

## Anti- $\beta_1$ -adrenergic receptor antibodies and heart failure: causation, not just correlation

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*J Clin Invest.* 2004;113(10):1379-1382. <https://doi.org/10.1172/JCI21748>.

### Commentary

Antibodies specific for the  $\beta_1$ -adrenergic receptor are found in patients with chronic heart failure of various etiologies. From work presented in this issue of the *JCI*, we can now infer that these antibodies actually contribute to the pathogenesis of chronic heart failure. This commentary discusses mechanisms by which these antibodies may engender cardiomyopathy.

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# Anti- $\beta_1$ -adrenergic receptor antibodies and heart failure: causation, not just correlation

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**Antibodies specific for the  $\beta_1$ -adrenergic receptor are found in patients with chronic heart failure of various etiologies. From work presented in this issue of the *JCI* (see the related article beginning on page 1419), we can now infer that these antibodies actually contribute to the pathogenesis of chronic heart failure. This commentary discusses mechanisms by which these antibodies may engender cardiomyopathy.**

Do anti- $\beta$ -adrenergic receptor (anti- $\beta$ -AR) antibodies play a role in the pathogenesis of chronic systolic heart failure (CHF)? This question emerged almost 30 years ago (1), when antibodies with  $\beta$ -adrenergic stimulating (agonist) activity were discovered in the serum of patients with Chagas disease, one of the most common causes of CHF worldwide (2). Since that time, IgGs with agonist activity for the  $\beta_1$ -adrenergic receptor ( $\beta_1$ -AR) have been found in sera not only from patients with Chagas disease, but also from patients with idiopathic dilated cardiomyopathy (3) as well as ischemic (4) cardiomyopathy. Whether these antibodies merely correlate with myocardial inflammation that leads to CHF, result from myocardial inflammation, or actually contribute to the pathogenesis of CHF could not be ascertained – until now. In this issue of the *JCI*, Jahns et al. employed isogenic injections of anti- $\beta_1$ -AR antiserum in inbred rats to produce a cardiomyopathy that appears to be non-inflammatory (5). In so doing, these authors conclusively demonstrated that agonistic, anti- $\beta_1$ -AR IgG – by itself – is sufficient to engender the sort of myocardial dysfunction characteristic of CHF. This finding fundamentally advances our understanding of CHF. However,

it should not really surprise us, because it represents a logical extension of diverse but congruent investigations conducted over several decades.

To provide historical and mechanistic perspectives for the elegant work of Jahns et al., we address several questions that relate their work to contemporary concepts of  $\beta_1$ -AR pathophysiology: How might IgG activate the  $\beta_1$ -AR, and how could chronic  $\beta_1$ -AR activation result in cardiomyocyte toxicity? What molecular mechanisms regulate the  $\beta_1$ -AR when it is chronically stimulated by IgG or other agonists, and how might these mechanisms affect the pathogenesis of CHF? Lastly, how can these perspectives elucidate the therapeutic efficacy of  $\beta$ -AR antagonists, or “beta blockers,” in CHF?

## Activation of cardiac $\beta_1$ -ARs

The  $\beta_1$ -AR constitutes approximately 80% of the cardiac  $\beta$ -AR complement. Like the  $\beta_2$ -AR (with 54% overall homology), the  $\beta_1$ -AR is a seven-membrane-spanning receptor, with three extracellular polypeptide sequences (“loops”) connecting the transmembrane  $\alpha$  helices (Figure 1). With their intracellular domains, the  $\beta$ -ARs couple to the stimulatory heterotrimeric GTP-binding protein ( $G_s$ ). Activation of the  $\beta_1$ -AR requires a specific receptor conformation – one that is stabilized by agonist (and, apparently the binding of certain IgGs to the second extracellular loop; ref. 5). It also appears that  $\beta_1$ -AR dimerization (6, 7) may be involved in receptor activation (and may underlie the agonist properties of anti- $\beta_1$ -AR antibodies observed in CHF patients and rats). Stimulation of  $\beta$ -ARs engenders a cascade of consequent activation: first  $G_s$ , then adenylyl cyclase

(which forms cAMP), then the cAMP-dependent protein kinase (PKA). Activated PKA subsequently phosphorylates molecules critical for regulating sarcoplasmic [ $Ca^{2+}$ ] (8) – thereby increasing cardiomyocyte inotropy, chronotropy, and lusitropy. Activation of  $G_s$  can also increase L-type  $Ca^{2+}$  channel currents directly (9, 10) (Figure 1). Over the last few years, this time-honored scheme has been modified in ways that illuminate the  $\beta_1$ -AR-specific findings of Jahns et al. (5).

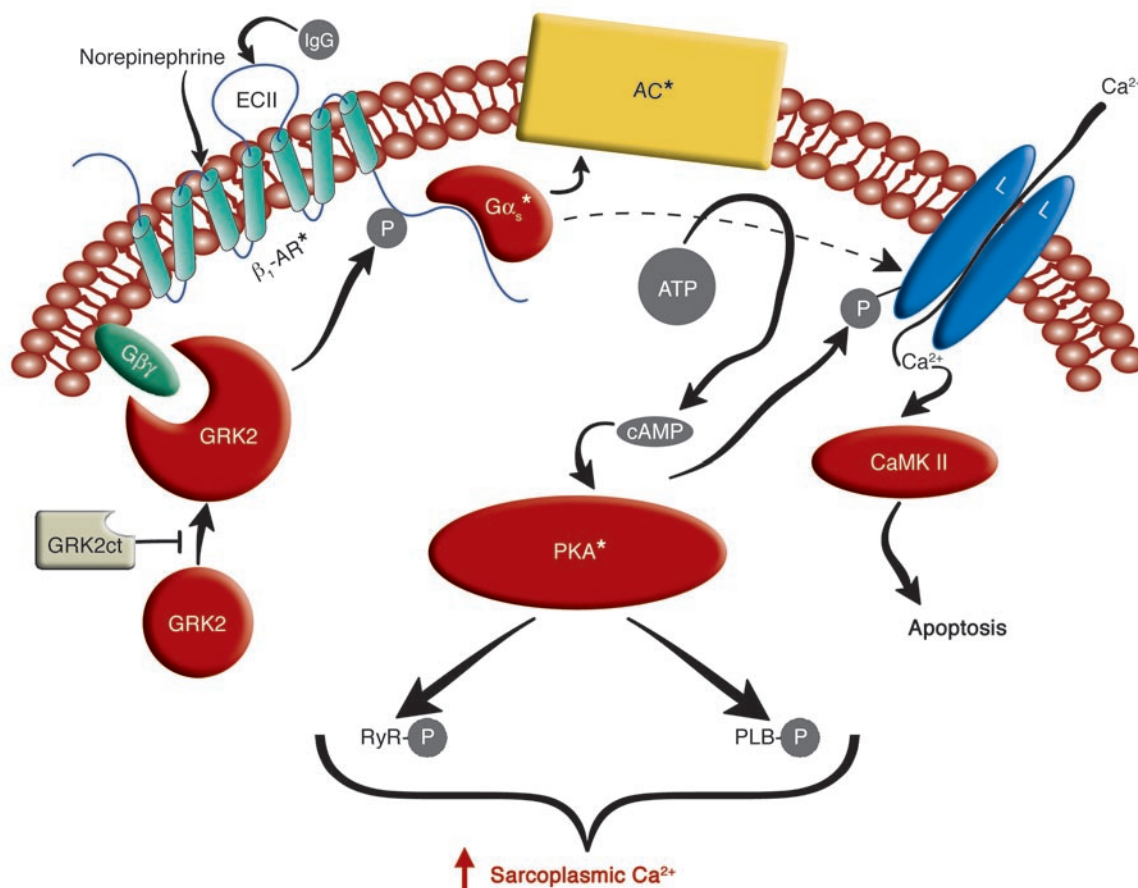
Genetic and pharmacologic approaches have demonstrated that the  $\beta_1$ -AR plays the predominant role in mediating cardiac inotropic and chronotropic responses to catecholamines (8). Cardiac preparations from  $\beta_1$ -AR knockout mice fail to augment contractility or their rate of contraction in response to isoproterenol, which stimulates both  $\beta_1$ - and  $\beta_2$ -ARs (11). Remarkably,  $\beta_1$ -AR knockout hearts have almost the same  $\beta_2$ -AR density as cognate wild-type controls, and can demonstrate contractile responses equivalent to controls – when contractility is augmented directly via adenylyl cyclase rather than via  $\beta$ -ARs (11). Even isoproterenol-induced adenylyl cyclase activity in cardiac homogenates is mediated overwhelmingly via the  $\beta_1$ -AR, in a manner disproportionate to the relative densities of  $\beta_1$ - and  $\beta_2$ -ARs (11). The failure of cardiac  $\beta_2$ -ARs to promote cAMP production to a degree commensurate with their expression level may be attributable to a signaling property that the cardiac  $\beta_2$ - and  $\beta_1$ -AR do not share: the ability to activate both the inhibitory heterotrimeric G protein ( $G_i$ ) and  $G_s$  (12, 13). (A  $\beta_1$ -AR subtype-specific intracellular binding protein appears to prevent  $\beta_1$ -AR/ $G_i$  coupling [ref. 14].) In human subjects,  $\beta_2$ -AR-selective agonists have been used to augment left ventricular inotropy (15). However, a large fraction of this inotropic effect is mediated by  $\beta_1$ -ARs, and the role of  $\beta_2$ -ARs in this process may be largely to augment norepinephrine release from cardiac sympathetic neurons (15).

**Nonstandard abbreviations used:**  $\beta$ -adrenergic receptor ( $\beta$ -AR);  $\beta_1$ -adrenergic receptor ( $\beta_1$ -AR); chronic systolic heart failure (CHF); cyclic AMP-dependent protein kinase (PKA); G protein-coupled receptor kinase-2 (GRK2); GRK2 carboxyl-terminal polypeptide (GRK2ct); inhibitory heterotrimeric G protein ( $G_i$ ); stimulatory heterotrimeric GTP-binding protein ( $G_s$ ).

**Conflict of interest:** R.J. Lefkowitz is cofounder of Norak, a company developing an inhibitor to GRK2.

**Citation for this article:**

*J. Clin. Invest.* 113:1379–1382 (2004).  
doi:10.1172/JCI200421748.



**Figure 1**

Schema for  $\beta_1$ -AR-mediated cardiomyocyte stimulation and  $\beta_1$ -AR desensitization. The seven-membrane-spanning  $\beta_1$ -AR is stimulated by the physiologic agonist norepinephrine or by IgG specific for the receptor's second extracellular loop (ECII) (in CHF subjects). The stimulated  $\beta_1$ -AR then activates the heterotrimeric  $G_s$ , which dissociates into its  $G\alpha_s^*$  and  $G\beta\gamma$  subunits. The  $G\alpha_s^*$  activates both adenylyl cyclase (AC), which catalyzes cAMP formation, and the L-type calcium channel (L), which then permits  $Ca^{2+}$  to enter the cardiomyocyte. This  $Ca^{2+}$  can both augment contractility and, on a slower time scale, promote cardiomyocyte apoptosis by activating  $Ca^{2+}$ /calmodulin kinase II (CaMK II). The cAMP produced by adenylyl cyclase activates PKA, which subsequently phosphorylates (P) numerous substrates important to sarcoplasmic  $[Ca^{2+}]$  regulation: the L-type  $Ca^{2+}$  channel, the ryanodine receptor (RyR), and phospholamban (PLB). The net effect of this activity in the short term is to augment sarcoplasmic  $[Ca^{2+}]$  and contractility. (In the long term, this activity engenders cardiomyocyte toxicity.) The activated  $\beta_1$ -AR is desensitized when it is phosphorylated by PKA (not shown) and by GRK2, the cellular expression of which increases with chronic  $\beta_1$ -AR stimulation. Translocation of GRK2 from the sarcoplasm to the sarcolemma requires  $G\beta\gamma$  subunits, and this translocation is inhibited when GRK2ct is expressed heterologously in cardiomyocytes. Asterisks denote activated proteins.

### Excessive $\beta_1$ -AR activation yields cardiomyocyte toxicity

Excessive isoproterenol stimulation has long been known to produce cardiomyocyte toxicity, myocardial scarring, and CHF (16, 17). More recently, chronic administration of submaximal isoproterenol doses has also been shown to produce cardiomyopathy, independent of myocardial scarring (18). That this isoproterenol-induced cardiomyopathy results primarily from  $\beta_1$ -AR activation can be inferred from the  $\beta_1$ -AR knockout mouse studies discussed above (11), as well as from a host of in vitro studies with rodent cardiomyocytes (13, 19). Further evidence that chronic  $\beta_1$ -AR hyperstimulation causes cardiomyocyte toxicity

has emerged from studies with transgenic mice displaying modest (~15-fold), cardiac-specific overexpression of the  $\beta_1$ -AR: these mice not only possessed enhanced  $\beta_1$ -AR activity, but also developed cardiomyopathy by age 4–9 months (20). In contrast, transgenic mice overexpressing the  $\beta_2$ -AR at higher absolute levels failed to develop any cardiomyopathy by this age (21).

The particular signaling pathways responsible for  $\beta_1$ -AR-induced cardiomyocyte toxicity remain somewhat enigmatic. Increasing cardiomyocyte PKA activity by as little as 2.4-fold can engender CHF (22), and cardiomyocyte overexpression of  $G\alpha_s$  engenders CHF (23). However, overexpression of adenylyl cyclase type VI

(which also augments cardiomyocyte cAMP levels) not only avoids CHF but also can alleviate CHF in the  $G\alpha_q$ -overexpressing mouse (24). In addition,  $\beta_1$ -AR-promoted cardiomyocyte apoptosis can result from  $Ca^{2+}$ /calmodulin-dependent protein kinase activity, independently from the PKA pathway (19). The diverse studies delineating molecular mechanisms responsible for  $\beta_1$ -AR-promoted cardiomyocyte toxicity have been reviewed recently (8). In light of these data, it is intriguing that levels of plasma norepinephrine (which activates the  $\beta_1$ -AR, like the anti- $\beta_1$ -AR IgG of Jahns et al. [ref. 5]) have been directly and independently associated with CHF mortality in human subjects (25).



### Regulatory mechanisms for subduing the $\beta_1$ -AR signaling system

In the face of persistent  $\beta_1$ -AR hyperstimulation (either in CHF or experimental systems), both receptor-based and non-receptor counter-regulatory mechanisms are engaged in the cardiomyocyte — perhaps to bridle cellular toxicity. These mechanisms result in approximately 50% downregulation of the  $\beta_1$ -AR itself (26) (through mechanisms that appear to involve PI3K [ref. 27]), a decrease in adenylyl cyclase activity, and upregulation of the multifunctional  $G_i$  (which can inhibit adenylyl cyclase) (23). In addition, myocardium from CHF patients demonstrates a two- to threefold upregulation of G protein-coupled receptor kinase-2 (GRK2) (26), which phosphorylates and desensitizes the  $\beta_1$ -AR (28, 29). This upregulation of GRK2 even precedes the onset of left ventricular failure in mice with transgenic myocardial overexpression of calsequestrin (30). Although these “desensitizing” mechanisms are insufficient to prevent CHF, some of them may, paradoxically, contribute to CHF pathophysiology. Perhaps the most thoroughly studied of these cases is the role of GRK2.

### Relieving excessive $\beta_1$ -AR desensitization

Inhibition of cardiomyocyte GRK2 activity has been shown to ameliorate CHF in several mouse models, including deficiency of muscle LIM protein (31) and myocardial calsequestrin overexpression (32). GRK2 inhibition in these studies was achieved by transgenic myocardial overexpression of a polypeptide that comprises the carboxyl-terminal third of GRK2. This molecule, termed GRK2ct, inhibits GRK2 activity on receptors by binding to the heterotrimeric G protein  $\beta\gamma$  subunits required for GRK2 recruitment to the receptors (33) (Figure 1). Because “GRK2ct therapy” presumably does not alter the fundamental cardiomyocyte problems leading to myocardial dysfunction in these mouse models, its success points to the possibility that CHF-related enhancement of cardiomyocyte GRK2 activity may itself be maladaptive, and contribute to the pathogenesis of CHF. Remarkably, GRK2ct-expressing myocardium demonstrates attenuation of CHF-related GRK2 upregulation (31),  $\beta_1$ -AR downregulation (31), and  $\beta_1$ -AR/adenylyl cyclase desensitization (31, 32). In interpreting these data, however, it is important to note that GRK2ct binds a large variety of  $G\beta\gamma$  subunits as well as phosphatidylinositol 3,5-bisphosphate

(33). These binding activities could, beyond inhibiting GRK2, also contribute to the effects observed with GRK2ct (27, 34).

### $\beta$ -AR antagonists: possible mechanisms underlying therapeutic efficacy

Improvements in myocardial contractility, exercise tolerance, and mortality have been observed in CHF patients treated chronically with the  $\beta$ -AR antagonists metoprolol, bisoprolol, and carvedilol (35). This clinical improvement cannot depend upon reversal of  $\beta_1$ -AR downregulation, since carvedilol does not promote such a reversal (35). Moreover, this clinical improvement seems unlikely to be mediated through  $\beta_1$ -ARs at all, since it can be observed under conditions precluding agonist stimulation of  $\beta_1$ -ARs (36). However, probably because it diminishes toxic hyperstimulation of the  $\beta_1$ -AR by elevated sympathetic tone, prolonged  $\beta$ -AR antagonist therapy reduces cardiomyocyte apoptosis in CHF (37) and effects a partial recovery of CHF-associated derangements in gene expression (38, 39) and PKA-mediated hyperphosphorylation of proteins (40, 41), including those constituting the signaling and  $Ca^{2+}$ -handling machinery downstream of the  $\beta_1$ -AR. These “receptor-distal” mechanisms can enhance myocardial performance despite ongoing  $\beta$ -AR antagonist occupancy (36).

As a specific example of how mechanisms distal to the receptors can affect cardiomyocyte function, let us again consider the role of GRK2 in CHF. Bisoprolol (42) and carvedilol (43) have been used in animals to reduce GRK expression. (We should note that bisoprolol was used by Jahns et al. to abolish the adenylyl cyclase response to anti- $\beta_1$ -AR IgG [ref. 5].) Because GRK2 can desensitize a multitude of heptahelical receptors (33), reduced GRK2 levels could enhance signal transduction, and thus inotropy, evoked by heptahelical receptors other than the (antagonist-occupied)  $\beta_1$ -AR, such as endothelin receptors (44). From the perspective of this hypothesis, it is intriguing that metoprolol and GRK2ct reduced calsequestrin-associated cardiomyopathy in a synergistic manner (32).

### Perspectives

From decades of CHF investigations, we should indeed have expected that agonistic, anti- $\beta_1$ -AR antibodies *would* promote the development of heart failure. Now that Jahns et al. have provided an important proof of principle (5), we have another excellent reason to increase the clinical use of  $\beta$ -AR antagonist therapy in CHF of all

causes. Whether immunoabsorption of anti- $\beta_1$ -AR antibodies (45) will provide CHF patients with benefits beyond those obtainable from  $\beta$ -AR antagonists alone, however, remains to be determined.

### Acknowledgments

R.J. Lefkowitz is an Investigator of the Howard Hughes Medical Institute. This work was also supported in part by NIH grants HL-64744 (N.J. Freedman), HL-16037, and HL-70631 (R.J. Lefkowitz).

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## Dissecting the functional role of different isoforms of the L-type $\text{Ca}^{2+}$ channel

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**There currently exist a great number of different mouse lines in which the activity of a particular gene of interest has been inactivated or enhanced. However, it is also possible to insert specific mutations in a gene so that the pharmacological sensitivity of the gene product is altered. An example of such an approach shows how the abolition of the sensitivity of an L-type  $\text{Ca}^{2+}$  channel isoform to dihydropyridines allows the investigation of the physiological role of these channels in different tissues (see the related article beginning on page 1430).**

**Nonstandard abbreviations used:** dihydropyridine (DHP); L-type  $\text{Ca}^{2+}$  channel (LTCC).

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Citation for this article:**  
*J. Clin. Invest.* **113**:1382–1384 (2004).  
doi:10.1172/JCI200421815.

### The LTCC family

L-type  $\text{Ca}^{2+}$  channels (LTCCs) are formed by different pore-forming  $\alpha_1$  subunit isoforms named  $\text{Ca}_v1.1$ ,  $\text{Ca}_v1.2$ ,  $\text{Ca}_v1.3$ , and  $\text{Ca}_v1.4$  associated to auxiliary subunits ( $\alpha_2\text{-}\delta$ ,  $\beta$ , and  $\gamma$ ) (1). The common pharmacological hallmark of all native and recom-

binant LTCCs is their sensitivity to dihydropyridines (DHPs). However, the small differences among the LTCC  $\alpha_1$  isoforms in their affinity for DHPs (agonists and antagonists) have limited the study of the functional role of these channels in various tissues, including the cardiovascular system, the brain, and the endocrine glands.

In this issue of the *JCI*, Sinnegger-Brauns and coworkers report that they have developed a new mouse model resulting from a knock-in mutation of the  $\text{Ca}_v1.2$  voltage-dependent LTCC subunit which abolishes the sensitivity of the channel to DHP (referred to herein as the  $\text{Ca}_v1.2\text{DHP}^{-/-}$  mouse) (see Figure 1) (2). Since  $\text{Ca}_v1.2$  is