, and cAMP levels were determined by radioimmunoassay. Individual data points for each treatment condition are means of replicate measurements from a single sample of myocardium normalized to cAMP levels of vehicle-treated tissue. Replicate samples from each of 6 hearts were tested for each condition. Average cAMP levels were calculated for each heart, and means of these values are shown as solid horizontal bars to right of each scatterplot (±SEM for n=6 hearts). *P<0.05, **P<0.01 vs vehicle-treated cells. B through D, Chamber-specific effects of β-adrenergic agents. cAMP responses of individual ventricular sites. Replicate determinations were made on samples from indicated site in each of 4 to 5 hearts. Values are normalized to levels in vehicle-treated strips and expressed as mean±SEM. *P<0.05, **P<0.01 for β-blocker-treated cells vs vehicle-treated cells. B, Left ventricular free wall. C, Interventricular septum. D, Right ventricle.
respectively, both \( P \leq 0.01 \). A wide range of cAMP levels was obtained at each concentration of bucindolol, suggesting individual variations in susceptibility to this effect (Figure 3A). Furthermore, concentration dependence appeared to vary significantly from the left to the right ventricle (Figure 3, B–D). Bucindolol was observed to increase cAMP above control levels, however, at all concentrations >0.01 \( \mu \)mol/L and in samples from each of the 6 hearts examined (cAMP-bucindolol > cAMPcontrol in 32 of 36 samples overall). In contrast, no ISA was observed at comparable concentrations of 3 other \( \beta \)-adrenergic antagonists (Figure 3, A–D). Metoprolol appeared to reduce basal tissue levels of cAMP by \( \approx 25\% \), consistent with its previously reported inverse agonist properties (0.75 ± 0.06-fold, \( P < 0.01 \)). Carvedilol (0.01 to 10 \( \mu \)mol/L) had no effect on myocardial cAMP levels (Figure 3). Propranolol likewise had no significant ISA by this detection method (cAMP 1.14 ± 0.11-fold of control, \( P = \text{NS} \))

**Discussion**

The studies presented here show that both bucindolol and xamoterol have ISA in human myocardium. Both of these compounds increased myocardial levels of cAMP, a hallmark of \( \beta \)-adrenergic agonist activity and a crucial effector of the potentially deleterious effects of \( \beta \)-adrenergic stimulation. Three other \( \beta \)-adrenergic antagonists did not show this activity. We also show that metoprolol significantly depresses basal myocardial cAMP levels in both human and rat heart, consistent with its previously reported inverse agonist properties.\(^{24,27}\) Inverse agonists exist for many classes of \( G \) protein–coupled receptors; these ligands appear to stabilize the inactive conformation of their receptors and thereby reduce basal, agonist-independent signaling.\(^{26,28}\) The clinical significance of this property of metoprolol is not clear, although inverse agonists have been shown to induce both homologous and heterologous receptor upregulation.\(^{28,29}\)

There have been 2 exceptions to the general observation that \( \beta \)-adrenergic antagonists are beneficial in heart failure. Xamoterol, an agent with partial agonist activity, improved clinical status, exercise capacity, and overall quality of life but significantly increased mortality in patients with congestive heart failure.\(^{31,36}\) Bucindolol also had significant beneficial effects on quality of life\(^{31,36}\) but failed to improve mortality in a long-term trial.\(^{12}\) The reasons for the failure of the BEST trial are not clear, but several explanations have been considered, including the possibility that \( \beta \)-adrenergic blockade is not beneficial for all subsets of patients. It is at least theoretically possible that racial or genetic differences influence the response to heart failure therapy. In the BEST trial, mortality benefits were observed only in nonblack patients, and the study population had a lower percentage of nonblacks than in many previous studies of \( \beta \)-blockers. A recent trial, however, identified no racial differences in the therapeutic benefits of carvedilol,\(^{8}\) suggesting that this observed differential effect of bucindolol may have occurred by chance or may be specific to bucindolol. Other possibilities include the marked central sympatholytic effects of bucindolol and a greater proportion of class IV patients vulnerable to the loss of sympathetic drive.\(^{12}\)

Another possibility is suggested by our finding that bucindolol has clinically relevant sympathomimetic properties. Bucindolol previously has been proposed to act as a partial agonist in animal models; it exhibited \( \beta \)-adrenergic agonist characteristics in anesthetized dogs and rats,\(^{13,16}\) increased heart rate in pithed rats,\(^{17}\) and increased cAMP levels in neonatal rat cardiomyocytes.\(^{18}\) Bucindolol also has agonist-like (guanine nucleotide–sensitive) receptor binding properties\(^{14}\) at the human \( \beta \)AR.\(^{22}\) In 3 separate clinical studies of hypertensive males, bucindolol failed to decrease heart rate at rest or during exercise,\(^{19,21}\) suggestive of a partial agonist effect. Bucindolol also increased HDL cholesterol levels, a characteristic of \( \beta \)-blockers with ISA.\(^{22}\) Direct evidence for bucindolol ISA in human myocardium, however, has been limited and inconclusive. Hershberger et al\(^{14}\) found that bucindolol did not increase myocardial membrane adenylate cyclase activity or increase the force of contraction in failing right ventricular papillary muscles. Conversely, bucindolol was reported to mediate a very small increased force of contraction in catecholamine-depleted human atrial trabecula\(^{23}\) and significantly increased contractile force in a subset of failing human left ventricular papillary muscle strips.\(^{23}\) Despite these conflicting findings, it is widely reported that bucindolol lacks clinically relevant ISA in human myocardium.\(^{12}\)

Using cAMP accumulation as a sensitive end point, we show here that bucindolol has a significant amount of intrinsic agonist behavior in clinically normal human myocardium. The apparent conflict of our results with those of Hershberger et al\(^{14}\) may be due to differences in the integrity of the material used, the conditions of the assay, the activation state of the receptors in each case, or a combination of the 3. Also, the use of failing myocardium in earlier studies may have led to underestimation of \( \beta \)-agonist activity, because more than half of the \( \beta \)-adrenergic signaling capacity is lost from the failing heart through desensitization and uncoupling. Our use of nonfailing myocardium was designed to enhance the sensitivity of these assays for detection of \( \beta \)-adrenergic agonist activity in candidate compounds.

Both qualitative and quantitative changes in \( \beta \)AR subtypes occur during the course of heart failure. Bucindolol binds nonselectively to \( \beta_1 \) and \( \beta_2 \) subtypes, but it is possible that its agonist activity is mediated by only one of these, with the result that changing receptor subtype populations would affect the behavior of bucindolol. Despite this uncertainty, the combined observations from our studies and those of Maack et al\(^{23}\) in failing myocardium indicate that bucindolol is likely to have significant partial agonist activity in human myocardium across a wide spectrum of contractile function.

Clinical experience with \( \beta \)-adrenergic agonists and partial agonists in chronic heart failure has been disappointing. These agents, like others acting downstream in the same pathway (eg, forskolin, phosphodiesterase III inhibitors), have short-term positive effects on hemodynamics and quality of life.\(^{39}\) None have reduced mortality, however, and most have worsened survival in heart failure.\(^{28}\) Stimulation of \( \beta \)ARs raises intracellular cAMP and predisposes the myocyte to calcium overloading by both cAMP-dependent and -independent means.\(^{39}\) Sustained \( \beta \)-adrenergic stimulation and elevation of cAMP have been linked to cardiac myocyte apoptosis both in vitro and in vivo.\(^{40,41}\) The presence of partial agonist activity would thus be likely to reduce the beneficial effects of a \( \beta \)-adrenergic agonist in long-term
use. The preponderance of the data now supports the “adrenergic hypothesis” that chronic heart failure is best treated by agents with the most comprehensive antiadrenergic properties.6 We would further propose that compounds with partial β-adrenergic agonist properties are likely to be detrimental in mortality studies and that drugs should be specifically screened for this activity in human myocardium before their deployment in heart failure therapy.

Acknowledgments

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References

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