Translational Success Stories highlight how basic discoveries have led to clinical advances (such as the use of new drugs or diagnostic modalities in patients). This initiative reflects the renewed emphasis of our journal on translational research. It is hoped that these articles will stimulate efforts to translate basic insights into clinical practice.

Treatment of Chronic Heart Failure With β-Adrenergic Receptor Antagonists
A Convergence of Receptor Pharmacology and Clinical Cardiology

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Abstract: Despite the absence of a systematic development plan, β-blockers have reached the top tier of medical therapies for chronic heart failure. The successful outcome was due to the many dedicated investigators who produced, over a 30-year period, increasing evidence that β-blocking agents should or actually did improve the natural history of dilated cardiomyopathies and heart failure. It took 20 years for supportive evidence to become undeniable, at which time in 1993 the formidable drug development resources of large pharmaceutical companies were deployed into Phase 3 trials. Success then came relatively quickly, and within 8 years multiple agents were on the market in the United States and Europe. Importantly, there is ample room to improve antiadrenergic therapy, through novel approaches exploiting the nuances of receptor biology and/or intracellular signaling, as well as through pharmacogenetic targeting. (Circ Res. 2011;109:1176-1194.)

Key Words: heart failure ▪ adrenergic receptors ▪ β-blockers ▪ norepinephrine ▪ drug development

“I love it when a plan comes together”
—Colonel John "Hannibal" Smith, The A-Team

β-Adrenergic blocking agents are now considered first-line therapy for chronic heart failure.2 Between 1971 and 2001, β-blockers as a drug class went from contraindicated to being universally declared a highly effective new therapy for chronic heart failure with systolic dysfunction. As depicted in Figure 1, this 180-degree turn required the convergence of initially disconnected lines of basic and clinical investigation, none of which was systematically planned by the usual purveyors of drug development, large pharmaceutical companies. However, as for the typical outcome of Colonel Hannibal Smith’s unconventional and dubious tactical schemes,1 the end result was highly successful. This review provides some of the historical highlights, summarizes current knowledge, and provides thoughts on the future of β-blocker and antiadrenergic therapy.

Historical Highlights

Discovery of Adrenergic Receptors
In 1948, Raymond Ahlquist reported evidence for two types of adrenergic receptors,3 which, “for convenience,” he termed alpha and beta. The evidence was based on 2 distinct rank orders of agonist potency for a variety of pharmacological responses, plus the ability to inhibit the vasoconstrictor or uterine contraction responses of the α group, with 3 different compounds (dibenamine, ergotoxine, or tolazoline) that would later be termed α-blocking agents. At the time of Ahlquist’s landmark work, there were no known compounds that could inhibit the second, or β group of receptors; such a substance, dichloroisoproterenol (DCI), was first reported by Powell and Slater in 1958.4 Later that same year, Moran and Perkins reported that the right ventricular positive inotropic β-response to β-agonists was blocked by DCI and introduced the term “β-adrenoceptor blocking agent.”5 This work with the intrinsic sympathomimetic activity (ISA)-containing compound DCI was the final proof of Ahlquist’s bipartite...
categorization of adrenergic receptors. In 1964, the first β-blocking agent that would ultimately reach the market for treating various cardiovascular disorders, propranolol, was introduced and characterized by Sir James Black and colleagues of ICI’s Pharmaceutical Division.6 As will be discussed, the first-generation, nonselective agent propranolol would play a negative role in the development of β-blocker therapy in heart failure, as when given in standard therapeutic doses it can precipitate clinically important worsening of heart failure, as stated in a warning in the drug’s Food and Drug Administration (FDA) prescribing information. This realization led to β-blockers being generally contraindicated in heart failure, as was stated in propranolol’s original Inderal package insert.7

The 1-Subtype is the Dominant β-Adrenergic Receptor in the Heart

My introduction to the field occurred as an MD/PhD student in the Pharmacology Department of University of Illinois Chicago under the supervision of Richard Green, a superb receptor and autonomic pharmacologist. In 1967 Al Lands, Aaron Arnold, and colleagues at the Sterling-Winthrop Research Institute in Rensselaer, NY, had just published evidence that there were two subcategories of β-adrenergic receptors (ARs): “1,” mediating lipolytic and heart rate responses to catecholamines, and “2,” mediating bronchodilation and vasodepression.8 Similar to Ahlquist’s original work on the beta general category of adrenergic receptors,3 Lands et al’s classification was based on the order of potency of agonists in both in vitro and in vivo bioassay systems derived from several species,8 indicating that the β-1 receptor had higher affinity than the β-2 for the adrenergic neurotransmitter norepinephrine. This approach to receptor characterization is fraught with methological issues, and my thesis work was designed to test the hypothesis that a single species, in this case the rabbit, had multiple subtypes of β-ARS that could be identified by the more stringent method of determining antagonist dissociation constants.9 Several new β-receptor antagonists with apparent specificity for cardiac responses had just been introduced, including I.C.I. 501725 (also known as Ay 21,011), later named practolol.10

The PhD work yielded evidence for multiple different β-ARs in the various rabbit tissues,11 including a high affinity/low KD for Ay 21,011 or propranolol receptor in the right atrium consistent with Lands et al’s 1 classification3 that subsequently proved to be the β1 subtype, a low affinity/high KD for Ay 21,011 and high affinity/low KD receptor for propranolol in aorta consistent with Lands et al’s β-2 AR that was confirmed later to be the β2 subtype, and a low affinity/high KD for propranolol receptor in fundic stomach smooth muscle that was later shown to be a β3 subtype. The β1 and β2 receptor subtypes would later play a major role in the development of β-blocker therapy for heart failure, based on the discovery in the 1980s that human ventricular myocardium contains a substantial number of functional β2-ARs.12–14

Cardiotoxic Potential of Biogenic Amines Including Norepinephrine

The cardiotoxic potential of high doses of exogenously administered catecholamines has been recognized for over a hundred years,15 and by the mid 1970s this observation had been extended to multiple animal models as well as humans. In 1975, I began a series of experiments that would emphasize the potential of endogenously released biogenic amines including catecholamines to mediate cardiac damage. As a postdoctoral fellow at Stanford University in a joint project between John Daniel’s oncology and Don Harrison’s cardiovascular laboratories, we demonstrated in rabbits that the cardiotoxic antitumor agent doxorubicin caused cardiac hist-
tamine, systemic histamine and norepinephrine (NE) release, and that the majority of doxorubicin-mediated myocardial and renal damage was prevented by combined H1- and H2-histaminic and α- and β-adrenergic receptor blockade.16 Relevant to future therapeutic strategies, a major component of histamine-mediated17 and catecholamine-mediated18 myocardial damage appeared to be reversible if the biogenic amine was withdrawn.

Pharmacological Characterization of the β-Adrenergic Neuroeffector System in the Failing Human Heart

In 1978, during our cardiology fellowships, Bob Ginsburg and I began a collaboration consisting of performing isolated tissue and biochemical pharmacological studies on the explanted hearts removed from end-stage heart failure patients in the Stanford Cardiac Transplant Program. The focus on explanted human hearts as a source of starting material for pharmacological and biochemical studies was unique, made possible by Stanford’s standing as the world’s only high-volume transplant program, and the project became part of the Stanford Cardiac Transplantation program grant led by Ed Stinson. Enamored by the pathophysiology potential of the histaminic system in the myocardium, I wanted to be able to directly measure histamine receptors in cardiac tissue. Although in the Green laboratory we had been working on methods for radiolabeling adrenergic receptors, the method that eventually proved to be generally useful, incubation with high-affinity antagonists labeled at ultra-high specific activity, was independently developed and reported in 1974 by three other laboratories.19–21 One of these was Bob Lefkowitz’s at Duke University,20 and in 1979 Bob graciously allowed me to work in his laboratory on receptor radioligand binding as a visiting Fellow. Because Bob did not work on histamine receptors, the training system used [3H]di-hydroalprenolol (DHA) binding to human leukocyte β2-ARs.

On mastering the technique of radioligand binding, I returned to Stanford to attempt to radiolabel H1 and H2 histamine receptors in human cardiac tissue, but was never able to do so using [3H]mepyramine or [3H]cimetidine. Part of the protocol was to use [3H]DHA binding as a positive control for a viable membrane fraction, and in every ventricular myocardial sample tested, saturable [3H]DHA-specific binding was obtained. After several months, the histamine receptor binding project was abandoned in favor of one to characterize β-ARs in failing and nonfailing heart. This project was worthy because a prodigious amount of work done at the National Institutes of Health (NIH) in the 1960s by Eugene Braunwald and colleagues had clearly shown that heart failure was accompanied by marked changes in the adrenergic nervous system. The alterations they identified were an increase in systemic NE levels22 and catecholamine excretion23 associated with a marked decrease in myocardial tissue NE20 and a reduction in force of contraction in isolated left ventricular papillary muscles.24 These observations were interpreted as representing increased generalized adrenergic activity in heart failure, which could progress to the point of causing tissue NE depletion profound enough to compromise the major contractile support mechanism of the heart.24 These investigators also emphasized that in heart failure patients, the impaired adrenergic support mechanism could be further compromised by the administration of antiadrenergic agents,25 including propranolol,26 which could lead to clinical decompensation.

However, it was also known at the time that reduction in neurotransmitter activity such as from denervation could cause agonist supersensitivity, and that prolonged exposure to high concentrations of catecholamines produced desensitization of β-AR responses. Our view of the NIH data was that the reduction in tissue NE in the failing human heart might represent a form of functional denervation, resulting in receptor upregulation and response supersensitivity. Indeed, there was evidence from an animal model that this would be the case.27 Such a regulatory change would predispose the failing heart to damage from the high circulating levels of catecholamines. On the other hand, there was evidence from lymphocyte β2-receptor density28 and agonist response29 measurements that systemic β-adrenergic receptor desensitization was present in human heart failure. Thus, in the late 1970s, the status of β-receptor signal transduction mechanisms in the failing heart was an open question, which could be readily investigated in explanted human hearts.

As shown in Figure 2 we found just the opposite of what we expected, an approximately 50% decrease in left ventricular total β-AR density and a similar degree of decline in functional responses to adenylyl cyclase and muscle contraction in isolated ventricular preparations.30 This meant that the failing heart’s β-AR signaling systems were indeed compromised, not from depletion of NE stores but from receptor downregulation. However, a distinguishing feature of this form of adrenergic dysfunction was that since there was still ample signal transduction capacity remaining, the increased adrenergic drive would likely remain damaging to the failing heart.30 Another critical feature of the interpretation of these data was that despite the known depletion in neuronal/tissue...
NE stores the observed β-AR regulatory changes almost certainly reflected generalized overexposure to catecholamines at the tissue, ie, cardiac myocyte level.30 This was proved a few years later in studies performed first by Karl Swedberg and Kanu Chaterjee, using transmyocardial NE measurements,31 and then by Colin Rose’s32 and Murray Esler’s33,34 groups using sophisticated NE radiotracer methods. These studies demonstrated that the decrease in tissue NE content was in fact a product of increased adrenergic activity in the failing heart, but there was never a point where there was decreased cardiac adrenergic activity at the cardiac tissue level.31–34 Moreover, the Esler group’s studies showed that adrenergic neurotransmitter (NE) activity is selectively increased in the heart in direct relation to the severity of elevation in left ventricular (LV) filling and pulmonary artery pressures and is associated with increased mortality.34 A portion of the increased cardiac adrenergic activity spills over into the systemic circulation, accounting for most of the increased systemic adrenergic activity in heart failure35 and becoming a biomarker associated with increased mortality.35

Importantly, the β-adrenergic receptor downregulation/desensitization changes were not simply a late stage phenomenon confined to transplant recipient explanted hearts, as was shown in an endomyocardial biopsy study of ambulatory heart failure patients with nonischemic cardiomyopathies and a range of New York Heart Association (NYHA) classes and LV ejection fractions (EFs).56 As heart failure and LV systolic dysfunction progress, β-AR downregulation, already present in Class II heart failure, worsens.36 Moreover, the receptor downregulation was associated with major compromise in the dobutamine dP/dT dose-response but no change in the calcium inotropic response.56 demonstrating that as predicted, β-AR desensitization phenomena do compromise cardiac reserve, and this loss of inotropic support is specific to β-AR responses. More generally, this study also demonstrated that endomyocardial biopsy can be safely and effectively deployed as a research tool in relatively large numbers of patients with heart failure and nonischemic cardiomyopathies and that β-AR measurements can be made in extremely small amounts of myocardial tissue using the recently introduced 2000 Ci/mmol specific activity radioligand125 [I]iodocyanopindolol.56 We then began to consider how to approach the failing heart from a long-term therapeutic standpoint, and the obvious conclusion was to use something to protect the β-AR systems from overexposure to catecholamines.37,38

Landmark Swedish Clinical Experience With β-Blocking Agents in Heart Failure
At the time we were performing our explanted human heart investigations, the Åke Hjalmarson led team in Göteborg, Sweden, which included Finn Waagstein and Karl Swedberg, was publishing its observations on the response of dilated cardiomyopathy patients to β-blocking agents. This work, whose importance cannot be overstated, began when Finn Waagstein successfully treated a tachycardic, acutely failing dilated cardiomyopathy patient with an anti-ischemic approach consisting of the administration of intravenous practolol. The Göteborg group went on in 1975 to describe successful results in seven dilated cardiomyopathy patients, six of whom who were treated with oral practolol.39 After removal of practolol from the market for oculomucocutaneous syndrome, in 1979 the Göteborg group reported results in 24 patients, the last 11 of whom were treated with the β1-selective blocker metoprolol,40 an approximately 80-fold β1 selective “hometown” drug developed in Astra’s Mölndal facility near Göteborg. In this study,40 mortality versus a constructed life table of patients not treated with β-blockers was apparently better, with a 55% versus 20% survival at 3 years. In 1980, Swedberg et al42 reported that 28 patients treated with β-blockade, 17 of whom received metoprolol, had a 10 EF unit improvement in LV ejection fraction, the first description of reverse remodeling by a β-blocker. Fifteen of these patients improved by functional class, and the mortality seemed “lower than expected.”42

The main problem with the early Göteborg experience was that it was not placebo controlled, and in 1981 a small, short-term (1 month), placebo-controlled trial using the β1-selective blocker acebutolol was reported by Ikram et al,43 with no evidence of benefit. The Göteborg group realized that a long-term, placebo-controlled multicenter trial needed to be performed, as did a small group of US investigators44–47 who had been evaluating β-blockade in chronic heart failure. The idea that the time had come to embark on such an ambitious endeavor was bolstered by the favorable experiences of all investigators who had been using metoprolol to treat nonischemic cardiomyopathy patients, as well as the now extensive evidence that the failing human heart was being exposed to excessive, harmful adrenergic stimulation. Trial planning meetings focused on the challenge of conducting the trial in a multicenter setting, and in particular whether β-blockade could be safely initiated and uptitrated by a variety of investigators, some of whom would have had little/no experience with delivering β-blockers to heart failure patients. US investigators44–47 had adopted an approach of having pharmacists encapsulate 6.25 mg of metoprolol and using this ultra-low dose formulation to initiate therapy on an outpatient basis. This approach was adopted, and all that remained was a commitment from the industry sponsors, Astra in Europe and CIBA-Geigy in the United States, to formulate immediate release metoprolol tartrate into a 5 mg tablet. That was accomplished, and it was left to the Göteborg group to develop a draft of the protocol, which was revised and approved in 1986.

The “Metoprolol in Dilated Cardiomyopathy” or MDC trial, comprised of 33 centers, enrolled its first patient in September 1986 and was completed in July 1992.48 The patient population was nonischemic/idiopathic dilated cardiomyopathy, LVEF of <0.40, NYHA class II–IV, and conventional background therapy of digoxin, diuretics, and ACE inhibitors. The trial was sample-sized on a realistic 2-year placebo event rate of 30%, but an in-retrospect unrealistic 50% effect size. The primary end point was time to death or listing for transplantation, with the latter component adjudicated by an end points committee. Between 1986 and 1991, 383 patients were enrolled with an acceptable overall (≈15%) nonfatal withdrawal rate that was lower in the metoprolol group.48 The primary end point was reduced by
34% (P=0.058) in the metoprolol group (Figure 3), with the reduction completely due to the transplant component.48 Of some concern was that there were 19 deaths in the placebo group and 23 in the metoprolol group, mostly (71%) due to sudden death.48 However, after 36 months of follow-up extended beyond the end of the trial, there were 39 deaths in patients randomly assigned to placebo, versus 35 in the metoprolol group.49

Although the MDC trial did not meet its primary end point, a 34% reduction in a legitimate morbidity-mortality measure was impressive and presaged the more favorable range of results subsequently obtained in Phase 3 trials using a mortality primary end point. In addition, the mortality component results contributed to the consideration of nonselective β-blocker–vasodilators as potentially more ideal therapy.46

Discovery of Functional β2-ARs on Cardiac Myocytes in Human Ventricular Myocardium and Relative Preservation of their Expression in Heart Failure

The realization that there were multiple β-AR subtypes naturally led to the question of whether the human heart had receptors other than β1, in particular the β2 subtype that was present in smooth and skeletal muscle and was the dominant β-AR in frog myocardium. This was an important question, since metoprolol was a β1-AR selective compound, and if β2-ARs were on cardiac myocytes in human myocardium, a β1-AR selective compound might not be the optimal antiadrenergic strategy. By the early 1980s, all the pharmacological tools were in place to examine this issue in detail, including highly selective β1- and β2-AR antagonists and agonists, and computer modeling methods that could resolve mixed populations of receptors from radioligand competition curve data. In 1983, three groups reported evidence of β2-AR receptor binding sites in human atrial cardiac tissue, two in right atrial surgical biopsies (estimated to be either 20%50 or 50%51 of the total β-AR binding) and one from a single organ donor, with a β2 population of 26%.12 In the organ donor, LV measurements were also reported, with a β1/β2 binding site distribution of 0.86/0.14, similar to results obtained in three autopsy-procured hearts12; in the year after, eight mitral valve surgery patients’ papillary muscle β1/β2 distribution was reported, at 0.69/0.31.52 However, none of these early reports provided evidence of a cardiac myocyte origin of the β2 receptor binding, and since the majority of cells in myocardial tissue are nonmyocytes and in two of the reports12,50 the fractional β2 binding was small, these reports were naturally met with a degree of skepticism.

In terms of functional coupling of β2-ARs in human myocardial tissue, the Robberecht-Christophe group reported in 1983 that isoproterenol stimulation of adenylyl cyclase in human right atrium and left ventricle was exclusively β2-AR mediated, with no evidence of β1-AR coupling.53 However, again these data did not establish the presence of β2-ARs on cardiac myocytes. Evidence for the coupling of β2-ARs to an inotropic response as de facto proof of their presence on cardiac myocytes was soon provided in tissue bath studies conducted on small numbers of atrial54 or right ventricular septal55 samples removed from cardiac surgery patients without heart failure, and in much larger studies in nonfailing13,14 and failing14 ventricular (Figure 4A and 4B) and nonfailing atrial53 preparations.

These studies unequivocally established that functional β2-ARs are present on human cardiac myocytes, in both atria...
and ventricles. However, an even more interesting and potentially clinically important finding emerged, that in failing human ventricles\textsuperscript{14} and atria\textsuperscript{57} $\beta_1$-AR contractile responses (Figure 4B) and receptor density (Figure 4C) selectively downregulate, with little or no change in $\beta_2$-AR density (Figure 4C). This meant that the ratio of $\beta_1/\beta_2$ increased from 0.75 to 0.80/0.20 to 0.25 in nonfailing LV myocardium to 0.60 to 0.65/0.35 to 0.40 in failing heart.\textsuperscript{14} Therefore, the percentage of $\beta_2$-ARs nearly doubles in advanced heart failure and approaches 40\% of the total of $\beta_1 + \beta_2$, a proportion that logically would need to be taken into account when considering $\beta$-blocker strategies for heart failure. Although $\beta_2$-AR–mediated responses for both muscle contraction\textsuperscript{14} and adenylyl cyclase stimulation\textsuperscript{58} exhibited a detectable amount of desensitization in failing ventricular preparations and therefore the $\beta_2$-AR was presumably exposed to increased levels of catecholamines, receptor subsensitivity was much less than for $\beta_1$-AR–mediated responses.\textsuperscript{14,58} In addition, in a pharmacological curiosity that remains unsolved, muscle contraction responses to $\beta$-agonists in both nonfailing and failing human heart preparations were proportional to the fraction of $\beta_1$- and $\beta_2$-ARs, whereas the adenylyl cyclase response were disproportionately mediated by $\beta_2$-ARs.\textsuperscript{53,58,59}

Clinical pharmacological studies conducted in patients without heart failure also supported functional cardiac $\beta_2$-AR signaling.\textsuperscript{60,61} In patients with heart failure, administration of $\beta_2$-agonists demonstrated evidence of arrhythmogenic\textsuperscript{62} and possibly adverse remodeling\textsuperscript{28} effects, providing evidence for functional $\beta_2$-ARs in the failing heart but also demonstrating the potential adverse effects of chronic $\beta_2$-AR signaling. The potential downside of chronic $\beta_2$-AR activation was further reinforced by transgenic overexpression of $\beta_2$-ARs in mice,\textsuperscript{63} which, when studied for longer periods, produced preliminary evidence of cardiomyopathic effects that were eventually fully characterized (see below). Taken together this information suggested that $\beta_2$-AR signaling could provide inotropic support to the failing heart.\textsuperscript{14,57} and, for optimal therapeutic efficacy, a nonselective $\beta$-blocker might be preferable to a selective $\beta_1$-AR antagonist.\textsuperscript{14,46,64}

### Introduction of Nonselective $\beta$-Blocker–Vasodilators for Heart Failure Therapy

The nonselective ($\beta_1/\beta_2$) blocker propranolol is not well tolerated by patients with advanced heart failure.\textsuperscript{26,65} In our early studies at Stanford, Mike Fowler, Bob Ginsburg, and I had tried to use propranolol in dilated cardiomyopathy pretransplant patients by initiating therapy at the very low dose of 2.5 mg bid, which was met with an unacceptable adverse event profile related to decreased cardiac output.\textsuperscript{46} In contrast, in these patients with very advanced heart failure, the $\beta_1$-selective antagonist metoprolol could be tolerated in low (6.25 mg bid) starting doses, presumably because the unblocked $\beta_2$-ARs were able to mediate some degree of cardiac functional support as $\beta_1$-AR stimulation was being withdrawn. In addition, nonselective agents with vasodilator activity are well tolerated in small initiating doses,\textsuperscript{46} because the afterload and preload reduction counteracts the increase in systemic vascular resistance and increase in LV filling pressure that accompanies blockade of both $\beta_1$ and $\beta_2$ myocardial adrenergic receptors.\textsuperscript{64} This realization paved the way for Phase 2 studies with the nonselective $\beta$-blocker/vasodilator compounds bucindolol\textsuperscript{66–69} and carvedilol.\textsuperscript{70–73} These small- to medium-sized, placebo-controlled trials produced uniformly positive results on ventricular remodeling,\textsuperscript{66–73} and several studies demonstrated improved symptoms\textsuperscript{66,67,70–73} or quality of life.\textsuperscript{70} Both compounds were well tolerated in mild-moderate (NYHA class II or III) heart failure.\textsuperscript{66–73} As would be expected by $\beta$-blocker effects on maximal heart rate, maximal exercise tolerance was not improved in the majority of these studies,\textsuperscript{66,67,69,70} but several showed improvement or favorable trends in submaximal exercise.\textsuperscript{67,70,72}

### Era of Phase 3 $\beta$-Blocker Clinical Trials, 1993–2003

As depicted in Figure 1, by 1993, basic and clinical investigation had established the plausibility that chronically increased cardiac adrenergic drive produced adverse biological effects on the failing human heart including maladaptive regulation of receptor-signal transduction mechanisms, and Phase 2 clinical trials had unambiguously demonstrated the reverse remodeling and potential clinical benefits of $\beta$-blocker therapy. These two lines of evidence converged to provide the impetus to begin the era of Phase 3 clinical trials, conducted with five different $\beta$-blocking agents beginning with the US Carvedilol Trials Program\textsuperscript{74–78} in 1993 and ending with the nebivolol-SENIORS trial in 2003.\textsuperscript{79}

In the 1980s and into the mid 1990s, the regulatory standard for approval of chronic heart failure drugs was improvement in exercise tolerance/functional capacity, a vestige of the inotrope/vasodilator era. Thus, in 1992–1993, when SmithKline Beecham made the decision to move to Phase 3 with the Boehringer-Mannheim-licensed $\beta_\omega/\beta_1$-blocker carvedilol, it was with a trial design based on submaximal exercise as the primary end point. Carvedilol failed to meet this primary end point in its two Phase 3 pivotal trials,\textsuperscript{74,75} but there was a trend for reduction in mortality and a reduction in cardiovascular hospitalizations in one (PRECISE)\textsuperscript{74} and statistically significant reductions in both these endpoints in the other (MOCHA)\textsuperscript{75} (Figure 5A and 5B). The reduction in mortality in MOCHA was the first demonstration that a $\beta$-blocker could reduce all-cause mortality in a placebo-controlled trial, and both the improvement in LVEF and reduction in clinical events (Figure 5A and 5B) were dose-related.\textsuperscript{75} The carvedilol Phase 3 program also included two supportive trials, in mild\textsuperscript{76} and severe\textsuperscript{77} heart failure. All 4 trials were being followed by a single data and safety monitoring board (DSMB), and in 1995 after both pivotal trials were completed but before the supportive trials had ended, the DSMB stopped the two supportive trials for a reduction in mortality in the entire US carvedilol program.\textsuperscript{78}

When the carvedilol NDA was initially reviewed by FDA in 1996, it was not approved, in part because the design was not intention-to-treat, and mortality was not a prespecified efficacy end point. SmithKline Beecham then submitted
additional data on the combined end point of mortality and cardiovascular hospitalizations in the four US Phase 3 trials74–77 and the multicenter Australia-New Zealand Phase 2 study,73 and, in May 1997, carvedilol became the first β-blocker approved for the treatment of heart failure. The next four Phase 3 or 4 placebo-controlled β-blocker studies were based on all-cause mortality as the primary end point, using intention-to-treat designs.80–83 These trials were conducted in heterogeneous patient populations using four different β-blockers, including carvedilol.83 The first of these trials to be planned and initiated was the Beta blocker Evaluation of Survival Trial (BEST),80 using the nonselective β-blocker/vasodilator/sympatholytic agent bucindolol, which enrolled its first patient in May 1995. BEST enrolled only NYHA class III or IV nonischemic or ischemic cardiomyopathy patients with LVEF ≤0.35, and 23% of the patients were African American.80 In contrast to the other Phase 3 β-blocker mortality trials, this VA Cooperative Studies and National Heart, Lung, and Blood Institute–sponsored trial was nearly exclusively conducted in the United States (91/93 sites and 2645/2708 patients), with the remaining two sites being Canadian. BEST was stopped by the DSMB on July 26, 1999, for the “totality of evidence regarding the usefulness of β-blocker treatment derived from BEST and other studies”80,84 and a “loss of equipoise in an increasing number of BEST trial investigators.”84 What these statements meant was that data from BEST patients who were NYHA Class III or non–African American were favorable and consistent with mortality reduction results in these same types of patients who were investigated in two other trials that had been stopped for a survival benefit in the previous 12 months, CIBIS-II81 and MERIT-HF,82 and an increasing number of the BEST investigators considered it unethical to continue to administer placebo. However, when the trial was stopped the BEST primary end point results were not statistically significant, with an all-cause mortality unadjusted hazard ratio (95% confidence intervals) of 0.90 (0.78, 1.02), P=0.10.80 As shown in Figure 5C, when the data were analyzed by the prespecified regulatory statistical analysis plan that included covariate adjustment for the four randomization stratification variables and censoring of outcomes at the time of cardiac transplantation, the hazard ratio was 0.87 (0.76, 1.00; P=0.053). Because BEST did not achieve a statistically significant reduction in mortality, the drug’s sponsor did not submit an NDA.

The first placebo-controlled mortality trial to be stopped by the DSMB for benefit, in March 1998, was CIBIS-II81 using the ~100-fold β1-selective41 compound bisoprolol...
(Figure 5C). The 2647-patient CIBIS-II trial resulted in a 34% reduction in all-cause mortality (hazard ratio, 0.66 [0.54, 0.81], \(P<0.0001\)). Figure 5C, using a target dose of 10 mg/d. There had been an earlier, underpowered Phase 3 placebo-controlled trial with a bisoprolol target dose of 5 mg/d, CIBIS, which enrolled 641 Class III or IV patients with either nonischemic or ischemic dilated cardiomyopathy and achieved a nonsignificant (\(P=0.22\)), 20% reduction in mortality.\(^{85}\) The next trial to be stopped was MERIT-HF,\(^{82}\) which investigated the long-acting CR/XL formulation of metoprolol succinate delivered at an average dose of 159 mg/d, in October 1998. MERIT-HF yielded a result nearly identical to CIBIS II, with an all-cause mortality hazard ratio of 0.66 (0.53, 0.81; \(P=0.00009\)).\(^{82}\) So, by the end of 1998, two \(\beta\)-blocker mortality trials had been stopped for benefit, one of which had enrolled NYHA Class II-IV (MERIT-HF)\(^{82}\) and one Class III or IV (CIBIS-II)\(^{78}\) patients. In 2000, metoprolol CR/XL was approved in Europe and the United States, and bisoprolol was approved in Europe for the treatment of chronic heart failure.

By the beginning of 1999, there were two \(\beta\)-blocker mortality trials left running, BEST\(^{80}\) and COPERNICUS.\(^{83}\) Both of them were enrolling patient populations different from CIBIS-II and MERIT-HF. Compared with the general US population, BEST was overenrolling African Americans and also was recruiting very advanced, NYHA Class III/IV patients who could have lower blood pressures than the entry criteria in other trials and who could be fluid overloaded.\(^{80}\) However, there was overlap of BEST with the two recently stopped trials, particularly CIBIS-II that also enrolled Class III or IV patients. COPERNICUS was enrolling a severe LV dysfunction population who also had to be euvolemic and who had to have exhibited the equivalent of Class III or IV symptoms sometime in the 2 months before screening.\(^{83}\) BEST was stopped by its DSMB in July 1999 as described above, and COPERNICUS continued until March 2000 when the DSMB stopped it for benefit.\(^{83}\) Carvedilol received a mortality reduction claim in the United States and Europe in 2001.

So, by April 2000, there had been four \(\beta\)-blocker mortality trials stopped by their DSMBs, three for benefit in the entire study population and one (BEST) for benefit in a large subpopulation that had been investigated in two of the other trials. The reduction in mortality in these trials was by 34% (CIBIS-II),\(^{81}\) 34% (MERIT-HF),\(^{82}\) 10–13% (BEST),\(^{80}\) and 35% (COPERNICUS).\(^{83}\) These results would seem to suggest that there are efficacy differences among these 4 \(\beta\)-blockers, that is, that the outcomes are drug-specific. Furthermore, because the results between the 2 \(\beta_1\)-AR–selective compounds (metoprolol CR/XL and bisoprolol) and the nonselective \(\beta_1\alpha_1\) blocker carvedilol are virtually identical, the results suggest that \(\beta_1\)-selective and carvedilol are equally effective but that bucindolol has lower efficacy. However, the data in Figure 5D argue otherwise.

When enrollment criteria for BEST are restricted to those for MERIT-HF and CIBIS-II by excluding class IV patients, black race, baseline blood pressures <85 systolic and heart rates <60, the results of the three trials are very similar, with a hazard ratio in the “BEST Comparison Group (BCG)”\(^{78}\) of 0.75 (0.63–0.90; \(P=0.002\)) (Figure 5D, left panel). Additionally, there is good evidence that geographic differences in patient populations influences outcomes in \(\beta\)-blocker heart failure trials,\(^{87}\) and some of these factors such as longer duration of heart failure or more antecedent revascularization in US-enrolled versus European patients would not have been accounted for in the adjusted population shown in Figure 5D. Therefore, if the tendency for better survival outcomes in \(\beta\)-blocker trials conducted ex–United States\(^{87}\) is considered, there is no apparent difference in outcomes between BEST and CIBIS-II or MERIT-HF. Figure 5D (right panel) also shows the results of restricting the BEST population to the COPERNICUS enrollment criteria of euvolemic at baseline and an LVEF of <25%. Here the hazard ratio is 0.69 (0.55, 0.88; \(P=0.002\)). The same US/ex-US regional geographic differences apply to COPERNICUS,\(^{87}\) and so it can be appreciated that the four \(\beta\)-blocker mortality trials presented in Figure 5 yield essentially the same outcomes if patient populations and regional differences in patient populations/practice patterns are considered.

The fifth Phase 3 placebo-controlled \(\beta\)-blocker trial, SENIORS,\(^{79}\) conducted with the highly (320-fold) \(\beta_1\)-AR–selective \(\beta\)-blocker/vasodilator nebivolol, utilized a primary end point of the combination of time to all-cause mortality (ACM) or cardiovascular hospitalization, with ACM alone a secondary end point. In SENIORS, nebivolol reduced the rate of the primary end point, by 14% (\(P=0.039\)) and is approved in Europe for the treatment of heart failure, but not in the United States. The ACM end point in SENIORS had a hazard ratio of 0.88 (0.71–1.08; \(P=0.21\)), again suggesting differences between nebivolol and three of the other \(\beta\)-blockers used in Phase 3 heart failure trials, especially since the trial was conducted in a European population. However, here again, differences in patient populations likely account for an apparent lower efficacy; the SENIORS population was older and 35% had an ejection fraction >35%. Somewhat unexpectedly, patients with lower ejection fraction (greater degree of pathological remodeling) tend to respond better to \(\beta\)-blockade than do subjects with higher ejection fractions,\(^{88}\) as long as the patient has compensated heart failure and is not fluid-overloaded (eg, Figure 5D right panel, COPERNICUS\(^{85}\)).

When corrected for heterogeneity of study populations, the reason why all these placebo-controlled, Phase 3 studies give similar results is that \(\beta_1\)-AR signaling is responsible for the vast majority of adverse effects on cardiac myocytes (see below), and all the \(\beta\)-blocking agents used in these trials are competitive \(\beta_1\)-AR antagonists.

Only one \(\beta\)-blocker mortality study, COMET,\(^{87}\) has attempted to compare agents. COMET randomized 3029 patients to either immediate release/short half-life metoprolol tartrate or carvedilol, with a primary end point of all-cause mortality. The trial was preceded by two medium-sized trials of metoprolol tartrate versus carvedilol that suggested the latter produced a greater degree of LV functional and remodeling improvement.\(^{90,91}\) In COMET, the metoprolol tartrate target dose of 50 mg bid was 20% less than for MDC. The delivered (average actual administered) dose was 21% less than for MDC\(^{48,89}\) and 47% less than for metoprolol CR/XL succinate (controlled release, long half life formulation) in
MERIT-HF. The carvedilol target dose was the same as in COPERNICUS, and the delivered dose was 13% greater. In COMET, carvedilol demonstrated a 17% (P=0.0017) lower mortality compared with metoprolol. However, based on differences in resting heart rate measured at 4 to 16 months from randomization and the doses of agents delivered, this trial appears to have compared a lower β1-blocking dose of immediate release metoprolol tartrate to a higher β1-blocking dose of carvedilol. Although it is possible that blockade of β1-ARs or other carvedilol characteristics could have provided small incremental benefit, because of the likely difference in degree of β1-AR blockade, it is not possible to conclude that the β1/β2/α1-blocker carvedilol has superior efficacy to the β1-selective compound metoprolol. Studies comparing β-adrenergic blocking agents should document equivalent degrees of β1-AR blockade by measurement of exercise heart rate responses or use doses and formulations of each agent that have been successful against placebo in other trials.

The results of three dose-response remodeling studies with three different β-blockers, the mortality results in MOCHA (Figure 5B), the comparison of mortality outcomes in CIBIS versus CIBIS-II, and the results of the COMET study indicate that the favorable biological and major clinical effects of β1-AR blockade by measurement of exercise heart rate responses or use doses and formulations of each agent that have been successful against placebo in other trials.

Current Concepts of β-Adrenergic Signaling in the Failing, Pathologically Hypertrophied Heart

The relatively simple pathophysiologic concepts described above drove the successful development of β-blocker therapy for heart failure. If there is to be further progress, recent discoveries elucidating important details and nuances of adrenergic signal transduction will need to be exploited. Some of the mechanisms that have led to or have suggested testable therapeutic hypotheses will be reviewed.

Major Differences in the Pathobiological Effects of β1-AR Versus β2-AR Signaling

In an extremely important series of observations made in cultured cardiac myocytes and in transgenic mice in the 1990s and early 2000s, it was found that compared with β2-ARs, signaling by β1-ARs produces greater adverse biological effects. As first demonstrated by Lands et al., the β1-AR is an NE receptor, and in 1992, Doug Mann, George Cooper, and colleagues reported that NE at concentrations present in the failing heart, 10 to 100 nmol/L, was overtly cardiotoxic in adult cultured cardiac myocytes through a cAMP-dependent β-adrenergic signaling pathway that produced Ca2+ overload. In cultured adult rat cardiac myocytes, β1-AR signaling is proapoptotic, compared with the antiapoptotic effects of β2-AR signaling. In neonatal rat cardiac myocytes, β1-signaling induces hypertrophy and the induction of the pathological “fetal” gene program, the hallmark of which is upregulation in the β-isoform and downregulation in the α-isoform of myosin heavy chain (MHC).

Myocardial overexpression studies in transgenic mice have also demonstrated the greater adverse biological effects of β1-AR versus β2-AR pathways. In transgenic overexpression of the human β1-AR, it takes 9 months of expression at ~7000 fmol/mg membrane protein to produce a dilated cardiomyopathy. In contrast, with the codon 389 glycine version of human β1-AR, a dilated cardiomyopathy is produced at ≥9 months, with an expression level of 8–24% of the cardiomyopathy-associated β1 receptor density levels. With overexpression of the higher function 389 arginine β1-AR a dilated cardiomyopathy is produced earlier, at 6–8 months, with 14% of the β2 cardiomyopathy expression level. However, even though as compared with β1-ARs it takes a higher level of β2-AR expression to produce a dilated cardiomyopathy, when rescues of genetic cardiomyopathies are attempted by creating β2-AR bitransgens, the genetic cardiomyopathy is worsened by lower levels of β2-AR overexpression. These data indicate that despite the lesser cardiomyopathic potential of β1-AR signaling, even lower levels of increased expression have adverse biological effects.

The molecular structures of the human β1-ARs versus β2-ARs do not provide specific insight into the reason why β1-AR is more adverse than β2-AR signaling to cardiac myocyte biological processes. Both are the products of intronless genes and exhibit the standard 7-transmembrane spanning region structure of G-protein–coupled receptors. The human β1- and β2-ARs are moderately homologous, 71% in the transmembrane regions and 54% overall. Differences in agonist and antagonist binding between the these two subtypes resides in structural differences in the transmembrane spanning regions, whereas differences in signaling characteristics probably reside in the respective third intracytoplasmic loops, which are important for G-protein coupling. When the recombinant receptors are expressed in cell systems, both exhibit Gs coupling to adenylyl cyclase and cAMP increases. At physiological levels of expression, the selective coupling of β2-ARs to the inhibitory G protein (Gi) provides a mechanism to counteract adverse β1-AR signaling, such as by blunting β1-AR L-type Ca2+ channel activation. Most importantly, as described above, the equivalence of β1-selective and nonselective β-blockers in Phase 3 clinical trials is even more powerful evidence that the vast majority of cardiomyopathic effects of adrenergic signaling in the failing heart is mediated through β1-AR signaling. This is the likely reason why there are no obvious differences between the therapeutic effects of selective β1-AR and nonselective β1/β2-AR blocking agents when administered to chronic heart failure patients at equal β1-AR blocking doses. In addition, an extension of the differential pathobiologic potential of β2- versus β1-ARs is the idea that the combination of β1-AR blockade and a small amount of β2-agonism may produce a greater degree of cardioprotection than a β1-AR blocker alone, support for which has been generated in an animal model. However, in a sense, this approach has already been attempted in a heart failure trial using the β1-selective
antagonist \( \beta_2 \) partial agonist celiprolol, and the results did not suggest enhanced efficacy.\(^{110} \)

An unanticipated effect of blocking \( \beta_2 \)-ARs that has major therapeutic implications is sympatholysis, defined as drug- or device-induced reduction in NE release and levels. In heart failure, this occurs through blockade of cardiac prejunctional \( \beta_2 \)-ARs, which are facilitatory for NE release.\(^{111} \) For first-generation, nonselective \( \beta \)-blockers such as propranolol, the myocardiial depression effects of oral administration in heart failure may lead to reflex adrenergic activation,\(^{64} \) but a sympatholytic effect can be demonstrated after graded doses of I.V. administration.\(^{111} \) With the nonselective \( \beta \)-blocker/vasodilator buclindolol, an increase in filling pressures and decrease in cardiac output does not occur on oral drug administration,\(^{65} \) and systemic sympatholysis can be detected.\(^{80,112} \) Potent \( \alpha_1 \)-blocking activity increases systemic adrenergic drive,\(^{113} \) and for the \( \beta_1/\beta_2/\alpha_1 \) blocker carvedilol, this effect may be what largely cancels the sympatholytic effect of \( \beta_2 \)-blockade. Carvedilol does not reduce systemic adrenergic drive compared with placebo in patients with heart failure, although it results in lower NE levels than metoprolol.\(^{90} \) In some studies in heart failure, measurements of coronary sinus NE\(^{90} \) or cardiac spillover\(^{114} \) have demonstrated that carvedilol lowers cardiac adrenergic drive compared with placebo or metoprolol.\(^{114} \) However, another study\(^{115} \) has not detected a difference in reduction of cardiac adrenergic drive between carvedilol and placebo or metoprolol. The sympatholytic effects of bucindolol and to a much lesser degree carvedilol add to these compounds’ antiadrenergic profiles, and in the case of bucindolol must be considered in the therapeutic targeting of the drug.\(^{116,117} \) Because NE is \( \beta_1 \)-AR selective, reducing adrenergic drive has the effect of preferentially inhibiting \( \beta_1 \)-AR signaling, in effect producing cross-talk from \( \beta_2 \)-AR blockade to \( \beta_1 \)-AR inhibition. Thus, in the treatment of chronic heart failure, the biggest contribution of \( \beta_2 \)-AR blockade may be inhibition of \( \beta_1 \)-AR signaling.

**Compartmentation of Intracellular Signaling in Microdomains That Are Regulated by A-Kinase Anchoring Proteins and Phosphodiesterases**

One of the most important observations of the past decade in adrenergic signaling is that the compartmentation of cardiac myocyte cAMP/PKA signaling originally described by Buxton and Brunton in 1983\(^{118} \) is due to clustering into microdomains of key downstream elements on a scaffold of A-kinase anchoring proteins,\(^{119} \) in the vicinity of specific effectors such as receptors, phospholamban, and ion channels.\(^{119-121} \) Within these various microdomains reside different phosphodiesterases, which control the local concentration of cAMP that is freely diffusible from its sight of synthesis by membrane bound adenylyl cyclases.\(^{120} \) This situation creates the potential to selectively activate cAMP/PKA-dependent signaling in specific cellular compartments by the use of selective phosphodiesterase inhibitors. That approach was recently attempted in a Phase 3 trial using the phosphodiesterase 3 selective inhibitor enoximone in combination with \( \beta \)-blockade to couple inhibition of \( \beta_1 \)-AR adverse biological signaling with restoration of \( \beta_1 \)-AR phosphorylation,\(^{122} \) which is reduced in the failing heart.\(^{123} \) Although this 1854-patient clinical trial was not positive on its primary end points, it demonstrated promise in patients with the most severe LV dysfunction.\(^{124} \)

**PKA-Dependent and PKA-Independent Signaling**

Initial work on the cell biology of adrenergically mediated cardiac myocyte toxicity suggested that cAMP/PKA-dependent \( \beta_2 \)-AR activation was the major offender.\(^{95} \) A recent development in \( \beta \)-AR signaling is the observation that cAMP/PKA-independent pathways may be more important in mediating adrenergic adverse effects in the cardiac myocyte. Two major myopathic downstream biological effects in cardiac myocytes are mediated by \( \beta_1 \)-AR activation of Ca\(^{2+} \)/calmodulin kinase II (CaMKII), independent of protein kinase A (PKA). Apoptosis in adult rat\(^{125} \) and fetal gene induction in neonatal rat cardiac myocytes are \( \beta_1 \)-AR/CaMKII mediated. Moreover, unlike PKA-coupled signaling, CaMKII is upregulated in the failing human heart.\(^{126-128} \) CaMKII signaling can lead to increased \( \beta_1 \)-adrenergic receptor phosphorylation and positive inotropic and lusitropic effects, and, unlike PKA-dependent signaling, this effect does not undergo desensitization after sustained exposure to \( \beta_1 \)-AR stimulation with NE.\(^{129} \) On balance, as depicted in Figure 6, the \( \beta_1 \)-AR/CaMKII signaling is adverse and is one of the ways in which CaMKII signaling can play an adverse role in the failing heart by promoting pathologic hypertrophy and arrhythmias.\(^{130,131} \)

On the other hand, PKA-dependent signaling can be beneficial, as evidenced by the remodeling prevention effects of overexpression of Type VI adenyl cyclase,\(^{132} \) the cardio-myopathic effects of CREB genetic inhibition,\(^{133} \) and the antihypertrophic effects of PKA on Class II histone deacetylases.\(^{134} \) To be sure, PKA-mediated signaling can also be adverse, both on the myocardium\(^{135} \) and in promoting arrhythmias,\(^{136} \) but, as depicted in Figure 6, PKA signaling has elements of both benefit and harm and encompasses most of the upside effects of \( \beta \)-adrenergic activation.

As shown in Figure 7, with progressive desensitization, PKA-dependent signaling with its favorable components diminishes, whereas PKA-independent signaling does not,
due to increased activity of CaMKII.126–128 Thus, sustained increases in adrenergic drive changes the character of cardiac myocyte \(\beta_1\)-AR signaling, to favor more adverse downstream effects. On theoretical grounds what is needed in \(\beta_1\)-adrenergic inhibition strategies is something that will inhibit the PKA-independent arm of signaling, such as a CaMKII inhibitor with/without a \(\beta_1\)-blocker, but relatively spare PKA-dependent pathways, which can be done by increasing cAMP levels in desirable microdomains through concomitant delivery of the appropriate selective phosphodiesterase inhibitor.122

**Mechanisms of \(\beta_1\)-AR Downregulation and Desensitization of Response to Catecholamine Agonists**

Extensive work has been done on the mechanism(s) of \(\beta_1\)-AR downregulation and other desensitization changes in \(\beta_1\)-AR signal transduction mechanisms in the failing heart. In terms of the first abnormality identified, downregulation of \(\beta_1\)-ARS, a decrease in cognate mRNA137,138 due to mRNA destabilization139 is an important mechanism, as is probably increased protein turnover from enhanced internalization.140 Both \(\beta_1\)-141 and \(\beta_2\)-ARS\(^{58}\) undergo uncoupling in the failing heart, a process likely explained by receptor phosphorylation\(^{142,143}\) and increased activity or expression of the inhibitory G protein Gi.144,145 Strategies for using peptide\(^ {146}\) or small molecule147 inhibitors of GRK2-mediated receptor phosphorylation have shown promise, and, in addition to restoring receptor function, may improve contractility and reflexively reduce neurohormonal signaling.146

**MAP Kinase ERK1/2 Agonist Effects of \(\beta_1\)-AR Antagonists**

In addition to blocking the effects on \(\beta_1\)-ARS, certain \(\beta\)-blocking agents activate the MAP kinase ERK1/2 pathway, through both \(\beta_1\)- and \(\beta_2\)-ARS.148,149 The \(\beta\)-blockers that produce the greatest degree of ERK1/2 activation at doses that also block \(\beta_1\)-ARS are carvedilol, bucindolol, and propranolol, with metoprolol and bisoprolol having little or no effect.148,149 ERK1/2 activation is mediated at least in part by recruitment of \(\beta\)-arrestin as a scaffolding protein, and, through this complex, \(\beta_1\)-ARS transactivate EGF receptors.150 ERK1/2 activation in cardiac myocytes is antiapoptotic and cardioprotective\(^ {51}\) but also plays a role in \(\beta_1\)-AR–mediated...
cardiac myocyte hypertrophy.152 Thus, this atypical property of some β-blocking agents has uncertain therapeutic significance but could be exploited in future drug design if ERK1/2 activation or EGFR transactivation proves net beneficial.

β2-ARs and the Response to β-Blocking Agents

The β2-AR is present in brown fat, gut, urinary tract, and vascular smooth muscle.153,154 In vascular smooth muscle, the β2-AR is coupled to vasodilation through eNOS and nitric oxide (NO) production.154 The β2-AR is also expressed in the heart and coupled through Gi to NO generation and a negative inotropic effect.155 In the failing human heart, β2-AR expression is upregulated,156 in contrast to β1-ARs. Based on its signaling pathway and data with a receptor knock-out model,157 β2-AR signaling may serve a counter-regulatory function, but the role of the β2-AR in the natural history of myocardial failure is not fully understood.

Two β-blockers that have been used to treat heart failure are β1 agonists nebivolol158 and bucindolol,153,159 β2-AR activation coupled to NO generation is the mechanism of vasodilation for nebivolol158 and probably contributes to bucindolol’s vasodilator effects.159 However, it is unclear if an effect on myocardial β2-ARs contributes to any of the therapeutic actions of either agent.

Polymorphic Variation in β1- and β2-ARs

The targets of β-blockers in the heart, β1-ARs, and perhaps β2-ARs, exhibit much greater genetic variation than most genes coding for cardiac proteins.160 For example, single nucleotide polymorphisms, the most common type of genetic variation, are estimated to be present in 0.1–0.2% of the human genome but are present at a ≈1% frequency in the human β1- or β2-ARs.160 Moreover, some of these polymorphisms, such as the β1-Thr1641le variant161 and the β1-Arg389Gly,162 produce major functional effects on receptor function and signal transduction. The BEST trial contained the first DNA bank established in a large multicenter heart failure trial.116,163 Using this starting material Steve Liggett, whose laboratory had by this time characterized the majority of the common genetic variation in human adrenergic receptors, and colleagues conducted an investigation of six candidate adrenergic receptor polymorphisms on β-blocker (bucindolol) clinical responses. As expected, the allele frequency of the β1-Thr1641le variant161 was too small (0.01) in the sample size of 1040 to generate enough clinical events for analysis, but the β1-389 Arg/Gly allele frequencies of 0.69/0.31 provided ample numbers of clinical events to test the prespecified hypothesis that the 389Arg variant would be associated with a greater therapeutic effect than the counterpart Gly polymorphism.163

The amino acid position 389 Arg version of the human β1-AR is markedly different pharmacologically from the Gly version, with the former having 3 to 4 times the signal transduction capacity,162,163 a higher affinity for β-agonists162 including NE,117,164 and a higher probability of being constitutively active101 and sensitive to the effects of inverse agonists.163 These polymorphic variants are at least as different from another as are lower species β1- and β2-AR. The minor Gly allele, which by chance was the first cDNA cloned,104 exerts a dominant negative effect on NE affinity (unpublished data), constitutive activity and inverse agonist response,163 and clinical responses,163 such that effects in heterozygotes are similar to effects in Gly homozygotes.

From the standpoint of NE affinity, the β1-389 Gly receptor is more like a β2-AR than a β1-AR, and the high NE affinity of the β1-389 Arg-AR means that agents that lower NE will predominately inhibit β1-389 Arg signaling.

Among β-blockers that have been tested in Phase 3 heart failure trials, only bucindolol exhibits inverse agonist, inactive state stabilization effects on the human myocardial β1-389 Arg receptor.163 In addition, bucindolol is the only β-blocker that is sympatholytic.112 Given these pharmacological considerations, it would be predicted that bucindolol would produce greater therapeutic effects in patients with β1-389 Arg/Arg genotypes versus Gly carriers and that other β-blockers would be associated with less or no β1-389 pharmacogenetic differentiation.163 Figure 7A gives the placebo and bucindolol responses in the BEST DNA substudy for the combined end point of time to all-cause mortality or heart failure hospitalization, by β1-389 Arg/Gly genotype.163 Hazard ratios are calculated by treatment group within genotype and by genotype within treatment group. The by-treatment group hazard ratio within the β1-389 Arg/Arg subgroup is 0.67 (0.50, 0.89; P=0.005), whereas it is 0.86 (0.66, 1.11; P=0.24) in the Gly carrier subgroup. The by-genotype, within-treatment group analysis indicates that the selective efficacy is likely due to effects within the bucindolol group, where the Arg/Arg versus Gly carrier hazard ratio is 0.77 (0.57, 1.03; P=0.08) versus 0.99 (0.76, 1.30; P=0.95) in the placebo group.

A DNA bank was also established in MERIT-HF, and DNA from 600 patients was analyzed.165 When outcomes for metoprolol CR/XL were examined by β1-389 Arg/Gly genotypes, there was no evidence of any differentiation of effect for the all-cause mortality/heart failure hospitalization end point; within the metoprolol group, the hazard ratio for β1-389 Gly carriers versus Arg/Arg was 0.93 (0.62, 0.87; P=0.04) and in the placebo group, 1.0 (0.61, 1.64; P=0.99).165 It could be argued that differences in enrollment criteria between BEST and MERIT-HF may have influenced these results. However, Figure 7B gives results in β1-389 Arg/Gly genotypic subgroups in a cohort of BEST patients who were selected for the MERIT-HF/CIBIS-II enrollment criteria, the same as for the “BCG” group in Figure 5D. Within the MERIT-HF enrollment criteria (BEST Comparison Group, BCG) the differential response between genotypes is even more pronounced, with an Arg/Arg versus Gly carrier hazard ratio of 0.59 (0.40, 0.87; P=0.007) in the bucindolol group versus a nonsignificant hazard ratio of 1.02 in the placebo group. Another relatively large study (n=637) also has shown no β1-389 Arg versus Gly differentiation of clinical response to metoprolol, or carvedilol,166 and there are no reports that metoprolol or carvedilol clinical end point responses are affected by the β1-389 Arg/Gly genotype. This likely means that the basis for bucindolol’s differentiation is its unique pharmacological properties of β1-389 Arg inverse agonism157 and NE lowering.111,116 Because of the higher affinity of the β1-389 Arg receptor for NE158 the latter property has the effect
Mechanism of Action of β-Blockers in Heart Failure

β-Blockers have multiple favorable mechanistic effects on the failing heart, most if not all of which are mediated through blockade of β₁-ARs. On the basis of our original work in failing explanted and intact human hearts, we assumed that one of the mechanisms responsible for improved clinical status of heart failure patients treated with β-blocking agents was restoration of β-AR signaling, which would be accompanied by improved cardiac reserve and functional capacity. This hypothesis received strong support from an uncontrolled study of metoprolol’s effects on β-ARs and dobutamine responses, led by Mike Fowler at Stanford and completed after I had moved to the University of Utah. In this study metoprolol tartrate administered at an average dose of 103 mg/d was associated with a 2-fold increase in total β-AR density and dobutamine maximal dP/dT, and an increase in resting LVEF from 0.26 to 0.39. The β-AR and dobutamine response data were likely related, and even the LVEF improvement could have some basis in receptor upregulation if a fraction of receptors was constitutively low ATPase activity increases toward the normal range in the human heart, and the expected to improve contractile function. These favorable changes in MyHC isoforms and Serca2a would be expected to improve contractile function. These favorable changes in the FGP are mediated entirely through antagonism of β₁-AR–CAMKII coupling. Moreover, in the FGP, upregulates into the normal range in LVEF responders but decreases in nonresponders. At the protein level these changes in MyHC isoforms and Serca2a would be expected to improve contractile function. These favorable changes in the FGP are mediated entirely through antagonism of β₁-ARs, as the effects of b.i.d.-administered metoprolol tartrate and carvedilol were the same at average doses (125±47 mg/d and 70±29 mg/d, respectively) that reduced exercise heart rate to the same extent (by 29 bpm), and FGP induction in cultured neonatal rat cardiac myocytes is mediated through β₁-AR–CAMKII coupling. Moreover, in a mouse model in which cardiac myocytes expressing β-MyHC can be identified by imaging a fluorescent tag, only cells expressing β-MyHC exhibit β-AR desensitization,
suggesting that increased local adrenergic drive and fetal gene induction are cause-effect, and the reversal of FGP induction by β-blockers would improve contractile properties of the ventricle.

Another likely therapeutic mechanism is inhibition of β₁-AR-mediated apoptosis, which, like FGP reversal is signaled through CaMKII.125 Favorable metabolic effects, mediated through heart rate reduction and potentially other mechanisms such as carnitine palmitoyl transferase I inhibition174 almost certainly contribute to the β-blocker therapeutic response. Anti-ischemic properties in patients with coronary artery disease and ischemic cardiomyopathy175 are no doubt important, as are antiarrhythmic effects including prevention of ventricular tachycardia/fibrillation176 and atrial fibrillation.177 Of these potential salutary mechanisms, the most important effect of β-blockers on the dilated/hypertrophied and failing heart is to improve the biological properties of diseased heart muscle by effecting favorable changes in the expression of myocardial genes responsible for the regulation of contractile function and hypertrophy.

Can Antiadrenergic Therapy Be Improved Beyond Standard β-Blockade?

β-Blockade by compounds that are competitive antagonists of β₁-ARs has proved to be a remarkably effective way of treating chronic heart failure with reduced LVEFs, with 80–90% of symptomatic patients able to tolerate a therapy that produces a 20–35% reduction in all-cause mortality and comparable reductions in heart failure or cardiovascular hospitalizations. Moreover, the beneficial effects of β-blockers appear to be additive with all other proven/approved therapies. However, the heterogeneity in results between as well as within167 patient populations, and the tolerability issues in very advanced heart failure72,77,178 have prompted efforts to improve on standard single agent β-blockade as antiadrenergic therapy, thus far without success.

The failed attempts to improve on β-blocker therapy include the use of compounds with ISA in isolated or intact human heart,110,179 the addition of PDE3 inhibitors,122,124 the use of β-blockers with β₂-agonist activity,79,80 and the use of pure sympatholytic agents.180 As-yet untested possible new approaches are the addition of a β₂-agonist to a β₁-blocker,109 the use of pharmacogenetics to modulate the effects of sympatholytics into a more ideal, mild NE-lowering range,116 CaMKII inhibition130,131 added to β-blockade, the addition to β-blockade of agents that dephosphorylate receptors and restore receptor function,146,147 and prospective pharmacogenetic targeting of β-blockers that distinguish between different genetic variants of β₁-ARs.116,117,163 Although it is likely that one or more of these approaches will be successful, the fact that it has proved difficult to improve on single agent β-blockade is a powerful testament to the effectiveness of this form of heart failure therapy.

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Dr Bristow is an officer, director, and has equity in Arca Biopharma, which is developing pharmacogenetically targeted bucindolol and other drugs; he has equity and is an SAB member in Miragen, which is developing microRNA-based therapeutics; and he has equity and is an SAB member in Nite Therapeutics, which is developing drugs for acute heart failure.

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